

https://dspace.org/

Comparing the effect of referral intervals on the severity of dysplasia at the colposcopy clinic

Saayman, Francois

2013-03-19
http://hdl.handle.net/10539/12564

Downloaded from a DSpace repository. https://dspace.org/

Comparing the effect of referral intervals on the severity of dysplasia at the Colposcopy clinic

Dr. Francois Saayman

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in the branch of Obstetrics and Gynaecology

Johannesburg 2012

I, Francois Saayman, declare that this dissertation is my own work. It is being submitted for the degree of Master of Medicine in the branch of Obstetrics and Gynaecology at the University of the Witwatersrand, Johannesburg. It has been submitted previously and accepted in partial fulfilment of the requirements for the Fellowship of The College of Obstetricians and Gynaecologists.



this 8th day of October, 2012

Abstract

***Background*:** Cervical cancer is the most common cancer in black South African women. Cervical cancer screening was initiated in South Africa in 2001, but limited infrastructure in the public health service result in a long delay between initial screening and colposcopy and treatment for women with abnormal Pap smears.

Objective: To determine the effect of the time interval between cervical cytology screening and histology at treatment on the grade of cervical disease in women at a colposcopy clinic, Chris Hani Baragwanath Hospital.

Methods: Women with cytological abnormalities were referred to the colposcopy clinic according to National Guidelines for referral. Data extracted from the colposcopy clinic database were analyzed to determine whether early (up to 180 days) or late (more than 180 days) referral and treatment had an impact on the grade of dysplasia of the cervical lesion from the time of initial diagnosis on cervical cytology to the definitive treatment at colposcopy.

Results: In the early (7 to 180 days) referral group 213 (13.43%) women compared to 201 (14.63%) in the late (181 to 1702 days) referral group had up-grading of cervical dysplasia (p=0.35). The number of women with down-grading of dysplasia or no change in grade of dysplasia was 1373 (86.57%) and 1173 (85.37%) in the early and the late referral groups respectively (p=0.35). In the

univariate analysis, risk factors for up-grading of dysplasia were HIV (OR=1.63, p=0.00) and condom use (OR=1.30, p=0.02). There were 4 cases (0.68%) of invasion in the LSIL group and 50 cases (2.11%) in the HSIL group that were not detected by cervical cytology. Risk factors for invasive disease on histology were age (OR=1.09 per year, p=0.00), parity (OR=1.32 per pregnancy, p=0.00) and HSIL on cervical cytology compared to LSIL (OR=3.17, p=0.03).

Conclusion: There was no difference in up or down-grading of cervical dysplasia between patients that were referred to colposcopy clinic within 180 days compared to those that arrived after 180 days. With the present restrictive infra-structure, older women, HIV positive women, those of higher parity, and especially those with HSIL on cervical cytology should be referred sooner. HIV positive women should be prioritised by grade of dysplasia but not by CD4 until its effect on cervical dysplasia has been clarified. Ideally all women should be seen at a colposcopy clinic at least within 180 days and even sooner because of other factors such as anxiety, loss to follow up and cases of invasion being missed by cytology.

Acknowledgements

- The NHLS for the cervical cytology and histology results.
- The nurses, doctors and patients at the colposcopy clinic.
- To Prof CJ van Gelderen and Dr Y Adam for use of the data.
- To Dr Y Adam for suggesting this research topic, teaching of research methods and her valuable assistance with statistical calculations.

Table of Contents

	Page
Declaration	ii
Abstract	iii
Acknowledgements	v
Table of contents	vi
Lists of figures and graphs	viii
List of tables	ix
Abbreviations	x
Chapter 1	
1.1. Introduction	1
1.2. Problem statement	2
1.3. Natural history and pathogenesis	4
1.4. HIV and cervical dysplasia	6
1.5. Study objectives	9
Chapter 2	
2.1. Methodology	11

2.2. Data Management and Statistical Analysis 12

2.3. Ethics 14

Chapter 3

3.1. Results 16

3.1.1. Study population 16

3.1.2. Cervical cytology findings 19

3.1.3. Age and parity 20

3.1.4. Contraception 20

3.1.5. Colposcopy 22

3.1.6. HIV 24

3.1.7. Referral time 25

3.1.8. Histology findings compared to cervical cytology 25

3.1.9. Comparison of referral groups 27

Chapter 4

4.1. Discussion 31

4.2. Strengths 37

4.3. Limitations 38

4.4. Conclusion 39

4.5. Recommendations for cervical cancer screening and colposcopy referral policies 40

4.6. Suggested improvements for future studies 41

Appendix A Ethics certificate 42

References 43

List of figures and graphs

3.1 Patients excluded from analysis

3.2 Incidence of cervical cytology abnormalities referred

3.3 Contraceptive methods

3.4 Colposcopy findings

3.5 Comparison of histology findings in LSIL and HSIL group

List of tables

3.1 Comparison of demographic and other variables in respective referral interval groups

3.2 Effect of demographic details and other variables on invasive disease and up-grading of dysplasia

Abbreviations

AIDS	Acquired immunodeficiency syndrome
AGC	Atypical glandular cells (Bethesda Classification)
ART	Anti-retroviral treatment
ASC-H	Atypical squamous cells, HSIL cannot be excluded (Bethesda Classification)
ASCUS	Atypical squamous cells of undetermined significance (Bethesda Classification)
CD4 cell	T lymphocyte with CD4 receptor that recognizes antigens on the surface of a virus-infected cell and secretes lymphokines that stimulate B cells and killer T cells; HIV binds to the CD4 glycoprotein receptor, gains entry into the cell to infect and kill it.
CDC	Centers for disease control
CIN	Cervical intra-epithelial neoplasia
COC	Combined oral contraceptive
Depo-Provera®	Depot-Medroxyprogesterone acetate
HAART	Highly active anti-retroviral treatment
HIV	Human immunodeficiency virus
HPV	Human papillomavirus

HSIL	High- grade squamous intra-epithelial lesion (Bethesda Classification)
IUCD	Intra-uterine contraceptive device
LLETZ	Large loop excision of the transformation zone
LSIL	Low-grade squamous intra-epithelial lesion (Bethesda Classification)
NHLS	National Health Laboratory Services
NSA	Not sexually active
Nur-Isterate®	Norethisterone enanthate
POP	Progesterone only pill
SA	South Africa
SIL	Squamous intra-epithelial lesion
STI	Sexually transmitted infection
UK	United Kingdom
US	United States (of America)

Chapter 1

This chapter discusses the impact of cervical cancer on the population and how screening has changed this. The problem of an ideal referral time between cervical cytology screening and colposcopy is addressed. We look at the role of HPV, HIV immunosuppression and ART usage on progression and regression of cervical dysplasia.

Introduction

Cervical cancer is a major health concern in South Africa and other developing countries. Overall the crude incidence rate of cervical cancer in South Africa is believed to be 22.8/100 000 compared to 15.8/100 000 globally (1). The last National Cancer Registry of 1999 estimated the lifetime risk of cervical cancer in black South African females to be 1 in 25, making it the most common cancer in black women in South Africa (2). The prevalence of pre-malignant cervical abnormalities in the Johannesburg Metropolitan area was equally high at 13.7% for any abnormality (3). Cervical cancer meets most of the WHO criteria for screening according to the Wilson-Jungner criteria (4). Screening and treatment of cervical cancer precursors have been shown to reduce the mortality from cervical cancer by 34-80% in developed countries (5).

Prevention of cancer of the cervix became a National health priority in SA, where screening for cancer precursors with the Papanicolaou smear began in 2001 (6). The South African program has been developed in accordance with the recommendation by the WHO for mid-income countries. The recommendation

being that cervical cytology screening should be performed 3 times in a woman's lifetime, starting at the age of 35 at an interval of between 5-10 years to achieve 80% coverage of the female population (7). (The SA program starts at the age of 30 with an interval of 10 years) (6).

For cervical cancer prevention to succeed, all aspects of the service need to be functional: screening, diagnosis (colposcopy and biopsy), treatment, and follow-up. Screening within the Johannesburg Metropolitan area is approximately 7% per year and this will translate into 70% coverage within 10 years (8). However, colposcopy services are overwhelmed by the large number of referrals which results in a delay in diagnosis and treatment. An audit of the clinic at Chris Hani Baragwanath Academic Hospital showed that the average time from cervical cytology screening to the colposcopy visit between April 2003 and December 2006 was 113 days. A quarter of the patients arrived within 51 days, and, by 104 days, half of the patients had attended. Three quarters of the patients had arrived within 142 days. The range was 14 – 706 (9).

Problem Statement

The ideal time for referral to a colposcopy clinic, and its effect on the eventual outcome of the disease is still unclear and results from studies evaluating the course of untreated cervical cytological abnormalities are conflicting:

Fakokunde has shown that amongst 316 women with high-grade smears, those seen after 180 days were less likely to need excisional treatment (33.8% vs. 55.8% ; OR=0.45; 95% CI, 0.25-0.78; P=0.0004) and less likely to have high-grade disease (24.3% vs. 45.9%; OR=0.37; 95% CI, 0.21-0.68; P=0.001) than women seen before 180 days. He found no significant difference between the groups in proportion of women with invasive disease (10). It is not known whether the same is applicable to our population and to the HIV positive population in whom more rapid progression has been demonstrated. Nappi found the risk of recurrence or progression of low-grade SIL to be about 4-5 times higher in HIV positive than HIV negative women (11).

