Long-term outcomes of women treated for high-grade squamous intraepithelial lesions at a University Hospital colposcopy unit in South Africa. A 5-year retrospective cohort study

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Dissertation presented in partial fulfilment of the requirements for the MPhil in Gynaecology Oncology

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LIST OF ABBREVIATIONS

- AIS- Adenocarcinoma in situ
- ALTS- ASC-US/LSIL triage study
- ASCCP- American Society for Colposcopy and Cervical Pathology
- ASC-US- Atypical squamous cells of undetermined significance
- ASIR- Age-standardised incidence rate
- CD4- Cluster of differentiation 4
- CI- Confidence interval
- CIN- Cervical intraepithelial neoplasia
- DNA- Deoxyribonucleic acid
- GSH- Groote Schuur Hospital
- HAART- Highly active antiretroviral treatment
- HDI- Human Development Index
- HIV- Human Immunodeficiency Virus
- HPV- Human papillomavirus
- hr-HPV- High-risk human papillomavirus
- HSIL- High grade squamous intraepithelial lesion
- IARC- International agency for research on cancer
- IMB- Intermenstrual bleeding
- IPC- Injectable progestogen contraceptive
- LBC- Liquid-based cytology
- LLETZ- Large loop excision of the transformation zone
- LMICs- Low and middle-income countries
- lr-HPV- Low-Risk human papillomavirus
- LSIL- Low grade squamous intraepithelial lesion
- NHS- National Health Service
- NHLS- National Health Laboratory service
- NHSCSP- National Health Service Cervical Screening Programme
- NPV- Negative predictive value

- OCP- Oral combined pill
- Pap- Papanicolaou
- PCB- Post-coital bleeding
- PPV- Positive predictive value
- pRB- Product of retinoblastoma
- RCT- Randomised controlled trial
- RCPAQAP- Royal College of Pathologists of Australia Quality Assessment Programs
- SA- South Africa
- SIL- Squamous intraepithelial lesion
- UK- United Kingdom
- USA- United States of America
- VIA- Visual inspection with acetic acid
- VIAM- Visual inspection with acetic acid under magnification
- WHO- World Health Organisation

ABSTRACT

INTRODUCTION: Worldwide, there is a paradigm shift in the screening for cervical cancer with the use of high-risk human papillomavirus (hrHPV) molecular testing. Before South Africa (SA) adopts this technology in the public sector, health funders will need data on the performance of the current cytology and colposcopy-based programmes. This study was done to establish baseline data on the performance of the cytology and colposcopy based cervical cancer screening programme at the Groote Schuur Hospital (GSH) colposcopy clinic.

METHODS: This was a retrospective cohort study of all the women with high-grade squamous intraepithelial lesion (HSIL) Pap smears seen at GSH colposcopy clinic between 01 January 2010 and 31 December 2015. The outcome measures were; diagnostic concordance between cytology, colposcopy and histology, large loop excision of the transformation zone (LLETZ) and cone biopsy complication rates, cure rate, treatment failure and invasive cervical cancer rates, median time from treatment to recurrence, follow-up default rates. Data were managed and analysed using IBM SPSS Statistics Version 25 and Microsoft Structured Query Language (SQL) version 2014. Regression methods were used to assess the independent effect of baseline sociodemographic characteristics and clinical covariates on treatment failure and clearance of disease in those who had persistent disease after treatment. Kaplan-Meier curves were used to represent the time from treatment to recurrence and from persistence to cure. Time-to-event methods were applied to determine factors associated with treatment to recurrence and persistence to cure.

RESULTS: A total of 7601 women were referred to the GSH colposcopy clinic during the study period. HSIL or worse lesions (≥HSIL) were confirmed histologically in 74.1% (2282/3081) women. At the four-month follow-up visit, 61.2% (742/1213) of the women were considered cured, and 17.0% (206/1213) had persistent/residual disease. In women considered cured at

four months, recurrence was very low, and it peaked at ten months at 1.5% (11/740). By 24 months the cumulative recurrence rate was 4.6% (34/742). In women with persistent disease at the four-month follow-up visit, only 0.5% (1/202) developed invasive cervical cancer. The default rate for follow-up was very high, at 81% at 24 months. LLETZ and cone biopsy complication rate was 7.2% (117/1628). Log-rank analysis showed that parity ≥ four was significantly associated with a higher risk of disease recurrence (p=0.0004). In a Cox-regression model, taking HAART was the only factor associated with a reduced risk of disease recurrence (p=0.0261).

CONCLUSION: LLETZ and cone biopsy are safe procedures. After cure, recurrence rates are low. In women who are treated for HSIL, cervical cancer is very rare. Taking HAART was associated with a reduced risk of disease recurrence. There is a need to mitigate on higher default rates to follow up.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1. Background (Epidemiology, aetiology, secondary prevention, colposcopy, treatment, follow-up and Groote Schuur hospital practice)

1.1.1. Epidemiology

According to the International Agency for Research on Cancer (IARC GLOBOCAN 2012 database), cervical cancer is the fourth most common cancer in women worldwide and the commonest in women aged between 15- 44 years (1). In 2012, 528 000 women were diagnosed with cervical cancer, and 266 000 of them died from the disease. Unfortunately, 85% of these deaths occurred in under-resourced countries where there are no effective cervical cancer screening programmes and the burden of human immunodeficiency virus (HIV) infection is high (1). Prevalence of cervical cancer and deaths are higher in countries with low human development index (HDI), with five-year survival rates being less than 20% in these countries compared to over 65% in countries with very high HDI (2). In countries that have introduced organised cervical cancer screening, mortality has fallen significantly. In countries with opportunistic screening or without screening programmes, mortality has remained high (3, 4). Currently, cytology through a call and recall system is the most effective screening method available (every 3 – 5 years women are reminded to go for a repeat Pap smear). Cytology-based screening requires a relatively sophisticated laboratory set-up with appropriate equipment and technical support, built-in quality control, trained staff and a health system underpinned by good referral pathways. This system has been shown to reduce significantly cervical cancer incidence, morbidity and mortality especially when linked to accessible colposcopy and treatment services. Before 2012, the cost of the cervical cancer screening programme in the United States of America (USA) reached 6 billion dollars (5). Most low-resourced countries have not been able to set up effective cytology-based screening programmes due to these astronomical costs, and screening remains opportunistic if available at all.

South Africa (SA) is an upper-middle- income country with huge disparities in cervical cancer screening practices in the public and private sectors. In a study done in three districts of SA, only 50% of women with high-grade squamous intraepithelial lesions

(HSIL) Pap smears had colposcopy and biopsy within six months (6). In the Free State Province of SA, only 4.1% of women aged between 15 to 65 years were screened in 2002, a decrease of 42% compared to 1985. Only 2.6% were black women utilising mostly the public sector compared to 18.8% white women who had their screening in the private sector (7). Although infection with the human immunodeficiency virus (HIV) is a known risk factor for HSIL and cervical cancer, only 13.1% of newly diagnosed HIV-positive women had at least one Pap smear in primary HIV clinics in Cape Town between 2006- 2008 (8). In 2001, the South African Department of Health introduced a cervical cancer screening programme. The programme offered all asymptomatic women three free Pap smears in their lifetime at the ages of 30, 40 and 50 years but women with gynaecological problems were offered a Pap smear regardless of their screening history or age (9). Incidence and mortality of cervical cancer remain high in South Africa. In 1998-1999, there were 6061 and 5203 new cases of cervical cancer respectively giving an age-standardised incidence rate (ASIR) of 42-35/100 000 respectively. Mortality in the two year period was nearly 60% (10). In this study, we describe the long-term outcomes of women treated for HSIL in the GSH colposcopy clinic.

1.1.2. Aetiologic factors

1.1.2.1. HPV infection

The human papillomaviruses (HPV) are small double-stranded deoxyribonucleic acid (DNA) viruses enclosed in a 72- sided icosahedral protein capsid. They have around 8000 nucleotide base pairs, and they are known to infect the human epithelia. The HPV genetic material is divided into E (early) or L (late) genes. During epithelial differentiation, the E and L genes are expressed early and late respectively (11). More than 100 HPV subtypes have been fully sequenced, and after sequencing, they are divided into phylogenetic trees which make it easier to understand their classification and behaviour (12). Human papillomaviruses are also divided into different groups depending on the pathology they cause; these groups are known as clades. Clades alpha-7 and nine are strongly associated with anogenital cancers. HPV 16, a clade alpha- 9 virus, is one of the most powerful human carcinogens known to date, is responsible for about 50% of cervical carcinoma cases (12). In total, 15 anogenital types are associated with an increased risk of developing cancer, and these are called

oncogenic or high-risk human papillomavirus (hrHPV) types. These oncogenic HPV subtypes include; 16, 18, 31, 33, 35, 45, 52, and 58 (13). The contribution of the other hrHPV types; 39, 51, 56, 59, 66, 68 and 73 is less certain (14). HPV 6 and 11 are clade alpha-10 viruses, they are called low-risk HPV (lrHPV) types. They mostly cause genital warts and low-grade squamous intraepithelial lesions (LSIL).

The HPV normally survives in the host nucleus separate from the host genome as a stable viral episome. However, to develop HSIL and invasive cancer, the HPV genome needs to be integrated into the host genome (15). This process of integration is controlled by the E1 and E2 genes (16). The L1 and L2 genes are responsible for encoding the common viral capsid proteins. The E6 and E7 genes of the hrHPV types encode for oncoproteins which disrupt the control of the cell cycle resulting in immortalisation of keratinocytes. The E6 oncoproteins of the hr-HPV types induce degradation of the tumour suppressor p53 protein which is encoded by the tumour suppressor gene p53, resulting in loss of p53 control of the cell cycle (17). The E7 oncoproteins interact and deactivate the product of the retinoblastoma gene (pRB). The RB gene is a tumour suppressor which inhibits the cell cycle (18). Therefore, the combined action of the E6 and E7 oncoproteins is a necessary step in the transformation of cells and the development of cervical cancer.

