The incidence and impact of Human Papillomavirus in HIV infected transplant patients

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ENGJAN004

SUBMITTED TO THE UNIVERSITY OF CAPE TOWN

In fulfilment of the requirements for the degree

Master of Medicine (MMed) in Surgery

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

August 7th, 2017

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Declaration

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Abstract

The incidence and impact of Human Papillomavirus in HIV infected transplant patients

Background

Human Papillomavirus (HPV) is a common sexually transmitted infection, associated with condylomata acuminata, anogenital squamous intraepithelial lesions, and ultimately invasive squamous cell carcinoma. HPV types 16 and 18 are the most common subtypes in individuals with cervical cancer. The association with these two subtypes in individuals with squamous carcinoma of the anus is fundamentally the same as with cervical cancer, and also affects the same high risk phenotype. Human imunnodeficiency virus (HIV) positive transplant patients have two modes of immunosuppression – the disease itself and the additional immunosuppression required after transplantation, which intuitively places them at a higher risk for this type of infection, if compared to their HIV negative counterparts.

Aim

The first aim is to determine the prevalence of HPV-associated cytological and pathological abnormalities of the anus in HIV positive kidney transplant recipients and the second aim is to determine if HIV positive solid organ recipients carry higher risk for having HPV of the anus than HIV negative solid organ recipients.

Materials and methods

This is a cross sectional study, conducted at the Transplant unit of Groote Schuur Hospital. 14 HIV positive renal transplant recipients and 14 age matched HIV negative renal transplant recipients with similar immunosuppression regimens and time from transplantation were selected. Ethical approval for the study was obtained from the UCT Ethics committee (HREC/REF: 595/2014). Informed

consent was obtained from all participants. Samples for cytology and histology were taken from the anal canal. Demographic data was collected, date of HIV diagnosis, duration on anti-retroviral drugs, time since transplant, type of immunosuppression, whether there was visible condylomata or not and if there were any lesions suspicious of cancer. Cytology and histology was correlated with clinical findings. The statistics were analysed with Stata® software.

Results

Mean age was 40.8y ±7.5 (range 27-52) in the HIV positive study group and 41y ±14.4 (range20-62) in the HIV negative control group. HIV positive patients were screened 40.1 months ± 21 (range 13-74.6m) post renal transplant. HIV negative patients were screened 55.9 months ± 23.3 (range 8.9-80 m) post renal transplant. Two HIV positive patients had anal warts, compared to 1 in the HIV negative group. No statistically significant difference could be demonstrated between the occurrence of intra-epithelial neoplasm on cytology in the HIV positive and negative groups. However, HIV positive patients had a higher incidence of HPV on histology that was statistically significant. There was no evidence of squamous intra-epithelial neoplasm found on histology in either group.

Conclusions

Evidence of HPV of the anus was demonstrated in both groups, there was no demonstrable statistical significance in occurrence between the two groups' cytology. Histology, however, yielded a significant number of patients with HPV in the HIV positive group. None of the patients had evidence of invasive malignancy.

Background

Introduction

Human papillomavirus deoxyribonucleic acid (DNA) is detected in near 100% of anogenital cancers.[1] 60-70% of all HPV infections are eradicated spontaneously, but the remainder are at risk of becoming chronic infections, and progressing to dysplasia and eventually invasive cancer. High risk subtypes (HPV 16 and 18) take longer to eradicate than low risk subtypes.[2 3] Individuals with impaired cellular immunity are at greater risk for HPV infections and anogenital cancer.

Human papilloma virus characteristics

HPV is a small non-enveloped virus, comprised of approximately 8000 base pairs of double stranded viral DNA and an icosahedral capsid comprised of proteins E1 and E2.[4]

The virus requires access to the basal layers of epithelium by either mechanical micro trauma or reduced mucosal barrier function. The virus is internalised into the basal cells, and the viral genome is integrated into the nucleus, mediated by the E2 protein.[5] As the infected cell moves towards the mucosal surface, the rest of the viral genome is activated, replicating it's various components, last of which is the E1 and E2 proteins which is required for the complete virus assembly, being shed with the top mucosal cell layers. This process takes 3 weeks from infection to shedding. [5 6]

The virus is completely dependent on the host for its DNA replication, which only a mitotically active cell is capable of doing. Both the low and high risk viruses express proteins E6 and E7 which act to induce DNA synthesis and cease

apoptosis in cells that are no longer mitotic.[1] E6 inactivates p53, a tumour suppression gene, which leads to failure in apoptosis in cells deficient in the retinoblastoma protein (pRb), also a tumour suppressor protein. E7 acts to inactivate pRb, hence the pRb deficient cells.[7] The action of E6/E7 differs between high and low risk HPV subtypes, in that the high risk subtype E6/E7 expression is able to induce immortality and uncontrolled proliferation in keratinocytes, leading to dysplasia and ultimately invasive cancer.[5-7]

There are over 150 viral types identified to date, of which more than 40 infect the human anogenital tract.[8] They are highly species specific and cannot be transmitted interspecies. [1] The most common types isolated from anogenital warts are 6, 11, 16 and 18. Subtypes 6 and 11 (low risk subtypes) are most often isolated from genital warts and low grade squamous intra-epithelial lesions. The WHO has identified 12 high risk cancer causing HPV subtypes, namely 16, 18, 31, 35, 39, 45, 51, 52, 56,58 and 59.[5] HPV 16 and 18 are the most common high risk types, 10-20% of cervical cancers have DNA evidence of HPV 18 and 45-65% of cervical and anal cancer have DNA evidence of HPV 16, comprising the bulk of anogenital cancers. [9]

HPV immunity

Viral clearance is dependent on the interaction between innate immunity and adaptive immunity. Cell death or injury sets off a cascade of inflammation, recruiting local innate immune cells, most importantly dendritic cells, that phagocytose the offending antigen, and presents a surface antigen to a T-cell for destruction. Mucosal dendritic cells are called Langerhans cells.

There are 2 major subsets of T-cells - CD4 which recognises exogenous antigens presented on the dendritic cell surface by the major histocompatibility complex (MHC) class II, and CD8 which recognises endogenous antigens bound to MHC class I on the dendritic cell surface.

B-cells are produced by bone marrow and stored in the spleen, lymph nodes and other immune reservoirs. They are able to recognise antigens directly without help of APC's. If a naïve B-cell is activated, plasma cells get activated, releasing a large amount of soluble immunoglobulin into the blood stream, these immunoglobulins are identical to those expressed by the B-cell. Immune memory develops and a subsequent exposure to the same antigen leads to higher affinity and more rapid binding and more effective clearing of the antigen. This is the basis for vaccination. [3]

HPV is dependent on the normal lifecycle of the keratinocyte for replication and there is no blood borne phase of the infection. The keratinocyte is destined for death, and the newly assembled virus is propagated to new victims as the cell desquamates. The keratinocyte's lifecycle is short and the virus has no need to destroy the host cell. Because of this, there is no viral induced cell death or injury, therefor no inflammation, which means no activation and migration of dendritic cells in the mucosal basal layer to infected cells. Most viruses entering into a basal cell should trigger Langerhans activity, by recognition of pathogen-associated molecular patterns. The Langerhans cell should then migrate to the nearest draining lymph node and present the antigen to a naïve T-cell, which should migrate back to the site of infection, and destroy the offending cell.

Host immunity to HPV had only been studied in animal models and on biopsies of regressing warts, and these studies demonstrated high numbers of T-cells (CD4 and CD8), B-cells and macrophages in the wart stroma. Failure of activation of this mechanism leads to persistent infection, and mounting an immune response is dependent on an intact innate and adaptive immunity. [1 3]

Sequela of HPV infection

HPV is a common sexually transmitted infection which is most prevalent in young women aged 18-25 years of age. The lifetime risk of contracting the virus is 70%. 60-70% of HPV infections (combined high and low risk subtypes) will be eradicated spontaneously in a 20 -30 month period, for low risk infections, this number is 90%. High risk (oncogenic) subtype infections appear to be more effective at evading host immunity. [2] It takes 5-6 months to eradicate a low risk HPV infection, and 8-14 months to clear a high risk HPV infection.[3]

Risk factors for contracting the virus are sexual activity related i.e., high number of sexual partners, presence of genital warts in partners and lifetime number of partners. Oral contraceptive use is associated not only with higher risk of warts, but with increased risk high grade squamous intra-epithelial neoplasm. Impaired cellular immunity associated with solid organ transplant and HIV infection result in higher rates of anogenital HPV infection.[10] The use of oral contraception and sexual behaviour do not appear to affect eradication of the virus.[2]

High risk subtypes of HPV can be diagnosed with PCR and are associated with squamous-, adeno- and adenosquamous carcinomas of the anogenital tract. Almost 100% of cervical cancer is associated with high risk subtypes of the HPV.[1 9] Patients that are unable to eradicate the virus (10% of high risk infections) are at risk of developing cancer and having persistent infections. Cervical cancer is the most common cancer in females in the developing world. [1]

Cervical and anal cancer are biologically similar, they share HPV as an etiologic agent, and they both arise in a mucosal transformation zone connecting columnar and squamous epithelium.[11 12]

Human immunodeficiency virus (HIV) and HPV

The HIV related decrease in the CD4 T cell population is partly responsible for impaired immunity to HPV.[1 3]

HIV infection causes reduced numbers of CD4 T cells by two mechanisms: 1) infecting activated CD4 T cells cause rapid burst proliferation which leads to cell death and 2) supressing the production of naïve CD4 T cells from the thymus by either virus induced thymocyte destruction or immunologically supressing production. Suppressing the host's viral load reverses these effects and result in a higher number of circulating CD4 cells.[13] In addition to the effects of immunosuppression in a transplanted patient which promotes the persistence of HPV infection, co-infection with HIV may directly promote HPV associated oncogenesis at the molecular level. In vitro studies suggest that the HIV-encoded tat protein may enhance expression of the HPV E6 and E7 proteins.