The UK's National Health Service Cervical Screening Programme referral guidelines for colposcopy state that women should be examined in a colposcopy clinic within two weeks if their tests are reported as suspected cancer or glandular neoplasia, within four weeks if their tests show high grade disease (moderate or severe dyskaryosis) and within eight weeks if their tests show low grade disease (mild dyskaryosis or borderline changes) (12). Kirby felt that mild or moderate dyskaryotic smears should not be an indication for immediate referral for colposcopy, since under a conservative management policy most women return to normal without treatment (13).

For study purposes it would be unethical to randomize patients into a referral interval of more than 180 days, but due to the excessive patient load and limited resources of doctors and nurses there is a long and variable waiting time in the

South African public sector. We do not know whether the time interval between the cervical cytology screening and treatment affects the ultimate progression of disease in women in this setting.

The current waiting time for an appointment at the colposcopy clinic at Chris Hani Baragwanath Academic Hospital is about 8 months. However, when cervical cytology reported the presence of malignant cells or when invasive disease couldn't be excluded the patient was given an appointment for colposcopic assessment as soon as possible. The ideal time within which a patient with a cervical abnormality on cytology should attend colposcopy is not known. The situation is complicated by the fact that not all lesions will progress, and in fact some will regress. This study will therefore investigate the impact of referral times on the final histology. We will investigate the proportion and characteristics of change in severity of cervical dysplasia over time.

Natural History and pathogenesis

This section gives a short summary of HPV and its involvement in the pathogenesis of cervical dysplasia. Previously quoted progression and regression rates are presented. The interaction between HIV and HPV and its effect on cervical dysplasia is discussed.

The Human Papillomavirus, of the Papovaviridae family, has been proven to be the most important causative factor in the development of cervical epithelial dysplasia and cervical cancer (14,15). HPV's detected in the cervix can be divided

into low risk types (HPV 6 and 11), intermediate risk types (HPV 31, 33 and 35) and the high risk types (HPV 16 and 18). One to 15 % of cervical cancers test negative for HPV (14,15), this may reflect false negative test results or indicate a different type of cervical cancer (16). HPV infects the developing immature metaplastic cells of the transformation zone of the cervix. This viral infection is more often than not transient, because most individuals develop an effective type-specific immune response. Pre-malignant and malignant cells arise as a result of HPV DNA integration into the host cellular genome, and overexpression of the viral E6 and E7 oncogenes. Cells acquire a proliferative advantage by escaping growth control exerted by p53 and p105Rb which are inactivated by E6 and E7 proteins respectively (14).

The long latency period between primary infection and emergence of invasive cancer, together with the finding that not all cases progress to invasive cancer, suggest that additional factors such as sexual behaviour, hormonal effects of oral contraceptives and pregnancy, dietary deficiencies, immunosuppression, chronic inflammation, genetic predispositions, tobacco use and socio-economical level are also involved in the process of tumour development (14,15).

A meta-analysis by Melnikow found 7.13% of ASCUS and 20.81% of low-grade SIL progress to high-grade SIL after 24 months (17). Ostor described a 10% risk of progression from CIN1 to CIN3 and a 1% risk of progression from CIN1 to invasive cancer (18). Melnikow showed 0.25% of ASCUS, 0.15% of low-grade SIL and 1.44% of high-grade SIL progressed to invasive cancer after 24 months (17),

whilst McCredie found that 31 to 51 % of untreated CIN3 progressed to invasive cancer after 30 years (19).

Conversely, Melnikow also described that 68.19% of women with ASCUS on cervical cytology, 47.39% with low-grade SIL and 35.03% with high-grade SIL regressed to normal after 24 months (17) Ostor found a higher (60%) regression rate for CIN1 (18).

Precursor lesions of the cervix persist longer and progress more quickly in women with high-risk HPV infections than in women with low-risk HPV infections, or without HPV. Schlecht et al found the mean times for regression from ASCUS to normal, from LSIL to ASCUS or normal, and from HSIL/CIN2 to ASCUS or normal were longer for women infected with high-risk HPV types (16.8 months, 13.8 months, and 17.1 months respectively) than for women with low-risk HPV types (7.7 months, 7.8 months, 8.9 months), or for women with no HPV infection (7.6 months, 7.6 months and 7.0 months respectively). Testing cervical lesions for high-risk HPV's may help identify those that are likely to progress rapidly (20).

HIV and cervical dysplasia

The interaction between HIV and HPV and its effect on cervical dysplasia is discussed below:

The burden of HIV in Sub-Saharan Africa is high, with an estimated prevalence of HIV infection of 29.8% amongst Gauteng antenatal attendees during 2009 (21).

HIV infection has changed the severity of HPV infection and the natural history of cervical dysplasia: In a US study, 60–70% of HIV-positive women tested positive for HPV, making them 2.3 times more likely to be infected than HIV-negative women. They were also 1.9 times more likely to be infected with multiple HPV strains, and 1.6 times more likely to have a higher HPV viral load (22).

Palefsky et al found the prevalence of high risk HPV in HIV positive women to be 20-34%, making them about 5 times more likely to have high risk HPV infection present than is the case in HIV negative women (23). Women with HIV were also more likely to have persistent infections with HPV (24). A low CD4 count has been found to increase the abovementioned likelihood ratios (22,23,24). Women infected with both HIV and high-risk HPV had a more than 40 fold higher risk of SIL than uninfected women (25).

It seems probable that this association between HIV infection and HPV infection plays an important role in the significantly higher prevalence of cervical cellular abnormalities observed in HIV positive women (26,27). However, most of these cytological abnormalities are LSIL; in HIV positive women in the US most abnormalities were ASCUS and low-grade SIL, with high-grade lesions diagnosed in less than 10% (28). Massad et al found in a group of 1639 HIV positive women that the proportion of women with high-grade lesions did not increase over a period of up to 5 years (29).

Ellerbrock found that one in five HIV-positive women developed biopsy-confirmed SIL's within 3 years compared to 1 in 20 HIV-negative women with no evidence of cervical disease (30). Nappi found the risk of recurrence or progression of low-grade SIL to be about 4-5 times higher in HIV positive than HIV negative women (11).

Immune deficiency in HIV is associated with faster progression and slower regression of SIL. Massad demonstrated an OR of 1.1 for progression over a maximum period of 5 years in HIV positive women with a CD4>200 cells/mm^3 and a viral load < 4000 copies/ml. When the CD4 was less than 200 cells/mm^3 and the viral load >4000 copies/ml the OR was 1.7 for progression (29). Six showed that over a period of one year, LSIL progressed to HSIL in 38.1% of HIV positive women with a CD4 count less than 500 cells/mm^3, but there was no progression if the CD4 was more than 500 cells/mm^3 (31). Regression of SIL was reduced by 22% for every $\log_{10}$ increase in HIV viral load according to Schuman (26).

Anti retroviral treatment may impact on the natural history of cervical disease in HIV-positive women, but the results of studies are contradictory so far. Minkoff found that women on HAART were 40% more likely to demonstrate regression and less likely to demonstrate progression (OR=0.68) over 6 monthly intervals (32). Lillo found no significant difference in progression over 6 months between 74 women receiving HAART, 62 treated with one or two reverse transcriptase inhibitors only and 27 women not on treatment after adjusting for CD4 cell count (33).

HIV positive patients may need more frequent screening and a lower threshold for treatment due to the different behaviour of cervical dysplasia in these patients. In developed countries it is recommended that HIV positive women have two cytological assessments within the first year of HIV diagnosis and annually thereafter, with referral for colposcopy for any smear showing an ASCUS or more severe lesion (34). Faro et al recommends that in HIV-positive women, more frequent screening is recommended if HPV infection is known, if there has been a previous abnormal smear, if CD4 < 200 cells/mm^3, or following any surgical treatment for cervical lesions (28).

Study objectives

Primary objective

- To determine whether there is down-grading, the same grade of disease or up-grading of disease associated with the two different referral intervals of less than 180 days compared to more than 180 days.

Secondary objectives

- To determine what proportion of patients have up-grading of dysplasia, have the same grade of disease, or down-grading of dysplasia between the predicted grade on cervical cytology and the actual grade on histology.
- To determine the effect of HIV and CD4 count on up or down-grading of dysplasia and on the risk for invasive disease.

- To determine other risk factors for up-grading of dysplasia and invasive disease.

Chapter 2

2.1. Methodology

Study Design

A cross-sectional analysis of data collected at the colposcopy clinic at Chris Hani Baragwanath Hospital was done. The data was prospectively recorded into the colposcopy database. Information on the demographic details (Age, parity, and contraception), HIV status, CD4 count, ART use, date of cervical cytology screening with results, colposcopy findings and the date of treatment with final histology result were used. Cervical cytology was assessed by conventional Papanicolaou smear.

Study population

The Colposcopy clinic at Chris Hani Baragwanath Hospital is a referral centre for Soweto and the Southern parts of Gauteng Province. Between 2003/04/03 and 2010/06/30, 3942 patients were referred to and seen at the clinic.

Women with a clinically normal cervix (on speculum examination) and cervical cytology showing HSIL or more were referred to the colposcopy clinic although HIV infected women with a cervix which appeared normal in the presence of any cytological abnormality were also referred for colposcopy. HIV negative women with a LSIL were only referred if the cytological abnormality persisted after 12 months. When the presence of malignant cells were reported or when invasive disease could not be excluded the patient was referred for immediate colposcopic

assessment. Women with a clinically abnormal cervix but normal cervical cytology or without any cervical cytology result were also referred.

Most of the cervical cytology specimens and all histology specimens were processed by the NHLS.

