Excision margins in Human Immunodeficiency Virus seropositive women undergoing Large Loop Excision of the Transformation Zone for cervical dysplasia.

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Declaration

I, Carolyn Joyce Noël, declare that this research report is my own original research and work.

It is being submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Masters in Medicine, in the branch of Obstetrics and Gynaecology

It was also submitted to the College of Medicine of South Africa in partial fulfillment of the requirements for the qualification of the Fellowship of the College of Obstetrics and Gynaecology.

This dissertation has not been submitted in part or in whole for any other degree or examination at The University of the Witwatersrand or at any other university.

Carolyn Joyce Noël

Abstract

Background: HIV accelerates the development of cervical cancer by up to15 years. South Africa is currently in the midst of an HIV epidemic. With limited facilities for colposcopy it is vital to identify risk factors within the HIV positive population resulting in positive margins after Large Loop Excision of the Transformation Zone (LLETZ) and persistence of cytological abnormalities on follow-up Pap smears.

Objective: The primary objective was to determine the patient risk factors, pre and during colposcopy and LLETZ biopsy, which resulted in the histological involvement of margins of the LLETZ biopsy and persistent cervical dysplasia on follow-up Pap smears. Secondary objectives included determining follow up rate of patients at the clinic as well as the correlation between the original Pap smear cytology grade and the histological grade found on histology of the LLETZ biopsy.

Methods: A retrospective review of the files of HIV seropositive patients was done at the colposcopy clinic at Charlotte Maxeke Johannesburg Academic Hospital after the roll out of antiretroviral treatment for the period 1 April 2004 to 31 October 2012. Patients with abnormal pap smears during this time were referred to the colposcopy clinic where a colposcopy and LLETZ biopsies were done. Demographic and clinical data in regards to age, gravidity, contraception, CD4 count, antiretroviral usage, and referral time was collected. Data from the clinical description of the colposcopy and histology of the LLETZ biopsy was also collected. Patients followed up again after 6 months when a repeat pap smear was done. The results of these Pap smears were also collected. Data was then analysed and variate and multivariate logistical regression was used to find statistically significant correlations.

Results: A total of 480 files were found to have complete clinical records. One hundred and sixty eight (42.71%) patients had both endo and ectocervical margins clear. Predictive factors for the involvement of endocervical margins was the doctor performing the procedure (p-value <0.01) cytology of the original Pap smear (p value <0.01) and the grade of histological abnormality found at time of LLETZ (p-value <0.01). The statistically significant predictive factors for ectocervical margin involvement was the visualization of the transformation zone at colposcopy (p-value <0.01), the size of lesion found at colposcopy (p-value <0.01), the use of combined oral contraceptive pill (OCP) (p-value 0.02) and the histological grade of abnormality found on the LLETZ biopsy. Age, parity, CD4 count, use of antiretroviral drugs, length of time from Pap smear to colposcopy and use of contraception other than OCP were not found to be statistically significant in our sample population for the involvement of either endo or ectocervical margins.

Statistically significant risk factors for the recurrence of intraepithelial lesions on follow up Pap smear was having both endo and ectocervical margin involvement on histology (p-value 0.01) The Ectocervical margin alone was found to have a p-value of <0.01. Abnormal cytology on follow up Pap smear was found in 58.69% of patients.

The follow up rate at the clinic was 46.04%.

Correlation of cytological grade and histological grade of cervical intraepithelial neoplasm in our sample population was found to be adequate (p-value <0.01).

Conclusion: Incomplete incision of the intraepithelial lesion was found to be a significant risk factor for the recurrence of cytological abnormality in patients undergoing LLETZ biopsy. Identifying patients at increased risk for recurrence is important to ensure close follow up in this patient population.

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Abbreviations

ASCUS	Atypical Squamous Cells of Undetermined Significance (Bethesda Classification)
CD4	T-lymphocyte with CD4 receptor
CDC	Centres for Disease Control
CI	Confidence Interval
CIN	Cervical Intraepithelial Neoplasia
CMAJH	Charlotte Maxeke Academic Johannesburg Hospital
DNA	Deoxyribonucleic Acid
HAART	Highly Active Antiretroviral Treatment
HGSIL	High Grade Squamous Intraepithelial Lesion(Bethesda Classification)
HIV	Human Immunodeficiency Virus
HPV	Human Pappiloma Virus
IQR	Intraquartile Range
IUCD	Intra Uterine Contraceptive Device
LGSIL	Low Grade Squamous Intraepithelial Lesion (Bethesda Classification)
LLETZ	Large Loop Excision of the Transformation Zone
N	Number in group
NCR	National Cancer Registry
NHLS	National Health Laboratory Services
OCP	Oral Contraceptive Pill
Pap test/smear	Papanicolaou test
PHC	Primary Healthcare Centre
SD	Standard Deviation
VCT	Voluntary Counselling and Testing
WHO	World Health Organization

Chapter 1

1.1 Introduction

Cervical cancer remains a major cause of cancer mortality among women in developing countries with up to 80% of newly diagnosed cervical cancer cases occurring within these regions.(1) Areas with the heaviest burden of disease include Sub-Saharan Africa, Latin America, the Caribbean and South East Asia; which have access to less than five percent of the world's cancer resources.(2).Data collection and access to health care in these areas are poor and the true incidence is likely to be vastly under reported.(2)

In South Africa there are an estimated 16.84 million women 15 years and older at risk for developing cervical cancer.(3) Cervical cancer is the second most common cancer diagnosed in South Africa.(4) Cervical cancer accounts for 23.3% of all cancers in women in Africa.(1) Data from the 1998 South African National Cancer Registry (NCR) Report 1998-1999, published in 2004(4) reported a lifetime risk of 1 in 26 (0-74years) in all women in South Africa and 1 in 21 (0-74years) in black South African women of developing Cervical Cancer. Black South African women are far more likely to develop invasive cancer with 84% of cervical cancers reported during this period found in this population group.(4) True cancer statistics are difficult to obtain as statistics rely on a passive and voluntary reporting and surveillance system (4)and only takes into account histologically confirmed cases. (5)

Unfortunately in poor resource settings the majority of these cancers are diagnosed at an advanced stage and mortality approaches 78% compared to the United States where mortality is almost half at 40%(6). In America cervical cancer is the 13th most common cancer among

women.(7) The incidence according to the WHO of new cases of cervical cancer in the United States of America is 7 per 100 000(7) versus South Africa where the incidence is 22.8 per 100 000(3) highlighting the massive discrepancy of disease burden between developed and developing countries..

The discrepancy in cervical cancer statistics between countries can be attributed to the implementation of widespread successful cervical screening programmes in developed countries.(1) Most developing countries have very few independent screening programmes not directly attached to research units.(2)

1.2 Screening for Cervical Cancer

Disease prevention can be divided into primary and secondary prevention.(1) This can be applied to cervical cancer.

The World health Organisation (WHO) Wilson-Junger criterion(8) for disease screening is almost completely met by cervical cancer screening programs.