However, due to humoral and cellular-mediated immunity, the majority of women exposed to HPV infection can clear the infection within 24 months (19, 20). Persistence of the oncogenic HPV infection is the biggest risk factor for developing HSIL (20). This risk is not dose-dependent, and therefore, it is not modified by measuring the HPV viral load in the genital tract (11). Compared to lrHPV types, persistent infections are more likely with the hrHPV groups (21). In a United States of America cohort, hrHPV 16 infection at baseline was associated with a 14.6% and 8.5% risk of developing ≥ HSIL in women less and greater than 30 years respectively. In the same cohort, in hrHPV 16 naïve women, the risk of developing ≥ HSIL was less than 2% in both groups of women (22). In one of the world's most notorious unethical studies, Professor Herbert Green working at New Women's Hospital in Auckland, New- Zealand withheld curative treatment from women with CIN 3 from 1965 to 1974. Analysis of this data showed that thirty per cent of women with HSIL would progress to cervical cancer in about 30 years if left untreated (23). This long latent period allows for the Pap smear to be frequently repeated and is responsible for the relatively high longitudinal sensitivity of cytology.

1.1.2.2. Associated co-factors

1.1.2.2.1. Tobacco

Both active and passive smoking have been shown to increase the risk of developing HSIL (24). The risk is dose-dependent, and it also depends on the age of initiating smoking (25). The mechanism of action could be due to direct effect from the breakdown products of tobacco which have been isolated in cervical secretions or the depletion of the epithelial antigen-presenting Langerhans cells which leads to failure in clearing the HPV infection resulting in viral persistence and ultimately HSIL (26). Smoking is not associated with an increased risk of developing cervical adenocarcinomas (25).

1.1.2.2.2 Hormonal factors

Higher rates of genital HPV infection especially type 16 have been found in pregnancy, which supports the theory that the hormonal milieu of pregnancy supports viral replication (27). The prevalence of HPV infection increases dramatically from the first to the third trimester followed by a sharp decline post-delivery (28). Pregnancy before the age of 17 years and multiparity have been associated with a higher risk of developing cervical cancer (29). Prolonged exposure to contraceptives is also a known risk factor for cervical cancer (30).

1.1.2.2.3. Immunosuppression

HIV infection increases the risk of developing HSIL which is related to the degree of immunosuppression (31). However, a recent study has questioned the association between the level of immunosuppression and prevalence of abnormal Pap smears (32). The risk of harbouring multiple HPV infections is also higher in HIV- infected women (33). Co-infection with both HIV and hr-HPV increases the risk of developing cervical squamous intraepithelial lesions (SIL) 40- fold (34). Due to the lifelong use of immunosuppressive therapy, renal transplant patients have a 16-fold higher risk of developing cervical SIL compared to the general population (35). However, a recent study has also questioned this association (36).

1.1.3. Secondary prevention

Secondary prevention or cervical cancer screening has been historically done by examination of fixed exfoliated cervical cells under a microscope (Pap smear). It needs to start at an appropriate age to maximise benefit and minimise harm due to the treatment of transient cervical changes due to HPV infections. In a population-based case-control study, screening for cervical cancer below the age of 25 years was not shown to reduce the incidence of cervical cancer at the ages of 25- 29 years (37). The International Agency for Research on Cancer (IARC) in 2006 recommended 3- 5 yearly screening intervals between the ages of 25 to 65 years (3). Shorter screening intervals have not been shown to be more effective (4). This recommendation is followed by the United Kingdom National Health Service Cervical Screening Programme (NHSCSP), one of the most successful cervical cancer screening programmes in the world (38). However, the American society for colposcopy and cervical pathology (ASCCP) still recommend screening from the age of 21 years (39).

1.1.3.1. Cytology

Due to the lack of a more sensitive alternative, the Pap smear has been the screening method of choice in most established cervical cancer screening programmes. Reviews have shown the sensitivity of cytology to be between 50-60% (40, 41). Regular screening increases the longitudinal sensitivity of cytology, but this makes screening more expensive. Liquid-based cytology (LBC) which uses a brush to collect cervical cells and transport them in a buffered alcohol media was introduced as a way to try and improve the sensitivity of conventional cytology. However, its performance is similar to conventional cytology (42, 43). LBC reduces the number of inadequate smears which makes its use cost-effective (44, 45). SA migrated to LBC screening in 2017. HPV testing or co-testing can be done with LBC, so the introduction of LBC paves the way for HPV testing as a primary screening method.

1.1.3.2. HPV DNA

Understanding the role of hrHPV in the development of cervical cancer has accelerated research in cervical cancer screening. Currently, hrHPV molecular testing is used as a test of cure, in triaging women with atypical squamous cells of undetermined significance (ASC-US) cytology results, co-testing with cytology and in primary screening.

A meta-analysis showed that hrHPV molecular test of cure was more sensitive than conventional cytology by 21-25% (46). Molecular testing of hrHPV also results in earlier detection of residual or recurrent disease (47). Test of cure using hrHPV molecular testing was introduced in the NHSCSP in the UK after successful pilot studies showed that this approach was safe and 82% of women could safely be referred back to routine screening (48). Modelling studies also confirmed the clinical and cost-effectiveness of this approach compared to follow-up with annual cytology (49). However, the use of a hrHPV molecular test of cure has not been internationally accepted. Some studies have shown this approach to be less cost-effective (50) and less reassuring than co-testing with cytology (51).

Another role of hrHPV molecular testing is in triaging women with ASC-US Pap smears. The ASC-US/LSIL triage study (ALTS) showed that hrHPV molecular testing in women with ASCU-US Pap smears was as sensitive as immediate colposcopy in detecting ≥HSIL with half as many women referred for colposcopy (52). Use of this strategy in women with LSIL Pap smears has a much lower specificity compared to cytology (46).

Molecular testing of hrHPV is more sensitive and reproducible compared to cytology and screening intervals can be safely increased to at least five years (53). As a primary screening tool, hrHPV molecular testing detects more ≥ HSIL in the initial round of screening with less disease being picked up in subsequent screening rounds (54). HPV based primary screening is more effective than cytology in reducing invasive cervical cancer (55, 56). New techniques which can partially genotype HPV types 16 and 18 which are responsible for 70% of cervical cancer cases are now available and have found use in stratifying care of some women (57). However, worldwide, the use of HPV testing faces resistance in some societies due to associated stigma and shame of a positive result (58).

The Netherlands was the first country to implement hrHPV molecular testing as a primary screening modality for cervical cancer.

1.1.3.3. Other screening techniques

Visual inspection of the cervix with acetic acid (VIA) utilises 3-5% acetic acid to visualise aceto-white lesions close to the transformation zone with a bright light using naked eyes alone or with the help of low-level magnification (VIAM). VIA is cheap and easy to perform and its sensitivity and specificity range from 49-96 per cent and 49-

98 per cent respectively (59). In one Indian study, after four rounds of cancer education and VIA screening at 24-month intervals, cervical cancer mortality was reduced by 31% after 12 years of follow-up (60). Using a conditional probability model, VIA combined with digital cervicography was shown to prevent one cervical cancer death for every forty-six HIV-infected women screened in Zambia (61). In Africa, the efficacy of VIA is comparable to cytology (62), but its major weakness is a low positive predictive (PPV) value of only 10% (63). VIA results in overtreatment of a large number of women without HSIL. VIA is also less effective in older women. The combination of VIA and visual inspection with Lugol's iodine (VILI) has been shown to be a cost-effective tool requiring minimal medical resources in remote areas (64).

1.1.4. Colposcopy

HSIL Pap smears and positive hrHPV molecular tests are the commonest indications for colposcopy referral. Symptomatic women with abnormal looking cervices or unexplained intermenstrual bleeding (IMB), post-coital bleeding (PCB) or persistent vaginal discharge should also be referred for colposcopy. Women with ASC-US/LSIL Pap smears can also be immediately referred for colposcopy, triaged with hrHPV molecular testing or followed up by cytology. In the Western Cape Province, women are referred for colposcopy if they have two consecutive Pap smears showing ASC-US or LSIL since hrHPV molecular testing is not available in the public sector. Colposcopy can be normal, show minor or major changes or lesions suspicious of microinvasive disease. There is an overall agreement of 92% between colposcopic assessment and histological diagnosis of ≥ HSIL and adenocarcinoma in-situ (AIS) (65). There is need to continuously audit colposcopy practice in cervical cancer screening programmes to minimise under or overdiagnosis of HSIL resulting in missed disease or unnecessary treatment by excisional methods which can lead to adverse obstetric consequences (66). Some units have adopted various scoring systems to improve accuracy in colposcopic assessment.

1.1.5. Treatment

Historically, hysterectomy was the standard of care for treatment of women with carcinoma in-situ. Cone biopsy was adopted after studies showed similar efficacy to hysterectomy in the prevention of invasive cervical cancer (67). Colposcopically

directed biopsies were later shown to be as effective as cone biopsies in the diagnosis of HSIL (68). The acceptance of colposcopically directed biopsies as an effective diagnostic tool led to the introduction of ablative treatment modalities so that women could retain their reproductive potential. Cryotherapy was shown to have lower cure rates in women with endocervical involvement (69). Electrocoagulation diathermy have HSIL cure rates >95% (70). Laser treatment is also used to treat HSIL effectively. The majority of cases of women who develop invasive cervical cancer after ablative treatment are due to pre-treatment misdiagnosis of micro-invasive disease (71).

Availability of histopathological specimens to exclude micro-invasive disease led to large loop excision of the transformation zone (LLETZ) gaining widespread popularity. Colposcopy can be combined with immediate treatment using LLETZ or ablative techniques. Treating women at the same sitting with colposcopy is commonly referred to as 'see and treat'. 'See and treat' is an effective and safe technique that can be used in low- resource countries where follow-up default rates are high (72).

1.1.6. Follow-up

For women with HSIL, AIS or micro-invasive (MI) disease, their risk of HSIL remains high for many years even after an initial negative post-treatment cytology result (73). The National Health Service Cervical Screening Programme (NHSCSP) in the UK allows women with negative, ASC-US or LSIL Pap smears to be returned to routine screening if the hrHPV molecular test of cure is negative at six months post-treatment (38). The ASCCP guidelines recommend co-testing at 12 and 24 months and referral to colposcopy if any one of the test results is abnormal (39).