HIV also causes disruption of the basal cell layer integrity via expression of the tat and pg120 proteins, which interfere with normal tight junction internalization in the basal cell layer of epithelium, leading to decreased integrity and enhanced access to basal cells for HPV.[14 15]

The incidence of HPV infection is 16 times higher in HIV positive patients than in HIV negative patients. The risk for invasive cancer (HPV associated) is 14 times greater. [16] The risk for developing invasive anal cancer in AIDS is 7 times higher in women and 38 times higher in men, compared to HIV negative patients.[17]

Multiple HPV type infections are more common in HIV positive patients, 73% compared to 23% in HIV negative patients. Patients with multiple HPV infections are at greater risk of developing progression from low grade to high grade lesions over time.[12]

Regression rates of lesions in HIV negative patients are significantly less than in HIV positive patients. A San Francisco study reports lesion regression in HIV positive patients 5-30% compared to 50 to 60% in HIV negative patients.[18]

Risk factors for developing high grade dysplasia are a high HIV viral load and lower CD4 T lymphocyte count. HIV positive patients with a CD4 count of < 200/mm^2 have a threefold increased risk for disease progression and a CD4 count of >500/mm^2 confers double the risk compared to HIV negative patients. In addition to this, HIV positive patients with high grade dysplasia have been shown on histology to have half the amount of Langerhans cells in their anal mucosa when compared to HIV negative patients, which significantly increases the risk for developing high grade dysplasia. [18 19]

Anti-retroviral therapy and HPV

By the end of 2015, there were 36.7 million people living with HIV in the world. It was estimated that 18.2 Million of them were on Highly Active Anti-retroviral Therapy (HAART).[20] The HAART program was rolled out in 1996 in the USA and had an enormous positive impact on overall survival in HIV positive individuals by decreasing HIV viral load and increasing CD4 lymphocyte numbers. In South Africa HAART was only approved in 2006 and a strategic plan to provide treatment to HIV positive patients in South Africa was approved in 2007.[21]) In middle to low income countries, life expectancy has increased by about 30 years in an individual starting HAART at the age of 20 and another 20 years for those starting HAART at 35 years old. Before 1996, the average life expectancy after HIV diagnosis was 10 years.[22] Since the advent of HAART, the mean age of onset of acquired immunodeficiency syndrome (AIDS) has increased, and 2 year survival after the onset of AIDS has improved from 49.2% in the pre-HAART era to 90.0% in the post HAART era. The current World Health Organization (WHO)

definition of AIDS is a CD4 count of less than 200, or any of a list of 20 opportunistic infections or cancers, including invasive cervical cancer, but not anal cancer.[20 23]

The incidence of invasive anal cancer has risen dramatically with the advent of HAART. Reported incidence of anal cancer in HIV positive males from 1980-1989 was 10.5 cases per 100 000 person-years, 20.7 cases per 100 000 person-years from 1990 to 1995 and 42.3 cases per 100 000 person-years from 1996 to 2004. In the period between 1990 and 1995, anti-retroviral drugs had become in use, but most often as mono and dual therapies, and were not yet protocol based, as with the roll out of HAART in 1996. Increased screening did not contribute to this increase in incidence, as the distribution of stages of anal cancer in both the pre-and post HAART era were the same. [24]

As previously stated, a low CD4 is associated with decreased disease regression and increased progression to invasive cancer. [17 18] This data is from the HAART era. Contrastingly, before 1996, there was no significant association between a low CD4 count and increased risk for developing invasive anal cancer. Life expectancy was too short to permit disease progression, and mortality was mostly from opportunistic infections.[24]

Immunosuppression in solid organ transplant

Murray and co-workers performed the first successful renal transplant in 1958, made possible because the recipient and donor were monozygotic twins. The graft survived for 11 months. [25 26]

For several years after that, survival after transplant was dismal. In the 50's, recipients received whole body irradiation, but apart from isolated success, mortality soon after transplant was common.[27 28] In France, steroids were used in conjunction with irradiation, without much success.[28]

In the early sixty's, Azathioprine was synthesized in a laboratory in Boston, and Starzl's work in the sixties, using dual therapy with azathioprine and steroids, increased 1 year survival to around 50%. [29 30].

In the late 70's, the first calcineurin inhibitor (CNI), cyclosporine was synthesised, [31] added as a third agent in as triple therapy and had increased 1 year survival to around 85-95% by the year 2000. [32]

By the late 80's another 2 drugs came into use – another CNI namely tacrolimus, along with the first mammalian target of rapamycin (mTOR) inhibitor, sirolimus. [33]

The 90's saw the development of mycofenolate mofetil and mycofenolate sodium, as well as the first monoclonal anti-bodies used in transplant, namely basiliximab and dacluzimab, both CD25 antagonists. [34]

There are four classes of transplant immunosuppressive drugs used for maintenance.

1. Anti-proliferatives

   Azathioprine is a prodrug that is converted to 6-mercaptopurine and then metabolised to cytotoxic 6-thioguanine nucleotides (6-TGN), which is incorporated into lymphocyte DNA and halts cell replication. 6-TGN is also reduced and phosphorylated to deoxy-6-thioguanine triphosphate, which binds to the GTPase Rac 1 receptor on lymphocytes. Blocking of Rac1 triggers cell cycle arrest via a mismatch repair pathway. An intermediate product in the path from AZA to deoxy-6-thioguanine triphosphate, thionosine monophosphate, is converted to s-methylthionosine-monophosphate, a powerful inhibitor of de novo purine synthesis, leading to halt in DNA replication. The effect of AZA is thus dual – halting lymphocyte proliferation and apoptosis of activated lymphocytes.[34 35]

   Mycofenolate mofetil and mycofenolate sodium inhibits purine synthesis by blocking inosine monophosphate dehydrogenase (IMPDH) type II, only

present on the surface of activated lymphocytes, therefor selectively inhibits proliferation of activated lymphocytes.[34]

2. Calcineurin inhibitors

   Calcineurin inhibitors bind to intracellular proteins (immunophilins) that block the effects of clacineurin effectively resulting in a reduction of interleukin-2 and reduced T cell proliferation. Tacrolimus is more potent than Cyclosporin and binds to a different immunophilin (FK-binding protein) to inhibit calcineurin.[34]

3. mTOR inhibitors

   Sirolimus: inhibition of mTOR results in blockade of T cell activation by halting cell cycle progression from the G1 to S phase. [34]

4. Steroids

   Prednisone and prednisolone: influence gene transcription resulting in a global reduction in cytokines, with a net result of impaired monocyte and macrophage function and reduced CD4 lymphocyte numbers.[34]

Thymoglobulin

The polyclonal antibody, rabbit anti-thymocyte globulin (ATG) is used as induction therapy in high risk renal transplant recipients. ATG is produced by introducing human thymocytes to rabbits. The resulting antibody is polyclonal, targeting all cells produced in the thymus. The thymus comprises 72% T lymphocytes, 6% B lymphocytes and the remainder antigen presenting cells and stromal cells. The net effect of ATG administration is rapid T cell depletion, but due to its polyclonal nature, also B cell, NK cell and dendritic cell depletion. [36 37]

In previous studies it was demonstrated that HIV positive renal transplant recipients treated with ATG induction therapy, had a significantly greater decline in CD4 count at one year post transplant than those not treated with ATG induction therapy, -239 vs. -135. [38]

All these drugs produce either decreased T-cell function or reduced T-cell numbers. None of them are T-cell specific, and all of them influence B-lymphocytes to varying degrees as well. HIV positive transplant recipients will therefore have a dual onslaught to their T-cells, HIV and transplant immunosuppression.

