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CERVICAL CYTOLOGY WITH A DIAGNOSIS OF AT LEAST LOW-GRADE SQUAMOUS INTRAEPITHELIAL LESION CANNOT EXCLUDE HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESION (LSIL-H) WHAT DOES THIS MEAN IN HIV POSITIVE WOMEN?

A cytologic-Histological Correlation

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STUDENT NUMBER: 1808152

Degree: For the purposes of partial fulfillment of the requirements for the degree of Master of Medicine in the branch of Anatomical Pathology, University of the Witwatersrand
Format: "Submissible" Format with the intention to be submitted for publication to Acta Cytologica.

DECLARATION

I, Lucretia Portia Mekoa, declare that this research report is my own work. It is being submitted in partial fulfillment for the degree of Master of Medicine in Anatomical Pathology. It has not been submitted before for any degree or examination at this or any other university.

X

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Article 1: Title: Cervical cytology with a diagnosis of LSIL-H, what does this mean in HIV +ve women?

Journal name, year, volume and page numbers: Currently undergoing peer review after cytology

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Article 2: Title: .....

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.....

.....

Article 3: Title: .....

Journal name, year, volume and page numbers: .....

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PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

Presentations:

Mekoa L, Chibeshwa C, Michelow P. Cervical cytology with a diagnosis of at least low-grade squamous intraepithelial lesion cannot exclude high-grade squamous intraepithelial lesion (LSIL-H) what does this mean in HIV positive women?
Accepted for poster presentation at the University of the Witwatersrand Faculty of Health Sciences Research day, Abstract number: CSTH-P-16, October 2020.

Publications: This manuscript was submitted to Acta Cytologica on 16 September 2020 where peer review is currently underway – Submission number: ACY-2020-9-7

DEDICATION

I dedicate this to Dimitri Maritz, my partner for his patience and support during the long hours spent preparing this project.

To my parents, Julia and Justice Mekoa.

ACKNOWLEDGEMENTS

I would like to thank the following people who have played an integral role in bringing this project into fruition.

Professor Pamela Michelow: For the conception of this topic, her time, patience and input throughout this process. I feel fortunate to have been guided by an esteemed figure in the field of cytopathology such as herself and have learnt an immense amount from her during this time.

Dr Carla Chibwesha: For her time and expertise.

Right to Care clinic and Helen Joseph Hospital for providing permission to conduct this study

Ms Gill Hendry for her assistance in interpretation of the statistical analysis.

Ms Fadilah Ebrahim for assisting with retrieval of data.

ABSTRACT

Introduction: The Bethesda System for reporting cervical cytology divides squamous intraepithelial lesions into low grade and high grade. Diagnostic dilemmas occur when the cytomorphology is intermediate between LSIL and HSIL [At least LSIL- cannot exclude HSIL/ LSIL-H]. HIV-infected women have high rates of HPV-related disease. The aim of this study was to determine the histologic outcome and best management strategy for HIV-positive women with a cytologic diagnosis of LSIL-H in a resource constrained setting.

Methods: A retrospective analysis was performed whereby histologic follow up was sought on 3678 abnormal Pap smears over a two-year period in HIV-positive women at a single urban institution in South Africa.

Results:

Of 3678 abnormal smears LSIL-H comprised 191 [5.2%], ASCH 217 [5.9%], ASCUS 602 [16.4%], LSIL 2147 [58,4%], HSIL 497 [13.5%] and 23[0.6%] squamous cell carcinoma. Histologic results were available in 70.3%. The most common histologic outcome for LSIL-H was LSIL in 61.8% while 31.8% showed HSIL. This is similar to ASCH with a histologic diagnosis of LSIL of 57.8% and HSIL of 26.7%. The histologic follow up of women with a cytologic diagnosis of LSIL was 75% LSIL and 13.1% HSIL while that for HSIL was 31.6% LSIL and 63.2% HSIL. The most frequent CD4 count in women with LSIL-H cytology was over 1000, ASCH, LSIL and HSIL was 800-900, 700-800 and less than 200 respectively. The majority of LSIL-H smears were in women aged 20-29 while that for ASCH, LSIL and HSIL was 60-69, 30-39 and 20-29 respectively.

Discussion/ conclusion: The majority of HIV-positive women with a cytologic diagnosis of LSIL-H will have histologic follow up showing LSIL with approximately one quarter showing HSIL. This is similar to those with ASCH. Thus, smears showing cytomorphology intermediate between LSIL and HSIL may be reported as ASCH and the appropriate management, even in resource constrained settings, would be colposcopy and directed biopsy.