1.1.7. GSH colposcopy clinic practice

SA has not yet introduced hrHPV molecular testing into its public cervical cancer screening programme. At Groote Schuur Hospital, women are mostly referred to the colposcopy clinic for HSIL, two consecutive ASC-US, and LSIL Pap smears. Women are also referred for any glandular abnormality on Pap smear or clinically suspicious cervices regardless of the cytology results. Women with adequate colposcopy confirming major changes after presenting with HSIL Pap smears are treated with an LLETZ procedure. Concordance between cytology and colposcopy is around 80% (74), so many women at GSH colposcopy clinic are treated at the same visit ('see and treat')

to minimise the impact of loss to follow-up. Women with HSIL Pap smears and inadequate colposcopic findings or have a discrepancy between cytology and colposcopy have a diagnostic cone biopsy done instead. Some colposcopists prefer doing a diagnostic punch biopsy before definitive treatment.

All the women above the age of 45 years with abnormal glandular cells on Pap smear also have their endometrium sampled to exclude uterine pathology. Women with colposcopic and histologically-confirmed LSIL are not treated because regression rates are high, especially in young women and progression to ≥HSIL is uncommon (75)

Every woman is brought back to the clinic after 4-6 months post-treatment, and if repeat cytology and colposcopy exclude HSIL, they are recalled every 6-12 months for repeat colposcopy and cytology for ten years before they go back to routine screening. If colposcopy shows major changes and or cytology shows HSIL, patients are treated again with an excisional procedure after diagnosis has been confirmed histologically via a punch biopsy specimen.

1.2. The justification for the study

At the moment, there is a paradigm shift in the world in how cervical cancer screening is performed. High-risk HPV molecular testing is being utilised as a primary screening tool, co-testing with cytology, triaging of ASC-US/LSIL Pap smears and as a test of cure in many high-income countries. The use of hrHPV molecular testing in cervical cancer screening programmes in low-income countries is feasible (76). In the private sector in South Africa, hrHPV molecular testing is already being widely used in cervical cancer screening. However, before this is adopted in the public sector, health funders will need robust data on both how the current cytology-based programme is performing, and the feasibility of using hrHPV testing in an SA population with high rates of co-infection with HIV and HPV. This study, although from a single institution, is essential as it will provide some baseline data on the performance of the current cytology-based cervical cancer screening programme in the local population. After the inevitable introduction of hrHPV molecular testing, its impact will need to be audited, and the baseline data of the current programme will be needed for comparison.

1.2.1 Research Question:

What are the oncological and operative outcomes of women with HSIL treated with an excisional procedure (LLETZ/cone biopsy) at GSH colposcopy unit?

2. STUDY OBJECTIVES

2.1 Main Objective

To evaluate the oncological and operative outcomes of women with HSIL treated with an excisional procedure (LLETZ/cone biopsy) at GSH colposcopy unit.

2.2 Secondary objectives of the study

- To document the number of women referred for HSIL Pap smears as a proportion of the total number of women referred to the GSH colposcopy clinic during the study period.
- To describe the baseline sociodemographic and clinical parameters of women referred for HSIL Pap smears to GSH colposcopy clinic during the study period.
- To document the proportion of women who had colposcopy followed by either immediate treatment with LLETZ (see and treat) or immediate diagnostic cone biopsy or a diagnostic punch biopsy before definitive treatment.
- To determine the diagnostic concordance between cytology, colposcopy and histology.
- To document the proportion of women who reported complications after LLETZ or cone biopsy.
- To document the proportion of women with treatment failure after excisional treatment which was defined as either residual/persistent or recurrent disease.
- To calculate the median time from treatment to recurrence.
- To document the proportion of women who developed invasive cervical cancer after excisional treatment of HSIL.
- To calculate the follow-up default rate.
- To define the baseline sociodemographic and clinical parameters associated with failure of HSIL treatment by an excisional procedure (residual/persistent or recurrent disease).

CHAPTER 2: MATERIALS AND METHODS

This was a retrospective descriptive study. The protocol was approved by the Human Research Ethics Committee of the University of Cape Town on 28 June 2017 (HREC Ref: 445/2017) (see appendix 1). Approval was also granted by GSH to retrieve old colposcopy folders and clinical notes as and when required to update missing data (see appendix 2).

2.1. Study outcomes measures

- Diagnostic concordance between cytology, colposcopy and histology
- Excisional procedure (LLETZ and cone biopsy) complication rate
- Post- HSIL treatment cure rate
- Median time from treatment to recurrence
- Post- HSIL treatment persistent or recurrence rates
- Post-HSIL treatment invasive cervical cancer rate
- Follow-up default rate

2.2. Data collection

Since 2007 all the women who were referred to the GSH colposcopy clinic have been entered into a computerised database which has the Human Research Ethics Committee (HREC) approval. After HREC approval for the study was granted, the database was screened to exclude women who did not meet the inclusion criteria. All those who met the inclusion criteria had their data extracted and analysed. We retrieved 900 colposcopy folders and clinical notes to update essential data which was missing from the database.

From the database, we extracted the following sociodemographic and clinical parameters; age, parity, menopausal status, contraception use, smoking status, HIV status, antiretroviral drug use, most recent CD4+ count, and medical comorbidities like previous or current infection with tuberculosis or diabetes mellitus. We also extracted the women's colposcopy, cytology and histology results for all their subsequent visits for the whole duration of the study.

Excisional procedures were either a LLETZ or a diagnostic cone biopsy and they were defined as follows; LLETZ (type 1 or 2 excision) is a therapeutic procedure done after colposcopic identification of a precancerous lesion and the following criteria has to be met before it is done; concordance between cytology and colposcopy results, the whole lesion has to be visualised, no suspicion of microinvasive disease and no glandular abnormality on cytology. When these criteria are not met, a diagnostic cone biopsy (type 3 excision) is done. A diagnostic cone biopsy can be therapeutic if the whole lesion is excised.

We also extracted the reported complications from the database. All this data were entered on IBM SPSS Statistics Version 25 for analysis.

2.3. Participants entry into the study

2.3.1 Pre-recruitment evaluations

The database was screened to exclude women who did not meet the inclusion criteria from the analysis.

2.3.2. Inclusion criteria

- ☐ Confirmed HSIL Pap smear at referral

2.3.3. Exclusion criteria

- No confirmed HSIL Pap smear at referral
- Women with no results recorded in the database

2.4. Statistics and data analysis

2.4.1. Sample size

We included all the women who were referred to GSH between 01 January 2010 and 31 December 2015 who had HSIL Pap smears and follow-up results captured in the database.

2.4.2. Data analysis

Data were managed and analysed in IBM SPSS Statistics Version 25 and Microsoft Structured Query Language (SQL) version 2014. Demographic characteristics and clinical factors were summarised using median and range, and percentages for

continuous and categorical variables respectively. We documented the management of the women on their first visit and calculated the proportion of those who had colposcopy followed by immediate treatment with LLETZ (see and treat) or a diagnostic cone biopsy and those who had a diagnostic punch biopsy before definitive treatment. We also documented the women's colposcopic findings and the final histology results for their initial referral visit. The final histology results were either from LLETZ, cone biopsy or diagnostic punch biopsy specimens. Diagnostic concordance between cytology and histology as the gold standard in detecting ≥HSIL was documented. Using the histological diagnosis as the gold standard, we also calculated the sensitivity, specificity, positive and negative predictive values and diagnostic accuracy of colposcopy in detecting ≥HSIL. We then calculated the diagnostic concordance between cytology and histology in detecting ≥HSIL in women who had colposcopic assessment followed by immediate LLETZ (see and treat) or diagnostic cone biopsy. We also calculated the concordance between colposcopy and histology in detecting ≥HSIL in women who had immediate treatment with LLETZ only. We then calculated the concordance between colposcopy and histology in detecting ≥HSIL in women who had a diagnostic cone biopsy. We also described the complications reported by the women on their four-month post-treatment follow-up visit. We used the women's four-month post-treatment cytology and or colposcopy results to put them into two categories; either persistent/residual disease or cured. We defined persistent or residual disease as any woman who had excisional treatment of HSIL and on the four-month follow-up visit had either major colposcopic findings and or ≥ ASC-H Pap smear. We defined a cure as any woman who had excisional treatment of HSIL, and on the four-month follow-up visit, had normal colposcopy findings and Pap smear results. Women who had minor colposcopic findings and or ASC-US or LSIL Pap smears were excluded since there was no hrHPV molecular testing to triage them into the persistent/residual disease or cured category. The colposcopy and or cytology and or histology results of all the subsequent visits of women in these two groups were extracted and analysed. We did not expect any woman to be discharged from follow-up during the study period because at GSH colposcopy clinic all women treated for HSIL are followed up closely for at least ten years. Women were defined to have recurred if they had any one of the following; major changes at colposcopy and or ≥ASC-H Pap smear and or HSIL histology after prior documented normal colposcopy

and histology results. Women who developed cancer and those who defaulted follow-up were also documented. Histology results in the database were searched for any hysterectomy histopathology results. Univariate association between treatment failure and sociodemographic and clinical parameters was evaluated using the chi-squared test, t-and non-parametric tests. Regression methods were used to assess the independent effect of baseline sociodemographic characteristics and clinical covariates on study outcome (failure of HSIL treatment by LLETZ or cone biopsy). The time from treatment to recurrence was determined and presented on a Kaplan-Meier curve. In women who had persistent/residual disease, the time to clearance of disease after retreatment was also presented on a Kaplan-Meier curve. Time-to-event methods were applied to determine factors associated with recurrence after cure and clearance of disease after retreatment in those who had persistent/residual disease.

2.5. Regulatory issues

2.5.1. Confidentiality

We complied with data protection legislation to preserve the confidentiality of women involved in the study. The colposcopy clinic database and all the computers used to store and analyse data were password protected.

2.5.2. Sponsor

The University of Cape Town and the South African Medical Research Council/Gynaecology Cancer Research Centre (SAMRC/GCRC) were the main sponsors for this study.

2.6. Publication policy

Authorship will be based on substantial contribution to conception, design, analysis, interpretation of data, drafting and approval of the version to be published.