HPV eradication requires an intact cellular immune response, most notable, normally functioning T lymphocytes.[1 3]

HPV and anal cancer in solid organ transplant

In 2003 Adami et al. reported a standardised incidence ratio (SIR) of 10.2 for squamous cell carcinoma of the anus, and a SIR of 4 for all cancers in patients from the Swedish Transplant Registry from 1970-1997.[39]

In 2011, Engels et al reported their study of the US Scientific Registry of Transplant Recipients from 1987-2008. The risk for all cancers were elevated two to four fold in solid organ transplant patients, translating into an overall incidence of 1375/100 000 person years and a standardised incidence ratio of 2.1. The highest risks were for cancers with viral aetiologies were anogenital cancers (HPV), Hodgkin and non-Hodgkin lymphoma (EBV), Kaposi's sarcoma (HHV8) and liver cancer (Hepatitis B and C). Anal cancer was nearly 6 times more common (SIR 5.7) in these transplant recipients than in the general population.[40]

In 2013, Madeleine et al. echoed Engels' findings in their publication on the incidence of HPV related cancers in solid organ transplant recipients of the US Transplant Cancer Match (TCM) Study, with a SIR of 5.4 for anal cancer.[41]

In a study by Ogumbiyi et al the overall incidence of HPV infection in solid organ transplant recipients was reported as 47%. In the same study it was reported that incidence of anal intra-epithelial neoplasm was 20% in these patients, compared to 1% in the patient group presenting for elective anogenital surgery [42-45] Other studies report that invasive anal cancer in transplant recipients is 6-10 times more common than in the general population. [40 44 46] The incidence rate of in situ anal cancer in solid organ transplant patients is 6.3/100 000 person years and 11-12/100 000 person years for invasive anal cancer.[40 41]

In the US it is estimated that the incidence of cancer 15 years after a solid organ transplant is around 15%. Madeleine et al reported 103 cases of invasive anal cancer amongst 187649 transplant patients. It is therefore estimated that 1 in 2000 of all cancers in transplant patients will be invasive anal cancer.[41 47]

In a large meta-analysis in 2007, the standardised incidence ratio (SIR) was significantly higher for anal cancer in HIV positive patients than for solid organ transplant recipients, 28.75 vs. 4.85. [48] It is well known that only 40% of transplant recipients with invasive anal cancer have a history of anal warts. [42]

Patients with HIV have 10 times the risk of cancer (all types) compared to the general population, but twice the risk of cancer (all types) when compared to solid organ transplant recipients. [49]

The use of older immunosuppressive drugs (cyclosporine and azathioprine) confers double the incidence of anal cancer compared to newer drugs (MMF and tacrolimus). Cyclosporin A promotes tumourgenesis and growth by inducing TGF-β and causing a dose dependent inability to repair DNA. MMF's anti-tumour properties are related to its negative influence on de novo purine synthesis.[50] Patients on sirolimus (mTOR inhibitor), showed a significantly decreased incidence of invasive cancer. The use of induction therapy does not impact the incidence of invasive anal cancer.[41]

Screening for HPV

The anus and the cervix share an embryological origin both develop dysplasia and cancer from the human papillomavirus and cytology for both can be classified according to the Bethesda system. [51]

The Bethesda system for reporting endo-cervical smear samples was first published in 1988. Its goal is to improve communication of cervical cytology results from the laboratory to the clinician. [52]

The 2001 Bethesda system firstly evaluated specimen adequacy, adequate cellularity (at least 10 endo-cervical or squamous metaplastic cells) is noted and the presence or absence of the squamo-columnar transformation zone is note. If a specimen is inadequate, the reason is noted in the report.[52]

The reporting system consists of the following categories:

1. *Negative for intra-epithelial lesion or malignancy*

   Reporting any non-neoplastic findings is optional

2. *Epithelial cell abnormalities: atypical squamous cells of uncertain significance (ASCUS)*

   To describe epithelial abnormalities that was too severe to be attributed to reactive changes, but was not sufficient to diagnose a squamous in situ lesion (SIL). Pathologists are encouraged to mention whether the diagnosis leans more towards reactive change or a SIL. 10-20% of women with ASCUS will have an underlying high grade dysplasia on repeat cytology.

3. *Epithelial cell abnormalities: low grade intra-epithelial lesion (LSIL)*

   This category presents a transient HPV infection that will self-eradicate in 90% of cases in a 20-30 month period. The HPV cytopathic effect cannot be reliably differentiated from cervical intra-epithelial neoplasm grade I (CIN I).

4. *Epithelial abnormalities: High grade intra-epithelial neoplasm (HSIL)*

   Subdividing HSIL into CIN II (moderate dysplasia) and CIN III (severe dysplasia) is poorly reproducible among pathologists.

5. *Epithelial abnormalities: Atypical glandular cells (AGC)*

   Like ASCUS, it is important to note, as 10-39% of patients at follow up will have high grade dysplasia.

Use of the CIN I, II and III system is still widely used and accepted, with the understanding that CIN I equals LSIL and CIN II and III represents two degrees of HSIL. [52]

Anal intra-epithelial neoplasm (AIN) shares many similarities with CIN, both are diseases of squamous epithelium, both occur at a squamo-columnar epithelial junction and both share a causative agent, HPV.

Because of these similarities between the anus and cervix, the Bethesda system is widely used to report anal cytology.

Anal cytology has 60-90% sensitivity for AIN, but only modest ability to distinguish between grades of AIN. [46] In 2007 Cranston demonstrated that any dysplasia on anal cytology has a 96% positive predictive value (PPV) for any grade of anal dysplasia, but a poor PPV (56%) for predicting the exact grade of dysplasia. [51] Palefsky compared anal cytology to anal biopsies in HIV positive and HIV negative patients, and showed 69% sensitivity in the HIV patients and 47% sensitivity in the HIV negative group. Sensitivity in the HIV positive group was comparable to that of cervical cytology sensitivity. [53]

De Ruiter showed a similar trend in 1994 – that anal cytology has good sensitivity (87.5%), but poor specificity (16.3%), a positive predictive value of 37.4 and a negative predictive value of 69.6%.[54]

The conclusion of these 3 studies is that positive anal cytology has to be followed up by anal biopsy.

Anal biopsy

Palefsky et al. performed anal biopsies after staining the anus with acetic acid via an anoscope. Acetic acid stains abnormal keratinised epithelium at the squamocolumnar junction in the anus. These areas were targeted to biopsy.[53]

Biopsy specimens are assessed for AIN on standard haematoxylin and eosin stain. In low grade dysplasia (AIN I and II) the proliferation zone above the basement membrane becomes thicker, and there is a decreased amount of mature keratinocytes. High grade dysplasia is characterised by full thickness proliferation and marked angioneogenesis. Once the basement membrane is breeched, invasive carcinoma is diagnosed.[55]

Study done at Groote Schuur Hospital, University of Cape Town September to December 2014

Methods

Patient Selection

This cohort study was conducted from September 2014 to November 2014. A total of 27 patients were enrolled in this study. 14 HIV positive renal transplant recipients, aged between 27 and 52 years old, 50% male and 50% female, that received a renal transplant (first episode) between 2008 and 2013 at the Groote Schuur Transplant Unit were contacted and screening for anal human papillomavirus offered to them. One of the patients that consented, did not attend the scheduled screening, and was therefore excluded from this study. All of the HIV positive renal transplant recipients were known to be HIV positive at the time of transplant and all them had received kidneys from HIV positive donors.

A similar group of 14 HIV negative renal transplant recipients were selected from the transplant record at Groote Schuur, aged between 20 and 62, 57% female and 43% male, that received a renal transplant (first episode) between 2007 and 2013. These patients were selected to match the age, gender and time of transplant of the HIV group, to negate any effect these 3 variables might have on results. The same screening offered to the HIV group, was offered to them.

Written informed consent was obtained from all participants by the principle investigator, and the study was approved by the University of Cape Town Faculty of Health Sciences' Human Research Ethics committee (HREC/REF: 595/2014).

Data Collection

Data collected for each patient was gender, age, months since transplant, HIV status, type and duration of ARV's and type of transplant immunosuppression. Appearance of the anus before and after acetic acid staining was documented. This date was collected on an excel spread sheet. Descriptive data was categorised into numbers, to facilitate the data processing.

Anal cytology

The sampling was conducted at the Obstetrics and Gynaecology outpatient Department at Groote Schuur Hospital. Before sampling was started, an external examination of the anus and perineum was performed and noted if there were any macroscopic lesions present.

Patients were asked to assume the lithotomy position, a nylon cervical cytology brush, moistened with 0.9% saline was inserted into the anal canal and rotated in either direction. The contents were then transferred onto 4 glass slides and fixed using an aerosol cytological fixative, and dispatched for cytological examination.

Anal histololgy

Subsequently, a gauze swab was soaked in 5% acetic acid and inserted with a forceps into the anal canal and left in place for two minutes. The anus was then examined for any white areas that may indicate dysplastic epithelium. If such areas were identified, the area was injected with a 1% lignocaine local anaesthetic agent, and biopsied. Samples were fixed in 10% formalin and sent for standard histopathological examination.

Laboratory

Cytological and histological examinations were done by two different pathologists, blind to each other's results and blind to the HIV status of the patients.