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NOMENCLATURE

Human Immunodeficiency Virus (HIV): A particular family of retroviruses that cause immunodeficiency.

Highly Active Antiretroviral Therapy (HAART): Cocktail of antiviral drugs used to treat HIV.

Papanicolaou smear: A screening process whereby epithelial cells are scraped from the cervix and placed on a slide to be stained and examined in order to detect epithelial abnormalities.

High grade Squamous Intraepithelial Lesion (HSIL): Moderate or severe dysplasia of cervical epithelium as determined on Pap smear.

Low grade Squamous Intraepithelial Lesion (LSIL): Mild dysplasia of cervical epithelium as determined on Pap smear.

Atypical squamous intraepithelial lesion cannot exclude High-grade squamous intraepithelial lesion (ASC-H): Cytomorphology suggestive of HSIL but qualitatively or quantitatively insufficient for a definitive diagnosis of HSIL.

LSIL-H: Defines a cytological category between LSIL and HSIL.

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2.1. Title page

Cervical cytology with a diagnosis of Low-grade squamous intraepithelial lesion, cannot exclude high-grade squamous intraepithelial lesion [LSIL-H]. What does this mean for HIV positive women?

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Short Title: Cervical cytology with a diagnosis LSIL-H in HIV positive women.

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Number of Tables: Five (5)

Number of Figures: Four (4)

Word count: 3706 words

Keywords: HIV, LSIL cannot exclude HSIL, Bethesda System for reporting cervical cytology, ASCH, LSIL

2.2 Abstract

Introduction: The Bethesda System for reporting cervical cytology divides squamous intraepithelial lesions into low grade and high grade. Diagnostic dilemmas occur when the cytomorphology is intermediate between LSIL and HSIL [At least LSIL- cannot exclude HSIL/ LSIL-H]. HIV-infected women have high rates of HPV-related disease. The aim of this study was to determine the histologic outcome and best management strategy for HIV-positive women with a cytologic diagnosis of LSIL-H in a resource constrained setting.

Methods: A retrospective analysis was performed whereby histologic follow up was sought on 3678 abnormal Pap smears over a two-year period in HIV-positive women at a single urban institution in South Africa.

Results:

Of 3678 abnormal smears LSIL-H comprised 191 [5.2%], ASCH 217 [5.9%], ASCUS 602 [16.4%], LSIL 2147 [58,4%], HSIL 497 [13.5%] and 23[0.6%] squamous cell carcinoma. Histologic results were available in 70.3%. The most common histologic outcome for LSIL-H was LSIL in 61.8% while 31.8% showed HSIL. This is similar to ASCH with a histologic diagnosis of LSIL of 57.8% and HSIL of 26.7%. The histologic follow up of women with a cytologic diagnosis of LSIL was 75% LSIL and 13.1% HSIL while that for HSIL was 31.6% LSIL and 63.2% HSIL. The most frequent CD4 count in women with LSIL-H cytology was over 1000, ASCH, LSIL and HSIL was 800-900, 700-800 and less than 200 respectively. The majority of LSIL-H smears were in women aged 20-29 while that for ASCH, LSIL and HSIL was 60-69, 30-39 and 20-29 respectively.

Discussion/ conclusion: The majority of HIV-positive women with a cytologic diagnosis of LSIL-H will have histologic follow up showing LSIL with approximately one quarter showing HSIL. This is similar to those with ASCH. Thus, smears showing cytomorphology intermediate between LSIL and HSIL may be reported as ASCH and the appropriate management, even in resource constrained settings, would be colposcopy and directed biopsy.

2.3 Manuscript

2.3.1. Introduction

Globally, cervical carcinoma is the fourth most frequent cancer affecting women and accounts for 12% of cancers in resource-constrained settings(1). It is currently the leading killer of women in Sub-Saharan Africa (1,2). Furthermore, South Africa has a surge of HIV and HPV infection rates compared to the rest of the world (1). Human Papillomavirus infection [HPV], more specifically persistent oncogenic HPV infection, is the principal source in the genesis of invasive cervical carcinoma(2). HIV is a well-recognized co-factor for persistent HPV infection. It not only augments the virulence of the virus but also increases its aggressiveness thus accelerating the progression to malignant transformation (1,2). In addition, HIV-positive women have a five times increased risk of being infected with high-risk HPV subtypes compared to HIV-negative women (1). Studies from the United States have shown that cervical cancer related mortality in HIV-positive women is double that of HIV-negative women(2). HIV-positive women in South Africa present with cervical lesions a decade earlier than HIV-negative women and usually experience increased risk of recurrences, advanced clinical stage and are more prone to develop treatment related complications (4). Women who have access to combined antiretroviral treatment are living longer lives and thus may be at higher risk for progression to cervical neoplasia and invasive cervical carcinoma (4). The effect of antiretroviral therapy [ART] on the progression of HPV related disease is controversial with data regarding the impact of ART on HPV showing inconsistent and inconclusive findings(2).