CHAPTER 3: RESULTS

Summary of table 1

The women's ages ranged from 18 to 83 with a median of 37, and their parity ranged from 0 to 10 with a median of 2. The group was predominantly premenopausal 84.3% (3384/4012). More than a third of the women were not using any contraception 42.1% (1435/3408) and 40.1% (1367/3408) were using injectable progestogen contraception (IPC). Less than a tenth 8.8% (300/3408) of the women had undergone tubal ligation. Oral combined contraceptive usage was very low at 3.8% (130/3408). A relatively large number of women were smokers 19.4% (769/3958). Just less than 60% of the women were HIV positive, and 77.1% (1768/2294) were on HAART with an overwhelming majority still on first-line treatment 89.1% (1505/1689). A majority had a CD4 count ≤ 500 74.3% (1321/1779). A fifth of the patients 20.3% (818/4036) had a positive history of tuberculosis (previous or active infection).

Table 1: Baseline characteristics: Demographics and medical history

Variables	
Median age (years), n=4035 and missing data= 0.02% (1/4036)	**37, range 18-83**
Median Parity, n=3946 and missing data= 2.2% (90/4036)	**2, range 0-10**
Menopausal status, n=4012 and missing data= 0.6% (24/4036)	**%(n/n)**
Premenopausal	84.3(3384/4012)
Post-menopausal	15.7(628/4012)
Contraceptive use, n= 3408	**%(n/n)**
None	42.1(1435/3408)
Injection	40.1(1367/3408)
Tubal ligation	8.8(300/3408)
Oral combined contraceptive (OCP)	3.8(130/3408)
Others	5.2(176/3408)
Duration of OCP use, n= 98 and missing data= 24.6% (32/130)	**%(n/n)**
≤ five years	71.4(70/98)
> five years	28.6(28/98)
Smoking status, n= 3958 and missing data= 1.9% (78/4036)	**%(n/n)**
Yes	19.4(769/3958)
No	80.6(3189/3958)
HIV status, N=4036	**% (n/N)**
Positive	59.7(2409/4036)
Negative	30.1(1215/4036)

Unknown	10.2(412/4036)
Taking anti-retroviral treatment, n=2294 and missing data= 4.8% (115/2409)	**% (n/n)**
Yes (n=1768)	77.1(1768/2294)
No (n= 526)	22.9(526/2294)
Anti-retroviral treatment, n= 1689 and missing data= 4.5% (79/1768)	**%(n/n)**
First line	89.1(1505/1689)
Second line	10.7(180/1689)
Third line	0.2(4/1689)
CD4 count, n= 1779 and missing data= 26.2% (630/2409)	**%(n/n)**
≤500	74.3(1321/1779)
> 500	25.7(458/1779)
Tuberculosis (previous or active infection), N=4036	**%(n/N)**
No	79.7(3218/4036)
Yes	20.3(818/4036)
Diabetes Mellitus, N=4036	**%n(N)**
No	96.2(3384/4036)
Yes	3.8(152/4036)

Summary of table 2

Between 01 January 2010 to 31 December 2015, 7601 women were referred to the Groote Schuur Hospital colposcopy clinic. Referral cytology was ≥HSIL in 53.1% (4036/7601) women. We excluded 0.2% (13/7601) women without any entries in the database.

Table 2: Indications for referrals to colposcopy clinic 2010-2015

Referral cytology	**n (%)**
≥HSIL Pap smears	4036(53.1)
Non- HSIL Pap smears, vulval HSIL, genital warts and others	3552(46.7)
Missing data	13 (0.2)
N (%)	7601 (100)

Summary of table 3

Colposcopic findings were not documented in 13.8% (555/4036) women. Colposcopic assessment data were available for 85.1% (3434/4036) women, and colposcopy was not indicated in 1.0% (40/4036) women because they had macroscopic cancer. Half of the women who had colposcopy 50.3% (1728/3434) had an excisional procedure (LLETZ or cone biopsy) performed at the same sitting. In a third 33.0% (1132/3434) of the women,

a punch biopsy was performed for histological confirmation of the diagnosis before definitive treatment.

Table 3: Management on the referral visit

Colposcopy examination performed, n= 3481, missing data= 13.8% (555/4036)	**%(n/N)**
Yes	85.1(3434/4036)
No (macroscopic cancer)	1.0(40/4036)
No	0.2(7/4036)
Management after colposcopy, N=3434	**%(n/N)**
Excisional procedure (LLETZ or cone biopsy) on the same day	50.3(1728/3434)
Punch biopsy	33.0(1132/3434)
*Other	16.7(574/3434)

**premarin, antibiotics, repeat Pap smear*

Summary of table 4

A quarter 27.7% (41/148) of the women with normal colposcopic findings had CIN2+ on histology. A third 33.3% (30/90) of the women with atrophic changes at colposcopy had CIN 2+ on histology. More than a third 38.1% (222/583) with minor colposcopic changes had CIN 2+ on histology. In the women with inadequate colposcopy, 41.5% (17/41) had CIN 2+ on histology. More than half 58.3% (14/24) of the women with inflammatory changes had CIN 2+ on histology. Colposcopic major changes were confirmed histologically in 76.5% (1528/1997) women. Suspicion of microinvasion at colposcopy was confirmed in 7.1% (6/84) of the women. More than half 51.2% (43/84) of those with colposcopic findings suggestive of microinvasion had CIN 2+ on histology.

Table 4: Colposcopic and [a]final diagnosis of the referral visit

Colposcopic diagnosis %(n/N)	**[a]Final diagnosis**			
	Histology (cervical biopsy, LLETZ or cone biopsy) %(n/n)		**Cytology %(n/n)**	
***Normal, n =148 and missing results= 31.8% (69/217)**	Normal	4.7(7/148)	Normal	23.6(35/148)
	CIN 1	13.5(20/148)	ASCU-S/LSIL	10.8 (16/148)
	CIN 2+	27.7(41/148)	ASC-H/HSIL	18.9(28/148)
***Minor changes, n= 583 and missing results= 12.6% (84/667)**	Normal	5.3(31/583)	Normal	6.3(37/583)
	CIN 1	42.0(245/583)	ASC-US/LSIL	4.3(25/583)

	CIN 2+	38.1(222/583)	ASC-H/HSIL	3.6(21/583)
***Major changes, n=1997 and missing results= 8.0% (173/2170)**	Normal	2.9 (57/1997)	Normal	4.1(82/1997)
	CIN 1	15.1(301/1997)	ASC-US/LSIL	1.0(19/1997)
	CIN 2+	76.5(1528/1997)	ASC-H/HSIL	0.3(5/1997)
***Suspicion of microinvasion, n= 84 and missing results= 14.3 % (14/98)**	Normal	2.4(2/84)	Normal	1.2(1/84)
	CIN 1	6.0(5/84)	ASC-US/LSIL	3.6(3/84)
	CIN 2/3	51.2(43/84)	ASC-H/HSIL	26.2(22/84)
	[b]MI	7.1(6/84)		
Inflammation, n=24 and missing results= 25% (8/32)	Normal	8.3(2/24)	Normal	12.5(3/24)
	CIN 1	8.3(2/24)	ASC-US/LSIL	4.2(1/24)
	CIN 2+	58.3(14/24)	ASC-H/HSIL	8.3(2/24)
Atrophy, n= 90 and missing results= 29.7% (38/128)	Normal	5.6(5/90)	Normal	26.6(24/90)
	CIN 1	12.2(11/90)	ASC-US/LSIL	6.7(6/90)
	CIN 2+	33.3(30/90)	ASC-H/HSIL	15.6(14/90)
***Inadequate, n=41 and missing data= 24.1% (13/54)**	Normal	14.6(6/41)	Normal	17.1(7/41)
	CIN 1	9.8(4/41)	ASC-US/LSIL	7.3(3/41)
	CIN 2+	41.5(17/41)	ASC-H/HSIL	7.3(3/41)
Other, n=62 and missing data=8.8% (6/61)	Normal	6.5(4/62)	Normal	21.0(13/62)
	CIN 1	29.0(18/62)	ASC-US/LSIL	4.8(3/62)
	CIN 2+	35.5(22/62)	ASC-H/HSIL	3.3(2/62)
***Colposcopic diagnosis missing, n= 536 and missing results= 3.4% (19/555)**	Normal	3.2(17/536)	Normal	21.5(115/536)
	CIN 1	10.8(58/536)	ASC-US/LSIL	3.9(21/536)
	CIN 2+	59.0(316/536)	ASC-H/HSIL	1.3(7/536)
Colposcopy not done, n=7	Normal	28.5(2/7)	Normal	0(0)
	CIN 1	28.5(2/7)	ASC-US/LSIL	0(0)
	CIN 2+	42.9(3/7)	ASC-H/HSIL	0(0)
Macroscopic cancer, n=40				
N= 4036				

[a]*Final diagnosis- histology or repeat cytology depending on colposcopic findings at referral*
[b]*MI- microinvasion*

**Non specified histology excluded from the table from each category; normal colposcopy 0.7% (1/148), minor changes 0.3% (2/583), major changes 0.3% (5/1997), suspicion of microinvasion 2.4% (2/84), inadequate 2.4% (1/41) & colposcopic diagnosis missing 0.4% (2/536).*

Summary of table 4a

The Pap smear correctly identified 74.1% (2282/3081) women with CIN 2+ on histology. Over a fifth 21.6% (666/3081) had CIN 1, and very few 4.3% (133/3081) had normal histology.

Table 4a: Concordance between cytology and histology in detecting CIN2+

Cytology	Histological diagnosis		
	CIN 2+ %(n/n)	**CIN 1 %(n/n)**	**Normal %(n/n)**
≥HSIL (histology results available) n= 3081	74.1% (2282/3081)	21.6(666/3081)	4.3% (133/3081)

Excluded 955 women from analysis; missing results n= 424, histological diagnosis not specified n= 13, cytology repeated n= 518

Summary of table 4b

Colposcopy sensitivity = 83.7% (95% CI 82.0%-85.4%), specificity= 47.0% (95% CI 43.2%-50.8%), positive predictive value (PPV) = 81.2 % (95% CI 80.1%-82.3%), negative predictive value (NPV) = 51.3% (95% CI 48.0%-54.5%) and diagnostic accuracy= 73.9% (95% CI 72.1%- 75.6%). It is important to note that women with two LSIL Pap smears who also had colposcopic examination were not included in this calculation since only women with HSIL Pap smears were analysed.