The colposcopy clinic practices a policy of "see-and-treat", where all women with cervical cytology showing HSIL or more and a colposcopic diagnosis of CIN 2 and more, or an inadequate colposcopy are treated with immediate LLETZ. HIV infected women with cervical cytology showing LSIL or more and colposcopy of CIN1 or more are also treated immediately. The Finesse I® and Finesse II® machines are used with the appropriate size of either a C-LETZ® or UtahLoop® to perform the LLETZ.

2.2. Data Management and Statistical Analysis

Exclusion criteria (see Fig. 3.1)

- Patients who were referred with normal cytology and an abnormal cervix
- Patients who had had a hysterectomy and were referred with a vault smear
- Patients who were referred with a biopsy result rather than a cervical cytology result
- When the date of the cervical cytology screening was unknown
- When the cervical cytology reported "malignant cells" or "cannot exclude invasion"
- When the cervical cytology was normal or showed ASCUS, ASC-H or AGC

Data cleaning and coding

Data was extracted from the database on 2010/11/10, all the variables were put into an excel spreadsheet and coded for analysis. Missing data and outliers were re-checked on the NHLS system and on the Chris Hani Baragwanath Hospital record database for accuracy. Duplicate records were deleted.

Missing HIV and CD4 results were updated from the NHLS system as explained: If the HIV result was unknown, then it was presumed to be positive if the CD4 was known and less than 500(n=20). Patients with an unknown HIV status at the first visit were regarded to be positive at that time if they tested HIV positive within a 6 month period after this visit. Women who reported having had a negative test within the 6 months prior to the colposcopy visit were regarded as negative in this database. If only the year was known when the patient was initially found to be HIV positive , then the month of diagnosis was entered as June (n=51). Only CD4 results within 6 months before or after the first visit were recorded into the database.

New variables were made for cervical cytology result, histology, types of treatment and contraception - these were categorized and coded.

Descriptive statistics

- Description of age, parity, contraceptive use, HIV status, CD4 count
- Description of all cervical cytology results
- Description of all the histological diagnoses

Analytical statistics

The patients were divided into 3 referral interval groups: early (≤180 days), intermediate (between 180 and 360 days) and late referrals (≥360 days). However only 263 (8.89%) patients were in the late referral group. We decided to add them to the intermediate group because the differences observed between the early and intermediate group did not change whether the data were analysed using 2 or 3 groups. The change in severity of dysplasia between the two groups was compared.

For comparisons of continuous data the T-test was used, for proportions Chi-square was employed. A Logistic regression was performed to find factors which are associated with up-grading of dysplasia and invasive disease on histology.

2.3. Ethics

All women at the colposcopy clinic were counselled and requested to sign an informed consent form that allowed their data to be used for audit, follow-up and research. Patients that declined to give consent received the same treatment as all other patients.

Permission to use information from the database was obtained from Dr Y Adam and Prof CJ van Gelderen. Ethical approval for the creation of the database had been obtained previously: Clearance certificate number M090676.

Ethics approval for secondary analysis of this data was sought from and granted by the Human Research Committee of the University of the Witwatersrand. Clearance certificate number M10530 (See Appendix A for copy of certificate).

Permission for this study was obtained from the CEO of Chris Hani Baragwanath Hospital.

Chapter 3

3.1. Results

After exclusion of certain patients, the final information extracted from the remaining 2960 patients is presented in detail below. Details on age, parity, contraceptive use, HIV status and CD4 cell count are summarized in table and diagram form. The reader is shown the proportions of cervical cytology results, colposcopy findings and the final histology results and how they compare with each other. Variables in the two referral interval groups are compared and p-values for each were calculated.

Finally, the result of logistic regression shows the risk factors for invasive disease and/or up-grading of dysplasia.

3.1. 1. Study population

From 2003/04/03 to 2010/06/30, 3942 women were referred to the Chris Hani Baragwanath Colposcopy clinic.

Eighty one patients with normal cervical cytology were referred because the clinician thought that the cervix was abnormal. ASCUS, AGC and ASC-H were excluded because there is no direct histological comparison/equivalent: high grade lesions may be seen in 10% to 39% of cases classified as AGC; 10% to 20% of women with Atypical Squamous Cells on cervical cytology are estimated to have underlying CIN2 or CIN3; underlying invasion may be present in 1 in 1000 (35).

Invasion or adeno-carcinoma in-situ patients would require urgent assessment and therefore looking at an ideal referral time would not make sense. Pregnant patients would only have a biopsy if the cervical cytology or colposcopy suggested a malignancy, LLETZ was thus postponed. Figure 3.1 shows the number of patients that were excluded from the analysis for the respective reasons:

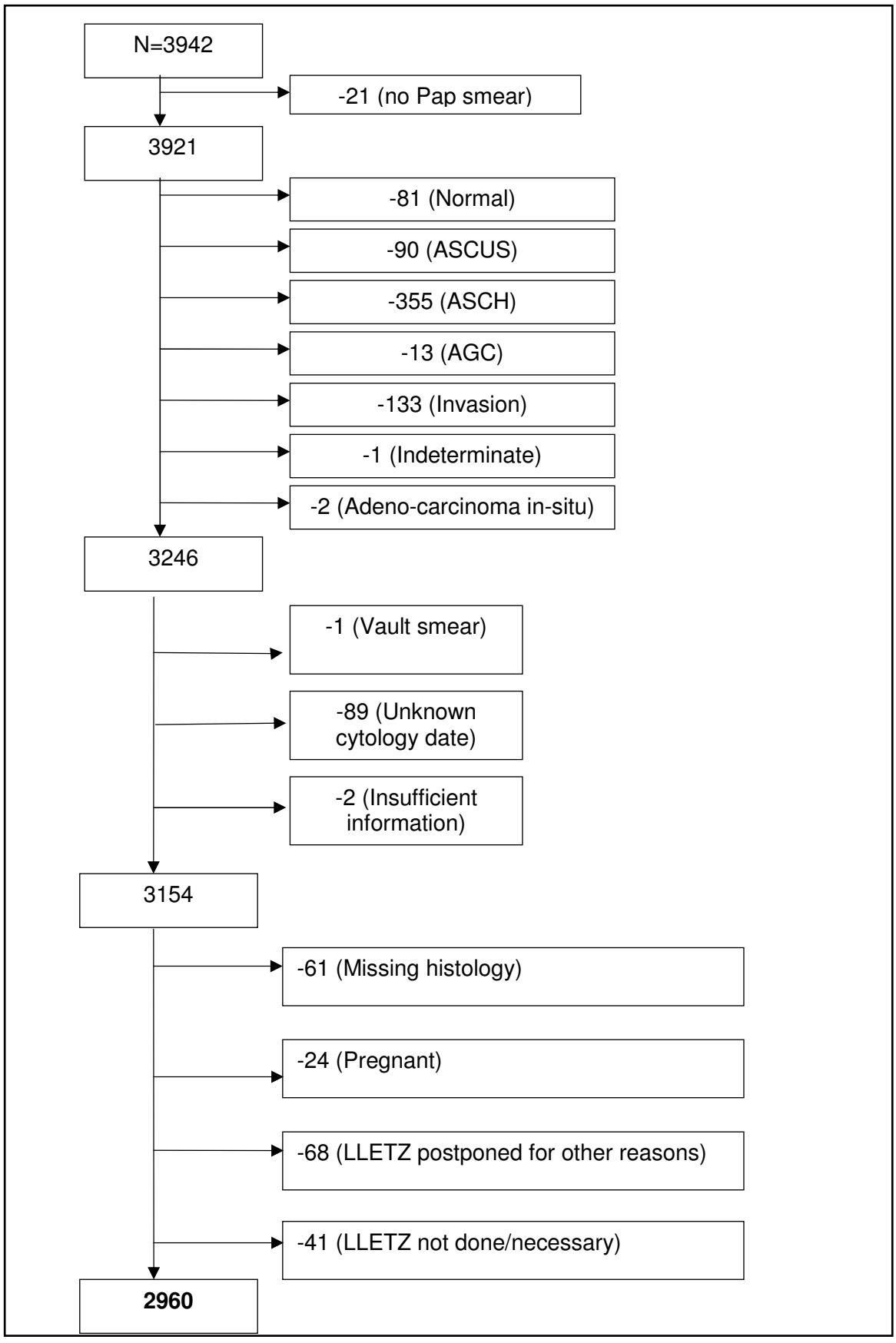


Figure 3.1 Patients excluded from analysis.

3.1.2. Cervical cytology findings

Figure 3.2 shows the proportion of abnormalities according to cervical cytology. Of note was the high percentage of cytology findings that suggested invasion, this is expected because the screening programme in SA only started in 2003 and SA is a country with a very high incidence of cervical cancer. A longer established screening service may have identified these women earlier, when they still had a pre-malignant lesion. A larger than expected number of patients with LSIL were observed; this was probably because all cases of LSIL in HIV positive patients were referred, whereas HIV negative women were only referred if they had persistent LSIL.