The cervical cytology Bethesda reporting system was introduced in 1988 and modified in 1991, 2001 and 2014(2). This system divides HPV-related disease into low-grade SIL [LSIL] and high-grade SIL [HSIL], which is equivalent to Cervical intraepithelial neoplasia [CIN] 1, and CIN 2 /CIN 3 respectively (3–6). This system divides HPV-related disease based on morphology, biologic differences and behaviors between these two lesions(2,3). The vast majority of LSIL lesions derive from short-lived infections with high-risk HPV that regress

spontaneously while HSIL represents pre-cancerous lesions, a third of which progress to invasive squamous cell carcinoma (3,4,6).

Despite these guidelines, diagnostic dilemmas occur in the interpretation of cervical cytology specimens which show intermediate morphological patterns in between LSIL and HSIL which has led to some authors calling this category "at least LSIL cannot exclude HSIL" (7,8). Other proposed definitions include [i] LSIL with occasional atypical cells suggestive but not definitive for HSIL, [ii](7) Atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion [ASC-H] without any inference to the LSIL, [iii] LSIL with ASC-H, [iv] HSIL or LSIL, and [v] LSIL: cannot exclude HSIL [LSIL-H] (7,9). Several studies in the literature are of the opinion that these lesions show different histological outcomes and rates of HPV prevalence and have researched the incidence of dysplasia and malignancy in LSIL-H and collated these findings with rates found in LSIL, ASC-H and HSIL(7). A common finding in these studies is that LSIL-H has a higher rate of high-grade lesions as compared to LSIL, similar rates to ASC-H and lower rates when compared to HSIL (10–12).

Despite the similarities in outcome, there is currently an undefined relationship between ASC-H and LSIL-H and some researchers have suggested including LSIL-H in the same reporting category as ASC-H. One study suggests LSIL-H is associated with an increased frequency of high risk HPV status as compared to ASC-H(12,13). Some studies have further gone to show that the development of high-grade lesions in LSIL-H patients is comparable to patients with HSIL and proposed that treatment algorithms for LSIL-H parallel those of HSIL(9,14,15). To date, however, no studies address this diagnostic dilemma in HIV-positive populations.

Even though the cytology literature has had several articles regarding LSIL-H as an independent entity, this category was not included in the latest [2014] Bethesda revision(2,16). This has been done to maintain the two-tiered classification system. Furthermore, this category is not recognized by current management guidelines (6,11). This can lead to confusion among clinicians regarding the suitable management of these patients if LSIL-H is reported(2). Furthermore, the significance of this entity in the HIV context has never been interrogated in the literature. In resource constrained settings, like South Africa,

clinical guidelines endorse close follow-up of patients with a cytological diagnosis of LSIL but women with ASC-H undergo colposcopy followed by sampling of the cervix(17,18).

The aim of this study was to determine the histologic outcome of LSIL-H in HIV-infected women compared to the histologic outcome of cervical smears reported as LSIL, ASC-H and HSIL in the same study population with a view to recommending the appropriate management of these women within a resource limited public health setting.

2.3.2. Materials and Method

A retrospective study was performed. The laboratory setting was the Cytology Unit, Anatomical Pathology Department, Faculty of Health Sciences, University of the Witwatersrand and National Health Laboratory Service, Johannesburg, South Africa. The clinical setting was an urban public-sector HIV clinic in Johannesburg, South Africa. The study population comprised all women aged 18-69 who had Pap smears performed for the time period 1st July 2013 to 30th June 2015. In July 2015, in line with the latest Bethesda Classification guidelines, the term LSIL-H was discontinued in this laboratory.

Cytology records were retrieved following a search of the laboratory database. All cases identified were subjected to a second computer-based search for the corresponding histology results. Histological specimens included cervical biopsy, large loop excisions of the transformation zone [LLETZ] and hysterectomy. Where more than one biopsy was available, the biopsy with the most severe lesion was selected. Histology results were categorized as negative [No HPV effect or dysplasia identified], Low-grade dysplasia [HPV cytopathic effect/ Cervical intraepithelial neoplasia [CIN] I] and high-grade dysplasia [CIN 2/3](10). Clinically significant histological findings which included adenocarcinoma in-situ [AIS], invasive adenocarcinoma and squamous cell carcinoma [SCC] were included in the final analysis.

