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A RETROSPECTIVE REVIEW OF CERVICAL SMEARS IN HUMAN IMMUNODEFICIENCY VIRUS INFECTED POSTNATAL WOMEN AT JOHANNESBURG HOSPITAL

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

Johannesburg, 2010

DECLARATION

I, Amy Juliet Wise declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Obstetrics and Gynaecology in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

_____

Amy Wise

Signed on the 20th day of October, 2010

DEDICATION

This work is dedicated to Adrian, Jessica with whom it was started, Thomas with whom it was completed and Sarah.

ABSTRACT

Introduction

Against the high background rate of HIV among our antenatal clinic attendees, 30.3% in Gauteng in 2007, and the importance of cancer of the cervix as a health issue; this study was undertaken to determine the rate of abnormality found in cervical smears performed on HIV positive women attending the postnatal clinic at Johannesburg Hospital. The degree of abnormality and where possible its management, was reviewed. Secondly it was determined whether the immune status, namely the WHO clinical stage, CD4 cell count and viral load, correlated with the Pap smear results. Lastly patients were also analyzed according to the treatment received for HIV and their Pap smear results.

Patients and Methods

The study is a retrospective record review. All the patients who attended the postnatal clinic (PNC) between October 2005 and the end of July 2006, who had a Pap smear, were included. Follow-up test results were collected to the end of June 2007. A total of 324 patients attended the clinic in the study time period, of which 248 (76.5%) had a Pap smear done and 76 (23.5%) did not.

Results

The main results of interest were as follows – 131 patients (52.8%) had normal Pap smears, 64(25.8%) had LGSIL, 32 (12.9%) had HGSIL, 10 (4.0%) had ASCUS and 11 (4.4%) had Pap smears that could not be classified. In total 47.2% of the Pap smears were abnormal. There was one case of malignancy developing after an abnormal Pap smear. Patients with abnormal Pap smears tended to have a lower mean CD4 cell count while the viral load and WHO Stage did not appear to have an impact on the final analysis of the Pap smears.

Conclusion

The rates of cervical abnormality in HIV sero-positive patients attending the Johannesburg Hospital postnatal clinic are much higher (47.2%) than would be expected in the general population (10%), with a significant portion requiring follow-up investigation and management. It is however preferable to deal with cervical cytological abnormalities comprehensively during the screening phase rather than trying to manage a potential increase in cervical cancer cases.

ACKNOWLEDGEMENTS

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1.0 INTRODUCTION

1.1 HIV and the antenatal population

The National HIV and Syphilis Prevalence Survey 2007 by the Department of Health, using antenatal clinic (ANC) attendees', estimated that 28.0% (95% CI of 26.9-29.1%) of pregnant women were Human Immunodeficiency Virus (HIV) sero-positive in 2007. This is an apparent decrease from the 30.2% and 29.1% in 2005 and 2006 respectively, although the sampling method is questioned. Gauteng had the third highest prevalence in 2005, - being 32.4% (95% CI of 30.6 – 34.3) while in 2006 and 2007 it was fourth highest with a prevalence of 30.8% (95% CI of 29.6-32.1%) and 30.3% (95% CI of 29.9%-32.8%) respectively [1, 2, 3].

1.2 Cervical cancer

The HIV epidemic co-exists with another major health concern, namely cancer of the cervix. The National Cancer Registry in South Africa reported in1997 that the risk of developing cancer of the cervix is one in 29 (age 0-74years). Black females showed the highest age standardised rate – 38.5 per 100 000, with a lifetime risk of one in 23 [4]. Newer data was expected out at the end of 2008 in which the cancer incidence for 2001/2 would be reported, this would however not take into account the results from the private health sector that stopped reporting at the beginning of 2001. As of February 2009 the data was not yet available [5]. The data from the Medical Research Council of South Africa showed that 3498 women died of cervical cancer in 2000, this represented 17.2% of cancer related deaths amongst women [6].

1.3 Cervical smears

Human Papilloma Virus (HPV) is the major aetiological factor for cancer of the cervix. HPV, potentiated by other co-factors, including smoking, induces changes in the cervical epithelium, which are detectable on Papanicolaou (Pap) or cervical smear. [7] The changes are classified according to the 2001 Bethesda System, importantly: atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intra-epithelial lesion (LGSIL) and high-grade squamous intraepithelial lesions (HGSIL) and squamous cell carcinoma (SCC). The presence of endocervical cells is not necessary in order to classify a Pap smear as adequate [8]. In 1993 the Centre for Disease Control (CDC) added invasive cancer of the cervix as an AIDS defining illness, highlighting the importance of screening programs in this high risk group [9]. The CDC has recently suggested that a Pap smear should be done routinely at the time of HIV diagnosis and should be repeated six months later. If these Pap smears are normal women should then have yearly cervical screening. Currently in South Africa the suggested screening program is three Pap smears in a lifetime starting at age 30, repeated at 10- year intervals, with no adjustment for HIV status. This recommendation has not been fully implemented and screening tends to be opportunistic [4].

1.4 Literature review

HIV sero-positive patients have higher rates of squamous intraepithelial lesions (SIL) than HIV negative patients [10]. The reported incidence of cervical carcinoma among HIV infected women is inconsistent; with some studies showing it is increased [11] and others not demonstrating this increase [12].

This may be due to the fact that patients with SIL, who are not receiving antiretroviral therapy (ART), partly because they are in resource poor areas, demise from HIV related disease before invasive cervical cancer develops [13]. The prevalence though of cervical carcinoma has been shown to be nearly twice that of HIV negative patients [10,13], and that HIV sero-positive patients present nearly 10 years earlier than HIV negative patients. Furthermore if their CD4 cell count is less than 200cells/μL at presentation they are significantly more likely to have advanced stage of cervical cancer [14]. Importantly, HIV sero-positive patients have an increased infection rate with monogenic HPV [13]. In a large prospective study conducted in Senegal, women with high risk HPV were more likely to have HGSIL or invasive carcinoma if they were HIV sero-positive compared to HIV sero- negative controls [15].

No studies were found which reported Pap smear findings on postpartum HIV sero-positive women. Table 1.1 summarizes 13 studies in which Pap smear results are available for HIV sero-positive women. For these studies the sample size ranged from 49 – 1200 women with the mean or median age ranging from 28.1y to 36y, and median CD4 cell count from 160 – 430cells/μL. Use of ART varied from all to none of the women among the nine studies that commented on it. The percentage of normal Pap smears was 6.7- 93.7% (13 studies) with a mean of 59.0%. Two studies combined their abnormal findings- with Anderson [16] finding a SIL rate of 26.1% and Joshi [17] 6.3%. The other 11 had a LGSIL range of 9.7- 43% and a HGSIL range of 2.8- 32.7%. In Zambia a squamous cell carcinoma (SCC) rate of 20 % was found by Parham [18]. Five studies did not demonstrate ASCUS, while the range in the remaining eight was 0.4- 20.7%.

Two studies had results which were out of keeping with the other ten studies. A Zambian study of women attending a well HIV care clinic demonstrated higher rates of HGSIL (32.6%) and possible SCC (20%). The median CD4 count of 165cells/μL was surprisingly low and may partly account for the high percentage of severely abnormal Pap smears. In addition Zambia has the second highest prevalence rate of invasive cervical cancer (61.1/100 000 women) in the world [18]. In comparison a study from India, where 20% of the world's new cervical cancer cases come from, only found a combined low grade and high grade SIL rate of 6.3% with 93.7% being normal [17]. The median CD4 cell count was not specified and one in 10 women was on ART compared with 76.3% of the Zambian cohort. No explanation was proffered for the low rate. With such variation between studies and regions, the importance of having information specific to one's own population is highlighted.

Table 1.1: Comparative results of Pap smears in HIV sero-positive women and selected variables in various studies

	Sample Size	Normal %	LGSIL %	HGSIL %	ASCUS %	Mean age *median years	Median CD4 cells/µL	ART Use %
Anderson USA 2006[16]	1200 (189)	53.3 (§)	SIL Combined 26.1 (§)		20.7 (§)	34.1	430	Unknown
Branca Italy 2003[19]	89	67.4	12.4	6.7	1.1	33.46	297	100
Chirenje Zimbabwe 2002[20]	207	74.3	9.7	3.4	12.6	28.1	Not done	None
Delmas Europe 2000[21]	467	75.8	21.0	2.8	0.4	31*	378	Unknown
Denny S. Africa 2008[22]	400	45	35	13	7	29.3	248	30
Heard France 1998[23]	49	31	43	26	-	33.9*	160	100
Heard France 2000[24]	307	48.2	13.7	13.3	-	32*	300	35.5
Joshi India 2005[17]	287	93.7	SIL Combined 6.3		-	29.1	Not specified	10.1
Levi Brazil 2002[25]	255	81	12	7	-	32.4	285	80
Lillo Italy 2001[26]	163	73.4	20.2	6.2	-	33.6	Not specified	83.5
Maiman USA 1998[27]	253	67.1	15.4	7.9	9.1	33.8	Not specified	Unknown
J.Moodley S. Africa 2006[10]	78	50	17.9	11.5	20.5	Unknown	Not done	Unknown
Parham Zambia 2006[18]	150	6.7	23.3	32.7 ?SCC20	17.3	36*	165	76.3

§- results are for the original 1200 HIV sero-positive patients, of which the 189 are a subgroup.

Patients attending the postnatal clinic were also classified clinically according to the World Health Organization staging system. Although the WHO guidelines were updated in November 2007[28], the older WHO staging criteria have been used in this study as they were applicable at the time patients were assessed. Cervical cancer in an HIV sero-positive patient stages the patient as stage 4 disease [9].

In studies which have looked at immune suppression markers and their significance in the context of cervical pathology and HIV status, it was found that, in general the lower the CD4 cell count the more likely the patient was to have an abnormality[22,29], and that the lesion was less likely to regress [30]. Patients with higher HIV viral loads were more likely to have oncogenic HPV [15,19,22,26, 31, 32], an association with the degree of cervical abnormality [15,22] and Pap smear abnormalities that were less likely to regress [30].

Smoking has been described as a risk factor in the development of genital warts and squamous intraepithelial lesions of the cervix which may progress to cancer of the cervix [33, 34]. The prevalence and incidence of HPV is also increased among HIV sero-positive women [35].

The advent of ART has precipitated studies looking at the effect of ART use on cervical dysplasia. Lillo [26] found that despite improved immunity in HIV sero-positive patients on ART and a rising CD4 cell count, the lesions and the persistence of oncogenic HPV were similar to those found in both HIV negative patients, and HIV sero-positive patients who were not treated with ART. However data from the Women's Interagency HIV Study found that the use of highly active anti-retroviral therapy (HAART) was associated with regression and that the likelihood was increased with higher CD4 cell counts [36].

This is supported by Del Mistro's work which emphasized the effectiveness of HAART over other ART regimen's in obtaining regression, noting that for HGSIL lesions this is in conjunction with surgical excision [37].

The management of HIV sero-positive patients with cervical pathology is not yet well defined in South Africa. The CDC suggests that if a Pap smear shows ASCUS, HPV typing should be done. If the HPV typing demonstrates an HPV with known oncogenic potential, then colposcopy should be performed. If no oncogenic HPV is demonstrated, then repeat Pap smears four to six monthly are recommended until there are three negative results. A repeat result of ASCUS needs colposcopy. LGSIL could be dealt with similarly, or immediately proceed to colposcopy. HGSIL requires colposcopy and invasive carcinoma treatment according to FIGO staging [11]. It should be noted that koilocytosis without SIL on a Pap smear has on colposcopy demonstrated SIL in 49.3% of HIV sero-positive patients compared to 28% in HIV negative patients. According to Moodley, this may argue against a conservative approach in sero-positive patients with this result [38]. Recently, a paper looking at the natural history of HIV sero-positive women and their Pap smear results, it was suggested that due to the low incidence of progression of LGSIL and ASCUS to HGSIL that after an initial colposcopy or normal smear follow-up can be biennial or triennial [22].

1.5 Objectives

By way of a retrospective review, the Pap smear results in the population of women attending the postnatal clinic were analyzed. The objectives were to firstly determine the number of Pap smears done, the percentage of abnormal results and the degree of abnormality as defined in the 2001 Bethesda classification [8]. Where possible the management of the abnormality was reviewed including the colposcopy results. The second was to determine whether the immune status, namely the WHO clinical stage, CD4 cell count and viral load, correlated with the patients' Pap smear results. Lastly patients were also analyzed according to the treatment received for HIV and their Pap smear results.

2.0 METHODS

2.1 Study design

The study is a retrospective record review. Records of all the patients who attended the postnatal clinic (PNC) between October 2005 and the end of July 2006, who had a Pap smear performed, were included. Follow-up test results, namely serology, cytology and histology, for this group were collected to the end of June 2007. The research protocol was submitted and approved by the ethics committee, number: M060842.

2.2 Study setting

The PNC was established at the former Johannesburg Hospital (now the Charlotte Maxeke Johannesburg Academic Hospital) in October 2005 to follow up HIV infected women who were either initiated on HAART during pregnancy, who had not been staged clinically according to the World Health Organization classification during pregnancy, or who had low CD4 counts (<200cells/μL) but did not have the opportunity to access HAART during pregnancy.

Patients attending the postnatal clinic were also classified clinically according to the World Health Organization staging system. Stage 1 patients are asymptomatic or had persistent generalized lymphadenopathy, and/or normal activity. Stage 2 is <10% weight loss, minor mucocutaneous conditions, herpes zoster in the last 5 years, recurrent upper respiratory infections, and/or symptomatic with normal activity. Patients in stage 3 have >10% weight

loss, unexplained diarrhea for more than 1 month, unexplained fever for more than 1 month, pulmonary TB in the last year, oral hairy leukoplakia, thrush, severe bacterial infection, and/or bedridden for less than half of the last month. Stage 4 is CDC-defined AIDS and/or bedridden for more than half of the last month [9]. Although the WHO guidelines were updated in November 2007 (Table 2.1), the older WHO staging criteria have been used in this study as they were applicable at the time patients were assessed [28].

Postnatal women who had recently tested positive and had not yet had a CD4 cell count done or needed further management of an HIV related problem were referred to the clinic in the month following delivery. Most of the latter group had received single dose nevirapine during labour as per the previous national Prevention of Mother-to-Child Transmission (PMTCT) program. The aim of the PNC is to ensure comprehensive management of HIV-infected women. Initially once women were more than six weeks post-partum they were required to pay a fee as they no longer qualified for free maternal services, this was later changed as the PNC qualified as an antiretroviral clinic to encourage adherence to the program. Part of management included screening for cervical lesions by Pap smear at or after 6 weeks post delivery. Once the patients have received their Pap smear results and their infants HIV status has been determined patients are referred to an appropriate site for ongoing management. If they are on antiretroviral therapy they are referred to the closest adult HIV clinic for follow-up and if they did not qualify for antiretroviral therapy they are referred to a primary health care clinic for ongoing monitoring.