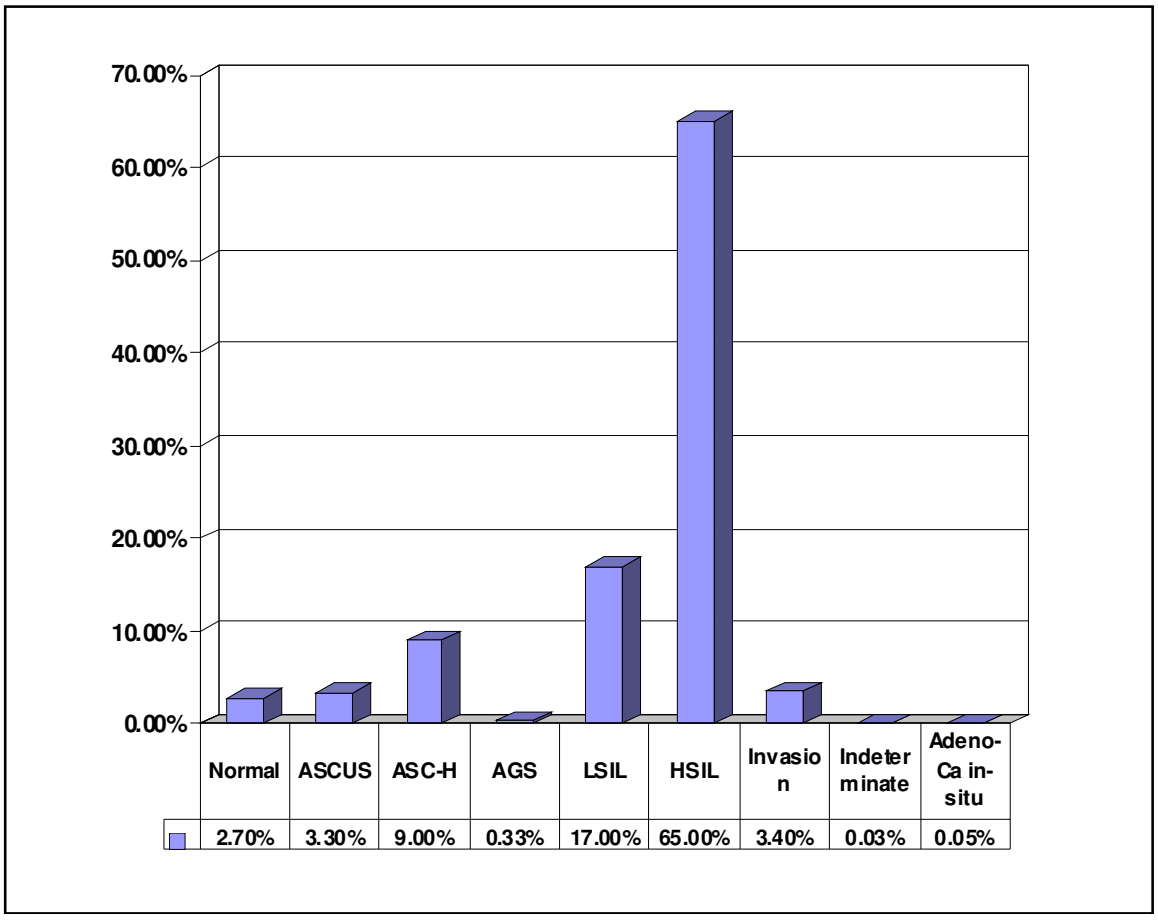


Figure 3.2 Incidence of cervical cytology abnormalities referred.

3.1.3. Age and parity

The mean age was 36.94 (SD= 8.34) with a median of 35.63 (IQR= 31.01- 41.76) and the range 18 to 81.The South African screening programme starts at the age of 30 (6), so the above values would be expected to be higher. This is possibly due to the referral of many HIV positive patients in whom cervical cytology screening was done at an earlier age. The mean parity was 2.11 (SD= 1.46) with a median of 2 (IQR= 1-3).

3.1.4. Contraception

The method of contraception used by a woman was also recorded to determine whether there was any association with up or down-grading of disease.

The age range in our patients was 18 to 81, so it must be kept in mind that this was not an accurate representation of women in their reproductive years. Condom use referred to both male and female condoms, although it was not specified on the database. Dual contraception referred to the use of a condom and any other modern method. Figure 3.3 gives a break-down of the contraceptive methods used by the women:

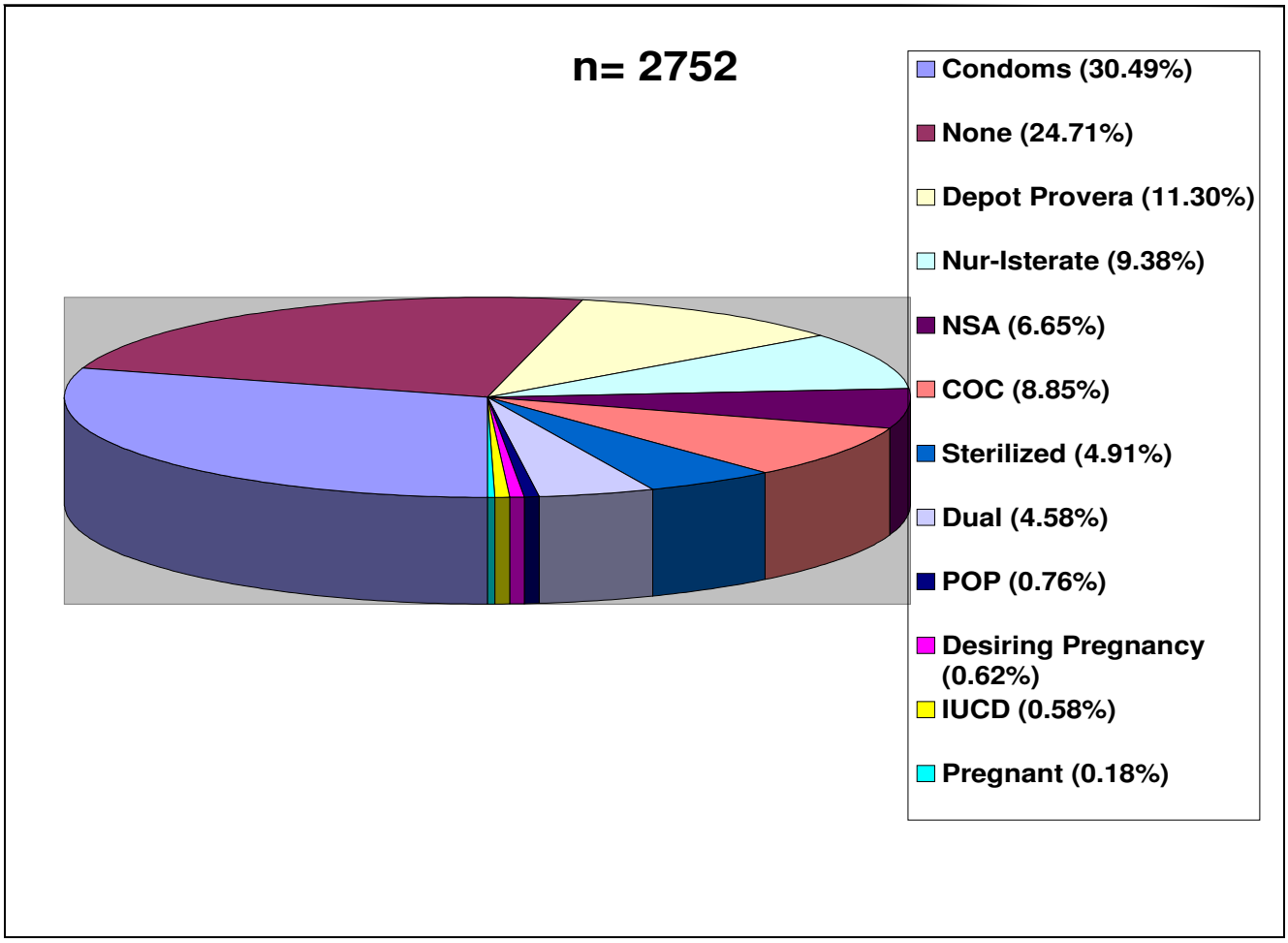


Figure 3.3 Contraceptive methods.

3.1.5. Colposcopy

Colposcopy is performed by specialist Gynaecologists or by supervised registrars in our service. Colposcopic diagnosis is performed using a method based on the Reid Colposcopic Index (36). This is a systematic, objective method of colposcopically grading the severity of premalignant cervical lesions. The index considers four attributes of premalignant cervical lesions. The first three signs are evaluated following application of 5% acetic acid to the cervix. The last sign is dependent on a preliminary score of the first three signs and is determined after Lugol's iodine application to the cervix.

The first sign considers the nature of the lesion margin. Low-grade lesion margins (0 points) are described as irregular, flocculated, feathered, angular or "geographic," indistinct, with "satellite" lesions or phytic micropapilliferous (condyloma-like) in contour. Intermediate lesion margins (1 point) are smooth and fairly straight. High-grade margins (2 points) exhibit raised peeling edges, or are proximally located within a larger low-grade lesion and have an internal border or demarcation between the area of low-grade change and the squamo-columnar junction.

The second sign considers the colour of aceto-whitening. Low-grade lesions (0 points) appear semitransparent or shiny snow white. High-grade lesions (2 points) are of a dirty oyster-gray color and dense appearing. An intermediate lesion (1 point) is off-white, between the two extremes in the color spectrum.

The third colposcopic sign assesses vascular changes. The low-grade category (0 points) is characterized by fine-caliber capillaries (punctation or a mosaic pattern). These vessels are not dilated and are configured in loose arcades. The intermediate category (1 point) displays an absence of superficial vessels. The high-grade category (2 points) features coarsely dilated vessels (coarse punctation or mosaic).

The final colposcopic sign is evaluated after staining of the cervix with Lugol's iodine. The low-grade category (0 points) may take up iodine (mahogany-brown color) or reject iodine (mustard-yellow color). The intermediate category (1 point) displays a variegated yellow-brown or "tortoise-shell" appearance resulting from partial or inconsistent iodine uptake. The high-grade category (2 points) rejects iodine (mustard-yellow). Some low-grade lesions, all high-grade lesions, columnar cells, and atypical immature squamous metaplasia reject iodine (mustard-yellow). Therefore, a preliminary summated index score of the first three colposcopic signs influences the score for the last colposcopic sign.