Primary prevention focuses on individual behaviour in an attempt to decrease the chance of Human Papillomavirus (HPV) infection- a highly infectious, sexually transmitted virus. Premalignant lesions of the cervix have been very closely linked to the infection of the cells of the transformation zone of the cervix with oncogenic strains of HPV which cause activation of oncoprotiens E6 and E7 which in turn inactivate tumour suppressor genes P53 and PRb.(5) Strategies include condom use, male circumcision, discouraging smoking, as well as the HPV vaccinations.(5) HPV vaccinations ideally need to be administered prior to exposure to HPV. The HPV vaccines currently available only provide immunisation against oncogenic HPV

subtype 16 and 18 despite there being numerous other oncogenic subtypes known to cause cervical cancer.(6) Human immunodeficiency virus (HIV) infected patients are at an increased risk of being infected by numerous subtypes of oncogenic HPV such as 33, 35, 52, and 81 but 16 and 18 remain the most common infective subtype even in this immune suppressed group.(1) There is some cross protection between the strains included in the vaccinations and those excluded.(5)

By extending coverage to the 8 most common oncogenic strains of HPV, it is postulated that up to 95% of cervical cancer could be prevented.(2) Because of the possibility of infection by numerous subtypes, cervical screening with cytology must be continued. One fear is that with the introduction of vaccination, women may not report for cervical screening.(1)

Secondary Prevention makes use of cervical cytology in the form of the Papanicolaou test (Pap test) to detect pre-malignant cervical intraepithelial neoplasia (CIN). These cytological findings are reported according to the Bethesda system from 2001.(9) Well established screening programmes in Scandinavia have shown that greater population coverage is more important than the frequency of screening.(10) Adequate screening could decrease the rate of cervical cancer by two thirds.(1,6) The cervical screening programme in South Africa was introduced in 2001.(11) These guidelines were developed based on the WHO guidelines for screening in middle income developing countries. (12)The South African National guidelines state that asymptomatic woman should receive 3 Pap smears in their lifetimes starting at the age of thirty, and then one ten years apart. Women over the age of 50 years who had never been screened would receive one Pap smear.(6,11) Uptake of these Pap smears has been poor with an estimated 20% coverage of the population of Johannesburg through the primary health care centres.(10)

Women with abnormal Pap smears showing precancerous lesions must then be referred to a centre offering colposcopy where the lesion can be visualised and excised. The most common

form of excision done is Large Loop Excision of the Transformation Zone (LLETZ). LLETZ is performed on all women with high grade lesions (CIN II and III),where the colposcopic finding and cytology finding are discrepant by more than one grade, or who have persistent low grade lesions.(10,13) One concern with an increasing level of screening by primary health care centres is the inability of referral centres to cope with the number of referrals received resulting in long waiting lists and delay of treatment.

Many referral centres have initiated a one step "Look and LLETZ" clinic.(14) In resource poor settings where follow-up is notoriously poor, LLETZ at time of first consultation has eliminated the risk of patients not returning for definitive treatment after colposcopic biopsies were performed. The results from the Look and LLETZ clinics have been satisfactory although 15-49% of patients have cytological abnormities on a follow-up Pap smears.(10,14) The risk of persistent disease seemed to be greater in HIV positive women, women over the age of 50 years, and incomplete excision at either one or both resection margins.(15,16) Although treatment of the pre-malignant lesion does decrease the risk of an invasive cervical cancer, patients undergoing treatment remain at a 5 times increased risk of developing invasive disease and so careful follow up is mandatory with possible retreatment if indicated.(16)

The advantage of LLETZ is the retention of fertility with minimal short term complications.(13,16) In immune-competent woman LLETZ has been shown to be highly effective (90%) in removing the CIN lesion in its entirety. LLETZ has been associated with a risk of preterm rupture of membranes and preterm birth.(13)

1.3 Cervical intraepithelial lesion and the Human Immunodeficiency Virus

The epidemic of Human Immunodeficiency Virus (HIV) infection in Sub-Saharan Africa is one of the worst in the world.(1) The HIV epidemic has hit where the incidence of cervical dysplasia and cancer is most prevalent.(1) It is estimated that there are approximately 5.9 million people in South Africa living with HIV.(17) In 2011 the HIV prevalence in antenatal clinics across South Africa was 29.5%.(18) This HIV prevalence has remained fairly stable since 2007. The HIV prevalence among the general population has remained at approximately 17.3% since 2005.(18)

Cervical cancer was added to the list of AIDS defining conditions by the Centres for Disease Control (CDC) in 1993. In the same document released by the CDC, emphasis was placed on the need for intensive screening to identify and treat cervical dysplasia in an attempt to prevent progression to invasive disease(19).

The incidence of HPV infection and intraepithelial lesions in HIV infected women is up to 5 times higher than the HIV-negative population with between 20-40% of women having CIN lesions ranging from LG-SIL to HG-SIL and up to 49% having concurrent infection with HPV.(15,20) The number of serotypes of HPV infection, including oncogenic subtypes is far more extensive in HIV positive versus HIV negative women. There is also increased persistence of the HPV infection with 24 % of HIV positive women having persistent HPV infection compared to only 4% in HIV negative woman.(21) Woman co-infected with HIV and HPV have shown a decreased regression of low grade lesions and a higher rate of progression to a high grade CIN.(21) The size of the CIN lesion is often found to be greater in diameter and this in itself may lead to the higher rate of unclear margins found in HIV positive women post LLETZ procedure.(22)

The progression from pre-malignant lesion to invasive cancer in HIV positive women is 10-15 years shorter than in HIV negative women. Invasive carcinoma is usually found in patients who are profoundly immune-compromised with CD4 counts below 200cells/µl.(1)

It is difficult to tell whether the incidence of cervical cancer has increased due to HIV as there is poor record keeping within most developing countries. In developed countries women living with HIV have an increased rate of CIN but because of the existence of vigorous screening programmes the overall incidence of cervical cancer has remained low.(21) In developing countries where the availability of Highly Active Antiretroviral Treatment (HAART) has only recently become available it is possible that HIV positive women were dying because of opportunistic diseases such as Tuberculosis and *Pneumocystis carinii* before the relatively longer transition from pre-malignant CIN lesions to invasive carcinoma became apparent.(21,23)

Studies looking at the introduction of HAART and its effect on HPV and CIN lesions have revealed mixed results (21,23) Some studies have shown there may be limited clearance or regression of HPV and CIN in HIV positive women on HAART. (21) The regression rates between women on HAART and those not on HAART were minimal. The small benefit seemed to be in woman who had the greatest response to HAART in terms of CD4 count and viral load suppression and those who initiated HAART at higher CD4 counts(21). This may imply that cellular genetic changes induced by prior HPV infection, more than the HPV itself, may be responsible for the persistence and progression of lesions.(21)

HIV positive woman should have intensive cervical screening with the HIV Management Policy suggesting that annual pap smears should be carried out.(5) (23) Although there is no fixed guideline the current opinion is that HIV positive woman should have a Pap smear and pelvic exam at time of first presentation and then again twice within the first year of diagnosis. If these show abnormal cytology, referral for colposcopy should be done immediately. If they

remain normal, Pap smears can then be done on an annual basis.(21) The South African cervical screening programme does not take HIV into account. The Western Cape Department of Health policy states that annual Pap smears should be undertaken once a diagnosis of HIV is made.(15) Unfortunately, research done in the Western Cape by Batra et.al shows poor adherence of this guideline, with only 13% of HIV positive patients having had 1 Pap smear. (15)

There are a number of areas of concern regarding HIV positive women and our current screening and treatment guidelines of HPV and CIN.

Firstly, Shah et al showed poor correlation between the initial cytological diagnosis and that found at time of histological diagnosis made at LLETZ in HIV positive patients. Shah's study found only a 42-45% correlation between the Pap smear and histological sample.(24) This study had significant limitation in that it was done on relatively small sample size of 71. Research done prior to this in 1994 by Wright et al which found the sensitivity and specificity of the cervical cytology to be adequate at 81 and 87% respectively when compared to histological samples.(25) The question raised by these findings is that of using alternative screening modalities in HIV positive patient such as HPV testing or colposcopy.