Cytology was reported as per the Bethesda criteria for cervical cytology, and was then categorised as either normal, LSIL or HSIL.[52] A smear was deemed adequate if columnar epithelium was present on the slide. Smears that did not contain columnar epithelium were repeated at the next outpatient visit. All smears in this study were adequate.

Histological examination was performed with standard haematoxylin and eosin staining, and examined for koilocytes, which are cells that have undergone morphological changes associated with HPV infection, namely peri-nuclear vacuolation (or halo), irregularity of the nuclear membrane contour, nuclear hyperchromasia and nuclear enlargement. The samples were then examined for any sign of dysplasia.

Statistical analysis

Demographic data, HIV status, time from transplant, time on ART, macroscopic appearance of the anus, cytology results and histology results were tabulated in Excell format. All descriptive data was categorised numerically to facilitate statistical calculations, and the statistics were determined using the Stata program.

Cytology was classified as 1) normal, 2) LSIL, 3) HSIL and 4) evidence of invasive cancer.

Histology was classified as 1) normal and 2) HPV, because there was no dysplasia detected in any of the histology samples.

Results

A total of 27 renal transplant recipients were examined and biopsied. 13 (48%) were HIV positive and 14 (52%) were HIV negative. The mean age in the HIV negative group was 40.8 years ±7.5 (range 20-62) and 41 years ± 14.4(range 27-52) in the HIV positive group. All study participants had only one renal transplant episode. There was no statistically significant difference in age between the two groups (p=0.89).

Mean time from transplant in the HIV positive group was 40.1 months ±21 (range 13-74.6) and 55.86 months ±23.3 (8.9-84) in the HIV negative group. There was no statistically significant difference in months since transplant between the two groups (p=0.08).

	n (percentage)	**Mean age (range)**	**Mean months since transplant (range)**
HIV +	13 (48.15%)	40.85 (27-52)	40.08 (12.99-74.6)
HIV -	14 (51.85%)	41.43 (20 – 62)	55.86 (8.98 – 84.03)

Table 1

All the patients in the HIV positive cohort were on the same immunosuppression regime, namely Tacrolimus, MMF and prednisone. The majority (n=7, 50%) of the HIV negative cohort was also on this regime. Of the remainder of the HIV negative cohort, 3 were on Tacrolimus, Azathioprine and prednisone, 2 were on Sirolimus, MMF and prednisone, one was on Cyclosporin, MMF and prednisone, one was on Sirolimus, Azathioprine and prednisone.

Immunosuppressive drugs were assigned into 5 numerical categories, representing all the drug combinations used in both cohorts:

1. Tacrolimus, MMF and prednisone
2. Tacrolimus, Azathioprine and prednisone
3. Sirolimus, MMF and prednisone
4. Sirolimus, Azathioprine and prednisone
5. Cyclosporine, MMF and prednisone

See Table 2 for distribution of immunosuppression amongst patients.

	HIV + (percentage)	**HIV- (percentage)**	**Both groups**
Immunosuppression 1	13 (100%)	7 (50%)	20 (74%)
Immunosuppression 2	0	3 (21.43%)	3 (11.11%)
Immunosuppression 3	0	2 (14.29%)	2 (7.41%)
Immunosuppression 4	0	1 (7.14%)	1 (3%)
Immunosuppression 5	0	1 (7.14%)	1(3%)
Total	**13**	**14**	**27**

Table 2

The mean time since HIV diagnosis in the HIV+ cohort was 8.92 (range 3-16) years, and the mean time since initiation of HAART was 7.15 (range 3-14 years)

	Time since HIV diagnosis in years (range)	**Time on HAART in years (range)**
HIV + males	9.8 (3-16)	7.3 (3-14)
HIV + females	7.8 (5-12)	7 (5-8)

Table 3

Anti-retroviral drugs regimes were varied amongst the HIV + cohort, as demonstrated in table 4. 23% of patients were on the combination of Lamivudine, Stavudine and Efavirenz, the most common combination.

	Lamivudine Stavudine Efavirenz	Lamivudine Stavudine Lopinavir/Rotinavir	Lamivudine Stavudine Nevirapine	Lamivudine Efavirenz Abacavir	Tenofivir Lamivudine Lopinavir/Rotinavir	Lopinavir/Rotinavir Emtricitabine Tenofivir	Lamivudine Efavirenz Tenofivir	Abacavir ZIdovudine Lopinavir/Rotinavir	Total
HIV + males	2	3	0	1	0	1	0	0	7
HIV + females	0	0	1	1	2	0	1	1	6
	2	3	1	1	2	1	1	1	13

Table 4

	n warts	*n* LSIL	*n* HSIL	*n* Normal cytology	*n* HPV on histo
HIV + Male	1	3	2	2	1
HIV + Female	1	2	1	3	3
Subotal HIV + (% of HIV+)	*2 (15.38%)*	*5 (38.46%)*	*3 (23.08%)*	*5 (38.46%)*	*4 (30.77%)*
HIV - male	1	1	1	4	0
HIV - Female	0	3	1	4	0
Subtotal HIV – (%HIV-)	*1 (7.14%)*	*4 (28.57%)*	*2 (14.29%)*	*8 (57.14%)*	*0*
Total (%of all)	***3 (11.11%)***	***9 (33.33%)***	***5 (18.52%)***	***13 (48.15%)***	***4 (14.81%)***

Table 5

Table 5 outlines the numbers of disease per cohort as per clinical, cytological and histological evaluation.

A total of 14 (51.8%) patients had abnormal cytological findings, 8 (61.5%) in the HIV positive cohort, and 6 (42.9%) HIV negative cohort, Fisher's exact test shows no significant difference (p = 0.806). On cytology, the most common result was a normal smear (n=13, 48.15%), followed by LSIL (n=9, 33.33%) and HSIL (n=5, 18.52%). There was no significant difference in this distribution between the two groups.

61.5% (n=8) of all the HIV patients had cytological evidence of HPV, compared to 42.9% (n=6) the HIV negative group.

HPV was proven histologically in 4 (30.77%) of the 13 HIV positive patients, and in none of the HIV negative patients. HPV positive histology in the HIV + cohort was significantly more common, p=0.014. There was no evidence of dysplasia in any of the histology specimens.

Two (15.38%) of the HIV positive group had anal warts and one (7.14%) of the HIV negative group had warts.

	HIV - with HPV on Histology	HIV - with no HPV on histology	HIV + with HPV on Histology	HIV + with no HPV on histology	Total
Normal Cytology	0	8	1	4	9
LSIL	0	4	1	4	9
HSIL	0	2	2	1	5
Total	0	14	4	9	23

Table 6

Table 6 demonstrates the relationship between histology and cytology findings. To calculate sensitivity, specificity, positive predictive value (PPV) and negative predictive value, cytology was grouped into normal and abnormal (combined LSIL and HSIL).

The sensitivity of anal cytology for detecting histologically proven HPV in the HIV positive group was 75%. Sensitivity for the HIV negative group could not be determined, due to no positive histology in the HIV negative group. Specificity in the HIV + group was 44.44% and 52.17% in both cohorts combined. The PPV of anal cytology was 37.5% in the HIV + group and 21.42% overall. The NPV of anal cytology for having HPV of the anus in the HIV+ group was 80% and 92% overall.

The relative risk for an HIV positive patient with abnormal anal cytology to have HPV on histology was 1.9 (95% CI 0.26 – 13.42, p=0.53), and 2.78 (95%CI 0.26-13.42, p=0.23) for a patient with positive cytology irrespective of HIV status.

Table 7 examines the relationship between warts and cytology results. It indicates that having warts, have a PPV of 66.66% for having positive cytology, and that only 50% of patients that don't have warts, will actually have negative cytology too. The relative risk when combining both cohorts for having positive cytology if a patient has warts is 1.3 (95% CI 0.54 – 3.26, *p*= 0.53), and 1.8 (95% CI 1.07 – 3.14, *p*= 0.027) in an HIV positive patient. The relative risk could not be calculated for the HIV negative group alone, because the patient with warts had normal cytology.

	HIV + positive cytology	HIV + negative cytology	HIV - positive cytology	HIV- negative cytology
Warts	2	0	0	1
No warts	6	5	6	7

Table 7

Table 8 examines the relationship between warts and histology outcomes. If a patient from any of the two cohorts did not have warts, they had an 83.8% probability of having negative histology too. None of the HIV positive patients with warts had positive histology, therefor the positive predictive value and relative risk to have abnormal histology if a patient had warts, could not be determined.

	HIV + Positive histology	HIV + negative histology	HIV – positive histology	HIV – negative histology
Warts	0	2	0	1
No Warts	4	7	0	13

Table 8

Table 9 examines the relationship between the acetic acid stain findings and the cytology outcomes. HIV positive patients with a positive stain had an apparently increased relative risk of 1.2 (95% CI 0.16 – 9.02, p=0.85) of having positive cytology, but was not statistically significant.