Inclusion criteria for the study included all HIV-positive patients over the age of 18 who received cervical screening within the stipulated time period and received a cytological diagnosis of ASC-H, LSIL, LSIL-H and HSIL. Exclusion criteria included HIV-negative patients, females younger than 18 years of age, reports with a cytological diagnosis of ASCUS, glandular abnormalities and malignancies, as well as patients with cervical cytology only without corresponding histopathology.

Smears were conventional in type as liquid based cytology was not yet available within this clinical setting. Also, HPV testing is not undertaken routinely in the South African public health sector (2). All slides were previewed by a cytotechnologist and the final report was authorised by a cytopathologist or senior cytotechnologist based on the Bethesda 2001 criteria(4,10).

Previous publications arising from this study population revealed a high proportion of LSIL and HSIL cases and it was anticipated that would be the case in this current study(6). A probability sampling method was thus selected to counteract this using a Confidence interval of 5 and a Confidence level of 95% which amounted to 191 and 238 cases, respectively. The final sample size was subjected to a random selection process. This brought the final cohort to 971 cases [n =971]. A random selection process using Excel® was subsequently utilized to procure a sample size for LSIL and HSIL cases. The data was descriptive and comprises frequencies that were expressed in the form of percentages, tables and histograms. Descriptive statistics in the form of standard deviation and mean were used to describe the different variables [Age, viral load and CD4 count]. Microsoft Excel 2010® was used to store data as a spread sheet and further statistical analysis was performed using STATISTICA, Statsoft® [www.statsoft.com]. Fisher's exact statistical test was utilised to determine the differences in categorical data. A p-value of <0.05 was selected to represent statistical significance. The Welch test with Games-Howell post hoc test was used to assess if the age of the patients differed significantly across cytology groups. This test was also used to assess for differences regarding the CD4 counts across the cytology categories. The ANOVA test was used to analyse the relationship between viral loads across cytology groups.

This study obtained approval from the Medical ethics committee [Study number – M190530] at the University of the Witwatersrand, Johannesburg.

2.3.3. Results

During the two-year study period, this cytology laboratory processed a total of 3678 abnormal conventional Pap smears from the participating clinic. This comprised 191 LSIL-H cases, 497 HSIL, 217 ASC-H, 602 ASCUS, 2148 LSIL and 23 SCC. All the ASC-H [n=217] and LSIL-H [n=191] cases were incorporated in the study. Of the final cohort of 971 smears, concurrent histology reports were available in 70.3% [total 683 cases, 137 LSIL, 180 ASC-H, 157 LSIL-H and 209 HSIL] and only these cases underwent further analysis [Figure 1].

Majority of the women fell in the 30-39 age category [n =249]. The least common age group was 60-69 [n=25]. Table 1 illustrates the abnormal Pap categories and age distribution. The Welch test and Games-Howell post hoc test showed a significant difference in average age, Welch [3; 358.424] = 6.466, $p < 0.0005$. In particular, the average age in the ASC-H group [43.4 +/- 10.28] was significantly higher than in LSIL-H [39.5+/- 9.68] and HSIL [39.4 +/- 8.9]. This was particularly so in post-menopausal women of the 60-69 age group accounting for 56% of cases. Both LSIL-H and HSIL were most prevalent in the 20-29 age group accounting for 34.2% and 37% of the cases, respectively. The most common cytological diagnosis overall was LSIL [2148 cases] and the least common was LSIL-H [191 cases].

Figure 2 provides the mean CD4 counts across the different cytological categories. Most of the women had a CD4 count between 201-400 cells/mm^3 [26.3%]. Figure 2 illustrates an inverse trend between the CD4 count and cytological category. Table 2 illustrates that a higher-grade lesion is most likely to be associated with a CD4 count of less than 200 [44.9% of cases] when compared to other cytological categories. In addition, CD4 counts of above 1000 are associated with only 14.8% risk of high-grade lesions(16). The mean CD4 count for subjects with a diagnosis of LSIL-H was 467.5 mm^3 [Figure 2](15).

Most women had a viral load of less than 1000 [63.7%]. Figure 3 provides the mean viral load across the cytological categories. Table 3 shows a minor difference in the prevalence of all squamous intraepithelial lesions in women who had a viral load lower than detectable limit [LDL] and viral loads between 1 and 1000. The frequency of high-grade lesions is seen to increase appreciably with viral loads exceeding 1000. Notably, for patients with a viral load of 10 000 or more, the most common cytological diagnosis was HSIL [48.3% of cases]. The mean viral load for LSIL-H was 9117. This was increased compared to the mean for ASC-H [n = 4714] and lower than HSIL [n=21 600].