Another area of concern is the higher rate of involved margins in HIV positive patients as shown in a study by Giles in 2005.(20) Positive margins were found in up to 40% of HIV positive patients with treatment failure as indicated by a CIN lesion on follow up Pap smears was found in as many as 56% of patients in this group.(20) Again this study was done with a small sample size of 68.

Foulot et al showed that careful selection of patients according to adequacy of colposcopy and size of lesion was important to obtain adequately excised lesions. In his study he

recommended the use of electrosurgical conisation over LLETZ for large lesions or an inadequate colposcopy. An adequate colposcopy was defined as full visualization of the transformation zone.(22)

1.4 Problem Statement

In our population, with a high prevalence of HIV infection, it is of upmost importance to ensure that CIN is adequately screened for and treated to prevent the development of invasive cervical cancer. It is still uncertain which patients despite positive margins at time of LLETZ will go on to develop persistent CIN or invasive disease.

Patients with certain risk factors which can be identified should possibly have closer surveillance. The difficulty is in attempting to identify this high risk group.

The involvement of histological margins after a LLETZ biopsy has been shown to influence recurrence of CIN post treatment. (16) Other factors shown to have an influence on CIN recurrence are HIV status and CD4 count.(16) The use of HAART does potentially have a protective effect against CIN. The greatest effect is in those patients with the greatest response in CD4 count and suppression of viral load.(23) Unfortunately in our clinic these parameters are not routinely collected as there is no integrated system of care between the antiretroviral clinics and the colposcopy clinic. This makes adequate surveillance of our patients almost impossible.

The higher the grade of CIN excised also is a risk for involved margins and therefore recurrence. Dobbs et al attributed this to higher grades having larger lesions as well as possible deeper crypt involvement leading to challenge at excision(26).

The international federation for Colposcopy and Cervical pathology endorsed a classification of the transformation zone. This was based on site, size and visibility.(27) Type 1 has a transformation zone that was fully ectocervical had the greatest chance of complete excision. In type 2, the full transformation zone is visualised but may have an ectocervical component. Type 3 the transformation zone has an endocervical component and is not fully visualized. Type 1 had the greatest chance of complete excision with Type 3 having the smallest percentage of clear margins.(28) As per Foulot(22) we may need to institute a system of careful selection of patients for LLETZ biopsy based on the visualization of the transformation zone.

1.5 Study Objectives

Primary Objectives

The primary study objectives are as follows:

1. To analyse the demographics of the sample population of women presenting for treatment at the Charlotte Maxeke Johannesburg Academic Hospital from 1 April 2004 to 31 October 2012.

2. To determine the risk factors for involved margins of the LLETZ biopsy.

Secondary objectives:

1. To determine the follow up rate of patients at the colposcopy clinic
2. To compare the results of the margins of the LLETZ biopsy to follow up Pap smears done after 6 months.

3. To correlate the cytology results of the Pap smear with the histology found at time of LLETZ biopsy.

Chapter 2

2.1 Methodology

Study design

This study was a retrospective review of case note files of HIV positive women attending the Colposcopy clinic at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in Johannesburg, South Africa. This clinic is an Academic unit and is part of the faculty of Health Sciences of the University of the Witwatersrand. It is a strictly referral unit accepting referrals from local primary health care clinics and general practitioners in the greater Johannesburg Metropolitan area. Patients are referred with high grade lesions or persistent low grade lesions found on Pap smear. The average waiting time for an appointment at this clinic is 3 months.

Population

The sample included all HIV positive patients referred for colposcopy and treatment from the roll out of HAART in Gauteng on 1 April 2004 to 31 December 2012. This cohort of patients is from a population that does not have access to private health care. HIV diagnosis is done by voluntary counselling and testing (VCT) at local primary health centres (PHC) followed by CD4 counts when appropriate. HAART is generally initiated at the PHC. All patients attending colposcopy clinic are again offered the opportunity for VCT if their HIV status is unknown at time of presentation. As the report of HIV status is verbal and relies on forthcoming disclosure, patients who claim to be HIV negative are encouraged to retest.

Procedure

Cervical smears done at PHC's are analysed by the National Health Laboratory Services (NHLS) according to the 2001 Bethesda System terminology.(9)

The Clinic offers a "Look and LLETZ" service and is staffed by dedicated nursing sisters and doctors. All procedures are performed by fellows in gynaecology oncology or specialist gynaecologists. Colposcopy is done using 3-5% acetic acid followed by the application of Lugol`s iodine. Twp percent lignocaine is used to perform a para-cervical block. An appropriate sized loop is then chosen to do the LLETZ.

LLETZ is performed on patients with a high grade CIN lesion (CIN II or higher), if there is inadequate colposcopy, or if there is a persistent CIN/ASCUS lesion on repeated Pap smears.

The LLETZ biopsy sample is then placed in formalin solution and is analysed by pathologists at the NHLS. Post LLETZ the base of the lesion is cauterised using a roller ball in the hope of ablating any remaining cells missed on excision.

Patients then return after 6 months for follow up which includes receiving the result of the LLETZ biopsy as well as a repeat pap smear. Involved margins were defined as either endocervical margin, ectocervical margin or both margins involved with CIN lesion of any grade. Regardless of the result of the LLETZ biopsy a repeat Pap smear is done at this time and the patients are asked to return in 3 months. If this repeat Pap smear shows a high or low grade lesion the patient has a repeat colposcopy and LLETZ.

The women's medical history and results: initial cytology report, HIV result, CD4 count, anti-retroviral regimen, contraception and histology are kept in a dedicated file in the colposcopy clinic thereby minimising the loss of files for review in this study.

Exposure and Outcome Variables

The factors that were reviewed included

- The experience of the attending doctor doing the LLETZ biopsy
- Age of patient at presentation
- Parity and gravidity
- HIV and immune status by way of CD_4
- Use and duration of HAART
- The grade of the original cytological lesion using the Bethseda classification system
- Time elapsed between the original pathological Pap smear and the LLETZ biopsy
- Use and method of contraception at the time of the consultation
- Involved margin of the biopsy: endocervical, ectocervical or both
- Histology of the LLETZ sample

2.2 Statistics

Data was collected and entered into a Microsoft Excel Software spreadsheet. Descriptive statistics were preformed and statements of frequency with percentages and mean with standard deviations, or medians with ranges were found. To determine associations between the outcome measure and possible determinates, variate and multivariate logistic regression were used with statements of odds ratios and 95% confidence interval. Determinants showing

p-values of <0.2 on variate analysis were included in the multivariate logistic regression model.

Sample size estimate was based on the possibility that clear margin rate may be decreased from 60% to 40% if the CD_4 count is lower than 200cells/μl. It was assumed that one third of women will have a CD_4 count of less than 200calls/μl. To achieve statistical significance for this difference at a p-value of 0.05 and with a power of 80%, a minimum of 240 patient files had to be reviewed.

2.3 Limitations

Poor record keeping by attending doctor led to a loss of data. Patient files that have missing data such as HIV result, CD4 count; poor note keeping around the colposcopic procedure by the attending physician were excluded.

2.4 Funding

As this is a retrospective review of files very little funding was required. This study was self funded

2.5 Ethics and consent

Ethics

Ethics permission has been obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand. (See Appendix A)

Hospital Permission

Hospital permission has been obtained from the Chief Executive Officer of Charlotte Maxeke Johannesburg Academic Hospital. (See Appendix B)

Consent

As this is an anonymous retrospective review of patient files, patient consent was not required.