	HIV + positive cytology	HIV + negative cytology	HIV – positive cytology	HIV- negative cytology
No white stain	0	0	3	2
Single white stain	6	4	1	1
Multiple white stains	2	1	0	1
Circumferential white stains	0	0	2	4
All stains	8	5	3	6

Table 9

Table 10 examines the relationship between acetic acid stain findings and histology outcomes. Of 27 patients screened, 5 (18.52%) had no anal areas of white staining with acetic acid. The most common finding was a single white stain in 12 patients (44.44%). The sensitivity of any positive acetic white stain for finding HPV on histology in the HIV positive cohort was 100% in this study. The specificity was 30.77%. The positive predictive value of a stain for having positive histology was only 30.77%, but the negative predictive value of no stain for having had normal histology was 100%.

	HIV + positive histology	HIV + negative histology	HIV – positive histology	HIV – negative histology
No white stain	0	0	0	5
Single white stain	4	6	0	2
Multiple white stains	0	3	0	3
Circumferential white stains	0	0	0	6
All stains	4	9	0	11

Table 10

In both groups there was no association between time on immunosuppression and the presence or severity of disease on cytology (p=0.44).

Discussion

There are no studies comparing the incidence of HPV of an HIV positive solid organ transplant cohort to that of an HIV negative solid organ transplant cohort. There is also currently no screening program for HPV in solid organ transplant recipients in South Africa.

In this cohort study, cytological evidence of HPV was more common in the HIV cohort (n=8, 61.5%) than in the HIV negative cohort (n=6, 42.9%) It may indicate that more HIV positive patients have HPV at the time of transplant than HIV negative patients, or that HIV positive patients are more likely to acquire new infections post-transplant than HIV negative patients.

HPV proven on histology was significantly more common in the HIV + cohort (4 vs 0). The risk for an HIV positive patient to have HPV is 16 times higher than that of a HIV negative patient [16]. Screening at time of transplant is likely even more important in HIV positive solid organ recipients.

The numbers of patients affected by HPV of the anus compares to those found in other studies, it highlights the need for screening in these patients after transplant. [12 42]

We did not know the HPV status of our patients at time of transplant, HPV is not routinely tested for in South Africa prior to transplant. The fact that that there was no significant association between disease severity on cytology and time from transplant, is reassuring. However, knowing whether the infections we encountered were acquired pre- or post-transplant, could offer more accurate insight into the natural history of the disease in transplant patients. Valuable information about new infections (post-transplant) and which patients are more likely to acquire new infections could be learnt.

The sensitivity of anal cytology for detecting HPV proven on histology could only be tested In the HIV positive group, as there was no HPV positive histology in the HIV negative group. Our cytology sensitivity was 75%, in keeping with 60-90% in

other published results. Also in keeping with other publications, the specificity of anal cytology was low, 44.4% for the HIV + group and 52.17% for both cohorts combined. Our positive predictive value was also low (37.5%), but with a higher negative predictive value, as in other studies. We will therefor also recommend that cytology always be followed up with biopsy. [46 53 54] Patients with no abnormal acetic acid staining, could potentially be followed up without initial biopsy.

The absence of warts was not a reliable indicator of disease, as was discussed by Kwak et al. in 2009.

Limitations of the study are a small sample size, with a correspondingly small control group, given the limited amount of HIV positive transplant patients. Histology may not be representative, the patients in this cohort generally did not tolerate anal biopsy under local anaesthetic very well, most found it uncomfortable, and biopsies were generally difficult. This is in contradiction with other studies describing anal biopsies. It may be that histology done under sedation, spinal or general anaesthetic will yield better results.[53]

Another limitation of the study is the heterogeneity of immunosuppression in the HIV negative group. Matching the cohorts for age, time from transplant and gender were given preference, given the small sample size of the HIV positive group.

Conclusion

It is encouraging that none of our patients have invasive cancer at this stage, but this study highlights the importance of early identification of HPV infections. Given the increased likelihood of disease progression in both groups, we propose HPV screening at the time of transplant and yearly follow up with acetic acid staining and cytological and histological assessment in those with positive acetic acid stains.

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APPENDIX 1: Consent form

CONSENT FOR HUMAN PAPPILOMA VIRUS SCREENING

Version 1.2

April 2014

Renal Transplantation

.

Study Conducted by:

Dr. Janie Botha, Principal Investigator (PI), Prof. Elmi Muller, Prof Helen Wainwright and Dr. Genevieve Learmonth

Collection of anal and cervical Pap smear for Storage and Testing

A. Who is conducting this sub-study?

Dr. Elmi Muller, Principal Investigator (PI), Dr. Janie Botha, Dr. Claire Warden and Dr. Genevieve Learmonth are conducting a research study.

B. Why am I invited to participate in this optional sub-study?

You are being asked to take part in this study because you had a transplant in the last 5 years. The data obtained from this study will be compared to data from a group of patients who are enrolled as a participant in "*Renal Transplantation in HIV positive patients: Using HIV positive cadaver donors for HIV positive recipients."*

Research studies include only people who choose to take part. Please take your time to make your decision and discuss it with your friends, family and/or physician. Remember that your participation is completely voluntary.

C. What is the purpose of this optional sub-study?

Patients who are immunosuppressed for any reason (after receiving a transplant) are thought to be at a higher risk for the development of HPV (a virus)-associated cancer in the cervix as well as in the anus. The purpose of the Pap smear(s) of the anus is to look for HPV itself. The pap smear of the anus also allows for early detection of any pre-cancerous changes in the cells.

The study investigators would like to learn more about anal HPV infection and the course of anal disease over time. Because HPV infection of the anus can be seen in both men and women, if you are sexually active, you will be asked to have an anal examination and a Pap smear of the anus to collect cells to test for anal HPV infection.

If a potentially pre-cancerous area is found, your primary medical care provider and the study doctors will discuss the results so that arrangements for appropriate follow-up can be made. If treatment is required, referrals will be made outside of the study.

You may choose not to participate in the studies of the anal region or you may stop participating in them at any time. Your decision will not affect you follow-uo transplant study. You may agree or refuse to participate in these HPV studies by checking the correct choice at the end of this consent form.

D. Are there potential conflicts of interest?

Groote Schuur Hospital investigators must satisfy legal requirements for identifying and managing potential conflicts of interest before a research study can be approved. The purpose of these requirements is to ensure that the design, conduct and reporting of the research will not be biased by any conflicting interests. If at any time you have specific questions about the financial arrangements or other potential conflicts for this sub-study, please feel free to contact any of the individuals listed in Section M.

The investigators of this research do not have any financial interest in the sponsor or in the study; this means that the investigator will not be financially affected by the results of the study (positive or negative).

The person inviting you to participate in this research may also be your treating doctor. In such cases, the doctor has an interest in both your care and promoting the successful conduct of this research. Sometimes these two interests may cause conflict. You can choose not to participate in the research and still receive treatment from your doctor. If you wish, you may also request to speak to another doctor who is not a member of the research team about your options.

E. How many people will take part in the sub-study?

In the first year of the study, about 25 people who had transplants in the last 5 years will be invited to take part in study.

F. What will I be asked to do?

For the anal Pap smear, you will have a swab inserted into the anal canal to collect cells and to look for HPV. If this is abnormal, you will be referred to a specialist for the following exams and tests:

You will have a visual examination of the anal region. An instrument called an anoscope will be inserted into the anal canal. The anoscope allows the specialist to look at the inside of the anal canal. Three percent acetic acid (diluted vinegar) will be applied to the surface of the anal canal as well as to the inside of the canal. During the anoscopy, if any areas of abnormality are seen, a biopsy (removal of a small piece of anal skin) may be required. To perform this biopsy, the anal skin will be numbed by injecting a numbing medicine (similar to that used by a dentist) with a small needle. After the skin becomes numb, a very small piece of anal skin will be removed. This biopsy skin will be sent to the Department of Pathology, at the University of Cape Town, for examination. If the area of abnormality is large enough, a second piece of anal skin may be needed for analysis. These procedures take about 30 minutes.

G. Are there any risks and discomforts?

Insertion of an anal swab, an anoscope and application of acetic acid may cause some discomfort. Anal biopsy may be associated with discomfort from the needle stick for anesthesia, bleeding, temporary discomfort after the anesthetic wears off and rarely, infection or allergic reaction to the anesthesia.

H. What other options are there?

You may choose to not participate the study. If you choose to not participate in this study, neither your medical care nor your ongoing participation in the main study will be affected by your decision.

I. What about confidentiality of my "identifiable" health information?

The research team will share information among themselves as part of the research study process. In addition, various institutional committees and governmental agencies that oversee research may request or require access to your identifiable health information. These include the Research Ethics Committee of Groote Schuur Hospital.

Why would my health information be shared as part of the sub-study?

Research involves the gathering and analysis of information. With medical research, the research team is gathering and analyzing health information about individuals in the hope that they will be able to answer specific questions about a bodily function, disease, or wellness. Those team members who act in a supportive role to the research study use health information when necessary for various administrative tasks, such as tracking data, making reports that are required by government oversight agencies or the study sponsor, and assisting the researchers with other data-related tasks. The Institutional Review Boards / Research Ethics Committees act as watchdog groups for the protection of the rights and interests of research subjects.