Table 4 provides values for histologic results in relation to the initial cytological diagnosis.

Two cases [0.6%] with a cytological diagnosis of LSIL showed adenocarcinoma in-situ on biopsy. Five cases [2.8%] of ASC-H and two cases [1%] of HSIL showed squamous cell carcinoma on follow up. No cases of LSIL-H showed adenocarcinoma-in-situ or invasive carcinoma on histology.

The cervical biopsy results in cases reported as LSIL-H were analogized to those reported as LSIL, ASC-H and HSIL. Table 5 surmises the figures in the literature regarding the detection of high-grade lesions in various cytology categories (8–10,12,19–22). In our study, the most common biopsy result for LSIL-H was LSIL comprising 61.8% of cases. This is lower than LSIL [75.2%], higher than HSIL [31.6%] and comparable to ASC-H [57.8%]. The presence of HSIL was seen in 31.8% of LSIL-H cases. This is higher than LSIL [13.1%], lower than HSIL [63.2%] but similar to ASC-H [26.7%]. The most common cytological diagnosis to have a negative histology result was ASC-H at 12.8%. A major proportion of women diagnosed with ASC-H [56%] were aged 60-69. The detection rate for high-grade dysplasia was found to have a similar rate in LSIL-H cases [31.8%] [Chi-Square test, $P < 0.0005$] when compared to ASC-H cases [26.7%] [$P < 0.0005$]. The prevalence of associated HSIL was significantly different from that associated with LSIL [13.1%, $P < 0.0005$] and HSIL [63.2%, $P < 0.0005$]. Figure 4 shows a conventional cervical smear reported as LSIL-H with the corresponding cervical biopsy.

In biopsies inclusive of the transformation zone, the pick-up rate for all lesions was significantly higher in all categories especially HSIL. A high proportion of HSIL cases and LSIL cases were inclusive of the transformation zone. This diagnosis was observed predominantly in pre-menopausal age groups. Patients with ASC-H were the least likely to have representation of the transformation zone compared to other cytology categories. The transformation zone was seen in 65.4% of LSIL-H cases.

2.3.4. Discussion

Our study introduces some of the first research regarding LSIL-H as a distinct category in an HIV-positive population. The most pertinent discoveries from this work include that LSIL-H cytology is associated with four times the risk of high-grade dysplasia on biopsy when compared to LSIL cytology [5.5% and 26.2%]. Moreover, LSIL-H is comparable in certain respects to ASC-H. This is signified in similar histological outcomes when the two are juxtaposed. The most common histological result for both ASC-H and LSIL-H is LSIL. The risk for a high-grade histologic lesion in LSIL-H and ASC-H is 26.2% and 22.1% respectively. A study conducted in 2018 by Segura et al [2018] showed similar detection of high-grade lesions in ASC-H [33.5%] and LSIL-H [31.5%](10). In contrast to our findings, two other

studies(11,14) reported that the most common histological outcome for LSIL-H and ASC-H was that of a high grade lesion. Particularly, LSIL-H had a high predictive value for CIN2 and ASC-H for CIN3(11,14). One study described a higher rate of LSIL in their LSIL-H cases [62%] when compared to ASC-H [33%](22). In the literature, the incidence of high-grade lesions varies from 7-23% for LSIL, 23-52% for ASC-H, 24-42% for LSIL-H and 65-81% for HSIL(3,8,9,12,19–22). In our study, high-grade dysplasia was found in 13.1% of LSIL cases, 26.7% ASC-H, 31.8% LSIL-H and 63.2% HSIL. The detection rate for high-grade dysplasia was found to have a similar rate in LSIL-H cases [31.8%] [Chi-Square test, $P < 0.0005$] compared to ASC-H cases [26.7%] [$P < 0.0005$]. This data mirrors the findings of other researchers (3,8,19–21). The rate of associated HSIL was significantly different from the rate of associated LSIL [13.1%, $P < 0.0005$] and HSIL [63.2%, $P < 0.0005$]. The risk for high-grade dysplasia was lowest in the LSIL category [13%]. These rates were comparable to Elsheik et al [2006] who assessed a total of 1033 cases and showed a rate of high-grade dysplasia in 13% of their LSIL cases and 74% of their HSIL cases. Similar to our study, their findings showed no statistical significance when comparing LSIL-H and ASC-H categories(19). Owens et al [2007] concluded that LSIL-H had an intermediate risk for high-grade dysplasia [40%] that was significantly greater than LSIL [10.8%] but less than that of HSIL [65.5%](3). They too demonstrated that the risk for high-grade dysplasia did not show statistical significance when compared with patients with LSIL-H [40%] and ASC-H [23%]. Ince et al [2011] had one of the largest sample sizes [n = 1713] in the literature and reported a 21% risk for high-grade dysplasia in LSIL, 43% in ASC-H, 40% in LSIL-H and 81% in HSIL.