2.3 Literature review

The literature search was conducted via Pubmed using the key words HIV, women, Papanicolaou/ Pap/ cervical smear/screening and postnatal.
Relevant references were accessed if available via the University of the Witwatersrand eJournal portal. Appropriate articles cited by other authors were also reviewed. Appropriate websites were also used and referenced.

2.4 Data collection

Data was obtained from patients' files at the clinic as well as the laboratory which is run by the National Health Laboratory Services (NHLS). Information collected included age, parity, gravidity, date of delivery, whether they smoked, WHO Clinical Staging of HIV, CD4 cell counts, viral loads, treatment regimen and date of initiation, and Pap smear results. Where colposcopy had been performed, colposcopy biopsy results and histology from large loop excision of the transformation zone (LLETZ) and hysterectomy the results were collected. (Appendix 7.3 Data sheet) As data was retrieved from file review, certain information which is known to be associated with cervical abnormalities such as coitarche, number of partners and previous STI's was not available.

2.5 Data analysis

Data was entered into Excel 2007. Variables were then coded to assist with analysis. The age category was divided into the following groups ≤19 years; 20-24; 25-29; 30-34; 35-39; >40

and unknown. The parity and gravidity categories were assigned codes corresponding to the actual number except for those that were unknown. Multiple CD4 cell count results were available for a number of patients, the most relevant being the initial count, the lowest count before the Pap smear and the count just preceding the Pap smear. In certain cases these were one in the same. The CD4 cell count category was divided as follows 0-50cells/μL; 51-200cells/μL; 201-350cells/μL; 351-500cells/μL and ≥500cells/μL. Viral loads were not tested when a patient's CD4 cell count was >200cells/μL and they therefore did not qualify for HAART. The viral load category was divided as follows: undetectable-40copies/ml; 41-400copies/ml; 401- 1 000copies/ml; 1 001-10 000copies/ml; 10 001-100 000copies/ml; ≥100 000copies/ml and not done. To work out a mean, all values recorded as <40 or undetectable were made '40' and those that were recorded as <400 were made '400'. The values under 40 and 400copies/ml are a function of differing laboratory tests and so are combined and analyzed as one group at times.

The South African National Antiretroviral Treatment Guidelines [39] suggest that pregnant women be started on stavudine (D4T), lamivudine (3TC) and nevirapine (NVP) - regimen 1b hence the majority of the patients in the study were on this regimen. Smaller numbers were on D4T, 3TC and efavirenz (EFV) – regimen 1a or D4T, 3TC and lopinavir/ritonavir (kaletra®) – mixed regimen. There were a few patients who were referred in from other healthcare providers or who were unable to take the standard regimens and were on individualized regimens.

For statistical purposes those that were on a combination of two nucleoside reverse transcriptase inhibitors (NRTI) and either a non-nucleoside reverse transcriptase inhibitor

(NNRTI) or a protease inhibitor (PI) were considered to be on HAART. These patients were then analyzed together with those on the standard regimens. Some women were not on HAART as either they did not yet qualify or they had yet to be initiated at the time of performing the initial Pap smear.

The World Health Organization clinical staging of HIV was recorded.

The smoking habits of the women were reviewed and were classified as unknown, no smoking, previously and currently smoking. For statistical purposes they were grouped into two groups for certain tests, namely 'never smoked' and 'ever smoked'.

The results of the Pap smears were collected from the files and the reports checked on the laboratory computer system as well as checking if any subsequent tests had been done and recording those results. Of importance for this study was the classification on the Pap smear report of no malignant cells, LGSIL, HGSIL, ASCUS and ASC-H (atypical squamous cells cannot exclude HGSIL). The presence or absence of endocervical cells and koilocytosis was also noted. Results that were classified as 'other' were excluded from analysis, depending on the statistical test used. The use of the term 'other' to describe one of the categories is for the purposes of this study only and does not reflect its use in the Bethesda classification.

Where LGSIL was reported as dual pathology with ASCUS or ASC-H the result was considered as LGSIL for analysis. The justification being that at colposcopy, which is recommended for LGSIL and not always immediately for ASCUS and ASC-H in HIV positive women, [9] ASCUS would be more likely to be confirmed as LGSIL rather than HGSIL, and

although the majority of ASCUS would be normal the management would reflect the potential abnormality [40].

The frequencies, means, medians and ranges for the data were determined. Specifically, the first objective to determine the number of Pap smears done, the percentage of abnormal results, the degree of abnormality as defined in the 2001 Bethesda classification and to review the management of the abnormality including the colposcopy results as well as the second objective to determine whether the immune status, namely the WHO clinical stage, CD4 cell count and viral load, correlated with the patients' Pap smear results, were addressed using contingency table summaries and Pearson chi-squared tests of independence. Concerning objective two a one-factor analysis-of-variance (ANOVA) test and non-parametric Kruskal-Wallis rank sum test using Box-Cox transformations were carried out. The Box-Cox transformations aim to create variables that are more normally distributed in order to meet the assumptions required for ANOVA, the transformations have no effect on the Kruskal –Wallis test. Objective three, to analyze patients according to the treatment received for HIV and their Pap smear results was investigated quantitatively using the chi-squared test. A p-value of 0.05 was considered significant. Most of the statistical analyses were performed using the software package R (2006) and the remainder using Microsoft Office Excel 97-2003 Worksheet. Missing variables are excluded to yield a complete data set, aid analyses and help prevent bias.

Regression was defined as an abnormal Pap smear result which on follow up either had downgraded or reverted to normal. A result was considered persistent if on follow up it had not changed. Progression was defined as the follow up result having upgraded to a more serious grade of pathology than the initial Pap smear.

3.0 RESULTS

3.1 Sample population

A total of 324 patients attended the clinic in the study time period, of these 248 (76.5%) had a Pap smear and 76 (23.5%) did not. The mean age of those who did have a Pap smear was 28.3years (data missing for one patient) with the mean parity and gravidity being 2.3 and 2.5 respectively (data missing for 18 and 26 patients respectively). Twenty-six of the 76 (34%) were on HAART, the mean initial CD4 cell count being 264.1 and initial viral load 427763 copies/ml (no result for five and 31 patients respectively). The WHO clinical stage was documented for 32 patients; in 44 patients (57.9%) the stage was unknown, 12 patients (15.8%) it was Stage 1, 4(5.3%) Stage 2, 14(1.8%) Stage 3 and 2(2.6%) Stage 4. The smoking habits of 27(35.5%) were not recorded while 46(60.5%) had never smoked and 3(3.9%) had smoked. Among the 76 women who did not have a Pap smear; one (1.3%) refused a Pap smear despite counseling as to its benefit, six (7.9%) no reason was specified, 22 women (28.9%) were referred to their local clinic for ongoing management prior to Pap smear and 47 women (61.8%) defaulted.

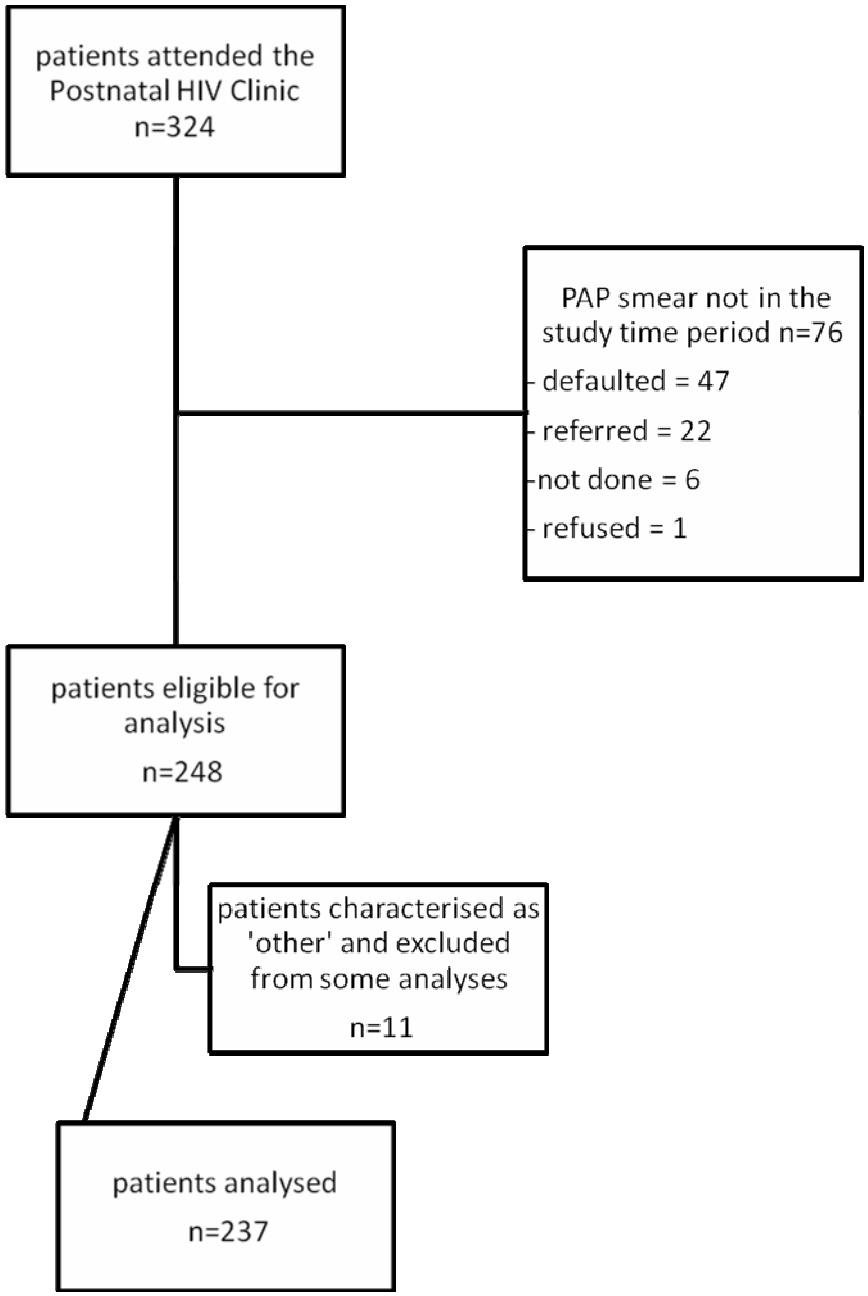


Figure 3.1: Flow diagram of patient group

Of the 248 patients who had a Pap smear 11(4.4%) were classified as ‘other’. This group comprised of one patient who had previously been referred to the outpatient gynaecology clinic and had a colposcopic biopsy which confirmed HGSIL, the original Pap result was not traceable, subsequent to the biopsy the Pap smear showed LGSIL. One patient needed a repeat Pap as the first had moderate squamous cell atypia favoring reparative and inflammatory changes, the repeat showed LGSIL. The remaining nine patients had insufficient ectocervical cells and were thus not fully representative and needed a repeat sample. Repeat Pap smears were found for five.

3.2. Cervical smear distribution

There were 131(52.8%) normal Pap smears, 64(25.8%) LGSIL, 32 (12.9%) HGSIL, 10 (4.0%) with ASCUS and 11 (4.4%) ‘other’. (Figure 3.2) Thus nearly half the Pap smears had some degree of abnormality – 47.2%. Five patients had Pap smear results which had dual diagnoses, three were reported as LGSIL and ASCUS and two as LGSIL and ASC-H. These were analyzed as part of the LGSIL group. One case of HGSIL was noted to be suspicious for microinvasion. This patient demised, the cause of death is unknown. One of the cases in which a patient had a Pap smear with a dual diagnoses – LGSIL and ASC-H – did not return for the result however did present to Urology the following month where a biopsy of the vagina showed a squamous cell carcinoma of female genital tract origin.

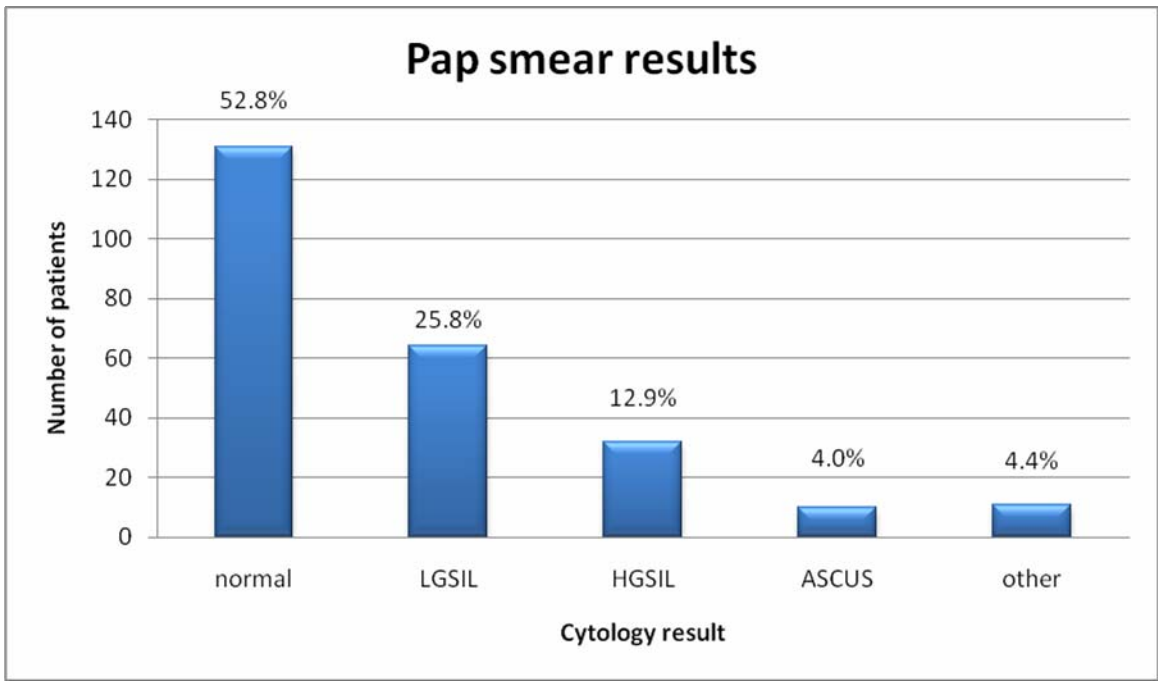


Figure 3.2: Histogram of Pap smear result distribution

Endocervical cells were not found in 49 of the 248 (19.8%) Pap smears. Of these 49 cases, 40 had no malignant cells and were thus analysed as part of the 'normal' group and nine had LGSIL. The recommendation from the laboratory for the timing of a repeat Pap smear was immediate for the normal smears without endocervical cells and either six weeks (one case) six months, 12 months or 6-12 months for those with LGSIL.