It is important for you to know that if your health information is used for teaching purposes outside the study, or to prepare a medical journal report about the research study, your identifiable health information will not be made public; your identity will be kept confidential in those circumstances.

Each time your identifiable health information is disclosed to any of the individuals listed above, precautions will be taken to minimize the possibility that the information shared could directly identify you. When possible, all identifying information will be coded. This means that the researchers will assign a unique code to represent your identifiable data so that people who see the coded data will not be able to identify you.

You may authorize the research team to share your identifiable health information in connection with the research study. Whenever we disclose any of your identifiable health information based on your authorization, your written statement will accompany the information.

J. Will I be paid?

You will not be paid for your participation in this study.

K. What happens if I need emergency care?

In the event of injury or illness resulting from this study, you should immediately contact one of the personnel listed in Section M "Whom do I call if I have questions or problems?"

L. What are my rights as a human research participant?

Taking part in this sub-study is voluntary. You may choose not to take part or may leave the study at any time. Your decision not to participate or to withdraw from the study means that you will not or will no longer undergo any research-related procedures. You will, however, still be able to receive treatment and services at Groote Schuur Hospital that are not related to this research. If you leave the study:

- We will no longer be able to allow you to participate in the research sub-study; and
- We will stop collecting any additional identifiable health information about you. However, we are allowed by law to continue to use the health information we already have about you, as necessary to maintain the integrity of the research study and make reports that oversight agencies require of us.
- You also have the right to revoke or withdraw your authorization for us to use your identifiable health information. If you wish to revoke or withdraw your authorization, you must do so in writing, and provide that written revocation to the investigator Elmi Muller, MD, whose mailing address is: Renal Unit, Groote Schuur Hospital, Observatory, Cape Town 7925.

During your participation in this sub-study, we will tell you about any new information that may affect your health, welfare, or willingness to stay in this study.

M. Whom do I call if I have questions or problems?

For questions about the study or a research-related injury, please contact the numbers below 24 hours daily:

Groote Schuur Transplant Unit 021 404 3327

Groote Schuur Renal Clinic 021 404 3311

Dr Elmi Muller 0829201111

For questions about your rights as a research participant, contact the Groote Schuur Hospital Research Ethics Committee (REC) office at E52 24 Old Main Building, Groote Schuur Hospital, Observatory (Tel: 021 406 6492/ 406 6626/ 4066338). The REC is a group of people who review the research to protect your rights and welfare.

N. Consent and Authorization Provisions

Your signature below means that: (1) you have carefully read and understood the information presented in this informed consent form; (2) the information concerning the research study and its involved procedures has been fully explained to you and your questions have been answered to your satisfaction; (3) you have received all of the information you desire; (4) you consent to your participation in the research sub-study, and (5) you authorize the use and disclosure of your identifiable health information as described in this form. If you have any additional questions during the course of your involvement in the research, you should contact the investigator(s), the REC Chair(s) and/or the REC Office at any time.

Your signature below reflects that, after considering both the potential risks, anticipated benefits and alternatives (and their relative risks and benefits) of participation, you voluntarily agree to participate in this research and authorize the research team to create, obtain, use or disclose your identifiable health information as described in Section L of this document, and in connection with this research study. By consenting to participate in the research, you are not giving up any of your legal rights. You will be given a copy of this signed and dated consent form.

O. What if something goes wrong?

The University of Cape Town (UCT) undertakes that in the event of you suffering any significant deterioration in health or well-being, or from any unexpected sensitivity or toxicity, that is caused by your participation in the study, it will provide immediate medical care. UCT has appropriate insurance cover to provide prompt payment of compensation for any trial-related injury according to the guidelines outlined by the Association of the British Pharmaceutical Industry, ABPI 1991. Broadly-speaking, the ABPI guidelines recommend that the insured company (UCT), without legal commitment, should compensate you without you having to prove that UCT is at fault. An injury is considered trial-related if, and to the extent that, it is caused by study activities. You must notify the study doctor immediately of any side effects and/or injuries during the trial, whether they are research-related or other related complications.

UCT reserves the right not to provide compensation if, and to the extent that, your injury came about because you chose not to follow the instructions that you were given while you were taking part in the study. Your right in law to claim compensation for injury where you prove negligence is not affected. Copies of these guidelines are available on request.

SIGNATURE BY THE PATIENT:

Name of Patient	Signature of Patient	Date of Signature
_____	___	_____

SIGNATURE BY THE INVESTIGATOR:

I attest that all the elements of informed consent described in this form have been discussed fully in non-technical terms with the subject. I further attest that all questions asked by the subject were answered to the best of my knowledge. The subject has been provided with the Participant Bill of Rights.

Signature of the Investigator Who Obtained Consent of Signature

Date

SIGNATURE BY THE WITNESS/TRANSLATOR

(Signature of a witness is only required when a non-English speaking subject is consented with the assistance of a translator. The signature of the witness below attests that the translator has presented the elements of consent to the subject, orally and in his/her preferred language, and that a summary of the oral presentation, in a language the subject can understand, has been given to the participant.)

Signature of Witness of Signature

Date

Appendix 2: Data analysis

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___    ____  ____  ____  ____ (R)
  /__    /   ____/   /   ____/
 ___/   /   /___/ /   /___/   11.2 Copyright 1985-2009 StataCorp LP
Statistics/Data Analysis            StataCorp
                                    4905 Lakeway Drive
                                    College Station, Texas 77845 USA
                                    800-STATA-PC        http://www.stata.com
                                    979-696-4600        stata@stata.com
                                    979-696-4601  (fax)

Single-user Stata perpetual license:
Serial number:  30110550580
Licensed to:  Kathryn Manning
Private

Notes:
      1.  10.00 MB allocated to data

. use "/Users/Kathryn/Desktop/Stats 2014/Janie Engelbrecht/database v1.dta"

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```

HIV status	Freq.	Percent	Cum.
Negative	**14**	**51.85**	**51.85**
Positive	**13**	**48.15**	**100.00**
Total	**27**	**100.00**	

```
. tab gender
```

Gender	Freq.	Percent	Cum.
Female	**14**	**51.85**	**51.85**
Male	**13**	**48.15**	**100.00**
Total	**27**	**100.00**	

```
. univar age
```

					Quantiles		
Variable	n	Mean	S.D.	Min	.25	Mdn	.75	Max
age	**27**	**41.15**	**11.39**	**20.00**	**34.00**	**40.00**	**52.00**	**62.00**

```
. univar tx_months
```

					Quantiles		
Variable	n	Mean	S.D.	Min	.25	Mdn	.75	Max

tx_months	**27**	**48.26**	**23.53**	**8.98**	**29.26**	**50.89**	**69.44**	**84.03**

. tab prestain

Macroscopic disease before stain	Freq.	Percent	Cum.
None	**21**	**77.78**	**77.78**
Warts	**3**	**11.11**	**88.89**
Other	**3**	**11.11**	**100.00**
Total	**27**	**100.00**	

. tab poststain

Post stain	Freq.	Percent	Cum.
No white areas	**5**	**18.52**	**18.52**
Small white area	**12**	**44.44**	**62.96**
Multiple white areas	**4**	**14.81**	**77.78**
Circum white	**6**	**22.22**	**100.00**
Total	**27**	**100.00**	

. tab immunosup

Immunosuppressives	Freq.	Percent	Cum.
T-M-P	**20**	**74.07**	**74.07**
T-A-P	**3**	**11.11**	**85.19**
S-M-P	**2**	**7.41**	**92.59**
S-A-P	**1**	**3.70**	**96.30**
C-M-P	**1**	**3.70**	**100.00**
Total	**27**	**100.00**	

. tab arv_regime

ARV regime	Freq.	Percent	Cum.
Lam-Sta-Eff	**1**	**7.69**	**7.69**
Lam-Efa-Sta	**4**	**30.77**	**38.46**
Lam-Sta-Nev	**1**	**7.69**	**46.15**
Lam-Efa-Aba	**2**	**15.38**	**61.54**
Ten-Lam-L/R	**2**	**15.38**	**76.92**
Ten-Lam-Efa	**1**	**7.69**	**84.62**
L/R-Emt/Ten	**1**	**7.69**	**92.31**
Aba-Lam-Efa	**1**	**7.69**	**100.00**
Total	**13**	**100.00**	

. tab hivdx_date

Year of Dx of HIV	Freq.	Percent	Cum.