Table 4b: *Specificity, sensitivity, PPV, NPV and diagnostic accuracy of colposcopy in detecting CIN2+

Colposcopic diagnosis	Histological diagnosis	
	≥CIN 2+ n	**Other (LSIL, atrophy, inflamed or normal) n**
≥ major changes (HSIL) n=1942	1577	365
Other (LSIL, atrophy, inflamed or normal) n=630	307	323

**Excluded 1464 women from analysis; inadequate colposcopy n= 41, other colposcopic diagnosis n= 62, no colposcopic diagnosis n= 536, colposcopy not done n= 7, macroscopic cancer n= 40, no histological diagnosis n=424, cytology repeated n= 344 and other histological diagnosis= 10*

Summary of table 5

The Pap smear correctly identified 81.5% (1311/1609) women with CIN 2+ on histology. Over a tenth 13.2% (212/1609) had CIN 1, and very few 5.3% (86/109) had normal histology.

Table 5: Concordance between cytology and histology in detecting CIN2+ in women who had an immediate LLETZ or cone biopsy

Cytology	Histological diagnosis		
	CIN2 + %n(n/n)	**CIN 1 %(n/n)**	**Normal %(n/n)**
≥HSIL n=1609	81.5(1311/1609)	13.2(212/1609)	5.3(86/1609)

Excluded 114 patients from the analysis; missing histological results n = 111 and histological diagnosis not specified n = 3

Summary of table 5a and 5b

Colposcopic major changes were confirmed histologically in 81.7 % (1198/1467) of the women after immediate treatment with LLETZ. The majority 80 % (8/10) of the women who had a diagnostic cone biopsy after a normal colposcopic examination had CIN2+ on histology. Of those with minor changes, more than two thirds 68.9% (31/45) had CIN 2+ on diagnostic cone biopsy. Microinvasion was confirmed after a diagnostic cone biopsy in 12.2% (6/49) of the women who had a suspicion of microinvasion at colposcopy. The majority of the women who had a suspicion of microinvasion on colposcopy, 79.6% (39/49) had CIN 2/3. Almost three quarters 71.4% (5/7) of the women with atrophic changes at colposcopy had CIN 2+ on diagnostic cone biopsy. In women with inadequate colposcopy, 58.8 % (10/17) had CIN 2+ on diagnostic cone biopsy. In women who underwent 'look and LLETZ', there was overtreatment in 18.2% (193/1467).

Table 5a: *Concordance between colposcopy and histology in detecting CIN2+ in women who had immediate treatment with LLETZ

Colposcopic diagnosis	Histological diagnosis		
	≥CIN2+ %(n/N)	**CIN 1 % (n/N)**	**Normal %(n/N)**
≥Major changes, n= 1467 and missing results= 6.3% (98/1565)	81.7(1198/1467)	13.2 (193/1467)	5.0 (74/1467)
Overtreatment	18.2% (267/1467)		

**Women with other histology excluded from analysis; n=2*

Table 5b: *Concordance between colposcopy and histology in detecting CIN2+ in women who had a diagnostic cone biopsy

	Histological diagnosis		
Colposcopic diagnosis	**≥CIN2+ % (n/N)**	**CIN 1 % (n/N)**	**Normal %(n/N)**
Normal N= 10 and missing results= 16.7% (2/12)	80 (8/10)	10 (1/10)	10 (1/10)
Minor changes N= 45 and missing results=6.3% (3/48)	68.9 (31/45)	22.2(10/45)	8.9 (4/45)
Atrophy N= 7 and missing results=12.5% (1/8)	71.4 (5/7)	14.3 (1/7)	14.3 (1/7)
Suspicion of microinvasion N= 49 and missing results=3.9% (2/51)	91.8 (45/49)	8.2 (4/49)	0 (0)
Inadequate N= 17 and missing results=19.8 (4/21)	58.8(10/17)	5.9 (1/17)	29.4 (5/17)

**Excluded 130 women from the analysis; other colposcopic diagnosis n= 7, no colposcopic diagnosis n= 10, other histology n=2, missing histological diagnosis n=111*

Summary of table 6

The overall complication rate was 7.2% (117/1628). The risk of infection 3.8% (54/1628) was similar to that of bleeding 3.3% (62/1628).

Table 6: *Excisional procedure (LLETZ or cone biopsy) complications

Excisional procedure (LLETZ or cone biopsy) complication, n= 1628 and missing results= 3.8% (64/1692)	**%(n/n)**
Yes No	7.2(117/1628) 92.8(1511/1628)
Complications n=117	**%(n/n)**
Bleeding, n=62 Infection, n=54 Other, n=1	53.0(62/117) 46.2(54/117) 0.9(1/117)

* *In women who had multiple excisional procedures, only complications of the last procedure were recorded*

Summary of table 7

Persistent disease was observed in 17.0% (206/1213) women, and 61.2% (742/1213) women were considered cured.

Table 7: Post-treatment results (using colposcopy and or cytology and or histology results)

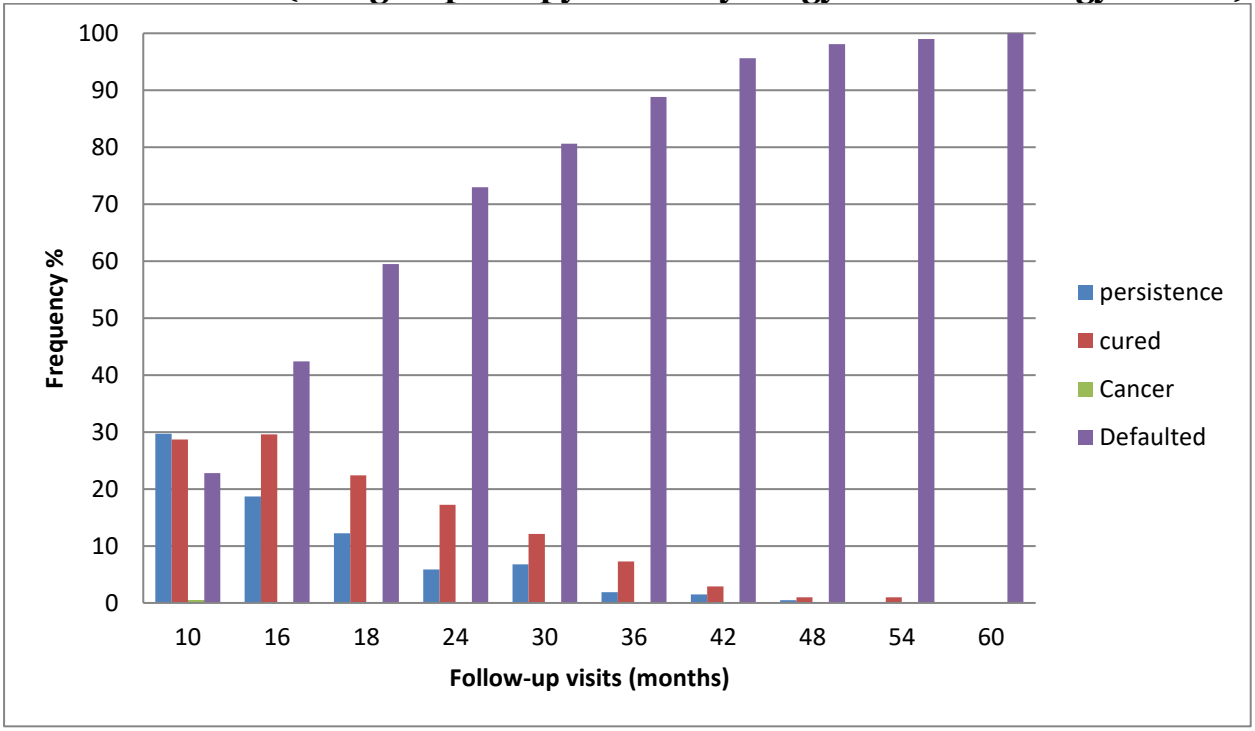
Result	**%(n/N)**
Persistent disease	17(206/1213)
Cured	61.2(742/1213)
ASC-US/ LSIL	21.5(261/1213)
Other histology	0.3(4/1213)
Total	1213

Excluded 426 women; 383 with no results, 14 with inadequate histology, and 29 who had a diagnostic cone biopsy and needed further treatment

Summary of figure 1

At 24 months 5.9% (12/204) women still had persistent disease. Cancer was a rare outcome; it occurred in 0.5% (1/202) of women in this group. The default rate at 24 months was very high at 73.0% (149/204).

Figure 1: Long-term outcomes of women who had persistent disease after initial treatment (using colposcopy and or cytology and or histology results)