Scores between zero and 2 are predictive of mild dysplasia (CIN I) or human papillomavirus infection. Scores between 3 and 5 are suggestive of mild or moderate dysplasia (CIN I or CIN II). Total scores of 6 to 8 are predictive of moderate or severe dysplasia. In our clinic iodine staining is not used as part of this staging and there is a degree of subjectivity. Figure 3.4 gives a breakdown of the different colposcopy findings.

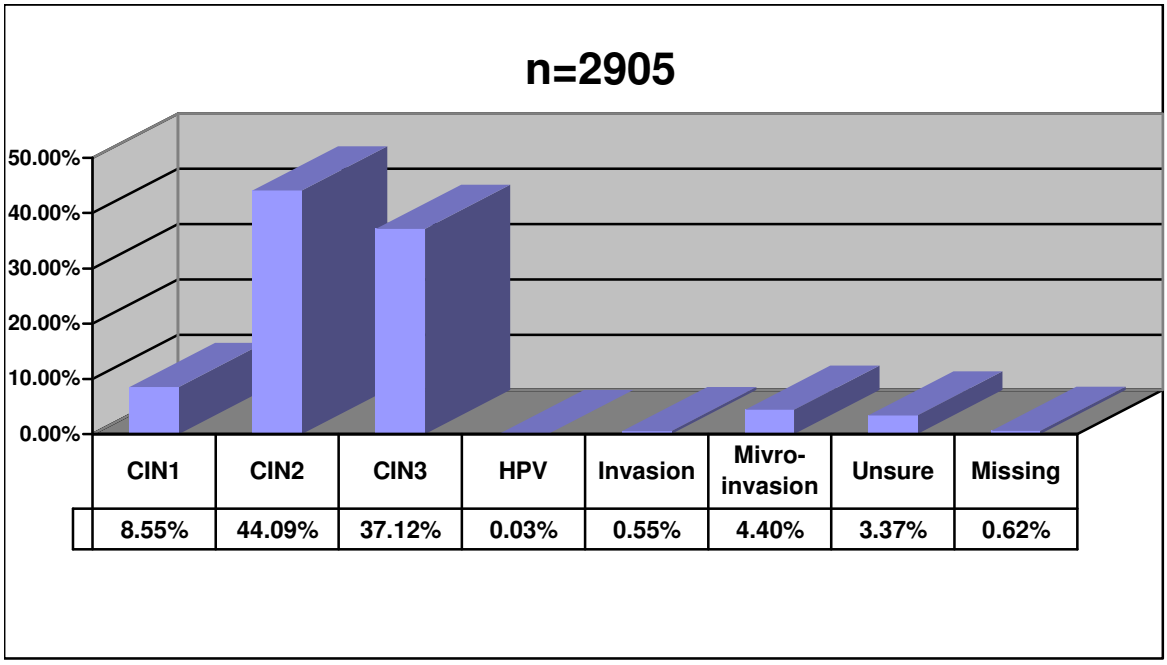


Figure 3.4 Colposcopy findings.

3.1.6. HIV

The accuracy of HIV negative status was limited by the fact that it was ascertained by self-report in many women, and could not be corroborated. The stigma surrounding HIV is still a big problem in SA and this further limits the accuracy of a reported negative test result. Women who reported a negative test within 6 months of the colposcopy visit were recorded as negative in the database. HIV status was recorded as unknown if the woman tested negative more than 6 months before. HIV testing had only been offered routinely to all women attending the clinic since 2006, and some women declined to be tested.

Of 2947 patients, 19.10% were HIV negative, 69.63% were HIV positive and in 11.27% the HIV result was unknown. Of the HIV positive patients, the mean CD4

count was 267.19 (SD= 181.76) and the median CD4 was 230 (IQR= 131-366). ART's were being used by 41.81% of the HIV positive patients.

The high proportion of HIV positive women may in part be due to HIV positive women having cervical cytology screening at an earlier age and due to the strong association between HIV and SIL's.

3.1.7. Referral time

The referral time was calculated from the date of cervical cytology screening until the day of the first Colposcopy clinic visit. The mean referral time was 199.29 days (SD= 140.64), median referral interval was 169 days (IQR= 112.50- 263) with a range of 7 to 1702.

The 2960 patients were divided into two arbitrary groups according to referral interval: those seen up to 180 days (group 1) and those seen after 180 days (group 2). The SA guidelines from 2003 do not give a specific timeframe within which a patient must be seen (6); women are given the first available appointment. Using these time scales, 1586 (53.58%) patients arrived within 180 days and 1374 (46.42%) patients were seen after 180 days.

3.1.8. Histology findings compared to Cervical cytology result

Amongst 2808 patients, treatment options included LLETZ in 2666 (94.94%), hysterectomy in 67 (2.39%) and cone biopsy in 40 (1.42%). In 35 (1.25%) patients, only a punch biopsy was done. The aim of treatment was complete excision of the

lesion, since positive margins have been shown to be a predictor of persistence (2). Amongst 2693 patients, 273 (10.14%) had both margins involved by dysplasia, 1143 (42.44%) had complete excision margins, 897 (33.31%) had involvement of the ectocervical margin, 253 (9.39%) had endocervical margin involvement and in 127 (4.72%) margin involvement was not known.

Four cases of invasion in the LSIL group and 50 cases in the HSIL group had not been detected on cervical cytology.

Figure 3.5 gives a breakdown of the histological abnormalities found in those patients where cervical cytology showed LSIL compared to those with HSIL:

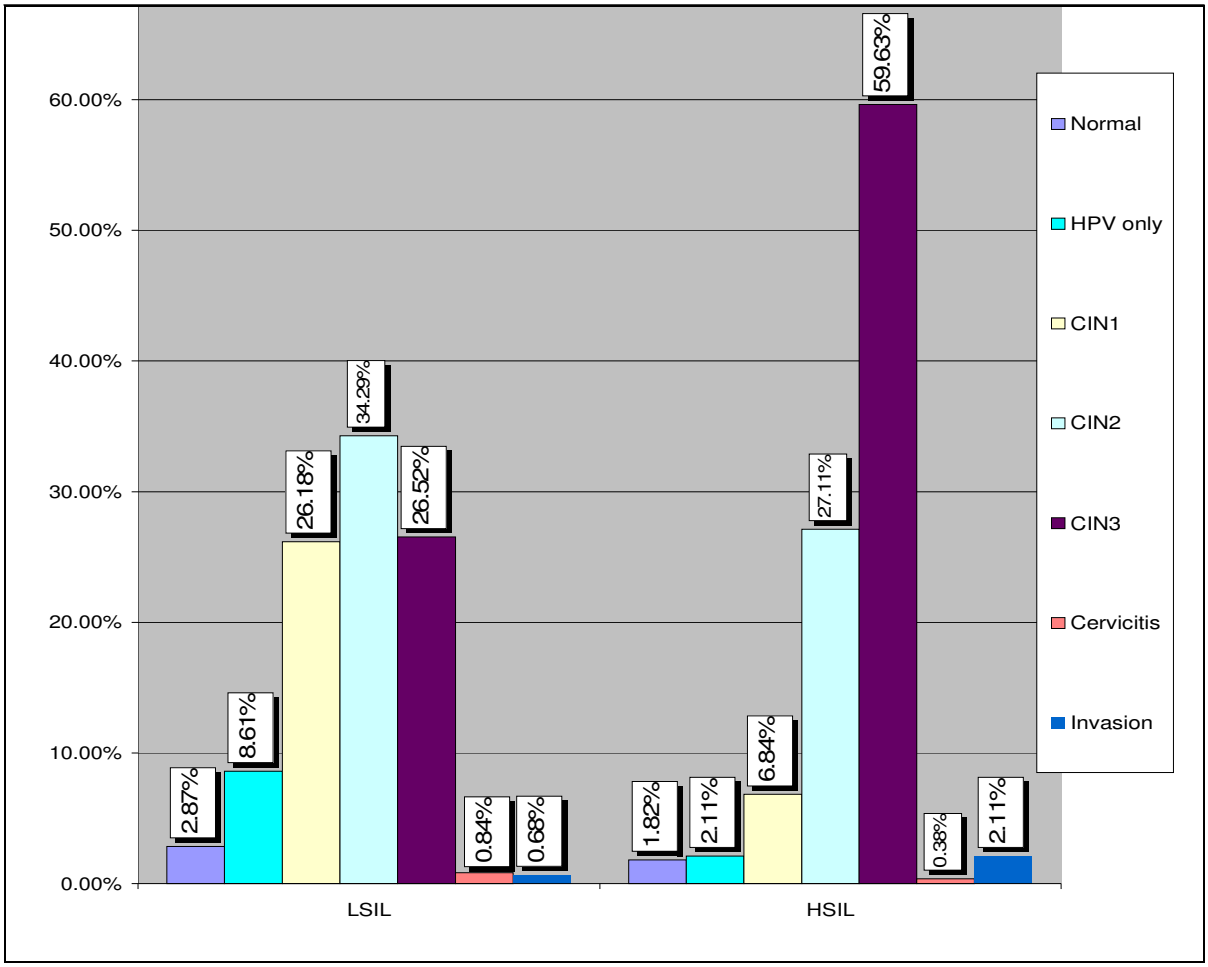


Figure 3.5 Comparison of histology findings in LSIL and HSIL group.