No minors were included in this study.

Chapter 3: Results

After the files of the CMJAH Colposcopy clinic were reviewed, 480 files were found to be complete with all demographic information as well as clinical data including results of HIV, CD4 count and histology results adequately recorded. The time frame for files reviewed was from 1 April 2004 to 31 December 2012.

As HIV is self-reported by the patients, the absolute number of patients in our study found to be HIV positive may well be under reported. All patients presenting with an unknown HIV result or who claim to be HIV negative were strongly encouraged to retest and sent for voluntary counselling and testing. In patients with unknown CD4 counts, CD4 counts are taken and patients sent for initiation of HAART if appropriate.

3.1 Demographics and Clinical features of Patients

Age and gravidity of study population

The mean age of the study population was 36.10(SD =7.77) with a median age of 35.5(IQR =31-40). The youngest patient in our sample group was 20 and the oldest 64 years of age.

For statistical analysis in this study the population was divided into 3 groups namely <35, 35-49, and >49.

Table 1: Age in years number(n)=480)

Age Group	Frequency(n)	Percentage(%)
<35	211	43.96
35-49	237	49.38
>49	32	6.67

The mean gravidity on our sample population was 2.35(SD=1.30) with a median of (IQR=1-3). There were 23 women in the study who had never fallen pregnant and 30 who had a gravidity greater than 5. The greatest gravidity was 9.

Again for statistical analysis the patients were divided into 3 groups: gravidity of 0, gravidity of 1-2, and gravidity >2

Table: 2 Gravidity (n=480)

Gravidity	Frequency(n)	Percentage(%)
0	23	4.79
1-2	269	56.04
>2	188	39.17

Contraception use

Contraception in the files was recorded according to what the patient was currently using. No mention was made of historic contraceptive use by the patients. Contraception was divided into none, combined oral contraception pill (OCP), injectable progesterone (Nur Isterate and Depo Provera), intra uterine contraceptive device (IUCD) and condom usage.

Table 3: Contraception use (n=480)

Contraception	Frequency(n)	Percentage(%)
None	262	54.58
Condom	119	24.79
OCP	23	4.79
Progesterone	74	15.42
IUCD	2	0.42

In this category are included patients who desire fertility, are not sexually active or have undergone surgical sterilization so may account for the high proportion not using contraception.

CD4 Count

CD4 is measured as an absolute value in cells/mm^3 at time of colposcopy

In our study population the mean CD4 was 316.20 (Standard deviation (SD)190.82). The median was 288.5 (IQR=170-423.5) with a range of 7 to 1000

For further analysis the CD4 count was divided into 3 groups namely <201, 201 to 350, and >350.

Table 4:CD4 count (n=480)

CD4 count	Frequency(n)	Percentage(%)
≤ 200	155	32.29
201-350	141	29.38
>350	184	38.33

Antiretroviral use

Table 5: Antiretroviral use (n=480)

On ARVs	Frequency(n)	Percentage(%)
Yes	232	48.33
No	248	51.67

The duration of HAART use nor patients response to the use of HAART in terms of absolute CD4 count or viral load are not recorded in the files at the colposcopy clinic.

Referral time

This was defined as the time taken from the day of first presentation for a Pap smear at the primary care centre to the day the first colposcopy was done at CMJAH Colposcopy clinic. The mean was 186.49 days (SD 150.55). The median was 152 (IQR 110-210.5). The range was 27-1769 days.

For analytical purposes this group was divided into 3 groups namely less than 180 days, 180 to 365 days, and greater than 365 days.

Table 6: Referral time from original Pap smear to colposcopy (n=480)

Time in days	Frequency(n)	Percentage(%)
<180	319	66.46
180-365	123	15.63
>365	38	7.92

3.2 Colposcopy and histology LLETZ biopsy samples

Cytology of referral Pap smear

Table 7: Cytological grade of CIN lesion on referral Pap smear (n=480)

Cytological grade	Frequency(n)	Percentage(%)
ASCUS	19	3.96
HGSIL	401	83.54
LGSIL	60	12.50

The vast majority of patients referred had HGSIL on their referral Pap smear which was to be expected considering the referral criteria for the colposcopy clinic.

Size of CIN lesion at colposcopy

The size of the lesion was judged completely objectively by the surgeon doing the colposcopy and was marked on a diagram within the patients file. There was no way to verify the size of the lesion or by which standard the surgeon sized the lesion.

Table 8: Clinical impression of size of CIN lesion (n=480)

CIN Size	Frequency(n)	Percentage (%)
Small	172	35.83
Medium	171	35.63
Large	137	28.54

Transformation zone visualized at time of colposcopy

The transformation zone was noted to be visualized in 340 (70.83%) of patients undergoing colposcopy. In 140 (29.17%) of the patients the transformation zone was not seen.

Histology of LLETZ biopsy

Table 9: Histology of LLETZ biopsies (n=480)

Histology Result	Frequency(n)	Percentage(%)
CIN1	86	17.92
CIN2	152	31.67
CIN3/Ca in-situ	242	50.42

Of these colposcopic LLETZ biopsies done, 168 (35.21%) had clear margins. 275 (57.29%) had involved margins.

In 36 (7.50%) specimens the pathologist was unable to comment on the involvement of the margins. Reasons given for the inability to comment on involvement of margins in the pathology reports included severely traumatised specimens, significant cautery artifact, failure to orientate the specimens, or the specimens being submitted in multiple small fragments.

Histological involvement of the excised margins

Table 10: Involvement of margins (n=480)

Margin involved	Frequency(n)	Percentage(%)
Unsure	36	7.50
Both margins clear	169	42.71
Endocervical	117	24.38
Ectocervical	57	11.88
Both margins involved	101	21.04

Patients with unsure margins were excluded from further analysis. Because of this our study population was reduced to 444 participants. The number of specimens with any positive margins was combined and found to be 275(61.94%) The endocervical margin was clear in 226(50.90%) of specimens. Ectocervical margin was found to be clear in 286 (64.41%) of specimens.

Treating Doctor

Table 11: Clear and involved margins obtained by each treating doctor (n=444)

Doctor code	Involved Margins		Clear Margins		Total of patients treated by Dr
	Frequency(n)	Percentage(%)	Frequency(n)	Percentage(%)	
1	74	69.81	32	30.19	106
2	40	43.48	52	56.52	92
3	23	71.88	9	28.132	32
4	108	68.35	50	31.65	158
5	8	72.73	3	27.27	11
6	9	56.25	7	43.75	16
7	2	22.22	7	77.78	9
8	11	55.00	9	45.00	20

Doctor 2 was used for further statistical analysis as they had the greatest clear margin rate and treated a significant number of patients.

3.3 Patient follow-up at CMJAH colposcopy clinic

Two hundred and twenty one (46.04%) patients did not come back to the colposcopy clinic for follow-up Pap smears. We cannot exclude that these patients did not follow up at other institutions or returned to their primary health centres for their follow-up Pap smears.

The table below demonstrates the follow-up Pap smear results of patients who did return to the colposcopy clinic.

Table 12: Follow up Pap smears (n=259)

Cytology Grade	Frequency(n)	Percentage(%)
Normal	107	41.31
ASCUS	12	4.63
HGSIL	60	23.17
LGSIL	80	30.89

3.4 Prediction of positive margins

This was divided into endocervical and ectocervical margin involvement to determine independent risk factors for each. An analysis of the data was done according to each of the risk factors for which we had available data. A p-value was then calculated with significance being set at <0.05.