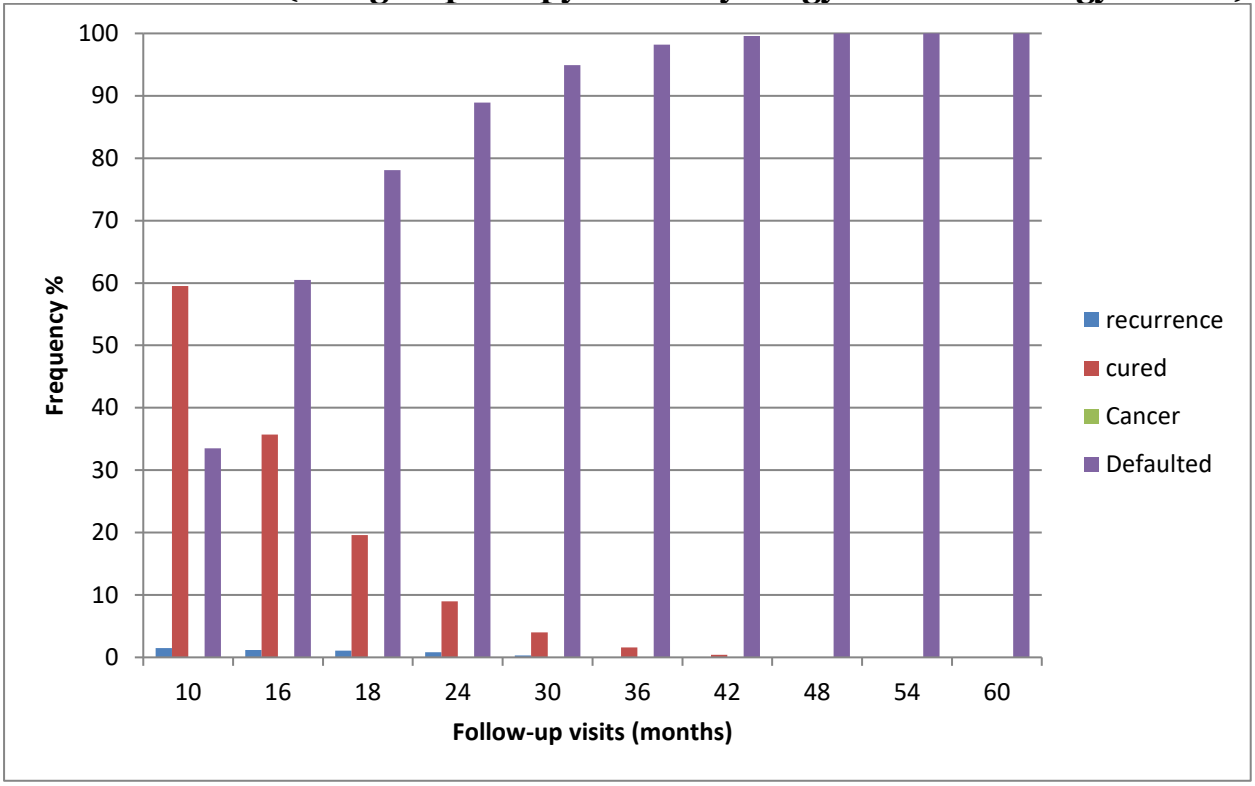
**Excluded from analysis women with inadequate or other histology; n= 4 at 10months, n= 3 at 16 months, n= 1 at 18 months and n= 2 at 24 months*

**Women with persistent disease had repeat excisions if their histology confirmed HSIL*

Summary of figure 2

Recurrence was very low, and it peaked at ten months at 1.5% (11/740), and by 24 months the cumulative recurrence rate was 4.6% (34/742). Default rate at 24 months was very high at 88.9% (660/742). No women developed cancer in this group.

Figure 2: Long-term outcome of women who were considered cured after initial treatment (using colposcopy and or cytology and or histology results)



**Excluded from analysis women with inadequate or other histology; n= 2 at 10months, n= 2 at 16 months, n= 1 at 18 months, n= 1 at 30 and 36 months*

Summary of outcomes in the two groups

The overall risk of developing cancer in the two groups was 0.5% after 60 months of follow up. The mean default rate at 24 months was very high at 81%.

Summary of table 8

In the univariate analysis, parity >=4 was associated with a higher risk of disease recurrence (p=0.0004). In the multivariate analysis, taking HAART was associated with a reduced risk of disease recurrence and being menopausal was associated with an increased risk of disease recurrence.

Table 8: Univariate & multivariate analysis of disease recurrence in women considered cured after the initial treatment.

Variable	***Univariate***			***Multivariate***		
Variable	**HR**	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**
Age						
30-65	2.83	0.379-21.15	0.311	-	-	-
>65	2.081	0.0-inf	0.997	-	-	-
Parity						
>=4	4.912	2.033-11.860	0.0004*	-	-	-
Postmenopausal						
Yes	1.748	0.578-5.282	0.323	13.03	1.190-142.4	0.0353*
HIV Status						
Positive	1.288	0.492-3.372	0.606	-	-	-
CD4						
>500	0.864	0.168-4.455	0.861	0.694	0.117-4.12	0.688
HAART						
Yes	0.283	0.076-1.054	0.06	0.109	0.015-0.768	0.0261*
Smoking						
Yes	0.839	0.305-2.306	0.733	-	-	-

Summary of table 9

In the univariate analysis, age > 65 years was associated with delayed clearance of disease after retreatment for persistent disease (p= 0.00036)

Table 9: Univariate & multivariate analysis for disease clearance after retreatment in women who had persistent disease after the initial treatment

Variable	***Univariate***			***Multivariate***		
	HR	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**
Age						
30-65	0.923	0.463-1.842	0.820	-	-	-
>65	62.968	6.477-612.120	0.00036*	-	-	-
Parity						
>=4	1.125	0.600-2.108	0.714	1.980	0.476-8.287	0.346
Postmenopausal						
Yes	0.975	0.594-1.601	0.920	1.120	0.421-2.992	0.818
HIV status						
Positive	0.777	0.519-1.162	0.218	-	-	-
CD4						
>500	1.061	0.550-2.048	0.860	0.905	0.440-1.861	0.785
HAART						
Yes	1.018	0.559-1.854	0.953	0.921	0.442-1.916	0.825
Smoking						
Yes	0.866	0.514-1.459	0.589	0.364	0.111-1.192	0.0951

CHAPTER 4: DISCUSSION

4.1 Colposcopic clinic workload

GSH colposcopy clinic is run by four gynaecology oncology subspecialists; three experienced colposcopists who are general practitioners and two gynaecology oncology subspecialist trainees. To remain proficient in colposcopy, some colposcopy quality control programmes recommend that a colposcopist must see more than 50 new referrals in a year (38). In the study period, 7601 women were seen in the GSH colposcopy clinic (1520 women/ year), and 4036 of those women had been referred for an HSIL Pap smear. Of the 4036 women with HSIL, 3434 women had documentation that they underwent a colposcopic examination. Therefore, each colposcopist attended to about 170 new referrals yearly (1520 women/year/ 9 colposcopists) during the study period. Since 3434 women were documented to have undergone colposcopic assessment, each colposcopist perfomed about 76 colposcopies/ year on women who had been referred for an HSIL Pap smear. This workload meets the recommendation of most colposcopy quality control programmes.

4.2. Sociodemographic and clinical characteristics

Cervical cancer screening in South Africa is still opportunistic. However, the South African cervical cancer screening policy recommends that from the age of thirty years, asymptomatic women should have three ten-yearly Pap smears free of charge (9). The median age of women referred with an HSIL Pap smear in this study was 37 years, range (18-83 years). In a group of unscreened South African women, the mean age of diagnosis of HSIL was 37.7 years, and this was higher in comparison to Europe or Northern USA (77). The mean age of diagnosis was lower in Europe or Northern USA because cervical cancer screening starts at the age of 25 years in most European countries and 21 years in Northern America. Earlier age of screening then translates to younger age at diagnosis. The median age in our study was similar to others done in resource-constrained environments. In a study conducted in Turkey, a middle-income country like SA, which looked at the age trends in HSIL and cancerous lesions of the uterine cervix, the peak-age

incidence of HSIL was in the 30–39 years age group (78). In the same study, the mean age at diagnosis of HSIL was the same as in this study, 37.7 years.

The median parity in the study was two, range (0-10). Between 2007 and 2017, fertility has declined from an average of 2,7 to 2,4 children in South Africa (79). The median parity in this study is therefore consistent with the South African census data. There is some published literature showing an association between parity of four or more with a high incidence of low and high grade squamous intraepithelial lesions (78).

Injectable progestogen contraceptives were the commonest form of contraception used by more than a third 40.1% (1367/3408) of women in this study. There is conflicting data on whether the use of injectable progestogen contraceptives especially medroxyprogesterone acetate increases the risk of HIV acquisition (80-82). Despite this, injectable progestogen contraceptives have remained the contraceptive of choice among South African women. Injectable progestogen contraceptives were used by 24% and 26% of married and unmarried sexually active women respectively (83). On the other hand, 45.4% and 35.8% of married and unmarried sexually active women respectively were not using any form of contraception (83). Similarly, in this study, 42.1% (1435/3408) of eligible women were not on contraception. The reason why such a high number of women in the reproductive age group were not using any form of modern contraception needs to be explored further. There might be an opportunity to link cervical cancer screening and family planning services to reduce the unmet need of contraception in South Africa which stands at 14.7% in married women and 24% in sexually active single women (83).

Smoking and HIV infection are known risk factors for developing cervical HSIL and invasive cancer. In this study, a relatively large number of women were smokers 19.4% (769/3958). The prevalence of smoking in black South African women is very low at 3% compared to 38% and 15% in mixed-race and white women respectively (83). Smoking is more prevalent in urban women compared to those living in rural areas (83). Western Cape Province has the highest prevalence (25%) of smoking women in the whole of South Africa (83). All these factors contribute to the relatively high prevalence of smoking among women attending the GSH colposcopy clinic during the study period. Unfortunately, in the GSH colposcopy database, the race of the women was not captured so it was not possible to show the distribution of smoking according to race.

Almost 60% of the women in this study were HIV positive, and an overwhelming majority were on first-line highly active antiretroviral treatment 89.1% (1505/1689). The prevalence of HIV in this study was much higher than the prevalence of 12.6% in the total South African population and 22% in women of reproductive age group (79). Cervical cancer screening has been incorporated as the standard of care in most HIV treatment programmes. Cervical cancer screening in HIV programmes leads to selection bias since more HIV-infected women are screened and referred for colposcopy compared to HIV uninfected women who may not be accessing health services. Most patients had a CD4+ count less than 500 cells/ mm ^{3}. HIV viral load data was not performed or entered into the GSH colposcopy database during the study period. In some colposcopy clinics in high-income countries, HIV-infected women are virtually unseen (84). A study in women attending colposcopy services at Chris Hani Baragwanath Hospital in the Gauteng Province of South Africa had similarly high rates of HIV infection (85). The high burden of HIV infection in women attending the colposcopy clinic during the study period was also highlighted by the high rates of tuberculosis (previous and active infection), an opportunistic infection which affected 20% of these women.