Koilocytosis was noted in 88 of the 248 (35.5%) Pap smears. No normal smears showed koilocytosis, 57/64 (89.1%) of the LGSIL, 28/32 (87.5%) of the HGSIL and 3/10 (30%) of the ASCUS smears.

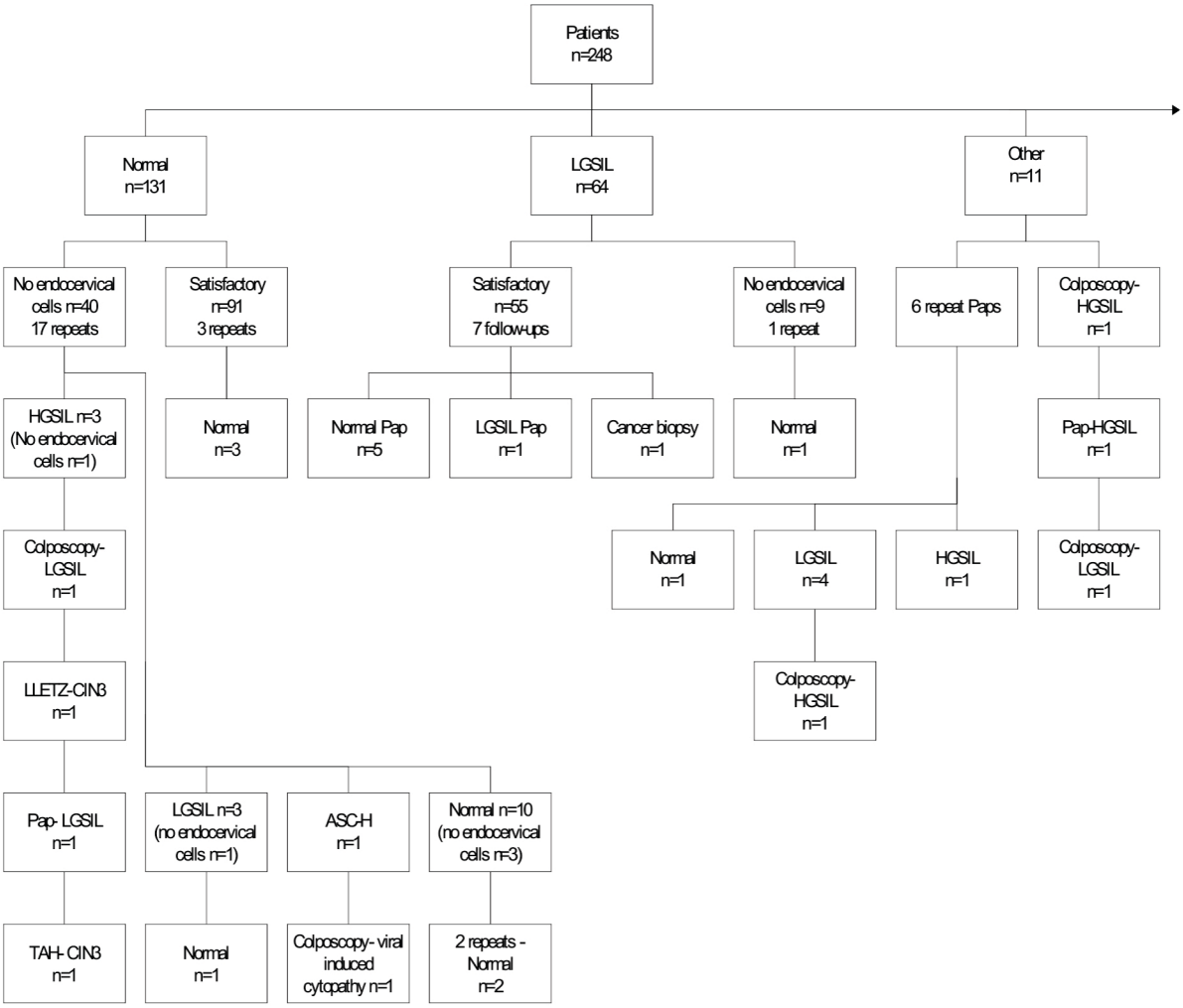


Figure 3.3: Flow diagram of Pap smear management

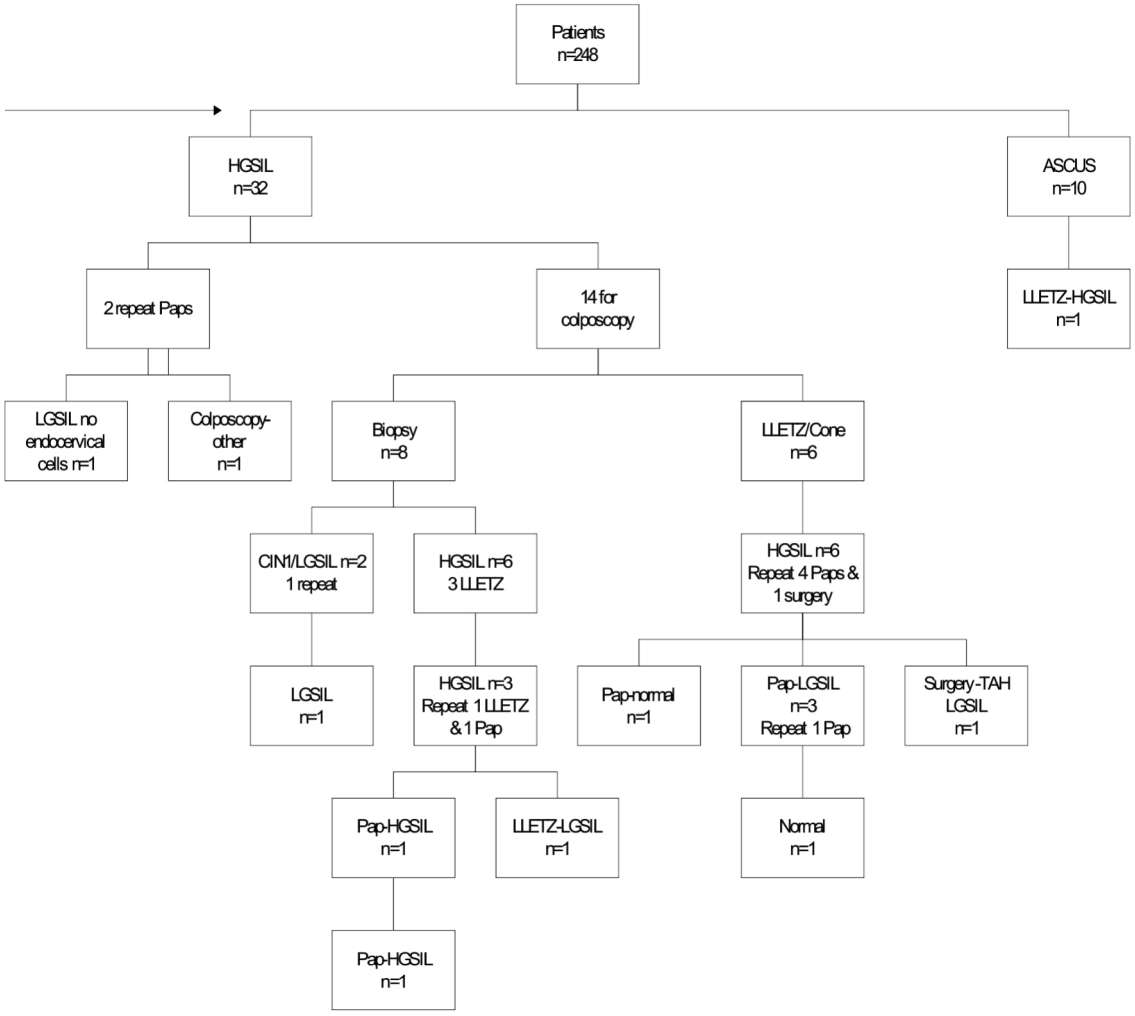


Figure 3.3: Flow diagram of Pap smear management continued

3.3Cervical smear follow-up results

3.3.1 Normal

Repeat Pap-smears were performed in 35 women. Of these 17 were on women who had normal Pap smear results with inadequate endocervical component, 10 were reported as normal, three showed LGSIL, three had HGSIL, and one ASC-H. Colcoscopic biopsies were performed for ASC-H which demonstrated viral induced cytopathic effect, and a HGSIL which was reported as inadequate but showed LGSIL and so went on to have a LLETZ which confirmed cervical intraepithelial neoplasia (CIN) 3 which was later confirmed following hysterectomy. The 91 normal and satisfactory smears had three repeats, all of which were normal again.

3.3.2 LGSIL

Among 64 smears showing LGSIL, nine had no endocervical cells, only one repeat was found and was normal. The 55 satisfactory smears had seven follow ups of which five were normal and one showed LGSIL on Pap smears while one biopsy confirmed female genital tract cancer.

3.3.3 HGSIL

The patients with HGSIL all had satisfactory smears. Two patients had repeat smears, one showed atypical cells of uncertain origin and was characterized as other – colposcopy was advised but no result was found. The other Pap smear showed LGSIL with no endocervical cells. Fourteen patients referred for colposcopy had results available; eight had a colposcopic directed biopsy and six a LLETZ/cone biopsy.

Of the biopsies, two showed LGSIL which was reconfirmed in one patient. The other six biopsies had HGSIL; three had a LLETZ done, which reconfirmed HGSIL but they were inadequate due to the lack of involvement of the endocervical margin. One of these had a repeat LLETZ which showed LGSIL, another had two follow up Pap smears which both showed HGSIL.

The six LLETZ/cone biopsies all confirmed HGSIL. Four follow up Pap smears were performed, three had LGSIL (yet another repeat of one of these yielded a normal smear) and one was normal. Of the six, one had a total abdominal hysterectomy and the histology showed LGSIL.

3.3.4 ASCUS

The patients with ASCUS also included those with ASC-H. One patient with ASC-H had a LLETZ which had HGSIL focally at the endocervical margin. No other follow up results were found.

3.3.5 Other

The 11 Pap smears designated as ‘other’ had one colposcopic biopsy with HGSIL. A follow up Pap confirmed the HGSIL, but the repeat colposcopy was LGSIL. Six repeat Paps were found, four with LGSIL of which one went on to have colposcopy showing a HGSIL. The other two Paps showed one HGSIL and one normal smear.

3.3.6 Summary of follow-up results

Thus among the 131 originally normal Pap smears, there were 20 patients who went on to have a repeat smear, of which six (30%) were abnormal. At the time of the final Pap smear, biopsy, LLETZ or surgery, 11 of the 20 were on ART. Four of the six patients with abnormal smears were on ART. Of the 64 patients with LGSIL, seven had a repeat smear – six regressed to normal and one showed persistent LGSIL. One smear which had a dual diagnosis, including ASC-H, had a biopsy which showed cancer of female genital tract origin. Of the eight follow-ups six were on ART – five that regressed and one that stayed the same. Sixteen of the 32 patients with HGSIL had follow up investigations and treatment. Ultimately six regressed to LGSIL, three to normal, six had persistent HGSIL and one joined the 'other' group and needed colposcopy. Thirteen of the 16 patients with HGSIL were on ART; seven regressed and six remained as HGSIL. Only one patient in the ASCUS group was followed up and was found to have HGSIL. This patient was not on ART. The group 'other' had seven follow ups out of 11 and finally showed two HGSIL, four LGSIL and one normal result. From the group of seven all but one (who had LGSIL) was on ART. A total of 52 patients had follow-up investigations and/or treatment, which is 21.0% of the sample. Thirty-six were on ART at the time of the final intervention – three for less than 12 weeks and 33 for longer. Thirty (83.3%) patients had full virological suppression at the time of their last intervention. (Table 3.1)

3.3.7 Regression, persistence and progression

A total of 52 patients had follow-up investigations and/or treatment, which is 21.0% of the sample. Thirty-six were on ART at the time of the final intervention – three for less than 12 weeks and 33 for longer. Thirty (83.3%) patients had full virological suppression at the time of their last intervention. The seven from the group 'other' who did not have an initial Pap smear

with which one could make comparisons were excluded as well as those with a normal Pap smear which remained normal. Within the normal Pap smears 14 remained normal and six progressed; in the LGSIL group six regressed, one persisted and one progressed to cancer. In the HGSIL group nine regressed (seven following surgical treatment) and seven persisted. In the ASCUS group an ASC-H was confirmed as HGSIL. This gives a rate of 51.6% for regression, 22.6% for persistence and 25.8% for progression for the group of 31. The 23 patients in the group of 31 who were on ART had a rate of 52.2% for regression, 30.4% for persistence and 17.4% for progression. The eight patients of the 31 not on ART had a rate of 50.0% for regression, none persisted and 50.0% for progression.

Twenty-two of the 31 patients whose follow-up results were looked at were on ART or for more than 12 weeks; 50.0% regressed, 31.8% persisted and 18.2% progressed. Nine of the 31 were not on ART or had been on for less than 12 weeks; 55.6% regressed, none persisted and 44.4% progressed.

3.4 Cervical smear analyses

3.4.1 Age

The age range was 16 to 43 years old, with a mean of 29.47 years and median of 30 years. Data was missing for one patient. (Figure 3.4 and Figure 3.5)

When age is cross-tabulated (Table 3.2.1) with the Pap smear results using the defined categories in a contingency table certain cells have a value less than five and so to avoid

problems with chi-squared approximation certain age categories (≤19 with 20-24 and 35-39 with ≥40) were combined and the group 'other' was excluded.

The *p*-value for the (marginal) test of independence between the age categories and Pap smear categories was not significant – 0.1526. A one-factor analysis-of-variance test for equality of mean ages across the Pap categories shown in Table 3.2.1 yields a *p*-value of 0.1278 (for the null hypothesis of equal mean age versus the alternative hypothesis that at least one mean age differs). Thus as the null hypothesis is not rejected the variation in mean ages is most likely due to chance alone. Similarly, a non-parametric Kruskal-Wallis test for equality of median ages across Pap scores yields a p-value of 0.0917 (for the null hypothesis of equal median age versus the alternative hypothesis that at least one median age differs).