3.1.9. Comparison of referral groups

The variables in the two referral-interval groups were compared (Table 3.2); HIV status, CD4 count, grade of dysplasia and invasion on cervical cytology showed significant differences, but histology findings did not.

It is interesting to see that there were more women with a CD4 count less than 200 cells/mm^3 in group 1, perhaps this was because they were more ill and sought medical care sooner or because the appointment system was biased towards

giving these women an earlier appointment. More HIV positive women in group 2 were on ART, this is probably because they have had more time to be initiated.

There were also more women with HSIL in group 1 (1292, 54.56%) than in group 2 (1076, 45.44%), again this could be because of the same bias mentioned above.

There were 33 (58.25%) women with invasive disease in group 1 compared to 20 (41.75%) in group 2. Although not statistically significant, this could be because invasive disease is more likely to be symptomatic; also, women with a clinically suspicious cervix are referred immediately.

The ideal would be to compare histology with histology because then the actual progression or regression of disease can be determined. Because we are comparing cytology to histology we can merely suggest progression or regression of dysplasia because it is well known that there are discrepancies between cervical cytology and histology. The sensitivity and specificity of the Papsmear is 78.2% and 85.6% respectively for CIN2 and worse as diagnosed on histology (37). This would obviously affect the accuracy of all our findings. For the purpose of this study, we use the terms “up-grading of dysplasia” when cervical cytology showed LSIL and the histology showed at least CIN2 or more, or when cervical cytology showed HSIL and invasion on histology. “Down-grading of dysplasia” was defined as a HSIL or LSIL on cervical cytology with normal histology or a HSIL with CIN1 or less on histology.

Table 3.1 Comparison of demographic and other variables in the two referral interval groups

	Referral interval < 180 days	**Referral interval >180 days**	
N (%) = 2960 (100%)	1586 (53.58%)	1374 (46.42%)	
Age	Mean= 36.71 (SD=8.39) Median=35.14 (30.84-41.60) Range= 18.25-81.68	Mean= 37.20 (SD= 8.27) Median= 36.16 (31.25-41.90) Range= 18.62-81.70	P= 0.11* P= 0.07**
HIV Positive	N= 1009 (34.09%)	N=1043 (35.24%)	P= 0.00 (Chi^2)
HIV Negative	N= 342 (11.55%)	N= 221 (7.47%)	
HIV Unknown	N= 235 (7.94%)	N= 110 (3.72%)	
Known CD4	N= 919 Mean= 253.19 (SD= 179.70) Median= 210 (114-355) Range= 2-980	N= 964 Mean= 280.55 (SD= 182.79) Median= 244.50 (145-377.50) Range= 1-1222	P= 0.00* P= 0.00**
CD4<200 N= 837 (44.08%)	N=446 (48.16%)	N= 391 (40.18%)	P= 0.00(Chi^2)
CD4= 200-350 N= 533 (28.07%)	N= 239 (25.86%)	N= 294 (30.22%)	
CD4>350 N= 529 (27.86%)	N=241 (26.03%)	N= 529 (27.86%)	
HIV positive using ART	N=365 (36.17%)	N=493 (47.27%)	
Cytology LSIL	N= 294 (49.66%)	N= 298 (50.34%)	P= 0.03 (Chi^2)
Cytology HSIL	N= 1292 (54.56%)	N= 1076 (45.44%)	
Histology CIN1	N=161 (50.79%)	N= 156 (49.21%)	P= 0.29 (Chi^2)
Histology CIN2	N= 478 (56.57%)	N= 367 (43.43%)	P= 0.04 (Chi^2)
Histology CIN3	N= 833 (53.09%)	N= 736 (46.91%)	P= 0.57 (Chi^2)
Micro-invasion	N= 8 (47.06%)	N= 9 (52.94%)	P= 0.59 (Chi^2)
Invasion	N= 25 (67.57%)	N= 12 (32.43%)	P= 0.09 (Chi^2)
Up-graded	N= 213 (13.43%)	N= 201 (14.63%)	P= 0.35 (Chi^2)
Down-graded/ same	N= 1373 (86.57%)	N= 1173 (85.37%)	

*T-test, ** Mann-Whitney

Table 3.2 shows some demographic and other variables and their effect on the likelihood for histology to show invasive disease which was not suspected on cervical cytology. It also shows the effect of these variables on the rate of up or down-grading from cervical cytology (LSIL or HSIL) to histology. Logistic regression was used to calculate the odds ratio, significant differences were highlighted and only these will be discussed.

Table 3.2 Effect of demographic details and other variables on invasive disease and up-grading of dysplasia

	Invasive disease (Univariate)	**Any up-grading**
Age	**1.09 OR p=0.00 (1.07-1.13)**	1.01 OR p=0.39 (0.99-1.02)
Age (Older than 30 compared to younger than 30)	**13.59 OR p= 0.01 (1.89-98.44)**	1.07 OR p=0.62 (0.82-1.39)
Cytology HSIL as compared to LSIL	**3.17 OR p=0.03 (1.14-8.81)**	Not done
Referral every 3 months	1.00 OR p=0.97 (0.85-1.19)	1.03 OR p=0.35 (0.97-1.10)
Parity	**1.32 OR p=0.00 (1.13-1.53)**	0.96 OR p=0.31 (0.90-1.04)
HIV positive compared to HIV negative	**0.37 OR p=0.00 (0.20-0.68)**	**1.63 OR p=0.00 (1.21-2.20)**
For every 100 cells increase in CD4, if HIV infected	1.15 OR p= 0.15 (0.95-1.40)	1.05 OR p=0.16 (0.98-1.12)
The use or ART in women who are HIV infected	0.54 OR p=0.17 (0.22-1.29)	0.99 OR p=0.91 (0.78-1.26)
Nur-Isterate®	0.51 OR p=0.26 (0.16-1.64)	0.76 OR p=0.14 (0.52-1.10)
Depo-Provera®	0.27 OR p=0.07 (0.06-1.10)	0.73 OR p=0.07 (0.51-1.03)
Combined Oral Contraceptives	0.22 OR p=0.23 (0.04-2.13)	1.02 OR p= 0.09 (0.66-1.58)
Condoms	0.54 OR p=0.06 (0.28-1.03)	**1.30 OR p=0.02 (1.05-1.62)**
Sterilization	1.47 OR p=0.47 (0.52-4.12)	0.86 OR p=0.55 (0.53-1.41)

Chapter 4

The extent of the burden of HIV infection and cervical cancer is reflected in our results. This chapter will begin with a discussion of these results, the limitations and strengths of the study and will end with recommendations regarding referral times and further studies.

4.1. Discussion

HIV status

The HIV infection prevalence among patients was 69.63 % which is much higher than the estimated 30.30% among antenatal attendees in Gauteng during 2007 (21). This is in keeping with the strong association between HIV and cervical dysplasia. Moodley found women infected with both HIV and high-risk HPV had a more than 40 fold higher risk of SIL than women infected with neither of these viruses (24). Schuman found the incidence rate for SIL's among HIV-positive women was 11.5 cases/100 person-years of observation, compared with 2.6 cases/100 person-years of observation among HIV-negative women (RR, 4.5; 95% confidence interval CI, 3.1–6.4; *P*<.001) (26).

However, this figure may also be high because HIV positive women were referred with a report of any cytological abnormality; HIV negative women were only referred if they had persistent equivocal results.

The mean CD4 count of 267 is quite low; this is also in keeping with the known association of a low CD4 count and cervical dysplasia. Firnhaber showed that the prevalence ratio for LSIL and HSIL were 2.5 if the CD4 was <200 compared to a prevalence ratio of 1 if the CD4 was >500 (38). Agaba found the median CD4 cell count was lower in women with dysplasia compared to those without (142 vs. 170 cells/mm^3; P=0.04) (39). In this study we were not able to control for ART use, duration of ART use and CD4 count. Of the HIV positive women, 41.81% were already on ART's. This is a high proportion, considering that national ART 'roll-out' only began in 2001 at a very slow pace.

Referral interval

The mean referral interval was 199.29 days, which is equal to approximately 28 weeks. Only 53.58% of patients had a referral interval of less than 180 days compared to 76.6% in the study done by Fakokunde (10). This interval is much longer than the intervals recommended in the UK (12). The longest referral time of 1702 days (approximately four and a half years) is shocking. Regrettably, the retrospective nature of the study resulted in individual reasons for such long referral intervals remaining speculative.

We do not know the actual proportion of patients that are lost to follow-up, but a longer referral interval would surely worsen this problem. We try to decrease loss to follow-up by using a "see-and-treat" policy and by not delaying colposcopy with repeat cervical cytology if a patient is seen after a very long referral interval.

Although there is a long waiting list due to poor infrastructure, poor education and poor socioeconomic conditions may also contribute to such long intervals. The lack of a policy in SA that addresses the referral interval is also an important contributing factor.

Histology

We found no significant difference in up or down-grading of dysplasia between the two referral-interval groups. This is in contrast to the findings of Fakokunde who showed that amongst 316 women with high-grade smears, those seen after 180 days were less likely to need excisional treatment (33.8% [25/74] vs. 55.8% [135/242]; OR=0.45; 95% CI, 0.25-0.78; P=0.0004) and less likely to have high-grade disease (24.3% [18/74] vs. 45.9% [111/242]); OR=0.37; 95% CI, 0.21-0.68; P=0.001) than women seen before 180 days. He found no significant difference between the groups in proportion of women with invasive disease (10).