Endocervical Margins

Table 13: Predictive factors for involved endocervical margins

Predictor		Involved margins n=218,(%)	Clear Margins n=226,(%)	P value
Age	**<35**	97(49.49)	99(50.51)	0.98
	35-49	107(48.64)	113(51.36)	
	>49	14(50)	14(50)	
CD4 count	**>350**	84(49.12)	87(50.88)	0.25
	201-350	56(43.75)	72(56.25)	
	<201	78(53.79)	67(46.21)	
Gravididty	**0**	8(36.36)	14(63.64)	0.47
	1-2	124(49.80)	125(50.20)	
	>2	86(49.71)	87(50.29)	
HAART	**No**	109(50.93)	105(49.07)	0.46
	Yes	109(47.39)	121(52.61)	
Doctor 2	**No**	196(55.68)	156(44.32)	<0.01
	Yes	22(23.91)	70(76.09)	
Cytology group	**LGSIL/ASCUS**	24(32.00)	51(68.00)	<0.01
	HGSIL	194(52.57)	175(47.43)	
CIN Size	**Small**	71(44.94)	87(55.06)	0.20
	Medium	76(48.10)	82(51.90)	
	Large	71(55.47)	57(44.53)	
TZ Seen	**no**	55(43.65)	71(56.35)	0.15
	yes	163(51.26)	155(48.74)	
Time to referral	**<180**	141(48.62)	149(51.38)	0.95
	180-365	58(49.57)	59(50.43)	

	>365	19(51.35)	18(48.65)	
Contraception	**None** **Condom** **OCP** **Progesterone** **IUCD**	107(44.96) 66(58.41) 12(52.17) 32(47.06) 1(50.00)	131(55.04) 47(41.59) 11(47.83) 36(52.94) 1(50.00)	0.22
Hormonal contraception	**No** **Yes**	174(49.29) 44(48.35)	179(50.71) 47(51.65)	0.87
OCP	**No** **Yes**	206(48.93) 12(52.12)	215(51.07) 11(47.83)	0.76
Depot Progesterone	**No** **Yes**	186(49.47) 32(47.06)	190(50.53) 36(52.94)	0.72
LLETZ Histology	**CIN 1** **CIN 2** **CIN 3**	7(9.09) 68(49.64) 143(62.17)	70(90.91) 69(50.36) 87(37.83)	<0.01

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Table 14 Logistic regression model for prediction of clear endocervical margins

	Adjusted Odds Ratio	**95% Confidence Interval**	**P value**
LLETZ histology CIN1 (reference)	1.00	-	-
LLETZ histology CIN2	0.10	0.41-0.23	<0.01
LLETZ histology CIN3	0.07	0.28-0.15	<0.01
Other Doctors (reference)	1.00	-	-
Doctor 2	3.84	2.21-6.66	<0.01

Ectocervical Predictors for Involved margins

Table 15: Predictors of positive ectocervical margins

Predictor		**Involved margins n=218 n(%)**	**Clear Margins n=226 n(%)**	**P value**
Age	**<35**	77(39.29)	119(60.71)	0.33
	35-49	71(32.27)	149(67.73)	
	>50	10(35.71)	18(64.29)	
CD4 count	**>350**	56(32.75)	115(67.25)	0.21
	201-350	42(32.81)	86(67.19)	
	<201	60(41.38)	85(58>62)	
Gravidity	**0**	8(36.36)	14(63.64)	0.77
	1-2	85(34.14)	164(65.86)	
	>2	65(37.57)	108(62.43)	
HAART	**No**	73(34.11)	141(65.89)	0.53
	Yes	85(36.96)	145(63.04)	
Doctor 2	**No**	133(37.78)	219(62.22)	0.06
	Yes	25(27.17)	67(72.83)	
Cytology group	**LGSIL/ASCUS**	24(32.00)	51(68.00)	0.47
	HGSIL	134(36.31)	235(63.69)	
CIN Size	**Small**	37(23.42)	121(76.58)	<0.01
	Medium	51(32.28)	107(67.72)	
	Large	70(54.69)	58(45.31)	
TZ Seen	**No**	30(23.81)	96(76.19)	<0.01
	Yes	128(40.25)	190(59.75)	
Time to	**<180**	101(34.83)	189(65.17)	0.51

referral	**180-365**	46(39.32)	71(60.68)	
	>365	11(29.73)	26(70.27)	
Contraception	**None**	80(33.61)	158(66.39)	0.09
	Condom	45(39.82)	68(60.18)	
	OCP	3(13.04)	20(86.96)	
	Progesterone	29(42.65)	39(57.35)	
	IUCD	1(50.00)	1(50.00)	
Hormonal	**No**	126(35.69)	227(64.31)	0.93
contraception	**Yes**	32(35.16)	59(64.84)	
OCP	**No**	155(36.82)	266(63.18)	0.02
	Yes	3(13.04)	20(86.96)	
Depot	**No**	129(34.31)	247(65.69)	0.19
Progesterone	**Yes**	29(42.65)	39(57.35)	
LLETZ	**CIN 1**	8(10.39)	69(89.61)	<0.01
Histology	**CIN 2**	40(29.20)	97(70.80)	
	CIN 3	110(47.83)	120(52.17)	

Table 16: Logistic regression model for the prediction of clear ectocervical margins

	Adjusted odds ratio	95% Confidence interval	P value
Non OCP Use(reference)	-	-	
OCP use	3.35	0.99-12.64	0.052
LLETZ histology	-	-	
CIN1(reference)	0.26	0.11-0.61	<0.01
LLETZ histology CIN2	0.13	0.06-0.29	<0.01
LLETZ histology CIN3			
CIN lesion small or medium (reference)	-	-	
CIN large	0.36	0.23-0.57	<0.01

3.5 Results of Secondary Objectives

Follow-up rate at CMJAH colposcopy clinic

Of the 480 patients seen at the clinic 221(46.04%) did not return for a follow-up Pap smear. Two hundred and fifty nine (53.95%) followed up and the results of their repeated Pap smear were analysed for the risk factors for recurrence of the intra epithelial lesion.

Risk factors for recurrence of intraepithelial lesion on follow up Pap smear

Table 17: Risk factors for recurrence of CIN lesion at time of follow-up Pap smear.

		Cytology of follow-up Pap smear				P Value
		Normal	**ASCUS**	**LGSIL**	**HGSIL**	
Both Margins clear	**No**	54(35.29)	4(2.61)	49(32.03)	46(30.07)	0.01
	Yes	40(47.62)	6(7.14)	27(32.14)	11(13.10)	
Endocervical margin clear	**No**	47(38.21)	4(3.25)	37(30.08)	35(28.46)	0.38
	yes	47(41.23)	6(5.26)	39(34.21)	22(19.30)	
Ectocervical margin clear	**No**	25(28.41)	1(1.14)	32(36.36)	30(34.09)	<0.01
	yes	69(46.31)	9(6.04)	44(29.53)	27(18.12)	
Histology of LLETZ biopsy	**CIN1**	20(45.45)	3(6.82)	18(40.91)	3(6.82)	0.142
	CIN2	29(36.71)	4(5.06)	23(29.11)	23(29.11)	
	CIN3	58(42.65)	5(3.68)	39(28.68)	34(25.00)	

Both margins clear on histology were found to be significant with a p-value of 0.01. A clear ectocervical margin was found independently to be significant in regards to follow-up Pap smear results.

Correlation of cytology and histology

Table 18: Correlation of cytology of original Pap smear cytology and histology found at time of LLETTZ.