4.3. Colposcopic assessment

The colposcopic examination is an integral part of any successful cervical cancer screening programme. An HSIL Pap smear is an indication for referral for colposcopic assessment. Colposcopic examination allows magnification and visualisation of the cervix for diagnostic biopsies or treatment to be done under direct vision. An overwhelming majority of women in this study had a colposcopic assessment, 85.1% (3434/4036). Of concern is that 1.0% (40/4036) of women did not need colposcopic assessment because they already had macroscopic cancer on pelvic examination. These women were not candidates for colposcopy clinic referral but should have been referred straight away to gynaecological oncology hospital services for assessment and treatment. Much time was wasted while they waited for their colposcopy appointment. Inappropriate referrals for women with macroscopic cancer highlights the fact that healthcare workers involved in cervical cancer screening programmes need adequate training in recognising macroscopic cervical cancer to avoid delays in women accessing appropriate cancer care.

4.4. The colposcopic assessment followed by immediate treatment (see and treat)

In low-resource settings, loss to follow-up in cervical cancer screening studies is unacceptably high (86-88). As one of the auditable standards, The National Health Service Cervical Screening Programme in the UK recommends that loss to follow-up should not exceed 15% (38).

In low-resource settings, one way to mitigate the high loss to follow-up is by treating women with concordant high-grade cytology and colposcopic finding at the same sitting without histological confirmation of HSIL ('see and treat'). Cervical cancer screening programmes initially involve three visits to a healthcare facility (first a screening visit, a second visit for triaging with colposcopy and taking directed biopsies and the third visit for treatment of biopsy-proven HSIL). In "see and treat' programmes, there are only two visits involved, and treatment is done on the second visit in those who are screen-positive after a colposcopic assessment without any prior histological confirmation of HSIL by a punch biopsy. This approach is different from "screen and treat' which entails only one visit for screening and treatment of all those who are screen positive on the same day. In 'screen and treat' the likelihood of overtreatment is high. 'See and treat' has been shown to be effective, acceptable and leads to a reduction in loss to follow-up by reducing the number of visits to a health-care facility (89-94). At GSH colposcopy clinic, women with discordant results, that is high-grade cytology, but normal and minor changes at colposcopy can have a diagnostic cone biopsy which turns out to be therapeutic in most of the cases as well when the lesion is fully excised with clear margins. In the study period, more than half of the women 50.3% (1728/3434) had either 'see and treat' or had a diagnostic cone biopsy performed on the referral visit. A third of the women had routine care that is having colposcopically directed punch biopsies before definitive treatment.

4.5. Concordance between cytology and histology in detecting CIN2+

One of the prerequisites of a good cervical cancer screening programme is reliable cytopathological services. One of the many reasons why cytology-based screening has failed in many low- and middle-income countries is the expense and complexity involved in setting up reliable cytopathological services and a shortage of well-trained

cytotechnicians and cytopathologists. In South Africa, all the Pap smears are analysed at the National Health Laboratory Service (NHLS). In the Western Cape Province, Pap smears are only analysed at the two tertiary hospitals, Groote Schuur and Tygerberg. Almost all the patients seen at GSH colposcopy clinic during the study period had their Pap smears processed at GSH NHLS. At the GSH NHLS, they have 11 cytotechnicians, and they have robust internal quality control procedures which include histology and cytology correlation every three to four months. The lab has external accreditation with the Royal College of Pathologists of Australia Quality Assessment Programs (RCPAQAP). At GSH NHLS they process about 60 000 Pap smears yearly. One of the key performance indicators of the cervical cancer screening programme in the UK is that cytology laboratories must screen more than 35 000 Pap smears annually to maintain proficiency (95). One way of assessing the quality of the cytopathological services in cervical cancer screening is by looking at the concordance between HSIL Pap smears and histology which is the gold standard in the diagnosis of CIN2+. In this study, the concordance between cytology and histology was high. Three-quarters 74.1% (2282/3081) of women with HSIL Pap smears had CIN 2+ confirmed on histology. One South African study found a similar concordance of 71.8% between cytology and histology (85). In a study done in Brazil, a middle-income country like SA, the concordance between HSIL cytology and histology was 76% which was also similar to ours (96). In this study, the rate of discordance between cytology and pathology was 25.9% which is in keeping with other studies that have found rates of discordance between 11-28% (97-99). In a study that was done in British Columbia, a high-income setting, the overall correlation between cytology and biopsy was 79.4% (100).

4.6. Sensitivity, specificity, PPV, NPV and diagnostic accuracy of colposcopy in detecting CIN2+

In this study, the sensitivity and specificity of colposcopy in detecting CIN2+ was 83.7% (96% CI 82.0%-85.4%) and 47.0% (95% CI 43.2%-50.8%) respectively. In a meta-analysis of nine studies, for the threshold of normal cervix and LSIL compared with HSIL and cancer, the weighted average sensitivity and specificity was 85% and 69% respectively (101). In a study done in British Columbia, colposcopy had a sensitivity and specificity of

90.3% and 57.3% respectively (100). In another study, the sensitivity and specificity of colposcopy in detecting any lesion was 89% and 52% respectively (102). In the same study, the sensitivity for CIN 2/3 was 56%(102). The PPV and NPV of colposcopy in detecting CIN2+ in this study was 81.2% (95% CI 80.1%-82.3%) and 51.3% (95% CI 48.0%-54.5%) respectively. One study found colposcopy to have a PPV and NPV of 72.3% and 47.7% respectively (103). Another study showed a PPV of 75.5% which is similar to this study (104).

More than half of the women in this study period had LLETZ or diagnostic conisation performed at the same sitting. This practice was implemented to minimise the impact of very high rates of loss to follow-up experienced in cervical cancer screening programmes in low-income countries (86-88). However excisional procedures are not without risk. Besides the relatively more common risks of haemorrhage and infection, there is published literature on the association of cervical excisional procedures and pregnancy-associated adverse events (105). Since these women are treated on the same day without histological confirmation of HSIL, it is imperative that their outcomes are regularly audited to avoid over-treatment of women without the disease. In women who had treatment on the same day, the concordance between cytology and histology was high at 81.5%. The concordance between colposcopy and histology in detecting CIN 2+ was 81.7% (1198/1467). The NHSCSP recommends that the diagnostic concordance between colposcopy and histology in detecting CIN2+ to be at least 90% in women who undergo 'see and treat' (38). In this study, the rate of overtreatment was 18.2% which is in keeping with other studies where it ranged from 4-18% (91, 93, 94, 106-109). With very high rates of default to follow-up particularly over time, the option of 'see and treat' is justifiable in the GSH colposcopy unit.

4.7. Detection of CIN2+ in women undergoing a diagnostic cone biopsy for the discrepancy between cytology and colposcopy

Discrepancy between cytology and colposcopy was defined as: minor or normal colposcopic findings in a woman with an HSIL Pap smear. At GSH colposcopy clinic, these women undergo a diagnostic cone biopsy which is also therapeutic in most of the cases particularly if the lesion is completely excised and the margins are clear. In this study, a majority of these women had CIN2+ on histology. In women who had normal, atrophic,

inadequate or minor colposcopic findings, the rates of CIN2+ on histology were 80%, 71.4%, 58.8% and 68.9% respectively. In a study looking at outcomes in women who had a diagnostic LEEP for the discrepancy between cytology and colposcopy, CIN2+ was confirmed in two-thirds of these patients (110). Since a majority of women with HSIL Pap smears harbour CIN2+ regardless of the colposcopy findings, another option is to skip colposcopy and treat all these women with an excisional procedure. The ASCCP guidelines deem an excisional procedure without colposcopy as reasonable in women with HSIL Pap smears when colposcopy is unavailable (39). The findings of this study justify the practice at GSH colposcopy clinic of offering a diagnostic cone biopsy in women with the discrepancy between cytology and colposcopy results.

4.8. Complications of colposcopy

Overall the morbidity of LLETZ and cone biopsies was low at 7.2% (117/1628). The risk of bleeding was 3.3%, and the risk of infection was 3.8%. Most of these complications were reported by the women on their subsequent post-treatment visit. Data on admissions for complications was not consistently captured on the database. The reported complication rate is combined for both LLETZ and cone biopsy. LLETZ and cone biopsy complications were combined because the numbers were small. The other reason for reporting them together is that some studies have shown that the hot loop cone biopsy and LLETZ complications are similar (111). One study done in a high resource setting had a similar risk of bleeding of 3.8% (112). However, the complication rate in this study might have been underestimated because, on the database, women who had multiple procedures only had morbidity of the last procedure captured. Although the burden of HIV infection was high in the study, the complication rates were comparable to other studies. In a study done in China, 10% of women who had LLETZ were HIV- infected and the rate of infective morbidity of 4.3% was comparable to this study (113).

4.9. Cure and treatment failure rates

A number of factors affect the cure and treatment failure (persistent or recurrent disease) rates in women treated for HSIL. We defined cure as any woman who had excision of HSIL and on the four-month follow up visit had both normal colposcopic findings and Pap smear result. Persistent or residual disease was defined as any woman who had

excision of HSIL and on the four-month follow up visit she had either major colposcopic findings and or ≥ ASC-H Pap smear. Recurrence was defined as any woman who had any one of the following; colposcopy showing major changes and or ≥ASC-H Pap smear and or HSIL histology after prior documented normal colposcopy and histology results. Risk factors for treatment failure include; positive margins on excision specimens, high grade of CIN, oncogenic HPV types, HIV infection, age, smoking and sexual behaviour (114-118). In this study, the cure rate at the four-month follow-up visit was 61.2% (742/1213). This is in keeping with other studies done in countries with a similar high burden of HIV but much lower than reported in countries with a lower burden of HIV (101, 119-121). However, this study probably underestimated the cure rate for various reasons. Until 2017, GSH hospital colposcopy clinic protocol recommended following up women at four months post-treatment and thereafter six or 12 monthly depending on colposcopy and cytology findings. The National Health Service Cervical Screening Programme in the UK recommends that women should be followed up at six months post-treatment (38). Women with HSIL are immediately referred back for colposcopy. Those with normal, ASC-US and LSIL Pap smears have reflex testing of hrHPV as a test of cure. Those with a negative test are considered as cured and referred back to routine screening, and those who are positive are referred back for colposcopy since they have a higher risk of having residual disease. Therefore, it is possible that if we had followed up the treated women at six months, the cure rate was going to be slightly higher as more women were going to clear the disease. A fifth of the women still had ASC-US, and LSIL Pap smears at the four-month follow-up visit. A high-risk HPV DNA test of cure on these patients would have triaged them into the cure or treatment failure categories. It is plausible that if we had done the high-risk HPV DNA test of cure, some women in this group would have been designated as cured. In this study, the risk of persistent disease post-LLETZ or cone biopsy at the four-month follow-up visit was high at 17.0%. In women cured at the four-month follow-up visit, recurrence peaked at ten months at 1.5% (11/740) and by 24 months; the cumulative recurrence rate was 4.6% (34/742).