It must be kept in mind that the study by Fakokunde (10) was done in a developed country with the resources to use histology, the gold standard, to determine whether excisional treatment was necessary or not. Our colposcopy clinic however, uses a “see-and-treat” policy because it saves costs and time and also decreases the number of patients that would be lost to follow-up. The disadvantage of this policy is that more women receive excisional treatment which is associated with short term morbidities such as bleeding, pain and infection. Long-term morbidity includes cervical stenosis and there may also be an association with pre-term labour.

It is possible that the referral-intervals between the two groups were too short to show any significant results because of the slow progression of cervical dysplasia. Two studies demonstrated this effect: McCredie showed that 31 to 51 % of untreated CIN3 progressed to invasive cancer after 30 years (19). Melnikow showed that 20.81% of low-grade SIL progressed to high-grade SIL after 24 months; 0.15% of LSIL and 1.44% of HSIL progressed to invasive cancer after 24 months (17).

Risk factors

One would expect risk factors for invasion to be the same as those for up-grading of cervical dysplasia, but this was not true for age, parity, HIV status or use of condoms. This could be because of the slow progression of cervical dysplasia to cancer and because some of these dysplastic lesions may regress over time.

Age

The national cancer registry showed that the risk of developing cancer of the cervix increased with age, peaking at 136.4 per 100 000 in women between the ages of 65 and 69 in 1999 (2). Lomalisa (40) et al and Moodley (41) et al however showed that HIV positive patients presented with invasive cervical cancer 10 to 15 years earlier than patients that were HIV negative. Fakokunde found that among those women who received treatment, age over 35 years was associated with an increased risk (6.1% [4/66] vs. 2.1% [3/74]; p=0.4) of invasive disease (10). This is confirmed in our study where for every year increase in age, a woman was 9%

more likely to have cancer on histology. Age was however not a risk factor for up-grading of dysplasia, but the reason for this is unclear

Parity

For each child a woman had, the risk for invasion on histology was 32% higher. Parity had no effect on the risk of up-grading of cervical dysplasia.

HIV

HIV positive women had a smaller risk for invasion but an increased risk for up-grading of dysplasia compared to HIV negative women, with odds ratios of 0.37 and 1.63 respectively. Progression of LSIL was about 4-5 times higher in HIV positive than HIV negative women according to Nappi et al (11). This contradiction is difficult to explain. The decreased likelihood of invasive disease in HIV positive women may be due to these women dying from opportunistic infections before dysplasia can progress to cancer (40) or because they have more regular cervical cytology screening and are referred for colposcopy sooner. The total number of cases of invasion were however too small to determine the actual relationship. The increasing uptake of ART's in SA may change this association in time.

CD4

The CD4 count was not a risk factor for invasive disease or for up-grading of dysplasia. This is in contrast to other studies. Massad showed an OR of 1.1 for progression in HIV positive women with a CD4>200 cells/mm^3 and a viral load < 4000 copies/ml. Women with a CD4 <200 cells/mm^3 and a viral load >4000 copies/ml had an OR of 1.7 for progression over a maximum period of 5 years

(29). Six showed that over a period of one year, LSIL progressed to HSIL in 38.1% of HIV positive women with a CD4 count less than 500 cells/mm^3, but there was no progression if CD4 was >500 cells/mm^3 (31). Our finding is very difficult to explain; one would have to do a longitudinal cohort study looking at HPV infection, grade of dysplasia, viral load, serial CD4 and ART usage to confirm this finding and explain it.

Hormonal Contraception

Nur-Isterate® and Depo-Provera® had no significant effect on up or down-grading of cervical dysplasia nor the risk for invasive disease on histology. Multiple studies have been done to assess the effect of injectable progestogen contraceptives on cervical neoplasia, there seems to be some association but not very strong (42).

Our study found no association between COC and up-grading of dysplasia or invasive disease. Multiple studies have found an association between COC's and cervical SIL and malignancy, but this is possibly due to a difference in sexual behaviour. Women who do not use COCs usually use barrier methods of contraception, so they have an extra protection against HPV. COC users usually start having sexual intercourse at an earlier age, have more sexual partners, and rarely use barrier methods of contraception as compared to women using other contraceptives (43).

Condoms

Condom use was protective for invasion (although not statistically significant) and also a risk factor for up-grading of dysplasia. This is difficult to explain, but perhaps this is due to reverse causality, where HIV positive women are more likely to use condoms when they know their status.

ART usage

The use of ART's had no effect on the risk for invasive disease or the up-grading of dysplasia in our study. Studies have been contradictory so far: Minkoff found that women on HAART were 40% more likely to demonstrate regression and less likely to demonstrate progression (OR=0.68) at 6 monthly intervals (32). Lillo found no significant difference in progression between 74 women receiving HAART, 62 treated with one or two reverse transcriptase inhibitors only and 27 women not on treatment after adjusting for final CD4 count at 6-monthly intervals (33). Lillo et al found cervical disease progression to be independent of HIV treatment (33).

4.2. Strengths

Our study has improved a lot on the study by Fakokunde et al; our study population was much bigger (2960 women vs. 316 women). Fakokunde only studied patients with HSIL, whereas we studied patients with both HSIL and LSIL (10). We also investigated the effect of other variables such as parity and contraception on cervical dysplasia. Fakokunde briefly mentions that there was no difference in immune status between the two referral interval groups, but it seems

that he did not investigate the effect of HIV on the need for excisional treatment or the likelihood of invasive disease (10).

4.3. Limitations

- Cytology (a screening test) was compared with histology (gold standard). Therefore actual progression or regression of cervical dysplasia can not be proven, but merely suggested.
- HIV negative status was ascertained by self-report. HIV infection is a stigmatized condition so women may say they are negative when they are positive. Our results may thus be biased in the under-reporting of HIV infection. HIV testing has only been offered to all women attending the clinic since 2006, and some women declined testing.
- Most CD4 counts were known, but viral loads were not.
- We do not have information on all risk factors for progression of dysplasia such as smoking, sexual history and HPV-subtype. Duration and consistency of contraceptive use was not explored.
- HIV negative women with LSIL were only seen at the colposcopy clinic if there was persistent equivocal cytology.
- ART regimen and period of treatment were not known.
- The indication for cervical cytology screening was not known and not all were necessarily for routine screening.
- The retrospective nature of this study

4.4. Conclusion

Our study has shown that a delay between cervical cytology screening and colposcopy of less than 180 days compared to more than 180 days had no effect on up or down-grading of cervical dysplasia. It is also extremely important to note that invasive disease not detected on cervical cytology was present in 2.79% of patients.

HIV was shown to be a risk factor for up-grading of dysplasia. Contrary to other studies and our own expectation, CD4 count had no effect on up or down-grading of cervical cytology. Also unexpectedly, the use of condoms was found to be a risk factor for up-grading of dysplasia.

In addition, risk factors for invasive disease were identified, these are: age, parity and HSIL on cervical cytology.

Finally, the exact factor that determines whether dysplasia will regress or become invasive remains elusive. We found the risk factors for up-grading of dysplasia differed from those for invasion. Perhaps long-term studies involving large cohorts of women may explain this contradiction.

4.5. Recommendations for cervical cancer screening and colposcopy referral policies

Although there was no significant difference in up-grading of dysplasia between the two referral-interval groups, women should all be seen at a colposcopy clinic at least within 180 days and even sooner because of other factors such as anxiety, loss to follow up and the finding of invasion being missed by cervical cytology.

With the present restrictive infra-structure, older women, HIV positive women, those of higher parity, and especially those with HSIL on cervical cytology should be referred sooner. HIV positive women should be prioritised by grade of dysplasia but not by CD4 until its effect on up or down-grading of dysplasia has been clarified

The South African cervical cancer screening program does not differentiate between HIV positive and HIV negative women, although HIV infected women are offered cervical cytology screening at the time of their diagnosis (as part of their package of care). Management of minor cytological abnormalities in HIV infected women is different mainly because of the uncertainty regarding their prognosis. HIV positive women are more likely to have cervical dysplasia and up-grading of dysplasia. The finding in this study that HIV positive women are less likely to have invasive disease needs to be corroborated in longitudinal cohorts. Certainly in SA, with more women on ART's this association may change.

4.6. Suggested improvements for future studies

Only long term cohort studies in both HIV infected and uninfected women will inform better management in all aspects of the “Cervical Cancer Prevention program”.

Cervical pre-malignancy and malignancy in HIV infected women require special study because of the known association between HPV and HIV. Accuracy of HIV status, serial CD4 counts, Viral loads, HPV sub-typing and ART use will aid in assessing the risk of invasive disease in women who are HIV infected.

Investigating the number of patients lost to follow up and the associated factors will aid in deciding which measures should be put in place to attempt to minimise this problem.

Appendix A

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

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Franscois Saayman

CLEARANCE CERTIFICATE	**M10530**
PROJECT	Impact of Referral Intervals on Progression of Grades of Cervical Intra-Epithelial Neoplasia in Women Attending Coloscopy Clinic at CH Baragwanath Hospital between the times of Screening and Treatment
INVESTIGATORS	Dr Franscois Saayman.
DEPARTMENT	Department of Obstetrics & Gynaecology
DATE CONSIDERED	28/05/2010
DECISION OF THE COMMITTEE*	Approved unconditionally

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

DATE 31/05/2010 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr Y Adam

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...