1998	**1**	**7.69**	**7.69**

1999	**1**	**7.69**	**15.38**
2002	**2**	**15.38**	**30.77**
2005	**1**	**7.69**	**38.46**
2006	**4**	**30.77**	**69.23**
2008	**2**	**15.38**	**84.62**
2009	**1**	**7.69**	**92.31**
2011	**1**	**7.69**	**100.00**
Total	**13**	**100.00**	

```
. tab cytology
```

cytology	Freq.	Percent	Cum.
1	**13**	**48.15**	**48.15**
2	**9**	**33.33**	**81.48**
3	**4**	**14.81**	**96.30**
4	**1**	**3.70**	**100.00**
Total	**27**	**100.00**	

```
. tab histology
```

histology	Freq.	Percent	Cum.
1	**23**	**85.19**	**85.19**
2	**4**	**14.81**	**100.00**
Total	**27**	**100.00**	

```
.
. **Differences between hiv status groups
.
.
. tab gender hiv_status, row col
```

Key
frequency row percentage column percentage

	HIV status		
Gender	Negative	Positive	Total
Female	**8**	**6**	**14**
	57.14	**42.86**	**100.00**
	57.14	**46.15**	**51.85**
Male	**6**	**7**	**13**
	46.15	**53.85**	**100.00**
	42.86	**53.85**	**48.15**
Total	**14**	**13**	**27**

51.85	48.15	100.00
100.00	100.00	100.00

```
. univar age, by (hiv_status)

-> hiv_status=Negative
                                                    Quantiles
Variable   n      Mean     S.D.           Min   .25     Mdn          .75 Max
-------------------------------------------------------------------------------
     age      14    41.43    14.38    20.00    32.00    38.00    53.00   62.00
-------------------------------------------------------------------------------

-> hiv_status=Positive
                                                    Quantiles
Variable   n      Mean     S.D.           Min   .25     Mdn          .75 Max
-------------------------------------------------------------------------------
     age      13    40.85     7.53    27.00    35.00    42.00    46.00   52.00
-------------------------------------------------------------------------------

. **age normally distributed
. ttest age, by(hiv_status)

Two-sample t test with equal variances

   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
Negative |      14    41.42857    3.844097    14.38329    33.12391   49.73324
Positive |      13    40.84615    2.087343    7.526023    36.29822   45.39408
---------+--------------------------------------------------------------------
combined |      27    41.14815    2.191478    11.38725     36.6435   45.6528
---------+--------------------------------------------------------------------
    diff |            .5824176    4.471307               -8.626411   9.791247
------------------------------------------------------------------------------
diff =     mean(Negative) - mean(Positive)                      t =  0.1303
Ho: diff = 0                                     degrees of freedom =      25

Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
Pr(T < t) = 0.5513         Pr(|T| > |t|) = 0.8974          Pr(T > t) = 0.4487

. univar tx_months, by (hiv_status)

-> hiv_status=Negative
                                                    Quantiles
Variable   n      Mean     S.D.           Min   .25     Mdn          .75 Max
-------------------------------------------------------------------------------
tx_months      14    55.86    23.24     8.98    36.39    62.50    73.02   84.03
-------------------------------------------------------------------------------

-> hiv_status=Positive
                                                    Quantiles
Variable   n      Mean     S.D.           Min   .25     Mdn          .75 Max
-------------------------------------------------------------------------------
tx_months      13    40.08    21.79    12.99    21.53    37.97    56.19   74.60
-------------------------------------------------------------------------------

. **tx_months normally distributed
. ttest tx_months, by (hiv_status)
```

```
Two-sample t test with equal variances
```

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
Negative	**14**	**55.85988**	**6.210602**	**23.23794**	**42.44269**	**69.27707**
Positive	**13**	**40.07924**	**6.042211**	**21.7855**	**26.91439**	**53.24409**
combined	**27**	**48.2618**	**4.528158**	**23.529**	**38.95403**	**57.56956**
diff		**15.78064**	**8.686401**		**-2.109337**	**33.67062**

diff = mean(**Negative**) - mean(**Positive**) t = **1.8167**
Ho: diff = 0 degrees of freedom = **25**

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0
Pr(T < t) = **0.9594** Pr(|T| > |t|) = **0.0813** Pr(T > t) = **0.0406**

. tab prestain hiv_status, row col

Key
frequency row percentage column percentage

Macroscopic disease before stain	HIV status Negative	Positive	Total
None	**12**	**9**	**21**
	57.14	**42.86**	**100.00**
	85.71	**69.23**	**77.78**
Warts	**1**	**2**	**3**
	33.33	**66.67**	**100.00**
	7.14	**15.38**	**11.11**
Other	**1**	**2**	**3**
	33.33	**66.67**	**100.00**
	7.14	**15.38**	**11.11**
Total	**14**	**13**	**27**
	51.85	**48.15**	**100.00**
	100.00	**100.00**	**100.00**

. tab prestain hiv_status, exact

Enumerating sample-space combinations:
stage 3: enumerations = 1
stage 2: enumerations = 2
stage 1: enumerations = 0

Macroscopi

c disease before	HIV status

stain	Negative	Positive	Total
None	**12**	**9**	**21**
Warts	**1**	**2**	**3**
Other	**1**	**2**	**3**
Total	**14**	**13**	**27**

Fisher's exact = **0.525**

```
. tab poststain hiv_status, row col
```

Key
frequency row percentage column percentage

	HIV status		
Post stain	Negative	Positive	Total
No white areas	**5**	**0**	**5**
	100.00	**0.00**	**100.00**
	35.71	**0.00**	**18.52**
Small white area	**2**	**10**	**12**
	16.67	**83.33**	**100.00**
	14.29	**76.92**	**44.44**
Multiple white areas	**1**	**3**	**4**
	25.00	**75.00**	**100.00**
	7.14	**23.08**	**14.81**
Circum white	**6**	**0**	**6**
	100.00	**0.00**	**100.00**
	42.86	**0.00**	**22.22**
Total	**14**	**13**	**27**
	51.85	**48.15**	**100.00**
	100.00	**100.00**	**100.00**

```
. tab poststain hiv_status, exact

Enumerating sample-space combinations:
stage 4: enumerations = 1
stage 3: enumerations = 5
stage 2: enumerations = 16
stage 1: enumerations = 0
```

	HIV status		
Post stain	Negative	Positive	Total
No white areas	**5**	**0**	**5**

Small white area	**2**	**10**	**12**
Multiple white areas	**1**	**3**	**4**

Circum white	**6**	**0**	**6**
Total	**14**	**13**	**27**

Fisher's exact = **0.000**

```
. tab immunosup hiv_status, row col
```

Key
frequency row percentage column percentage

Immunosuppressives	HIV status		
	Negative	Positive	Total
T-M-P	**7**	**13**	**20**
	35.00	**65.00**	**100.00**
	50.00	**100.00**	**74.07**
T-A-P	**3**	**0**	**3**
	100.00	**0.00**	**100.00**
	21.43	**0.00**	**11.11**
S-M-P	**2**	**0**	**2**
	100.00	**0.00**	**100.00**
	14.29	**0.00**	**7.41**
S-A-P	**1**	**0**	**1**
	100.00	**0.00**	**100.00**
	7.14	**0.00**	**3.70**
C-M-P	**1**	**0**	**1**
	100.00	**0.00**	**100.00**
	7.14	**0.00**	**3.70**
Total	**14**	**13**	**27**
	51.85	**48.15**	**100.00**
	100.00	**100.00**	**100.00**

```
. tab immunosup hiv_status, exact

Enumerating sample-space combinations:
stage 5: enumerations = 1
stage 4: enumerations = 2
stage 3: enumerations = 2
stage 2: enumerations = 2
stage 1: enumerations = 0
```

Immunosuppressives	HIV status		
	Negative	Positive	Total

T-M-P	**7**	**13**	**20**
T-A-P	**3**	**0**	**3**

S-M-P	**2**	**0**	**2**
S-A-P	**1**	**0**	**1**
C-M-P	**1**	**0**	**1**
Total	**14**	**13**	**27**

Fisher's exact = **0.014**

```
. tab arv_regime hiv_status, row col
```

Key
frequency row percentage column percentage

	HIV status	
ARV regime	Positive	Total
Lam-Sta-Eff	**1** **100.00** **7.69**	**1** **100.00** **7.69**
Lam-Efa-Sta	**4** **100.00** **30.77**	**4** **100.00** **30.77**
Lam-Sta-Nev	**1** **100.00** **7.69**	**1** **100.00** **7.69**
Lam-Efa-Aba	**2** **100.00** **15.38**	**2** **100.00** **15.38**
Ten-Lam-L/R	**2** **100.00** **15.38**	**2** **100.00** **15.38**
Ten-Lam-Efa	**1** **100.00** **7.69**	**1** **100.00** **7.69**
L/R-Emt/Ten	**1** **100.00** **7.69**	**1** **100.00** **7.69**
Aba-Lam-Efa	**1** **100.00** **7.69**	**1** **100.00** **7.69**
Total	**13** **100.00** **100.00**	**13** **100.00** **100.00**

```
. tab arv_regime hiv_status, exact
```

ARV regime	HIV status Positive	Total
Lam-Sta-Eff	**1**	**1**
Lam-Efa-Sta	**4**	**4**
Lam-Sta-Nev	**1**	**1**
Lam-Efa-Aba	**2**	**2**
Ten-Lam-L/R	**2**	**2**
Ten-Lam-Efa	**1**	**1**
L/R-Emt/Ten	**1**	**1**
Aba-Lam-Efa	**1**	**1**
Total	**13**	**13**

```
. tab hivdx_date hiv_status, row col
```