HSIL smears were the least likely to have negative histology on follow-up, while ASC-H was associated with the highest frequency of negative histology compared to other cytological categories [10.6%]. This finding was noted in a previous study which reported the highest rate of negative histology [12.8%] in the ASC-H category. Extrapolating from our data this may be attributed to ASC-H being predominantly diagnosed [56%] in a postmenopausal age group. Furthermore, smears with a diagnosis of ASC-H were least likely to be inclusive of the transformation zone, which may arguably have an impact on the detection of a squamous intraepithelial lesion [SIL]. 68.7% of our cases were inclusive of the transformation zone with the highest rates seen in HSIL [82,8%] and LSIL-H [65.4%] categories. This may reflect the younger age group in these two categories which were 39 and 39.5 years respectively compared to a perimenopausal population group in the LSIL and ASC-H groups.

A number of researchers have looked into the incidence of high-risk HPV subtypes in both ASC-H and LSIL-H cases. Many of these studies have brought data to the forefront that LSIL-H is indeed associated with an increased risk of high risk HPV [HR-HPV](3,16,20,23). A study by Owens et al [2007] showed a significant difference in the prevalence of HR-HPV when comparing LSIL-H and ASC-H categories. Owen's study showed that all LSIL-H cases were associated with HR-HPV and only 59% of the ASC-H cases showed this phenotype (3). Zhou et al [2012] demonstrated similar findings with LSIL-H showing a higher frequency of HR-HPV [92%] when compared to ASC-H [78%], LSIL [74%] or LSIL and ASC-H combined [74%]. A striking similarity was found regarding the HPV infection pattern and infection rates of HR-HPV between LSIL-H and HSIL(16). A later study corroborated these findings and reported the prevalence of high-risk HPV in LSIL-H [86.9%] was comparable to the high risk HPV prevalence in HSIL [92.6%] when compared to ASC-H [68.8%] and LSIL [78.8%](10). This has subsequently steered some authors proposing similar management algorithms for LSIL-H and HSIL patients(16). A limiting factor of many of these studies was that HPV testing was performed on small sample sizes. In addition, many of the studies used participants who were HIV-negative making it difficult to extrapolate these findings into our population where a high proportion of women in the general South African population with cervical lesions are HIV positive.

In our study, women with a CD4 count of less than 200 had the highest risk [44.9%] of acquiring or being diagnosed with a high-grade lesion. The most common CD4 count was above 1000 mm^3 for women with a diagnosis of LSIL-H [29.6%] and most [26.5%] of these women had a viral load that was lower than detectable limit(7). The CD4 count and viral loads of ASC-H and LSIL-H patients showed no statistically significant differences. Overall, there was an increased risk of a high-grade lesion [48.3%] with viral loads of over 10 000. Even though HIV seropositivity and lower CD4 count have been shown to be the strongest factors predisposing to the development of persistent HPV infections, some studies have shown no relationship of Pap smear abnormalities among HIV-positive women with their immune status or duration of treatment(24,25). One study acknowledged that despite the lack of differences in the prevalence and persistence of high risk HPV in treated and untreated women or the natural history of the related cervical lesions, a significant reduction in the incidence of new HPV-16 and 18 infections were observed in the ART-treated women(26). Firnhaber et al [2012] reported increased clearance rates of SIL and oncogenic HPV with consistent adherence to HAART(6). In their study, they showed that HAART had a significant effect on any SIL with CD4 counts

of <350 cells/mm^3. There was no significant difference seen in the effect of HAART in women with CD4 counts >350 cells/mm^3.

The limitations of this study include the lack of HPV testing which is not currently standard of care in the South African public sector(27,28). This was a retrospective study and as such, there were challenges in obtaining information regarding the follow-up of these patients. Data regarding initiation, duration and adherence to HIV treatment was not incorporated. In view of this, CD4 counts and viral loads were used as surrogate markers for compliance. The lack of follow up creates difficulty in interpreting results for lesions that may have regressed or progressed. The strengths of this study include that it was performed in a government HIV clinic, providing an opportunity to extrapolate the findings more closely to the real-world experiences and clinical setting in which many HIV infected women are seen.