If one compares the age group 20-29 versus the 30-39 year old group, a statistically significant difference is found (*p*-value is 0.009). The main differences are more normal and 'other' Pap smears among the older cohort while the ASCUS group was bigger in the younger cohort. The results for the LGSIL and HGSIL groups were similar. However if the 'other' group is not included in the analysis the *p*-value is 0.066 and thus no longer significant. (Table 3.2.2)

3.4.2 Parity

For the 215 women with data, the parity had a range of one to seven children, the mean being 2.34 and median of two. There were 33 missing values. (Figure 3.6 and Figure 3.7)

The contingency table (Table 3.3) for the Pap smear categories with the patients' parity yields a *p*-value for the (marginal) test of independence between the two of 0.6065. Again to avoid

problems with chi-squared approximation, categories were combined – parity of one with two and four with five and six. The missing values and 'other' Pap smears were excluded from analysis.

3.4.3 Gravidity

For the 211 women with data, the gravidity had a range of one to seven, a mean of 2.56 and a median of two. There were 37 missing values. (Figure 3.8 and Figure 3.9)

The contingency table (Table 3.4) for the Pap smear categories with the patients' gravidity yields a *p*-value of 0.7936. To avoid problems with chi-squared approximation the extremes of gravidity were combined – one with two and four with five and six. The missing values and Pap smears classed as 'other' were excluded.

3.4.4 WHO classification

The raw data for the WHO classification was largely incomplete with 45.2% missing. Stage1 accounted for 30.6% of the 248 patients, Stage 2 for 8.9%, Stage 3 for 9.3% and Stage 4 for 6.0% of all the patients. Of the 15 patients who made up Stage 4, who by virtue of their stage qualify for ART according to SA Guidelines [39], 13 of them had initiated therapy whilst two still had not at the time of their Pap smear. Subsequently one of these patients was initiated on ART. The initial CD4 cell count in the patient who was not started was 246cells/μL. (Figure 3.10 and Figure 3.11)

The WHO classification was compared to the Pap smears using a contingency table (Table 3.5). Excluding the unknown values and 'other' Pap smears a *p*-value for the (marginal) test of independence is 0.6679.

3.4.5 Smoking

Smoking was unusual, with only five patients (2.4%) who smoked or had ever smoked and 201 of the remaining 206 (97.6%) were non-smokers. Data was missing for 42 patients. Four of the five smokers had abnormal Pap smears.

In this study, abnormal Pap smears are not significantly associated with smoking. A contingency table (Table 3.6.1) to cross-tabulate the Pap smear results against that of smoking and ignoring the missing values and 'other' Pap smears results supports that smoking is not significant $p = 0.1875$. A modified version (Table 3.6.2) in which the abnormal Pap smears (LGSIL, HGSIL and ASCUS) are grouped together creates a 2 by 2 table and yields a *p*-value of 0.1192 using a chi-squared test which is not statistically significant.

3.4.6 CD4 cell count

Only one patient did not have an initial CD4 cell count. The range was 3- 1193 cells/μL, with a mean of 257.2cells/μL and a median of 190cells/μL. (Figure 3.12) When looking at the last CD4 cell count prior to the Pap smear the range is 4-1193cells/μL, with a mean of 305.6cells/μL and a median of 267cells/μL. (Figure 3.13 and Figure 3.14)

The test for equality of mean response of the initial CD4 cell count and the Pap smear results using an ANOVA (a one-factor analysis-of-variance) test was carried out.

Box-Cox transformed versions of the CD4 cell count were used to create more normally distributed variables in order to meet the assumptions required for ANOVA. Tests for homogeneity of the variances across the Pap smear results after applying the Box-Cox transformations all failed to reject the hypothesis of equal variances which is an assumption required for the one-factor ANOVA test. The *p*-value was significant at 0.0423. A Kruskal-Wallis rank sum test for the equality of median response of the CD4 cell count across the Pap smear results was done. Box-Cox transformations have no effect on the nonparametric Kruskal-Wallis test. The *p*-value was again significant 0.0062. (Figure 3.15) Thus both the mean and median initial CD4 cell counts have a statistically significant relationship with the Pap smear results, with lower CD4 cell counts being related to worsening atypia.

For both the initial CD4 cell count and the last count before the Pap smear was performed, contingency tables were drawn up. The single case with a missing value was combined with group with counts greater than or equal to 500cellsμ/L during analyses. Concerning the initial CD4 cell count, the *p*-value for the full table (Table 3.7.1) is 0.1125. If the lowest two categories are combined to give a category of CD4 cell counts less than 200, then the *p*-value is 0.0684. However the chi-squared approximation may be poor due to the lower than expected frequencies, notably in the ASCUS and 'other' groups. Once the 'other' Pap smears are ignored the *p*-value is 0.0819. Both values are approaching significance. When the CD4 cell counts are cross-tabulated against whether or not the Pap smear was normal or abnormal the p-value is 0.0293.

The table (Table 3.7.2) for the CD4 closest to the Pap smear yields a *p*-value of 0.168. When the lowest two categories are combined for a CD4 cell count of less than 200 the *p*-value is

0.180. If the 'other' Pap smears are ignored, then the *p*-value is 0.114. However if the CD4 cell count are grouped into less than 200, 201-350 and greater than 350 and analyzed against the Pap smear results, including the 'other' Pap smears, the *p*-value is 0.075. If the 'other' Pap smears are ignored (Table 3.7.3), the *p*-value is significant – 0.039. Comparing the CD4 to normal versus abnormal Pap smears, which was significant for the initial CD4 cell count, the result is not significant whether including ($p = 0.063$) or excluding 'other' ($p = 0.101$).

In summary the contingency tables showed both significant and non-significant *p*- values for both the initial and last CD4 cell count depending on the grouping of the data. A lower CD4 cell count was associated with an abnormal Pap smear.

3.4.7 Viral load

The initial viral load results had 49 missing values. The range was from undetectable to 4000000copies/ml. The mean initial viral load was 122697.6copies/ml (which is a slight over estimation due to the rounding off of '<40' and '<400') and the median was 28000copies/ml. (Figure 3.16) The viral load before the Pap smear had 54 missing values and the range was from undetectable to 4000000copies/ml. The mean viral load was 41503.9copies/ml (again a slight over estimation) and the median was 400copies/ml. (Figure 3.17)

As for the initial CD4 cell count an ANOVA test and a Kruskal-Wallis rank sum test were carried out on the initial viral load. The ANOVA test for equality of mean response of the Box-Cox transformed versions of the initial viral load across the Pap smear result categories was not statistically significant ($p = 0.3702$) and neither did the Kruskal-Wallis test on the median viral load play a significant role ($p = 0.2891$). (Figure 3.18)

The cross-tabulation of the Pap smear results against the initial viral loads, but ignoring the missing values, gives a *p*-value of 0.6191. Owing to the lower than expected frequencies in certain cells the chi-squared approximation may be poor (Table 3.8.1). If the 'other' Pap smear results are ignored then the *p*-value for the test of independence of classifications is 0.6723. If the viral load values under 1000copies/ml are combined and then reanalyzed including and then excluding the 'other' Pap smear results the *p*-value is 0.8242 and 0.9285 respectively. A similar *p*-value of 0.866 is obtained where the values under 400copies/ml are combined and the missing and 'other' results are ignored.

When cross-tabulating the Pap smear results against the last viral load before the Pap smear, but ignoring the missing values, one gets a *p*-value of 0.841. Due to the lower than expected frequencies in certain cells (Table 3.8.2) the chi-squared approximation may be poor. If the 'other' Pap smear results are ignored then the *p*-value for the test of independence of classifications is 0.699. If the viral load values under 1000copies/ml are combined and reanalyzed including and then excluding the 'other' Pap smear results the *p*-value is 0.955 and 0.862 respectively. As above a *p*-value of 0.920 is obtained where the values under 400copies/ml are combined and the missing and 'other' results are ignored.

In summary neither the initial nor the last viral load showed a statistically significant relationship with the Pap smears results.

3.4.8 ART

Prior to their first Pap smear, 139 women (56.0%) had initiated ART. Of the 109 women (44.0%) who had not, 20 were eligible and ten of these subsequently initiated therapy. A further three patients had CD4 cell counts which decreased later and were thus eligible and then initiated treatment, bringing the total of those starting after the initial Pap smear to 13. In the group of 139 women, 135 were on HAART and four were on individualized regimens. By the end of the study time period a total of 152 women (61.3%) were on therapy. One hundred and forty-eight were on HAART and four on individualized regimens.

The Pap smear results were cross-tabulated with the ART usage of the patients prior to the Pap smear. The *p*-value for the full-table (Table 3.9.1) is 0.0597, with possible problems with chi-squared approximation. The *p*-value if the 'other' group is excluded is 0.0475, with possible problems with chi-squared approximation. If the table is collapsed (Table 3.9.2) to compare ART usage versus no ART usage before the Pap smear both including and then excluding the 'other' group then the *p*- values are 0.0874 and 0.0788 respectively.

A 2 by 2 table (Table 3.9.3) comparing ART use versus no ART's and normal versus abnormal Pap smears gives a statistically significant *p*-value of 0.0110. However recognizing the fact that different patients had been on ART's for different lengths of time, the table (Table 3.9.4) was recalculated. The ART category was re-divided into two groups, firstly no ART use and those on for less than 12 weeks versus those who had been on for 12 weeks or more. Viral suppression to less than 500copies/ml would be expected by 8 to 16 weeks [(9)]. The *p*-value was 0.067 which was no longer statistically significant.

The data was again refined (Table 3.9.5) by removing those not on ART's and who were not eligible i.e. a CD4≥200cells/μL. The *p*-value was not statistically significant at 0.424. It remained not significant (0.342) if the abnormal Pap smears were returned to their original diagnoses (Table 3.9.6).

The data was tabulated (Table 3.9.7) in order to characterize the normal versus abnormal Pap smear results by the use of ART's and duration of treatment as well as by their immunological status prior to the Pap smear being performed.

4.0 DISCUSSION

4.1 Summary of the main results

4.1.1 Study objective one

Among 324 women who attended the post natal clinic, 248 (76.5%) had a Pap smear, 22 (6.8%) were referred to their local clinic for a Pap smear and 47 (14.5%) defaulted their follow-up appointments. Only one woman (0.003%) refused a Pap and in 6 (1.9%) no reason was found for it not being done. As a health intervention the postnatal clinic was successful in screening over 75% of these high risk women for cancer of the cervix. However to ensure wider coverage, monitoring those who were down-referred for Pap smears and ensuring those in the referral areas are up-referred appropriately would improve the impact of the program. The expansion of this initiative more broadly would also be of great public health benefit in view of the serious consequences of cancer of the cervix and the high prevalence among post natal HIV infected women of cervical smear abnormalities.

Of the 248 patients 47.2% had abnormal pap smears, 25.8% had LGSIL, 12.9% had HGSIL and 4.0% had ASCUS. Of further importance were the facts that 80.2% of the Pap smears were satisfactory for evaluation and 35.5% were affected by koilocytosis.

In terms of the management of the Pap smear results (Figure 3.3) there were a few deviations from the standard. Regarding the three normal and satisfactory Pap smears that were repeated, the time interval was very short. It is possible that when the clinician reviewing the patient looked for a result and nothing was initially found, the test was repeated.

Unfortunately there was not a protocol that consistently recommended the follow-up of patients with LGSIL, namely six weeks (one case), six months, 12 months or 6-12 months. There were also five patients where colposcopy was suggested, however no such results were found although one did have a normal repeat Pap smear six months later. Patients with HGSIL were all referred for colposcopy at which either a biopsy was taken or a see and treat approach was adopted using LLETZ at the clinicians' discretion. One deviation from standard practice was noted in a patient who after a LLETZ showing HGSIL went on to have a follow- up Pap smear which again had HGSIL, and again repeated six months later, the result of which remained unchanged. In the 'other' group a patient had a colposcopic biopsy which showed HGSIL the Pap smear was again repeated six months later which also showed HGSIL. After three months she had a colposcopy and this confirmed the presence of LGSIL. These patients needed a definitive excisional procedure to prevent the occurrence of cervical cancer. Increased awareness of the correct management of abnormal cytology and histology results is needed. A thorough review of a patient's history and previous investigations as well as a reliable method of retrieving results would help prevent unnecessary duplication of investigations and delay in appropriate management.

4.1.2 Study objective two

The second objective was to correlate the immune status of the patient with their Pap smear result. There was no statistically significant association between the WHO classification and the Pap smear results. Concerning the CD4 cell count the *p*-value approaches significance when one compares the CD4 cell count categories with the Pap smear results. Once the CD4 cell count categories are combined such that they reflect the level at which ART would be initiated (<200cells/ml in the South Africa, <350cells/ml in well resourced settings) then the

p-value is significant at 0.039. The analysis of the viral load against the Pap smear did not yield a significant *p*-value.

4.1.3 Study objective three

The third objective concerning the use of ART and the patients' Pap smear results yields significant *p*-values on the data (*p*-value 0.0475). ART use is associated with abnormal Pap smear results reflecting the poor immune function of the patients who were initiated. However once it is refined by excluding those not on ART and not eligible - so that the comparison is between those not on ART and eligible or on ART for less than 12 weeks, versus those on for more than 12 weeks the *p*-value is not significant (0.424).

The follow-up results had a rate of 51.6% for regression, 22.6% for persistence and 25.8% for progression for the available group of 31. Of the twenty-two patients on ART or for more than 12 weeks; 50.0% regressed, 31.8% persisted and 18.2% progressed. Nine of the 31 were not on ART or had been on for less than 12 weeks; 55.6% regressed, none persisted and 44.4% progressed. . While these patients were not prospectively randomized and the follow-up results only represent a biased 21.0% of the sample, they do appear to suggest that the use of ART is beneficial. However the numbers are small especially of the no ART group.

4.2 Limitations

The 248 cases that were analyzed reflect two different data generating mechanisms, those that were being followed up having been initiated on ART's during their pregnancy and those that

were referred post-delivery for further management of conditions relating to their positive status and who were not necessarily on ART's.

Thus the group is not a random sample of the general population, and the results obtained cannot be generalized as such. The population is however reflective of the aims of the postnatal clinic itself and results may be generalizable to interventions proposed to similar groups of women. .

The limitations of this study are largely due to its retrospective nature. Certain demographic variables were not recorded and the definitions used may have differed between health care workers. Of concern, results for the gravidity appeared to be reported inconsistently between health care workers, and may not reflect a true result.

It must be noted that except for the use of ART's, every other data set had a variable number of missing values. This is a known weakness of retrospective studies. When gathering the results from the laboratory computer system, it was frequently necessary to search using different combinations of the name, surname, hospital number and birth date to find all the relevant results. Frequently names were misspelled or hospital numbers incorrectly recorded. As far as possible the final body of data was as accurate as possible, acknowledging the inherent problems of the system.