		Histology of LLETZ			P value
		CIN 1	**CIN 2**	**CIN3**	
Cytology of original pap smear	**ASCUS**	9(47.37)	4(21.05)	6(31.53)	<0.01
	LGSIL	30(50.00)	17(28.33)	13(21.67)	
	HGSIL	47(11.72)	131(32.67)	223(55.61)	

The correlation of original cytology with the histology was found to be adequate.

Chapter 4: Discussion of Results

4.1 Study group demographics

Age and gravidity

The mean age of our study population was relatively low at 36. This is especially true when one takes into account that the South African cervical screening programme states that cervical screening should only begin at age 30(11). There were 116(24.16%) patients seen at the colposcopy clinic who were 30 years and younger. Although this shows that national guidelines may not be being followed it does perhaps suggest that patients who are diagnosed as HIV positive are having Pap smears done at their primary health care centres despite their age. This does follow suggestions by the Western Cape Department of Health as well as Palefsky et al(21) who recommend a pap smear be done at time of first diagnosis of HIV and then annually thereafter.(15)

The younger age of this referred population is also important because invasive cervical cancer seems to present 10-15 years earlier in the HIV infected population(29) indicating the need for earlier and more intensive screening. It may also indicate that there are a significant number of women with cervical dysplasia under the age of 30 and that including this group into a national screening programme may be beneficial.

The gravidity of the study group was also relatively low with a mean of 2.35. This again may be attributed to the relatively young nature of our population group still within their reproductive years.

Contraception

Barrier method contraception in the form of condoms was low at 24.79%. In a population group infected with sexually transmitted infections such as HIV and HPV, one would hope that condom use would have been significantly higher. MacPhail et al found that despite good education and knowledge of HIV and its route of transmission, there are still many factors preventing condom use. These included lack of perceived risk of infection, peer norms, lack of condom availability, economic context of sexual relationships and gendered power relations. In his study 69% of respondents never used condoms with 16.7% reporting occasional use and only 14.3% reporting regular condom usage.(30) Lack of condom use has also shown a greater chance of persistence of HPV oncogenic strains and so may lead to higher rates of intraepithelial neoplasia and invasive cancers.(31)This is clearly an area of concern and indicates a clear need for further intensive education programmes.

OCP which has a known association with cervical cancer (32) was used by only 4.79% of our study population.

CD4 and HAART use

Almost half our study population was using HAART (48.33%). We did not have access to information as to the duration of treatment; regimens or response to HAART as it was not recorded within the patient files. This is unfortunate as it is the response to HAART in terms

of viral load suppression and improvement in CD4 count which is thought to be significant in the clearance of intraepithelial lesions and HPV infections.(21)

The mean CD4 count was 316.20(SD 190.82). The lower the CD4 count the greater the incidence of HPV persistence on CIN lesions. Moodley et al(33) found a 41-fold increased risk of any grade of CIN lesion if women were infected with HPV and HIV. Firnhaber et al found that if CD4 was < 200cells/cm^3 the prevalence ratio of HGSIL and LGSIL was 2.5 versus if the CD4 count was >500cells/cm^3 and did not find any significant affect of HAART on the outcomes.(34) This emphasises the need for intensive cervical screening of our HIV positive patients who have had a CD4 count fall below 200cell/cm^3 and the need to initiate HAART at a higher CD4 count.

Referral time

It is encouraging to see that the majority of our patients (66.46%) were seen at the colposcopy clinic in under180 days. A study by Saayman et al (35) did not show any significance in a greater time delay in the progression of CIN lesions at more than 180 days. Our range was very large with the longest time delay of 1769 days. Once again educating patients in the purpose and results of the Pap smear may help to significantly decrease the time it takes the patients to collect results from the primary health care centres and to make an appointment to get seen at the colposcopy clinic itself.

Fully booked clinics may also contribute to time delay from Pap smear to colposcopy and so assessment the available resources in terms of adequately trained staff as well as equipment needs to carried out.

Description of referral cytology and colposcopic lesions seen

The majority of patients (83.54%) who presented to the CMJAH colposcopy clinic had high grade lesions on their original Pap smears done at their primary health care providers. This is in keeping with the clinic protocol that will see patients either with a high grade intraepithelial lesion or with repeated low grade lesions.

On colposcopy the clinical impression of the lesion size was documented as well as the visualization of the transformation zone. This is deemed important as Foulot et.al (22) has shown that careful patient selection for a LLETZ biopsy is important for good outcomes and an adequately excised lesion. In our study population, the transformation zone was not visualized in 29.17% of cases. The lesion was deemed "large" in 28% of patients. One must question whether these patients may have been more appropriately treated with a cone biopsy. It must also be taken into account the impression of size of the lesion is purely objective and there is no way to verify the actual size of the lesion seen.

Treating Doctor

Doctor 2 had significantly lower involved margin rates compared to all other doctors working at the colposcopy clinic (56.52%). Doctor 7 had excellent clear margin rates but saw a small number of patients and so the significance of his/her success rate cannot be varified. What became apparent was that years of experience did not seem to impact on the clear margin rate but perhaps it is more the individual doctors' technique which plays a greater role.

4.2 Risk Factors for Involvement of Margins on Histology

In the analysis of our data we found that 169(42.71%) had clear margins. The clear excised margins is similar to those reported by Adam(16) at Chris Hani Baragwaneth Hospital colposcopy clinic: a sister unit within the University of the Witwatersrand, where the clear margins were 42.96%. The incomplete excision rates vary widely in published data from 5-51%.(28) Margin involvement is far more common in HIV infected women versus their HIV negative counterparts. (20,24) Higher margin rate involvement is likely due to the larger size of lesions and perhaps crypt involvement in HIV positive women.(24)

The results were divided in to clear margins for endocervix versus ectocervix as it was found that there were different risk factors that were significant for each group.

Risk factors for endocervical margin involvement

When the data was analysed, age, gravidity, CD4 count, use of HAART was not found to be significant. A higher CD4 count has in other studies been shown to decrease the positive margin rate and it is suspected that the stronger the immune system the more likely it would be that local immunity at the cervical level would be able to keep the size and depth of the CIN lesion small.(24) A lower CD4 count may contribute to the development of the lesion but once the lesion is established the progression of the disease process is not affected by the CD4 count.(36) Patients older than 50 years of age are also thought to be at risk for recurrence and involved margins. In our study the age of the patients was not significant but our group of patients older than 50 was small with only 28 patients and so may have been underpowered to obtain a significant result.

Surprisingly the visualization of the transformation zone and size of lesion did not appear to play a significant role either. This is against data that suggests that larger type 3 transformation zone with extension into the endocervix has twice the risk of incomplete excision at the endocervical margin.(28)

The cytological grade of the original pathological Pap smear was a found to be statistically significant with a p-value of <0.01. The higher the grade of intraepithelial lesion on the original Pap smear resulted in a greater chance of having involved margins. High grade intraepithelial lesions are a well-known risk factor for positive margins as well as recurrence. (37)

The rate of clear margins obtained by Doctor 2 was statistically (p-value <0.01). Doctor 2 was not the most experienced doctor in the study which suggests that his/her technique was the significant factor. There is a possibility that this doctor removed a greater volume of cervical tissue at time of the LLETZ or that he/she extended his biopsy higher up in to the endocervix. Unfortunately neither the depth of tissue nor volume of biopsy sample is reported as a standard on our pathology reports. The danger with overzealous excision is that there may be long term consequences for the patients in terms of preterm labour with an increase risk of 1.7 (95%CI 1.2-1.4).(28) The pregnancy associated risks have been shown to increase once the depth of excision exceeds 10mm with a 3 fold increase if the volume exceeds $6cm^3$.(28) There is also an increased risk of small gestational babies and preterm rupture of membranes. Despite this there is a smaller chance of adverse outcomes, short and long term, after LLETZ as compared to a cone biopsy. The risk of cervical stenosis post LLETZ is less than 1% and quoted as 1.3% if he excision depth is deeper than 14mm.(37) Despite the risks associated with LLETZ biopsy it is still the preferred method of treating high grade lesions in young women of child bearing age.(28)

Contraception of any type (hormonal or non-hormonal) did not provide significance at the endocervical margin.