2.3.5. Conclusions

In this study of HIV-infected women, most LSIL-H proved to be LSIL on histologic follow up with a rate of HSIL of 31.8%. The majority of women had a CD4 count above 1000 and an undetectable viral load. Similar findings were observed on biopsy follow up of women with ASC-H diagnosed cytologically(10). Thus HIV-infected women, in line with their HIV-negative counterparts, who have cytomorphology intermediate between LSIL and HSIL can have their Pap smears reported as ASC-H with the recommended management being colposcopy and directed biopsy.

2.3.6. Acknowledgement

Authors thank the contribution by Fadilah Ebrahim for assistance with data retrieval. We also thank Gill Hendry for assisting with the biostatistics and explanation of statistical analysis.

An extended thank you to Right to Care Clinic for kindly providing permission for use of their data.

2.3.7. Statement of Ethics

Ethics clearance has been granted by the Human Research Ethics Committee [Medical] and Faculty of Health Sciences of the University of Witwatersrand, Postgraduate Office: certificate number Study number – M190530

2.3.8. Disclosure Statement

The authors have no conflicts of interest to declare.

2.3.9. Funding Sources

No funding was received for the purposes of this study.

2.3.10. Author Contributions

Lucretia Mekoa, Conceptual organization as senior author, collection of data, data analysis and writing the manuscript.

Pam Michelow, Supervisor, reviewing and editing manuscript

Carla J. Chibwesha, review and editing of manuscript.

2.4. Figure Legends

Fig 1. Overview of study plan

Fig 2. Mean CD4 counts across cytological categories

Fig 3. Mean viral loads across cytological categories

Fig 4. Conventional cervical smear showing LSIL (thin arrow). At the thick arrow, cells with a higher nuclear: cytoplasmic ratio are noted that may represent HSIL i.e. LSIL-H (Papanicolaou stain, x400). A2: Corresponding cervical biopsy showing LSIL (H&E, X100). B1: Conventional cervical smear showing LSIL (thin arrow). At the thick arrow, cells with a higher nuclear: cytoplasmic ratio are noted that may represent HSIL i.e. LSIL-H (Papanicolaou stain, x400). B2: Corresponding cervical biopsy showing HSIL (H&E, X200).