Follow-up was not consistent and only results that were available retrospectively were included. There is no information as to why those who were supposed to have repeat

investigations did not return. There is little bias in terms of the sample as every patient who had a Pap smear in the time frame was included.

The accuracy of a retrospective study can be improved in a number of ways. Standardized clerking forms, especially if they were computerized would go a long way to improving data collection. Staff training in the field of interest, its management and appropriate referrals also contributes. Compliance can be improved with active follow-up and reminders to patients via cellular phone messaging.

4.3 Interpretation

The primary aim of the study has been fulfilled in that the range and rate of Pap smear abnormalities for our post partum HIV positive women attending the post-natal clinic has been described. Through rigorous follow-up 76.5% of the attendees were screened for cancer of the cervix. . In 2007 only 22% of women who needed a screening Pap smear received one in South Africa's cancer of the cervix screening programme [41].

The study was then looked at in light of the available literature. (Table 1.1) The average sample size from the literature review was 223 (range 49 to 467) women, so the 248 in this study compared favorably. The mean age of the sample was 29.47 years and the median was 30 years. In the literature all but four had means or medians slightly greater than ours, the oldest being 36 years. The youngest was from the Zimbabwean study at 28.1 years (mean) [20]. The median CD4 was available from eight of the studies and was generally higher than our

190cells/μL, only two were lower – 160 and 165cells/μL although both had higher ART use than our 55.8% at 100% and 76, 3% respectively.

The result of 52.8% normal Pap smears is lower than the majority of studies identified in the literature review. Five studies were lower, including both South African studies, which had rates of 45% [22] and 50% [10]. Moodley however had a very large proportion of ASCUS in their sample – 20.5% versus our 4.0%. Overall then 47.2% of this sample had an abnormality that is in contrast to prevalence at about 10 % in the general American population [42].

The study had a rate of 25.8% LGSIL which was the third highest in comparison with the literature. The study by Heard in 1998 being the highest (43%), their median CD4 cell count was lower than this study though – 160cells/μL versus 190cells/μL [23]. The HGSIL rate of 12.9% was fifth highest with the rest being less than 8%. The reporting of ASCUS varied widely with five studies not reporting it all and a wide range amongst those that did (0.4-20.7%), this study's finding was on the lower end with 4.0%. None of the studies mentioned had a default category of 'other' as this study did which mainly included those with no ectocervix represented for interpretation.

In keeping with the literature the abnormal Pap smears tended to have lower mean CD4 cell counts than the normal Pap smears [43]. The results of the contingency tables and the tests for equality of mean response looking at the viral load and Pap smears did not find any significant association which is in contrast to the literature where higher viral loads were associated with a greater risk of abnormality [15]. Possibly this study was not powered sufficiently to detect an association.

Neither the WHO Staging for HIV disease nor the use of cigarettes yielded a significant *p*-value, although the results regarding the staging were limited by a large amount of missing data and that of smoking by the small numbers. The literature does however note the link between smoking and abnormal Pap smears but no association has been noted between the WHO stage and abnormal Pap smears.

The use of ART versus the Pap smear results produced some statistically significant *p*-values which suggested that there was an association between the use of ART and the risk of an abnormal smear; however once confounding factors were controlled for this association is lost and suggests that ART usage is a marker of immune status/advanced disease.

4.4 Generalisability of results

While this study specifically looked at postnatal HIV infected women predominantly with advanced HIV disease, the findings can still be generalized to similar groups of women particularly those with advanced disease and those found at ART roll-out sites.

4.5 Summary of the implications for clinical practice and research

With an estimated 2.8 million women living with HIV in South Africa in 2007 according to the Department of Health's National HIV and Syphilis Prevalence Survey [(1)] the implication of this study is that if 47.1% were to have a cervical smear abnormality as they became progressively immunocompromised then our gynaecological services would be placed under

severe strain. As can be seen in the flow diagram on those patients that had follow- up, often multiple visits are required to health services in order to manage the patient appropriately.

Knowing the enormity of the problem, implementation of a national screening programme for women in general with a special focus on those that are HIV sero-positive is needed. It is important to note that the mean age in this study was 29.47years which is almost the recommended age for commencing screening in South Africa. This finding suggests that screening should be started earlier and possibly more frequently. Protocols for the management of Pap smear abnormalities in HIV sero-positive women need to be standardized and added to those that already exist. Once this is done research into the effectiveness of the programme can be done and improvements made. The HPV vaccines and HPV testing are yet to be widely used within the South African context and their addition to a government funded initiative against cervical pathology will be some time in coming.

5.0 CONCLUSION

The rates of cervical abnormality in HIV sero-positive patients attending the Johannesburg Hospital postnatal clinic are much higher (47, 1%) than would be expected in the general population, with a significant portion requiring follow-up investigation and management. It is however preferable to deal with the problem comprehensively during the screening phase rather than trying to manage a potential increase in cervical cancer cases.

6.0 FUNDING

This study had no direct costs associated with it besides printing and this was borne by the researcher.

7.0 APPENDIX

7.1 Tables

Table 2.1: Modified WHO Staging System for HIV infection and disease in adults and adolescents after the WHO 2007 guidelines

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Stage 1	Asymptomatic Persistent generalized lymphadenopathy
Stage 2	Unexplained weight loss < 10% body weight Recurrent respiratory tract infections Herpes Zoster Angular chelitis Skin lesions Oral ulceration
Stage 3	Unexplained weight loss > 10% body weight Chronic diarrhea > 1 month Persistent fever > 37.5^{o}C > 1 month Oral candidiasis, leukoplakia Pulmonary tuberculosis Severe bacterial infections Ulcerative stomatitis, gingivitis, periodontitis Unexplained anaemia, neutropenia, thrombocytopenia

Stage 4	Wasting syndrome: weight loss > 10% plus either chronic diarrhea, prolonged fever 1 > month or chronic weakness *Pneumocystis jeroveci* (previously carinii) Chronic herpes infection Oesophageal candidiasis Extra pulmonary tuberculosis Non tuberculosis mycobacteria infection Recurrent infections or septicaemia Cytomegalovirus retinitis HIV encephalopathy Cryptococcal meningitis Disseminated mycosis Kaposi's sarcoma Invasive cervical cancer

Table 3.1: ART usage amongst patients with follow-up results

Initial Pap Result	Final Pap/Biopsy or LLETZ/TAH	Repeat Result	No ART*	ART <12w*	ART >12w*	Total
Normal	Pap	Normal	7	1	6	14
	″	LGSIL	0	0	2	2
	″	HGSIL	1	0	1	2
	″	other	1	0	0	1
	TAH	CIN 3	0 (9)	0 (1)	1 (10)	1(20)
LGSIL	Pap	Normal	1	1	4	6
	″	LGSIL	0	0	1	1
	Biopsy	Cancer	1 (2)	0 (1)	0 (5)	1 (8)
HGSIL	Pap/Biopsy	LGSIL	2	0	0	2
	″	HGSIL	0	0	3	3
	″	other	1	0	0	1
	LLETZ/TAH	Normal	0	0	3	3
	″	LGSIL	0	0	4	4
	″	HGSIL	0 (3)	0 (0)	3 (13)	3(16)
ASCUS	LLETZ	HGSIL	1 (1)	0 (0)	0 (0)	1(1)
Other	Pap/Biopsy	Normal	0	1	0	1
	″	LGSIL	1	0	3	4
	″	HGSIL	0 (1)	0 (1)	2 (5)	2 (7)
TOTALS			16	3	33	52

*refers to ART usage at the time of the final result

Table 3.2.1: Pap smear results versus age categories

	Pap	Normal	LGSIL	HGSIL	ASCUS	other	Total
Age							
Missing		1	0	0	0	0	1
≤19		2	0	1	0	0	3
20-24		14	10	6	1	0	31
25-29		43	25	9	7	1	85
30-34		55	20	14	0	7	96
35-39		13	8	2	1	2	26
≥40		3	1	0	1	1	5
Total		131	64	32	10	11	248

Table 3.2.2: Pap smear results versus age selected categories

	Pap	Normal	LGSIL	HGSIL	ASCUS	other	Total
Age							
20-29 (%)		57 (49.1)	35 (30.2)	15 (12.9)	8 (6.9)	1 (0.9)	116
30-39 (%)		68 (55.7)	28 (30.0)	16 (13.1)	1 (0.8)	9 (7.4)	122
Total		125	63	31	9	10	238

Table 3.3: Pap smear results versus parity

	Pap	Normal	LGSIL	HGSIL	ASCUS	other	Total
Parity							
Missing		19	8	0	3	3	33
1		19	9	4	2	1	35
2		55	25	16	2	3	101
3		26	16	11	3	3	59
4		9	4	1	0	0	14
5		3	1	0	0	0	4
6		0	1	0	0	0	1
7		0	0	0	0	1	1
Total		131	64	32	10	11	248

Table 3.4: Pap smear results versus gravidity

	Pap	Normal	LGSIL	HGSIL	ASCUS	other	Total
Gravidity							
Missing		23	8	0	3	3	37
1		14	6	3	2	1	26
2		50	24	12	1	1	88
3		26	18	11	3	5	63
4		13	5	5	1	0	24
5		4	2	1	0	0	7
6		1	1	0	0	0	2
7		0	0	0	0	1	1
Total		131	64	32	10	11	248

Table 3.5: Pap smear results versus WHO clinical stages

	Pap	Normal	LGSIL	HGSIL	ASCUS	other	Total
WHO							
Missing		59	28	18	1	6	112
1		39	23	6	6	2	76
2		10	6	4	1	1	22
3		15	4	1	1	2	23
4		8	3	3	1	0	15
Total		131	64	32	10	11	248

Table 3.6.1: Pap smear results versus smoking

	Pap	Normal	LGSIL	HGSIL	ASCUS	other	Total
Smoking							
Missing		23	11	2	2	4	42
Never smoked		107	51	29	7	7	201
Ever smoked		1	2	1	1	0	5
Total		131	64	32	10	11	248

Table 3.6.2: Pap smear results (collapsed) versus smoking

	Pap	Normal	Abnormal	Total
Smoking				
Never smoked		107	87	194
Ever smoked		1	4	5
Total		108	91	199

Table 3.7.1: Pap smear results versus first CD4 cell count results

	Pap	Normal	LGSIL	HGSIL	ASCUS	other	Total
CD4 cell count (cells/μL)							
0-50		8	4	3	0	0	15
51-200		49	36	17	5	9	116
201-350		35	16	5	1	1	58
351-500		19	2	4	3	0	28
≥500		20	5	3	1	1	30
Missing		0	1	0	0	0	1
Total		131	64	32	10	11	248

Table 3.7.2: Pap smear results versus last CD4 cell count prior to Pap smear results

	Pap	Normal	LGSIL	HGSIL	ASCUS	other	Total
CD4 cell count (cells/μL)							
0-50		7	1	1	0	0	9
51-200		35	32	9	1	5	82
201-350		40	19	12	4	4	79
351-500		21	5	5	3	0	34
≥500		28	6	5	2	2	43
Missing		0	1	0	0	0	1
Total		131	64	32	10	11	248

Table 3.7.3: Pap smear results versus last CD4 cell count (collapsed) prior to Pap smear results

	Pap	Normal	LGSIL	HGSIL	ASCUS	Total
CD4 cell count (cells/μL)						
0-200		42	33	10	1	86
201-350		40	19	12	4	75
≥351		49	12	10	5	76
Total		131	64	32	10	237

Table 3.8.1: Pap smear results versus initial viral load

	Pap	Normal	LGSIL	HGSIL	ASCUS	other	Total
VL (copies/ml)							
Missing		34	8	5	1	1	49
Undetectable-40		5	3	0	1	1	10
41-400		6	3	5	1	0	15
401-1000		5	2	0	0	0	7
1001-10000		20	14	4	3	0	41
10001-100000		38	21	9	3	5	76
≥100000		23	13	9	1	4	50
Total		131	64	32	10	11	248

Table 3.8.2: Pap smear results versus viral load before Pap smear

	Pap	Normal	LGSIL	HGSIL	ASCUS	other	Total
VL (copies/ml)							
Missing		38	9	5	1	1	54
Undetectable-40		16	14	6	4	2	42
41-400		35	15	10	1	5	66
401-1000		7	6	3	0	0	16
1001-10000		13	9	4	3	1	30
10001-100000		13	8	2	1	1	25
≥100000		9	3	2	0	1	15
Total		131	64	32	10	11	248

Table 3.9.1: Pap smear results versus ART usage before the Pap smear

	Pap	Normal	LGSIL	HGSIL	ASCUS	other	Total
ART (before Pap smear)							
Nil		68	25	10	3	3	109
Regimen 1a & b		61	36	19	7	7	130
Other		2	0	2	0	0	4
Regimen 2		0	3	1	0	1	5
Total		131	64	32	10	11	248

Table 3.9.2 Pap smear versus ART usage (collapsed) before the Pap smear

	Pap	Normal	LGSIL	HGSIL	ASCUS	other	Total
ART (before Pap smear)							
No ART's		68	25	10	3	3	109
ART		63	39	22	7	8	139
Total		131	64	32	10	11	248

Table 3.9.3 Pap smear results (collapsed) versus ART usage (collapsed) before the Pap smear

	Pap	Normal	Abnormal	Total
ART (before Pap smear)				
No ART's		68	41	109
ART's		63	76	139
Total		131	117	248

Table 3.9.4: Pap smear results (collapsed) versus ART usage duration before the Pap smear

	Pap	Normal	Abnormal	Total
ART (before Pap smear)				
No ART's or <12w use		79	57	136
ART's for ≥12w		52	60	112
Total		131	117	248

Table 3.9.5: Pap smear results (collapsed) versus ART usage duration or eligibility before the Pap smear

	Pap	Normal	Abnormal	Total
ART (before Pap smear)				
No ART's but eligible or <12w use		19	29	48
ART's for ≥12w		52	60	108
Total		71	89	156

Table 3.9.6: Pap smear results versus ART usage duration or eligibility before the Pap smear

	Pap	Normal	LGSIL	HGSIL	ASCUS	other	Total
ART (before Pap smear)							
No ART's but eligible or <12w use		19	17	9	0	3	48
ART's for ≥12w		52	31	16	7	6	112
Total		71	48	25	7	9	160

Table 3.9.7: Distribution of Pap smear results according to time on ART's and immunological status