Key
frequency row percentage column percentage

Year of Dx of HIV	HIV status Positive	Total
1998	**1** **100.00** **7.69**	**1** **100.00** **7.69**
1999	**1** **100.00** **7.69**	**1** **100.00** **7.69**
2002	**2** **100.00** **15.38**	**2** **100.00** **15.38**
2005	**1** **100.00** **7.69**	**1** **100.00** **7.69**
2006	**4** **100.00** **30.77**	**4** **100.00** **30.77**
2008	**2** **100.00** **15.38**	**2** **100.00** **15.38**
2009	**1** **100.00**	**1** **100.00**

	7.69	7.69

2011	**1**	**1**
	100.00	**100.00**
	7.69	**7.69**
Total	**13**	**13**
	100.00	**100.00**
	100.00	**100.00**

```
. tab hivdx_date hiv_status, exact
```

Year of Dx of HIV	HIV status Positive	Total
1998	**1**	**1**
1999	**1**	**1**
2002	**2**	**2**
2005	**1**	**1**
2006	**4**	**4**
2008	**2**	**2**
2009	**1**	**1**
2011	**1**	**1**
Total	**13**	**13**

```
. tab cytology hiv_status, row col
```

Key
frequency row percentage column percentage

	HIV status		
cytology	Negative	Positive	Total
1	**8**	**5**	**13**
	61.54	**38.46**	**100.00**
	57.14	**38.46**	**48.15**
2	**4**	**5**	**9**
	44.44	**55.56**	**100.00**
	28.57	**38.46**	**33.33**
3	**2**	**2**	**4**
	50.00	**50.00**	**100.00**
	14.29	**15.38**	**14.81**
4	**0**	**1**	**1**
	0.00	**100.00**	**100.00**
	0.00	**7.69**	**3.70**
Total	**14**	**13**	**27**

51.85	48.15	100.00
100.00	100.00	100.00

```
. tab cytology hiv_status, exact

Enumerating sample-space combinations:
stage 4: enumerations = 1
stage 3: enumerations = 2
stage 2: enumerations = 2
stage 1: enumerations = 0
```

	HIV status		
cytology	Negative	Positive	Total
1	**8**	**5**	**13**
2	**4**	**5**	**9**
3	**2**	**2**	**4**
4	**0**	**1**	**1**
Total	**14**	**13**	**27**

Fisher's exact = **0.806**

```
. tab histology hiv_status, row col
```

Key
frequency row percentage column percentage

	HIV status		
histology	Negative	Positive	Total
1	**14**	**9**	**23**
	60.87	**39.13**	**100.00**
	100.00	**69.23**	**85.19**
2	**0**	**4**	**4**
	0.00	**100.00**	**100.00**
	0.00	**30.77**	**14.81**
Total	**14**	**13**	**27**
	51.85	**48.15**	**100.00**
	100.00	**100.00**	**100.00**

```
. tab histology hiv_status, exact
```

	HIV status		
histology	Negative	Positive	Total
1	**14**	**9**	**23**
2	**0**	**4**	**4**

Total	**14**	**13**	**27**

```
          Fisher'sexact  =                0.041
    1-sided Fisher'sexact  =                0.041

.
.
. **I think the main questions are:
. **1) does longer immunosuppression equal SIL?
. **2) do the HIV patients have a bigger chance of having it?
. **3) does having warts put you at risk?
.
.
. univar tx_months, by (cytology)

-> cytology=1
                                                        Quantiles
Variable     n    Mean     S.D.           Min   .25     Mdn          .75 Max
-------------------------------------------------------------------------------
tx_months      13    52.45    25.33     8.98    34.36    62.27    73.02   84.03
-------------------------------------------------------------------------------

-> cytology=2
                                                        Quantiles
Variable     n    Mean     S.D.           Min   .25     Mdn          .75 Max
-------------------------------------------------------------------------------
tx_months       9    48.24    21.72    18.38    36.39    50.70    61.68   77.42
-------------------------------------------------------------------------------

-> cytology=3
                                                        Quantiles
Variable     n    Mean     S.D.           Min   .25     Mdn          .75 Max
-------------------------------------------------------------------------------
tx_months       4    43.51    21.32    21.53    25.40    43.82    61.63   64.87
-------------------------------------------------------------------------------

-> cytology=4
                                                        Quantiles
Variable     n    Mean     S.D.           Min   .25     Mdn          .75 Max
-------------------------------------------------------------------------------
tx_months      1    13.02        .    13.02    13.02    13.02    13.02   13.02
-------------------------------------------------------------------------------

. oneway tx_months cytology

                        Analysis of Variance
Source                  SS         df      MS            F    Prob > F
------------------------------------------------------------------------
Between groups     1560.14303       3   520.047675      0.93     0.4412
Within groups      12833.8182      23   557.992094
------------------------------------------------------------------------
Total              14393.9612      26   553.613892

Bartlett's test for equal variances: chi2(2) =   0.2658  Prob>chi2 = 0.876

note: Bartlett's test performed on cells with positive
variance: 1 single-observation cells not used
```

```
. tab cytology hiv_status, row col
```

Key
frequency row percentage column percentage

cytology	HIV status Negative	Positive	Total
1	**8**	**5**	**13**
	61.54	**38.46**	**100.00**
	57.14	**38.46**	**48.15**
2	**4**	**5**	**9**
	44.44	**55.56**	**100.00**
	28.57	**38.46**	**33.33**
3	**2**	**2**	**4**
	50.00	**50.00**	**100.00**
	14.29	**15.38**	**14.81**
4	**0**	**1**	**1**
	0.00	**100.00**	**100.00**
	0.00	**7.69**	**3.70**
Total	**14**	**13**	**27**
	51.85	**48.15**	**100.00**
	100.00	**100.00**	**100.00**

```
. tab cytology hiv_status, exact

Enumerating sample-space combinations:
stage 4: enumerations = 1
stage 3: enumerations = 2
stage 2: enumerations = 2
stage 1: enumerations = 0
```

cytology	HIV status Negative	Positive	Total
1	**8**	**5**	**13**
2	**4**	**5**	**9**
3	**2**	**2**	**4**
4	**0**	**1**	**1**
Total	**14**	**13**	**27**

Fisher's exact = **0.806**

```
. tab cytology histology, row col
```

Key

frequency

row percentage *column percentage*

cytology	histology 1	2	Total
1	**12** **92.31** **52.17**	**1** **7.69** **25.00**	**13** **100.00** **48.15**
2	**8** **88.89** **34.78**	**1** **11.11** **25.00**	**9** **100.00** **33.33**
3	**3** **75.00** **13.04**	**1** **25.00** **25.00**	**4** **100.00** **14.81**
4	**0** **0.00** **0.00**	**1** **100.00** **25.00**	**1** **100.00** **3.70**
Total	**23** **85.19** **100.00**	**4** **14.81** **100.00**	**27** **100.00** **100.00**

```
. tab cytology histology, exact

Enumerating sample-space combinations:
stage 4: enumerations = 1
stage 3: enumerations = 2
stage 2: enumerations = 4
stage 1: enumerations = 0
```

cytology	histology 1	2	Total
1	**12**	**1**	**13**
2	**8**	**1**	**9**
3	**3**	**1**	**4**
4	**0**	**1**	**1**
Total	**23**	**4**	**27**

Fisher's exact = **0.179**

```
. tab prestain cytology, row col
```

Key
frequency row percentage column percentage

Macroscopic disease before stain	cytology 1	2	3	4	Total
None	**10**	**6**	**4**	**1**	**21**
	47.62	**28.57**	**19.05**	**4.76**	**100.00**
	76.92	**66.67**	**100.00**	**100.00**	**77.78**
Warts	**1**	**2**	**0**	**0**	**3**
	33.33	**66.67**	**0.00**	**0.00**	**100.00**
	7.69	**22.22**	**0.00**	**0.00**	**11.11**
Other	**2**	**1**	**0**	**0**	**3**
	66.67	**33.33**	**0.00**	**0.00**	**100.00**
	15.38	**11.11**	**0.00**	**0.00**	**11.11**
Total	**13**	**9**	**4**	**1**	**27**
	48.15	**33.33**	**14.81**	**3.70**	**100.00**
	100.00	**100.00**	**100.00**	**100.00**	**100.00**

```
. tab prestain cytology, exact

Enumerating sample-space combinations: stage 4:
enumerations = 1
stage 3: enumerations = 1 stage 2:
enumerations = 2 stage 1: enumerations =
0
```

Macroscopic disease before stain	cytology 1	2	3	4	Total
None	**10**	**6**	**4**	**1**	**21**
Warts	**1**	**2**	**0**	**0**	**3**
Other	**2**	**1**	**0**	**0**	**3**
Total	**13**	**9**	**4**	**1**	**27**

Fisher's exact = **0.864**

```
.
.
end of do-file

.
```