References

1. WHO/ICO Information Centre on Human Papilloma Virus and Cervical Cancer. South Africa Human Papillomavirus and Related Cancers. 2010. <http://apps.who.int/hpvcentre/statistics/dynamic/ico/country_pdf/ZAF.pdf> [accessed 22nd September 2011].
2. Mqoqi N, Kellett P, Sitas F, Jula M. National cancer registry. Johannesburg; 2004. Incidence of histologically diagnosed cancer in South Africa, 1998-1999.
3. Adam Y, van Gelderen CJ, Guy de Bruyn G, McIntyre JA, Turton DA, Martinson NA. Predictors of persistent cytological abnormalities after treatment of cervical intraepithelial neoplasia in Soweto, South Africa: a cohort study in a HIV high prevalence population. BMC Cancer 2008; 8: 211.
4. Wilson JMG, Jungner G. Principles and practice of screening for disease. Geneva: WHO; 1968.
5. Lăără E, Day NE, Hakama M. Trends in mortality from cervical cancer in the Nordic countries: association with organised screening programmes. Lancet 1987 May 30; 1(8544): 1247-1249.
6. National guidelines for cervical cancer screening in South Africa (cited 29 Jan 2010) http://www.doh.gov.za/docs/facts-f.html. 2000.
7. WHO. Cervical Cancer Screening in developing countries. Report of a WHO consultation. France. http://www.who.int/cancer/media/en/cancer_-cervical_37321.pdf. 2002 (cited 20 February 2010).

8. DoH SA, editor. Cervical Cancer Screening: PHC Facilities. Precancerous and Cancerous Cervical smears, July 2009 - February 2010. City of Johannesburg: 2010.
9. Adam Y, van Gelderen CJ, Newell K. 'Look and Lletz' – a Chris Hani Baragwanath Hospital Experience. SAMJ 2005; 95(3): 630-633.
10. Fakokunde B, Selo-Ojeme D. Impact of prolonged referral interval on colposcopic outcomes in women with moderate or severe dysplasia. Int J Gynaecol Obstet 2008; 101: 245-247.
11. Nappi L, Carriero C, Bettocchia S, Herrerob J, Vimercatia A, Putignanoa G. Cervical squamous intraepithelial lesions of low-grade in HIV-infected women: recurrence, persistence, and progression, in treated and untreated women. Euro J Obstet Gyn RB 2005; 121: 226–232.
12. NHS Cancer Screening Programmes. Colposcopy and Programme Management: Guidelines for the NHS Cervical Screening Programme. Sheffield, UK: NHS Cancer Screening Programmes, 2004.
13. Kirby AJ, Spiegelhalter DJ, Day NE, Fenton L, Swanson K, Mann EM, et al. Conservative treatment of mild/moderate cervical dyskaryosis: long-term outcome. Lancet 1992; 339: 828-831.
14. Mougin C, Dalstein V, Prétet JL, Gay C, Schaal JP, Riethmuller D. Epidemiology of cervical papillomavirus infections. Recent knowledge. Presse Med 2001 Jun 9; 30(20): 1017-1023.
15. Cox JT. Epidemiology of cervical intraepithelial neoplasia: the role of human papillomavirus. Baillieres Clin Obstet Gynaecol 1995 Mar; 9(1): 1-37.

16. Arends MJ, Buckley CH, Wells M. Aetiology, pathogenesis, and pathology of cervical neoplasia. J Clin Pathol 1998; 51: 96-103.
17. Melnikow J, Nuovo J, Willan AR, Chan BK, Howell LP. Natural history of cervical squamous intraepithelial lesions: a meta-analysis. Obstet Gynecol 1998: 92(4 Pt 2): 727-35.
18. Ostor AG. Natural history of cervical intraepithelial neoplasia: a critical review. Int J Gynecol Pathol 1993; 12(2): 186–92.
19. Mcredie MRE, Sharples KJ, Paul C, Baranyai J, Medley G, Jones RW et al. Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. Lancet Oncol 2008; 9: 425-34.
20. Schlecht NF, Platt RW, Duarte-Franco E, Costa MC, Sobrinho JP, Prado JCM et al. Human Papillomavirus Infection and Time to Progression and Regression of Cervical Intraepithelial Neoplasia. J Natl Cancer Inst 2003; 95: 1336–1343.
21. DoH SA, editor. National HIV and syphilis antenatal sero-prevalence survey in South Africa, 2010.
22. Jamieson DJ, Duerr A, Burk R, Klein RS, Paramsothy P, Schuman P, et al. Characterization of genital human papillomavirus infection in women who have or who are at risk of having HIV infection. Am J Obstet Gynecol 2002; 186(1): 21–27.
23. Palefsky JM, Minkoff H, Kalish LA, Levine A, Sacks HS, Garcia P, et al. Cervicovaginal human papillomavirus infection in human immunodeficiency

virus-1 (HIV)-positive and high-risk HIV-negative women. Journal of the National Cancer Institute 1999; 91(3): 226–236.

24. Ahdieh L, Klein RS, Burk R, Cu-Uvin S, Schuman P, Duerr A, et al. Prevalence, incidence, and type-specific persistence of human papillomavirus in human immunodeficiency virus (HIV)-positive and HIV-negative women. J Infect Dis 2001; 184(6): 682–690.
25. Moodley JR, Hoffman M, Carrara H, Allan BR, Cooper DD, Rosenberg L, et al. HIV and pre-neoplastic and neoplastic lesions of the cervix in South Africa: a case-control study. BMC Cancer 2006; 6: 135.
26. Schuman P, Ohmit SE, Klein RS, Duerr A, Cu-Uvin S, Jamieson DJ, et al. Longitudinal study of cervical squamous intraepithelial lesions in human immunodeficiency virus (HIV)-seropositive and at-risk HIV-seronegative women. J Infect Dis 2003; 188(1): 128–136.
27. Duerr A, Kieke B, Warren D, Shah K, Burk R, Peipert JF, Schuman P, et al. Human papillomavirus-associated cervical cytological abnormalities among women with or at risk of infection with human immunodeficiency virus. Am J Obstet Gynecol 2001; 184(4): 584–590.
28. Faro S, editor. Proceedings From the First Asia-Oceania Research Organization on Genital Infections and Neoplasia (AOGIN) Meeting. Infect Dis Obstet Gynecol 2006; 2006: 59089.
29. Massad LS, Ahdieh L, Benning L, Minkoff H, Greenblatt RM, Watts H, et al. Evolution of cervical abnormalities among women with HIV-1: evidence from surveillance cytology in the women's interagency HIV study. J Acq Immun Def Synd 2001; 27(5): 432–442.

30. Ellerbrock TV, Chiasson MA, Bush TJ, Sun XW, Sawo D, Brudney K, et al. Incidence of cervical squamous intraepithelial lesions in HIV-infected women. JAMA 2000; 283(8): 1031–1037.
31. Six C, Heard I, Bergeron C, Orth G, Poveda JD, Zagury P, et al. Comparative prevalence, incidence and short-term prognosis of cervical squamous intraepithelial lesions amongst HIV-positive and HIV-negative women. AIDS 1998; 12(9): 1047–1056.
32. Minkoff H, Ahdieh L, Massad LS, Anastos K, Watts DH, Melnick S, et al. The effect of highly active antiretroviral therapy on cervical cytological changes associated with oncogenic HPV among HIV-infected women. AIDS 2001; 15(16): 2157–2164.
33. Lillo FB, Ferrari D, Veglia F, Origoni M, Grasso MA, Lodini S, et al. Human papillomavirus infection and associated cervical disease in human immunodeficiency virus-infected women: effect of highly active antiretroviral therapy. J Infect Dis 2001; 184(5): 547–551.
34. Centers for Disease Control. 1997 USPHS/IDSA Guidelines for the prevention of opportunistic infections in persons infected with human immunodeficiency virus. MMWR 1997; 46: 1-26.
35. Solomon D, Davey D, Kurman R, Moriarty A, O' Connor D, Prey M, et al. The 2001 Bethesda System: Terminology for Reporting Results of Cervical Cytology. JAMA 2002 Apr; 287(16): 2114-2119.
36. Daron G. Ferris. "Reid's Colposcopic Index". J Fam Practice. FindArticles.com. 28 Apr, 2012.

37. Firnhaber C. Right To Care, Helen Joseph Hospital and Clinical HIV Research Unit, Faculty of Health Science Center, Department of Medicine, University of Witwatersrand, Johannesburg, South Africa. Email to Yasmin Adam (yasminadam@gmail.com) 2012 April 10 [cited 2012 April 26].
38. Firnhaber C, Van Le H, Pettifor A, Schulze D, Michelow P, Sanne IM, et al. Association between cervical dysplasia and human papillomavirus in HIV seropositive women from Johannesburg South Africa. Cancer Causes Control 2010 Mar; 21(3): 433-443.
39. Agaba PA, Thacher TD, Ekwempu CC, Idoko JA. Cervical dysplasia in Nigerian women infected with HIV. Int J Gynecol Obstet 2009 Nov; 107(2): 99-102.
40. Lomalisa P, Smith T, Guidozzi F. Human immunodeficiency virus infection and invasive cervical cancer in South Africa. Gynecol Oncol 2000 Jun; 77(3):460-3.
41. Moodley M, Moodley J, Kleinschmidt. Int J Gynecol Cancer. Invasive cervical cancer and human immunodeficiency virus (HIV) infection: a South African perspective. Int J Gynecol Cancer. 2001 May-Jun; 11(3):194-7.
42. La Vecchia C. Depot-medroxyprogesterone acetate, other injectable contraceptives, and cervical neoplasia. Contraception 1994 Mar; 49(3): 223-230.
43. Deligeoroglou E, Michailidis E, Creatsas G. Oral contraceptives and reproductive system cancer. Ann N Y Acad Sci 2003 Nov; 997: 199-208.