Patients	Pap smear result	ART usage	ART duration	CD4 (cells/µL)	VL (copies/ml)
248 patients	131 normal	63 ARTs	11 < 12w	10 < 200	4 ≤ 400
					6 > 400
				1 ≥ 200	1 ≤ 400
			52 ≥ 12w	24 < 200	16 ≤ 400
					7 > 400
					1 Missing
				28 ≥ 200	24 ≤ 400
					4 > 400
		68 no ART's		8 < 200	2 ≤ 400
					3 > 400
					3 Missing
				60 ≥ 200	4 ≤ 400
					22 > 400
					34 Missing
	117 abnormal	75 ART's	15 < 12w	11 < 200	3 ≤ 400
					8 > 400
				4 ≥ 200	4 ≤ 400
			60 ≥ 12w	27 < 200	17 ≤ 400
					10 > 400
				33 ≥ 200	29 ≤ 400
					4 > 400
		42 no ART's		11 < 200	1 ≤ 400
					8 > 400
					2 Missing
				31 ≥ 200	3 ≤ 400
					14 > 400
					14 Missing

7.2 Figures

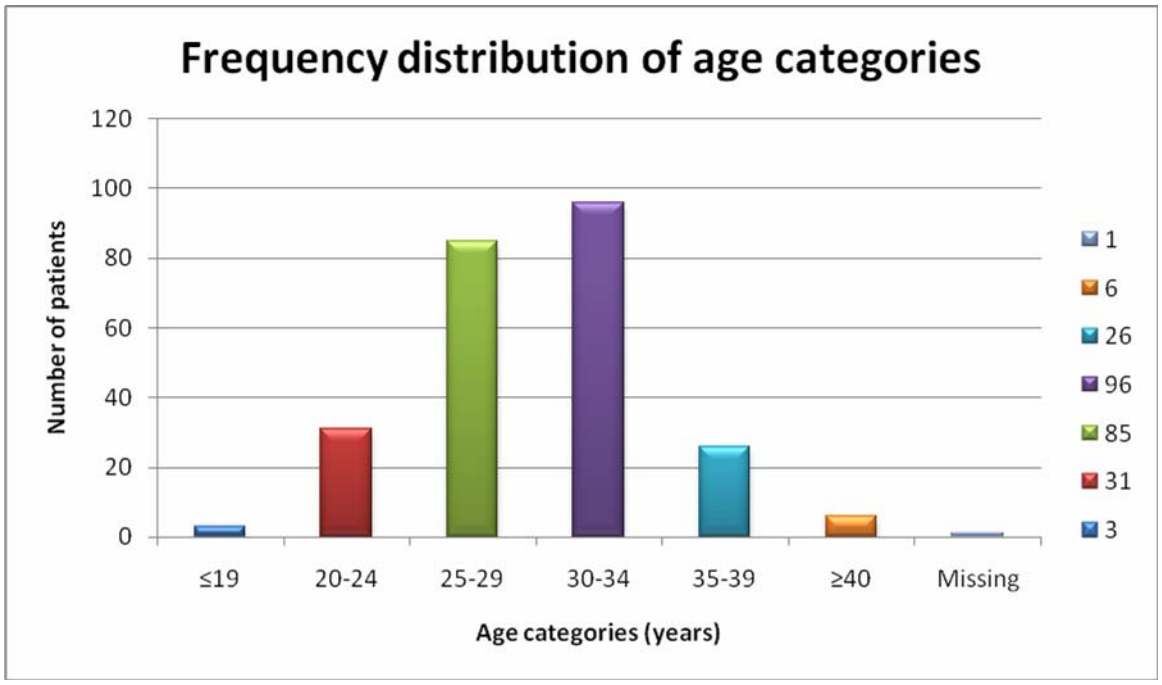


Figure 3.4

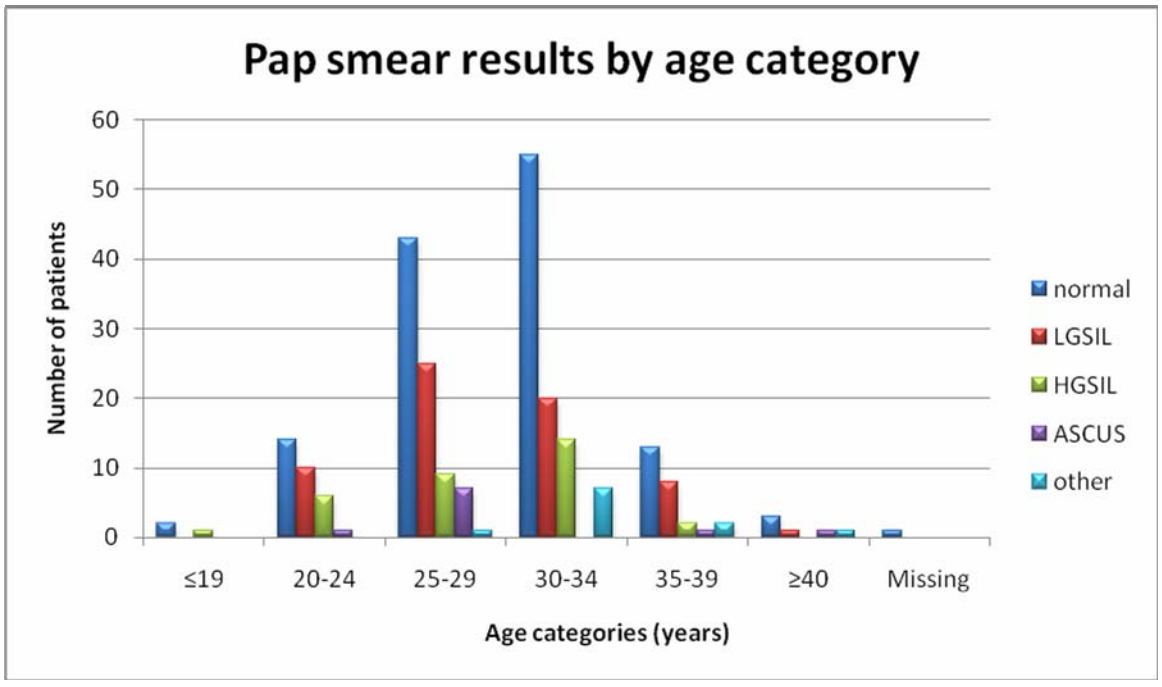


Figure 3.5

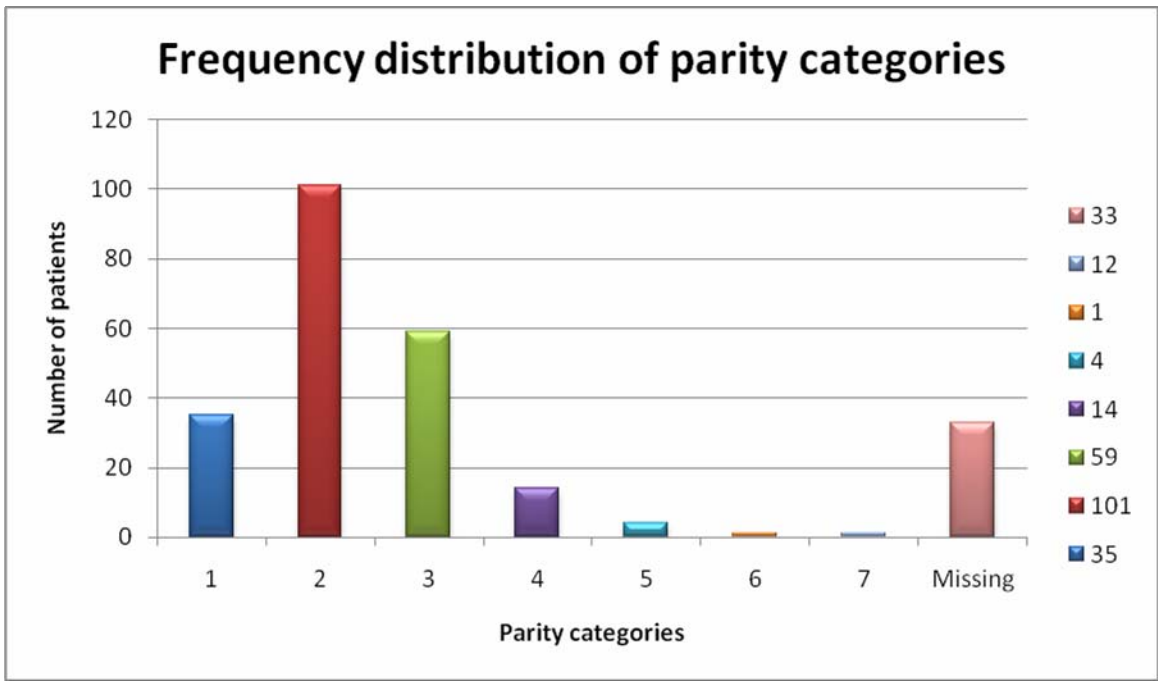


Figure 3.6

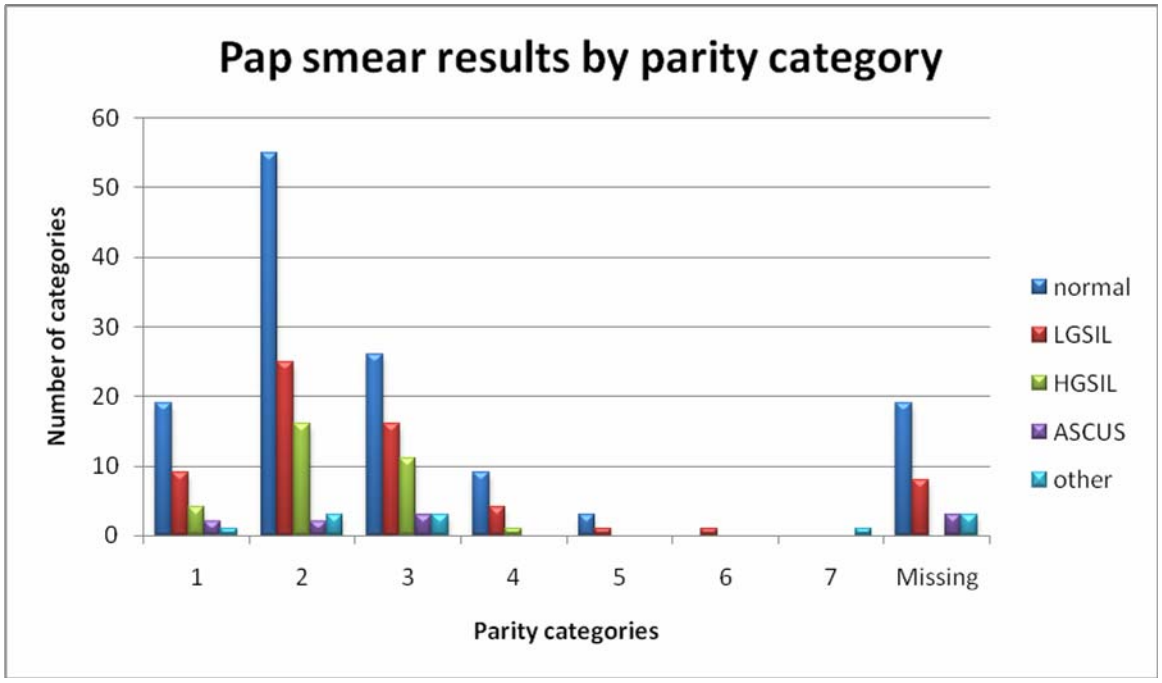


Figure 3.7

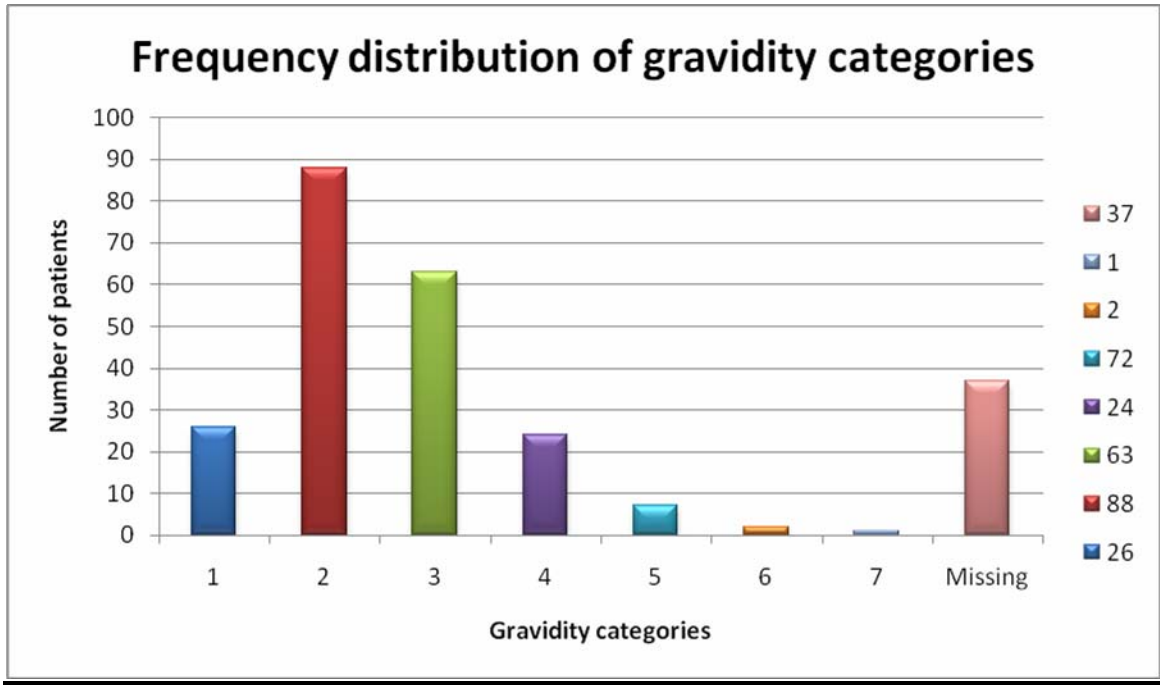


Figure 3.8

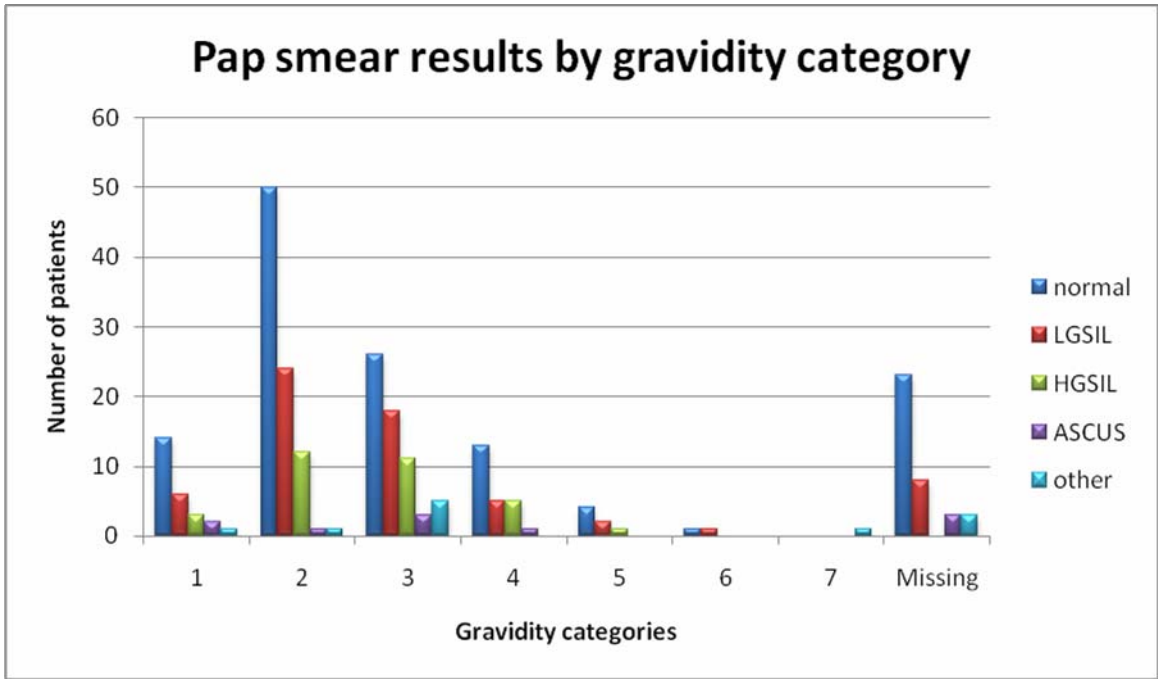


Figure 3.9

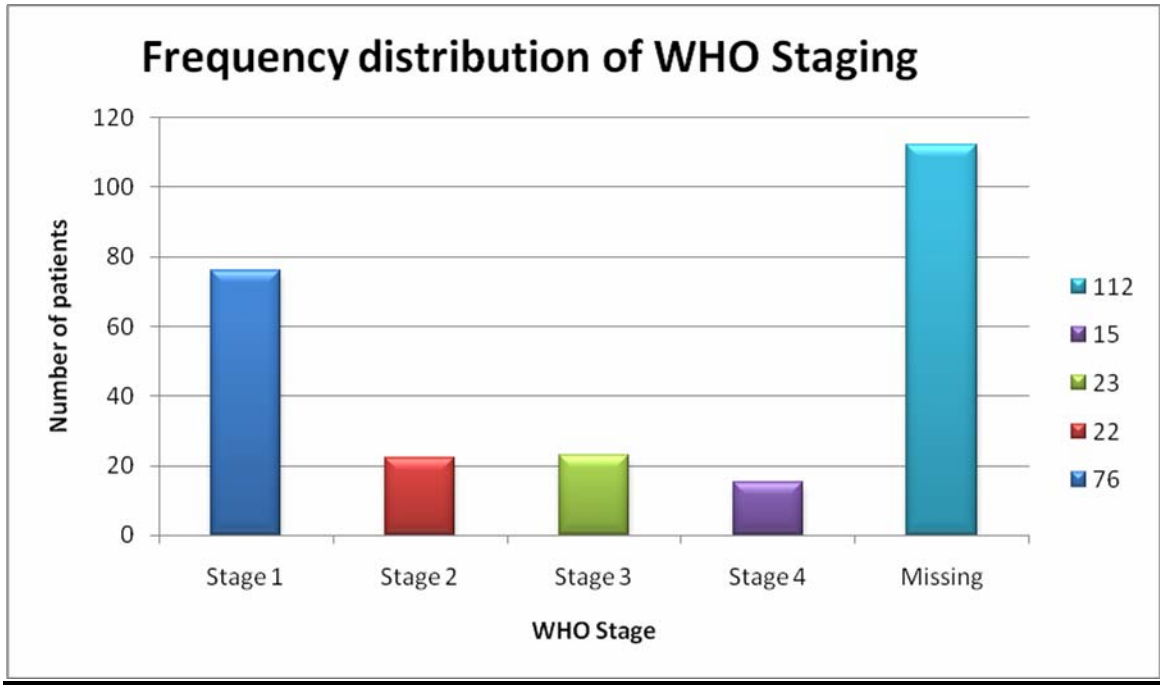


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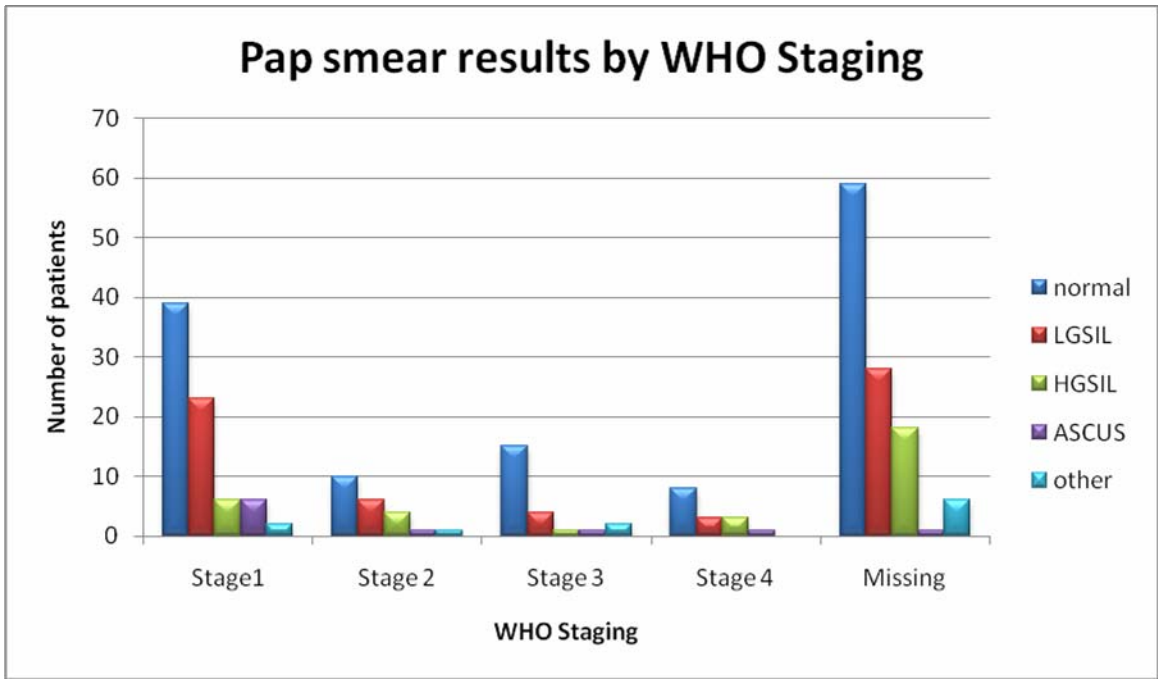


Figure 3.11

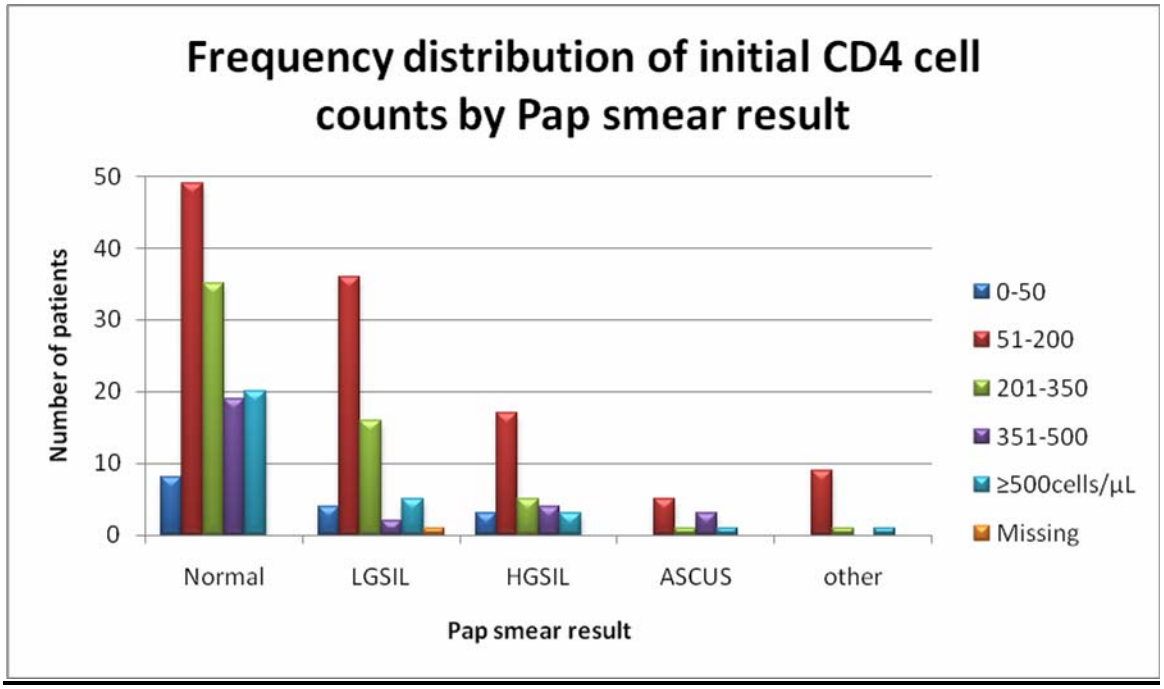


Figure 3.12

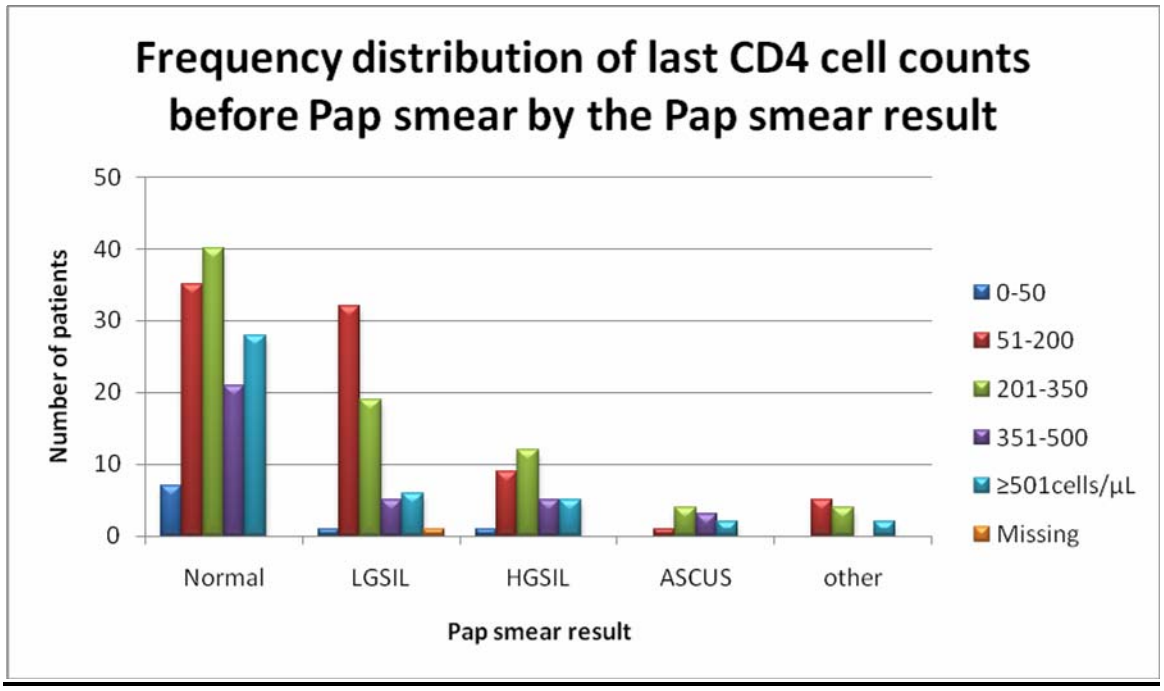
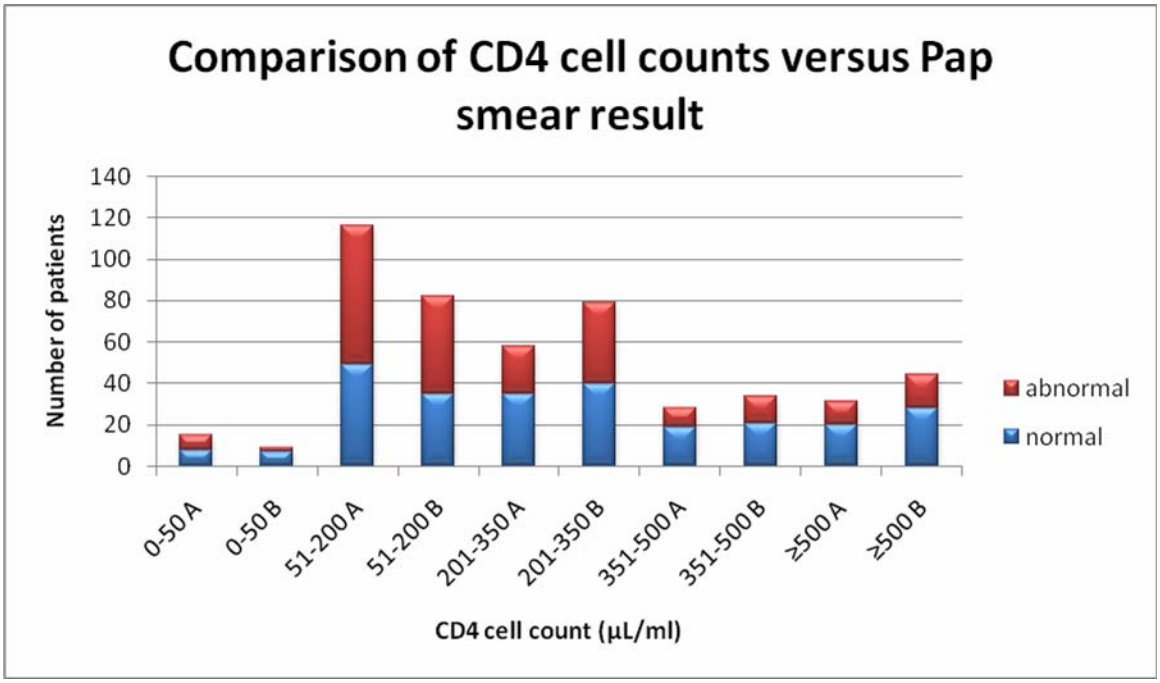