Our findings were in keeping with other studies which found that post excisional treatment, 4% to 17% of women have CIN2+ due to either residual or recurrent disease (119-122). One study done in South Africa showed much higher rates of persistent disease especially in HIV infected women (118).

Women with the persistent HSIL at the four-month follow-up had more excisional treatment, and in those who did not default follow-up, the development of invasive cervical cancer was a rare outcome. Only one patient, 0.5% (1/202) developed invasive cancer. At 24 months 5.9% (12/204) women still had persistent disease.

4.10. Impact of high loss to follow up rates

The default rate in this study was very high, at 81.0% at 24 months. We do not have any way to predict the outcome in these women. The possible outcomes include; persistent/residual disease, recurrence, cure and invasive cancer. As shown in this study, the risk of developing cervical cancer after treatment is very low. Therefore, if all these women had been accounted for, there was going to be an increase in all our outcome measures. Therefore, there is a strong possibility that we might have underestimated the outcome measures. The problem of high default rate in low-income countries has been alluded to before.

4.11 Univariate & multivariate analysis of disease recurrence in women considered cured after the initial treatment

Parity >=4 was associated with a higher risk of disease recurrence. Another study also found an association between high parity and increased risk of recurrence (78). In the multivariate analysis, taking HAART was associated with a reduced risk of disease recurrence. Although menopausal status was associated with a higher risk of recurrence, the result needs to be interpreted with caution because the 95% CI was too wide (1.19-142.40). This is most likely due to the very few numbers of women who had the outcome of interest.

4.12 Univariate & multivariate analysis for disease clearance after retreatment in women who had persistent disease after the initial treatment

In the univariate analysis, age > 65 years was associated with delayed clearance of disease after retreatment for persistent disease (p= 0.00036). This result also needs to be interpreted with caution due to the wide 95%CI of 6.477-612.120

4.13. Mitigating loss to follow-up

Effective ways to reduce loss to follow up also need to be deployed. Home visits by community health care workers to track women attending cervical cancer screening has been shown to reduce default rates (123). The use of community healthcare workers is cost-effective as it increases the average per-woman costs by only 14-47 SA Rand (ZAR) (124). Educating and effective communication with women about the importance of adherence to follow-up will also go a long way in reducing the problem of loss to follow-up. The other interventions that have been found to be moderately effective include intensive follow-up by mail and telephone to contact patients and providing economic vouchers to offset out-of-pocket expenses (125).

CHAPTER 5: CONCLUSION, RECOMMENDATIONS, LIMITATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

5.1. Conclusion

GSH colposcopy clinic is a high volume centre with the requisite workload to maintain the proficiency of colposcopists or trainees working in the unit. LLETZ and cone biopsy are safe procedures. Majority of women who did not default follow up after being considered cured at four months did not recur. In women who are treated appropriately and do not default follow-up, cervical cancer is very rare. Taking HAART was associated with a reduced risk of disease recurrence. However, default to follow-up is very high, and there is a need to improve this for cervical cancer screening to have an impact on the incidence of cervical cancer in South Africa.

5.2. Recommendations

There is need to link cervical cancer screening and contraception services to cater for a large number of women who are not taking contraception who are seen at the GSH colposcopy clinic. Smoking cessation programmes must be introduced in the GSH colposcopy clinic given the relatively large number of women who were smokers. Healthcare workers involved in cervical cancer screening need to be taught how to identify macroscopic cancer to avoid doing unnecessary Pap smears in these women and delaying referring them for cancer treatment. Given the high default rates, there is need to increase the number of women who are treated at the same sitting ('see and treat'). The practice of doing diagnostic cone biopsies in women with HSIL Pap smears and normal or minor colposcopic changes should be maintained. Funding should be sought to employ community healthcare workers who track women at home to reduce the high default rates. The follow-up visit should be maintained at six months and introducing high-risk HPV DNA test of cure should be considered as this will help in triaging women with a normal, ASC-US and LSIL Pap smear post-treatment.

5.3. Limitations

Inherent limitations of retrospective studies like missing and incomplete data and high loss to follow-up were our biggest problems.

5.4. Suggestions for further research

There is need for qualitative research to explore further why a lot of women are lost to follow up

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ANNEXURES

Appendix 1: HREC approval letter

Appendix 2: GSH approval letter

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6626
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

28 June 2017

HREC REF: 445/2017

Prof L Denny
Obstetrics & Gynaecology
H-Floor, OMB

Dear Prof Denny

PROJECT TITLE: LONG TERM OUTCOMES OF WOMEN TREATED FOR HSIL AT A UNIVERSITY TEACHING HOSPITAL COLPOSCOPY UNIT IN SOUTH AFRICA: A 5 YEAR RETROSPECTIVE COHORT STUDY. (MPhil candidate- Dr BT Guzhal)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 June 2018.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur.

The HREC acknowledge that the student, Dr Bothwell T Guzha will also be involved in this study.

Yours sincerely

Signature Removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki (2013) guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC 445/2017

RESEARCH ANNEXURE 2
PROPOSAL SUMMARY

For Official Use:
Research Proposal Number
_____

ANNEXURE 2 PROPOSAL SUMMARY	
Name of Institution/organisation conducting research	**Groote Schuur Hospital**
Name of Investigators	Prof L Denny, Dr B.T Guzha, Dr L Rogers
Postal Address	University of Cape Town, Groote Schuur Hospital, H-45 Old Main Building, Groote Schuur Hospital, Observatory,
Telephone Number	021-404485
Fax number	021-448 6921
Mobile Number	0723049829
Email Address	bothwellguzha@gmail.com
Institution which gave ethical approval	University of Cape Town HREC
Date of Ethical approval	28 June 2017
Date research expected to commence	28 June 2017
Proposed data collection dates at requested facilities	01 December 2017
Date research expected to end	28 June 2018
Date research reports should be expected	31 October 2018
Western Cape Districts where research will be done: (Please mark with an X)	**Metro** X **Westcoast** **Cape Winelands** **Overberg** **Central Karoo** **Eden**
WC DOH Facilities where research will be done: **(Please list the name of the facility under appropriate category)**	**Tertiary Hospitals:** X **District Hospitals:** **Community Health Centres:** **Clinics:**
Other facilities in the WC DOH where research will be done (Please specify)	**Psychiatric Hospitals:** **TB Hospitals**

GSH Research Annexure 2

	Other: **Databases : X**
Research title	Long term outcomes of women treated for HSIL at a University Hospital Colposcopy unit in South Africa: A 5 year retrospective cohort study.
Research aim	To evaluate operative and oncological outcomes of women with HSIL treated with an excisional procedure (LLETZ/cone biopsy) at GSH colposcopy unit.
Research objectives	1. To document the number women referred for HSIL Pap smears as a proportion of the total number of women referred to the GSH colposcopy clinic during the study period. 2. To describe the baseline sociodemographic and clinical parameters of women referred for HSIL Pap smears to GSH colposcopy clinic. 3. The proportion of women who had colposcopy followed by immediate LLETZ or cone biopsy treatment (see and treat) and those treated for HSIL confirmed on colposcopically directed biopsies. 4. To determine the diagnostic concordance between cytology, colposcopy and histology. 5. The proportion of women who reported complications after LLETZ or cone biopsy treatment. 6. The proportion of women with persistent and recurrent disease

	after treatment. 7. To calculate the median time from treatment to recurrence. 8. The proportion of women who needed a hysterectomy for post-treatment disease. 9. The proportion of women who developed invasive cancer after treatment. 10. To calculate the default rate from follow-up 11. To define the baseline sociodemographic and clinical parameters associated with failure of HSIL treatment by LLETZ or cone biopsy.
Key Words	HSIL, LLETZ, recurrence, defaulters
Brief description of methodology **(Please specify estimated sample size and duration of contact with each participant e.g. interview length, clinical exams)**	Data will be extracted from an anonymised electronic data base approved by HREC and into which data has been entered since 2008. The database was set up to audit the clinic on a regular basis and to provide clinicians with feedback.. Data for this study will be managed and analysed in Stata version 15.0. Demographic characteristics and clinical factors will be summarised using median and range, and percentages for continuous and categorical variables respectively. The proportion of women with persistent or recurrent disease after treatment will be calculated. The proportion of women who had a hysterectomy or who developed invasive cervical cancer will also be calculated. Univariate association between study outcome (failure of HSIL treatment by LLETZ or cone biopsy) and demographics will be evaluated using chi-squared test or t-test were appropriate, otherwise the non-parametric tests will be used. Regression methods will be employed to assess the independent effect of baseline demographics characteristics and clinical covariates on study outcome (failure of HSIL treatment by LLETZ or cone biopsy). The time from treatment to recurrence or development of invasive cervical cancer will be determined and presented on a Kaplan-

	Meier curve. Time-to-event methods will be applied to determine factors associated with treatment to recurrence or development of invasive cervical cancer.	
Type of Study Design: e.g. Case Control, RCT, Survey	Retrospective cohort study	
Budget for research	**None**	
Source of funding for the research	Departmental funds	
The research will have implications for the requested facilities regarding:	**Yes or NO**	**If Yes what are these implications and how does your project plan to mitigate the impact**
1. Additional load on nursing	NO	
2. Support services	NO	
3. Consumables	NO	
4. Laboratory tests	NO	
5. Equipment	NO	
6. Space	NO	
7. Communications	NO	
8. Additional OPD visits	NO	
9. Admission of patients	NO	
How will the sites be prepared to participate in your research?	To make the folder room available	
Results dissemination plan **1. Tick which groups will be affected by your research findings**	Provincial managers ☐X District Directors ☐X Facility manager and staff ☐X Patients ☐ Community ☐	

4

	Other (please specify) None
	Within one month ☐ Within one to three months ☐ Within three to six months ☐ Longer than six months X

GSH Research Annexure 2