A prolonged pap to colposcopy time did not influence the clear margin rate (p-value =0.95).

A direct significant relation was found when comparing the histological grade of CIN from the LLETZ biopsy to the clear endocervical margin. Approximately 90% of patients with CIN1 on histology had clear endocervical margins versus 37.83% with CIN3. This is concerning as the progression of CIN 3 to invasive cancer is 31-51% over 30 years.(38) McCredie's study was not on HIV positive patients and as discussed earlier the progression from CIN to invasive cancer appears to occur 10-15 years earlier in immune-compromised patients.(29) Compounded by our poor follow up rate, these patients are at significant risk for the development of invasive cancer in their future.

After the logistical regression and adjustment of confounding factors, (See table 15) the adjusted odds ratio was 0.07 for having clear endocervical margins if the histology was CIN3. The adjusted odds ratio for Doctor 2 was 3.84 and so remained significant for clear margins at the endocervical border.

Risk factors for the involvement of margins at the ectocervix

The predictive risk factors for endocervix were similar to those for the ectocervix.

Once again age, CD4 count, use of HAART, gravidity, and time to referral were not significant.

The grade of cytology of the original Pap smear was found not to be significant in the group with involved ectocervical margins with a p-value of 0.47. The cytology of the Pap smear was significant for involved endocervical margins.

The findings associated with Doctor 2 approached significance with a p-value of 0.06 which may imply that his LLETZ biopsies may have been deeper than they were wide.

CIN lesion size as perceived by the operating doctor was found to be significant with a p value of <0.01. The larger the lesion the more difficult it would be to excise completely without being overly radical in the process. This may imply that perhaps in patients where the lesion is very large a different mode of treatment such as a cone biopsy should be employed.

Strangely enough the visualization of the transformation zone was significant for clear ectocervical margins but not for endocervical margins. One way of explaining this may be that because the transformation zone was visualized, that perhaps the full extent of the lesion could be seen and targeted at the time of the LLETZ biopsy and so achieved complete excision.

Contraception as a whole (barrier and all hormonal methods included) was not significant. But when the combined oral contraceptive pill was analysed as a standalone variable it was found to be significant with a p-value of 0.02. This implied that the group using OCP had a higher rate of clear margins. This was an interesting finding as the long term use of OCP has been shown to be a risk factor for the development of invasive cervical cancer in patients who are exposed to HPV.(39) The exact mechanism of hormonal influence is unclear. Exogenous hormonal influence may promote the integration of HPV DNA into the host genome which in turn causes deregulation of E6 and E7 which are related to the oncogenic potential of HPV 16 as discussed earlier. Exogenous estrogen may also cause reactivation of HPV or persistence of the infection.(40) Despite this marginally increased risk it is still thought that the benefits of

contraception with OCP outweigh the risk it poses. There should be an effort to actively include long term users of OCP in screening programmes. (39)

One way of explaining our results in regards to OCP use may be that the squamocolumnar junction migrates under the influence of oestrogen, both endogenous and exogenous, causing ectropion. Because of this the transformation zone may have been better visualized and so a greater chance of achieving clear margins at time of LLETZ biopsy.

The histology of the LLETZ biopsy was found to be significant for ectocervical margin involvement (p-value <0.01). This would be for similar reasons as discussed above.

On the multivariate regression model that was done (see table 17), oral contraceptive use remained a significant predictive factor with an adjusted odds ratio of 3.35 and a confidence interval of 0.99-12.64,.As for the endocervical margins, the LLETZ histology also remained significant despite the size of the lesion or OCP use in the regression model with CIN 3 having the adjusted odds ratio of 0.13 with a 95% confidence interval of 0.06-0.29 and a p value of <0.01. A large intraepithelial lesion seen at time of colposcopy also remained statistically significant in the logistical regression.

4.3 Patient Follow-up

Follow-up of Patients at CMJAH Colposcopy clinic

The protocol for follow-up at our clinic is for the patients to return 6 months after the LLETZ for the results of their biopsy as well as a repeat Pap smear. Our follow up rate was

disappointingly low with only 259 (53.95%) of patients returning after 6 months. Both local and international studies have shown similarly low rates of follow-up in HIV infected populations.(10,41) This can be explained perhaps by a generally lower education level, and poorer socioeconomic background in this group of patients.(41)

In patients who have clear margins at time of LLETZ, cytological follow-up is adequate. In those with positive margins follow up with colposcopic and cytological methods is recommended.(26) A meta-analysis of 35 109 women in 66 different studies done by Ghaern-Maghami et al advised that patients who had deep or endocervical margins involved at time of LLETZ should have a repeat procedure as they are at significant risk for recurrence of high grade lesions and, in the long term, invasive cancer.(42)

By identifying pre-procedure risk factors or risk factors at time of LLETZ for positive margins, patients can be counselled accordingly for their risk of positive margins and recurrence at the time of their colposcopy appointment. Hopefully with better education and counselling at this first point of contact, follow-up rates can be improved.

Risk Factors for recurrence of cervical intraepithelial lesions on follow-up Pap smear

Our study found that if both margins of the LLETZ biopsy were found to be clear on histology, the recurrence rate at follow up cytology was significantly decreased with a p-value of 0.01. When analysing the margins divided into endo or ectocervical margins only, the ectocervical histology was found to be significant. This does go against other studies where the involvement of the endocervical margin appeared to be more significant in the persistence or recurrence of CIN.(16,41) There is a possibility that the group in this study was too small to yield statistical significance.

The grade of lesion found at histology was found not to be statistically significant (p-value 0.14).

HIV in itself is an independent risk factor for recurrence or reactivation of CIN. The management of CIN in these patients is extremely difficult and more intensive follow up of these patients is required from the time of diagnosis. Some studies have suggested that there is a decrease in recurrence in patients on HAART.(20) It is therefore important to encourage the initiation and adherence to HAART in this high risk group of patients.

Correlation of results of Pap smear cytology to histology of the LLETTZ Biopsy

There are findings that the cytological screening in HIV positive patients is not reliable with differing grades of intraepithelial lesion found when doing colposcopiclly directed biopsies.(24) This may be due to severe cervicovaginal infections which are common within this group. Wright et al (25) found that correlation between the cytology and the histology does appear to be adequate. The results of the analysis of our data echoed findings of local studies by Adam et al(10) who found an 80.2% correlation between the cytology and histology.

In my study the correlation was found to be appropriate with a p-value of <0.01.