Figure 3.13



A = initial CD4 cell count B= last CD4 cell count before Pap smear

Figure 3.14

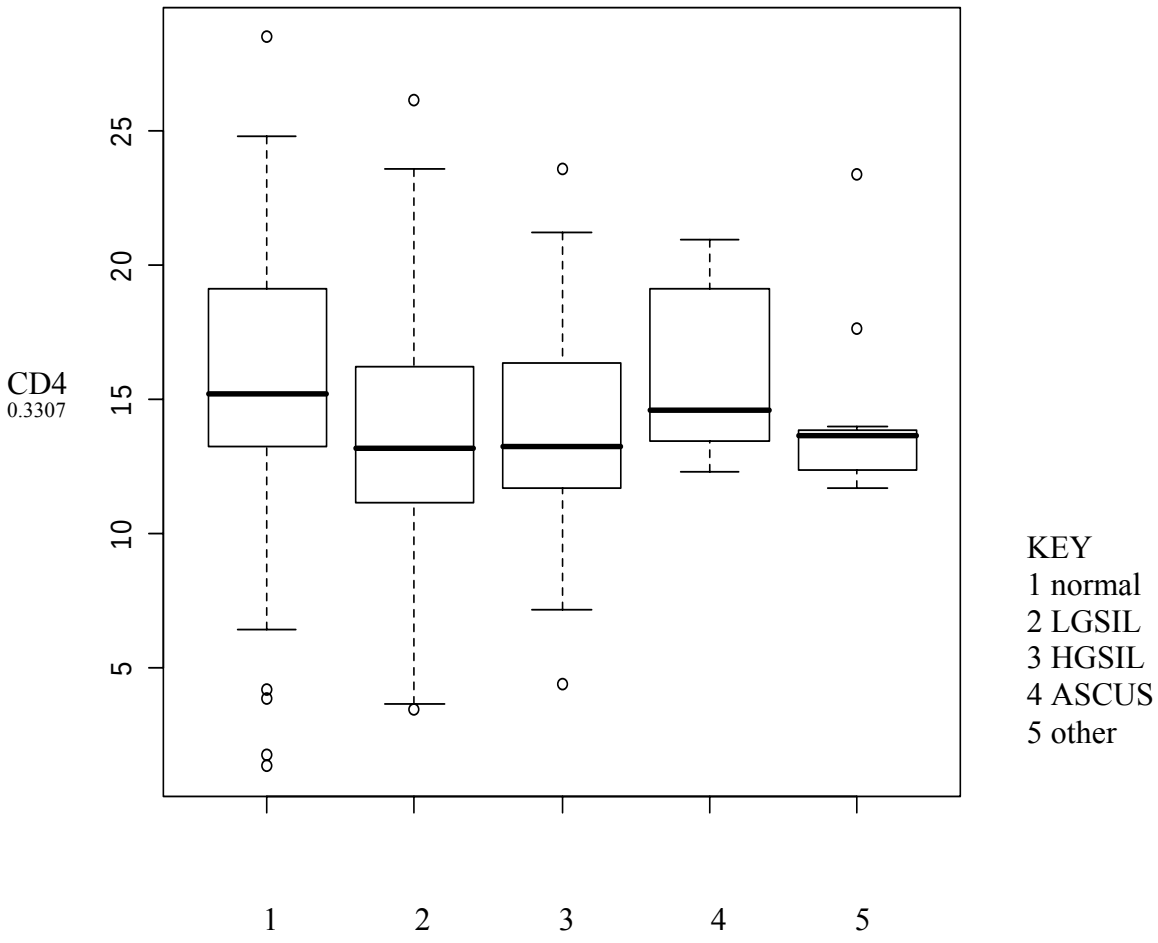


Figure 3.15 Boxplot of $CD4^{0.3307}$ versus Pap smear result

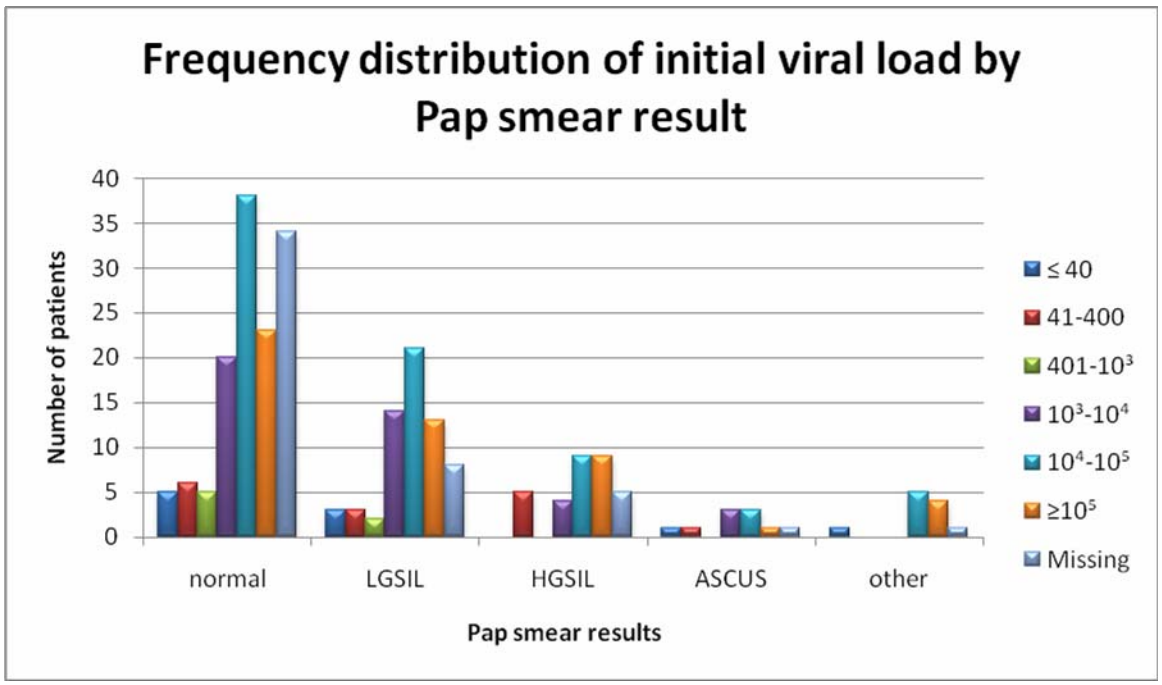


Figure 3.16

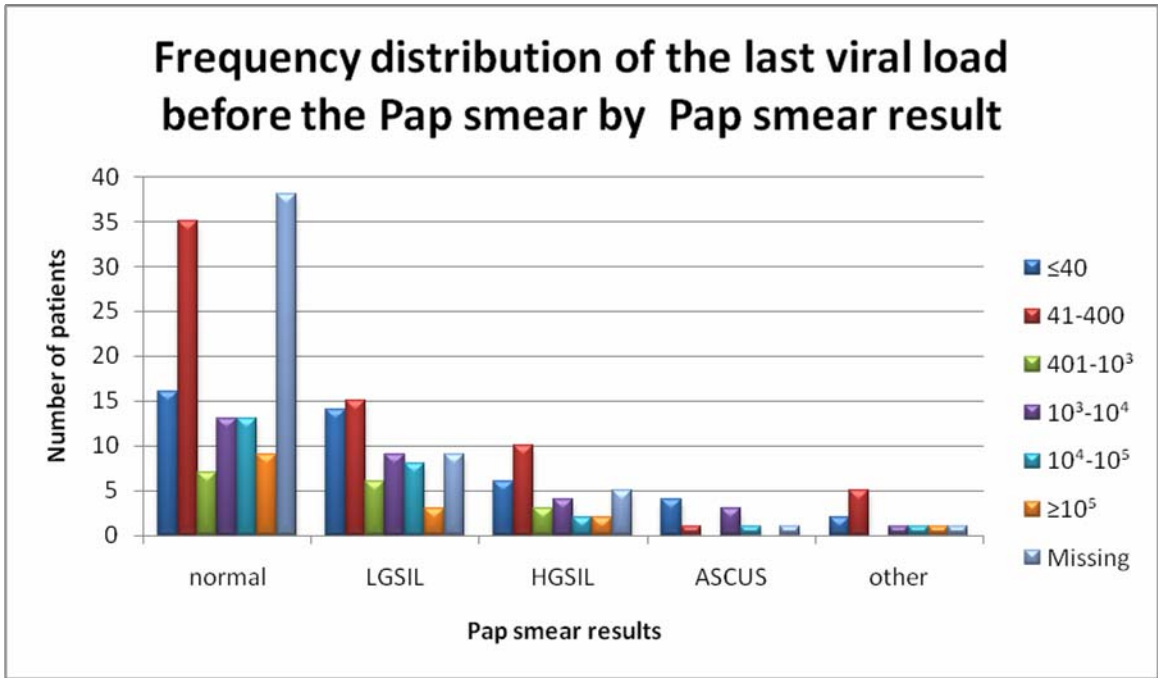


Figure 3.17

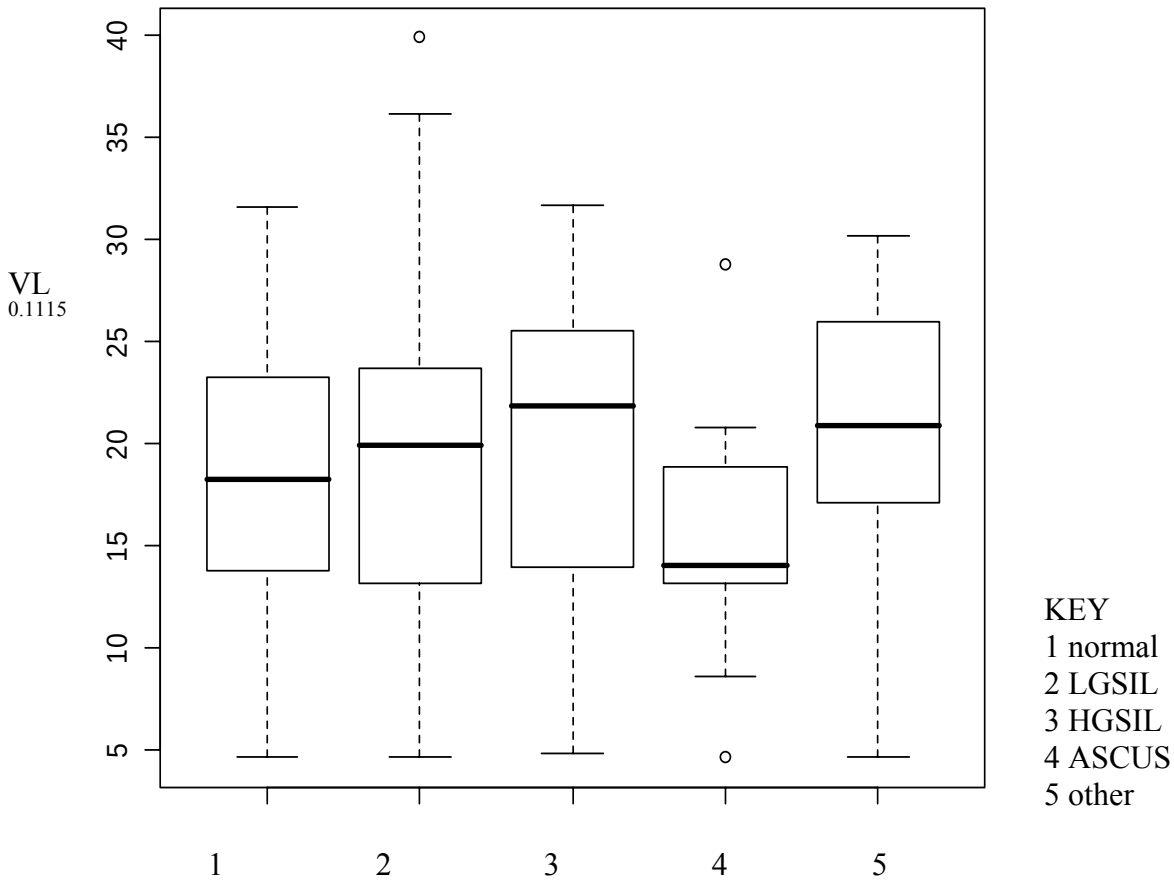


Figure 3.18 Boxplot of $VL^{0.1115}$ versus Pap smear result

7.3 Data sheet

Study number		Age	
Parity		Gravidity	
Date of delivery		HIV test date	
CD4 cell count date(1)		CD4 result (1)	
CD4 cell count date (2)		CD4 result (2)	
CD4 cell count date (3)		CD4 result (3)	
CD4 cell count date (4)		CD4 result (4)	
CD4 cell count date (5)		CD4 result (5)	
CD4 cell count date (6)		CD4 result (6)	
CD4 cell count date (7)		CD4 result (7)	
CD4 cell count date (8)		CD4 result (8)	
CD4 cell count date (9)		CD4 result (9)	
CD4cell count date(10)		CD4 result (10)	
CD4cell count date(11)		CD4 result (11)	
VL date (1)		VL result (1)	
VL date (2)		VL result (2)	
VL date (3)		VL result (3)	
VL date (4)		VL result (4)	
VL date (5)		VL result (5)	
VL date (6)		VL result (6)	
VL date (7)		VL result (7)	
VL date (8)		VL result (8)	
VL date (9)		VL result (9)	

VL date (10)		VL result (10)	
VL date (11)		VL result (11)	
VL date (12)		VL result (12)	
VL date (13)		VL result (13)	
ARV initiation date		ARV regimen	
ARV amendment date		Amended regimen (1)	
ARV amendment date		Amended regimen (2)	
ARV amendment date		Amended regimen (3)	
ARV amendment date		Amended regimen (4)	
WHO Stage		Other illnesses	
Habits			
Cervical smear date (1)		Lab no. (1)	
Result			
Cervical smear date (2)		Lab no. (2)	
Result			
Cervical smear date (3)		Lab no. (3)	
Result			
Cervical smear date (4)		Lab no. (4)	
Result			
Cervical smear date (5)		Lab no. (5)	
Result			
Colposcopy date (1)		Lab no.(1)	
Result			
Colposcopy date (2)		Lab no. (2)	
Result			

LLETZ date		Lab no.	
Result			
Surgery date		Lab no.	
Result			

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