4.4 Strengths of this study

The strength of this study lays in the large number of HIV positive patients records that underwent analysis. With the HIV endemic in South Africa, the more information we can gather on the effects of HIV and HAART on disease progression the more likely we will be to offer our patients appropriate management

4.5 Limitations

- This was a retrospective study. There were a significant number of patient records which could not be included in the study because of missing data, poor record keeping and inadequate clinical notes made by the attending doctors.
- Antiretroviral use: there was no information recorded in most of the files on the duration of use of HAART or the regimen the patient may have been on. Also the response to HAART was not documented either in improvement of CD4 count or the suppression of the viral load. Most patients receive their HIV treatment at dedicated HIV clinics either within CMJAH or at their local primary health care centres spread across the greater Johannesburg metropolitan area. This information may well be held at these locations and attaining this information and reassessing the clear margins and recurrence in light of these factors could be an area for future research in our unit.
- Other risk factors for CIN: HPV testing is not available within our setting of public practice. Smoking was not recorded in the patients' files. A detailed sexual history with regards to safe sexual practices or number of sexual partners was not recorded.
- HIV: the HIV result is self-reported by the patient. In South Africa there is still significant stigma around the disease and patients may not be willing to disclose a positive result even to a health worker. Those who claim to be negative are sent for VCT

at time of colposcopy. If the result was then found to be positive, these patients were then included into the study. There is a possibility of significant under reporting of HIV status by patients and so there may have been patients who would have inadvertently been left out of our study.

- Contraception: was recorded as current contraception. No information was available with regards to the historic use of different methods.

- Clinical description of the lesion and TZ: this is an objective impression by the different doctors and was recorded on a diagram in the patients' files or described by the doctor within the file as a small, medium or large lesion. There was no method to standardise their findings or to determine the actual measurements used as "small", "medium" or "large".

- The indication for doing the original Pap smear was not known.

- We do not have the depth or volume of LLETZ specimen excised. This may have given us some idea as to why certain doctors seemed to have improved clearance margin rates versus others.

- The poor follow-up rate after 6 months cause a possible under powering of statistics surrounding recurrence rates and correlation of cytology to histology.

- The correlation of initial cytology to histology appears to be good. But the gold standard would be to compare histology of colposcopically directed biopsy specimens done before the LLETZ to the histology of the LLETZ sample.

- Follow-up at 6 months is a relatively short follow up period. Ideally all patients who are HIV positive should be followed up for a number of years to ensure the clearance CIN.

Chapter 5:

Conclusion

South Africa is in the midst of an HIV epidemic. The HIV prevalence in antenatal clinics in 2011 was 29.5%.(18) This is a cross section of the sexually active proportion of our population and is also the proportion of women at risk for the development of CIN and eventually invasive cervical cancer. The burden on gynaecological and oncology services is overwhelming. Countries with well-established screening and treating facilities have managed to massively reduce the burden of invasive cancer.(2)

Intensive follow up of high risk patients is vital. It is already well accepted that HIV in itself is a risk factor for the development of CIN as well as invasive cervical cancer. In our study we attempted to define further which of these patients may fall into a group of even higher risk for recurrence of CIN. Of significance were involved margins (either endo or ectocervical) with involved ectocervical margins providing the greatest predictive factor.

The involved margin rate was relatively high when compared to international figures in patients who were HIV negative but appeared to be on par with studies done with a similar patient demographic.

A lower CD4 count or the use of HAART did not have a significant impact on the involvement of margins at histology. But due to the retrospective nature of our study, there was a significant amount of data around the use of HAART and response to HAART which was not available to us.

The use of the combined oral contraceptive pill improved clear margin rates at the ectocervical margin quite significantly. A well visualized transformation zone, and a small or medium size lesion was also found to improve clear margin rates at time of LLETZ biopsy.

The operating doctor made a statistical significant difference. This was not related to years of experience and was thought to be due to operative technique. A review of operative technique within the department may be warranted when faced with such a vast range in the clear margin rates. A follow-up of these patients in years to come may also prove to be interesting to determine if the patients seen by the various doctors may have higher complication rates in future pregnancies.

Our poor follow up rates may be an indication of the poor counselling and knowledge of our patients regarding the purpose of procedures undertaken and the need for intensive follow up. This may be an indication that even within an exceptionally busy colposcopy unit, a few extra minutes should be taken to ensure the patient understands the condition and the need for follow up or repeat procedures over a number of years.

Chapter 6:

Recommendations

Our study has confirmed risk factors found in other studies done on population groups with similar demographics both internationally and locally for involved margins and recurrence.

We could improve our service by doing HPV serotyping. In this way we could identify those patients who are at higher risk for invasive disease. These patients could then be kept in a high risk clinic and perhaps offered more intensive follow up. The HPV vaccines, although available in South Africa, are not yet a part of our Extended Programme of Immunization (EPI) as offered by the department of Health due to cost. Including the HPV vaccine may be a way to significantly reduce the burden of CIN as well as invasive cancer in future generations. The South African Department of Health is hoping to start the roll out of the vaccine in some of the poorest communities starting in Feburary 2014 and has encouraged parents who can afford the cost of the vaccine to purchase the vaccine for their daughters. The impact of this vaccine will be fascinating to witness in the years to come.

A review of the current National Guidelines for Cervical Cancer Screening should be undertaken taking into account the HIV status of the patients. Most HIV positive patients are offered one Pap smear at time of diagnosis but this should be formalized into a national guideline to ensure greater coverage and adherence. The frequency with which these patients should receive Pap smears and how to manage abnormal results should also be formalized with clear routes of referral to colposcopy clinics manned with well-trained colposcopists.

A prospective study in which all variables could be adequately and completely collected may provide a better understanding of how HIV, HPV subtypes, CD4, HAART and different

regimes, and response by way of viral load has on the clear margins as well as recurrence rates of CIN.

Appendix A: Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Carolyn Noel

CLEARANCE CERTIFICATE	**M121144**
PROJECT	Treatment of Cervical Intraepitheleal Neoplasia using "See and Treat" Approach in Human Immunodeficiency Virus Positive Women: Do We Get Clear Margins? A Retrospective Review of a Single Institution
INVESTIGATORS	Dr Carolyn Noel.
DEPARTMENT	Department of Obstetrics & Gynaecology
DATE CONSIDERED	30/11/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 30/11/2012 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr Trudy Smith

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

Appendix B: Permission From CEO CMJAH

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Office of the CEO
Enquiries:
Ms. L. Mngomezulu
(011): 488-3793
(011) 488-3753
31st October 2012

Dr. Carolyn Joyce Noël
Department of Obstetrics and Gynaecology
University of the Witwatersrand

Dear Dr. Noel

RE: "Treatment of cervical intraepithelial neoplasia using "see and treat" approach in human immunodeficiency virus-positive women: Do we get clear margins?"

Please note that your above request is provisionally approved. Your study can only commence once ethics approval is obtained.

Yours sincerely

Dr. T.E. Selebano
Chief Executive Officer

Appendix C: Data Capture Sheet

Record Number:

Date of LLETZ Preceedure:

Age								
Parity								
Gravidity								
Doctor code	A	B	C	D	E	F	G	H
Contraception	Yes				No			
Type of Contraception	None	OCP	Depo provera	IUCD	Condoms			
CD4								
HAART	Yes				No			
Duration of HAART								
Date of original Pap smear								
Days elapsed between original pap and LLETZ procedure								
Grade of original CIN Lesion	ASCUS		LGSIL		HGSIL			
Clinical size of CIN lesion	Small		Medium		Large			
Involvement of Transformation Zone	Yes				No			
Clear resection margins	Yes		No		Unsure			
Reason for unsure margins								
Histological margin involvement	Ectocervical		Endo Cervical		Both			
Histology of LLETZ Biopsy	CIN I		CIN II		CIN III/Ca-in situ			
Patient follow up	Yes				No			
Grade of CIN on Follow up Pap smear	Clear		ASCUS		LGSIL		HGSIL	

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