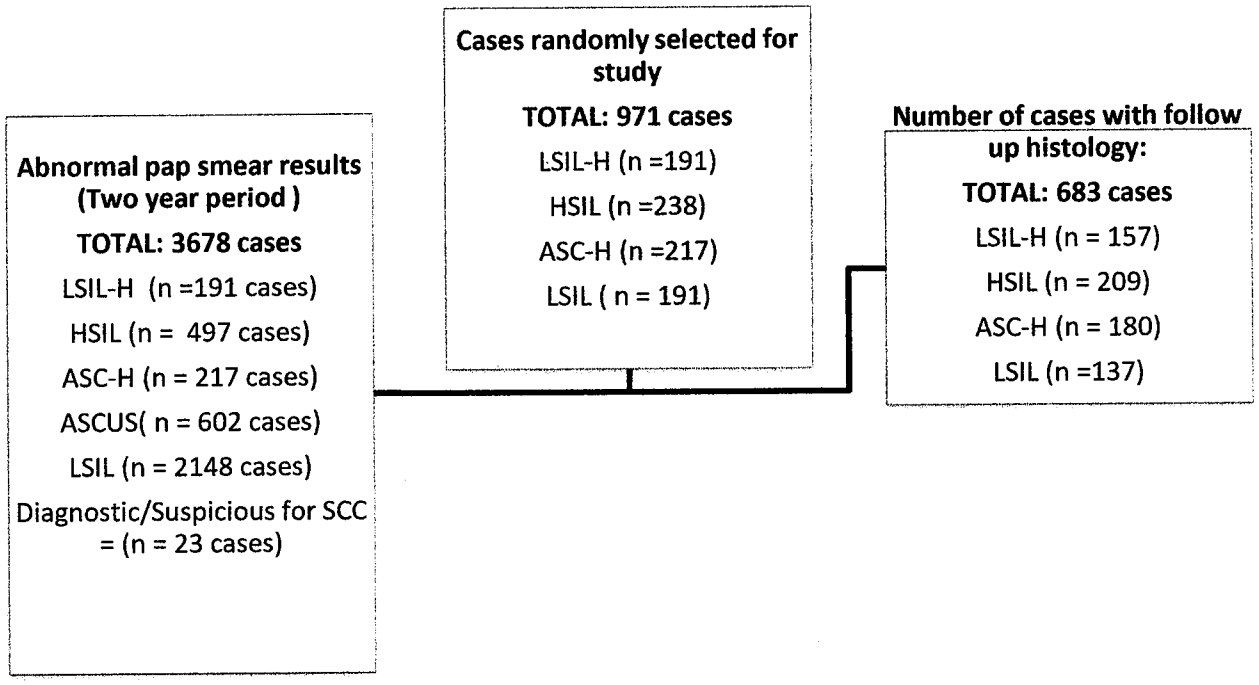

FIG 1. OVERVIEW OF STUDY PLAN

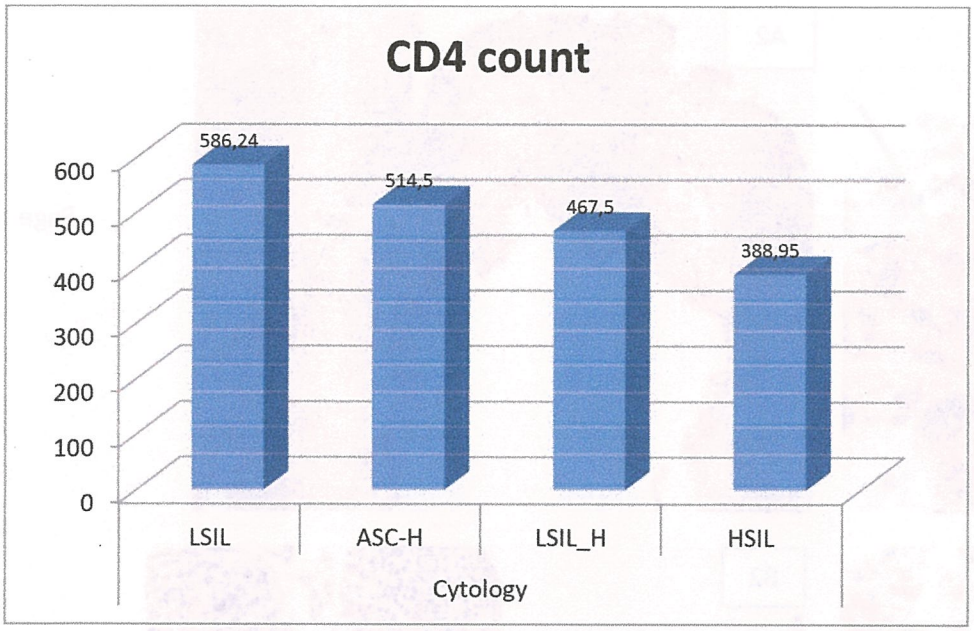

Fig 2. Mean CD4 counts across cytological categories.

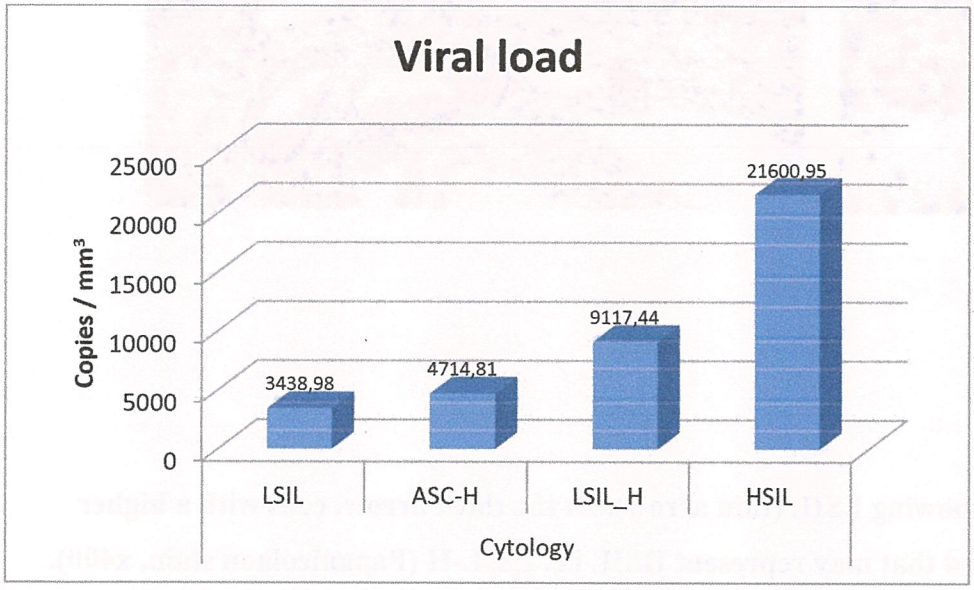

Fig 3. Mean viral loads across cytological categories

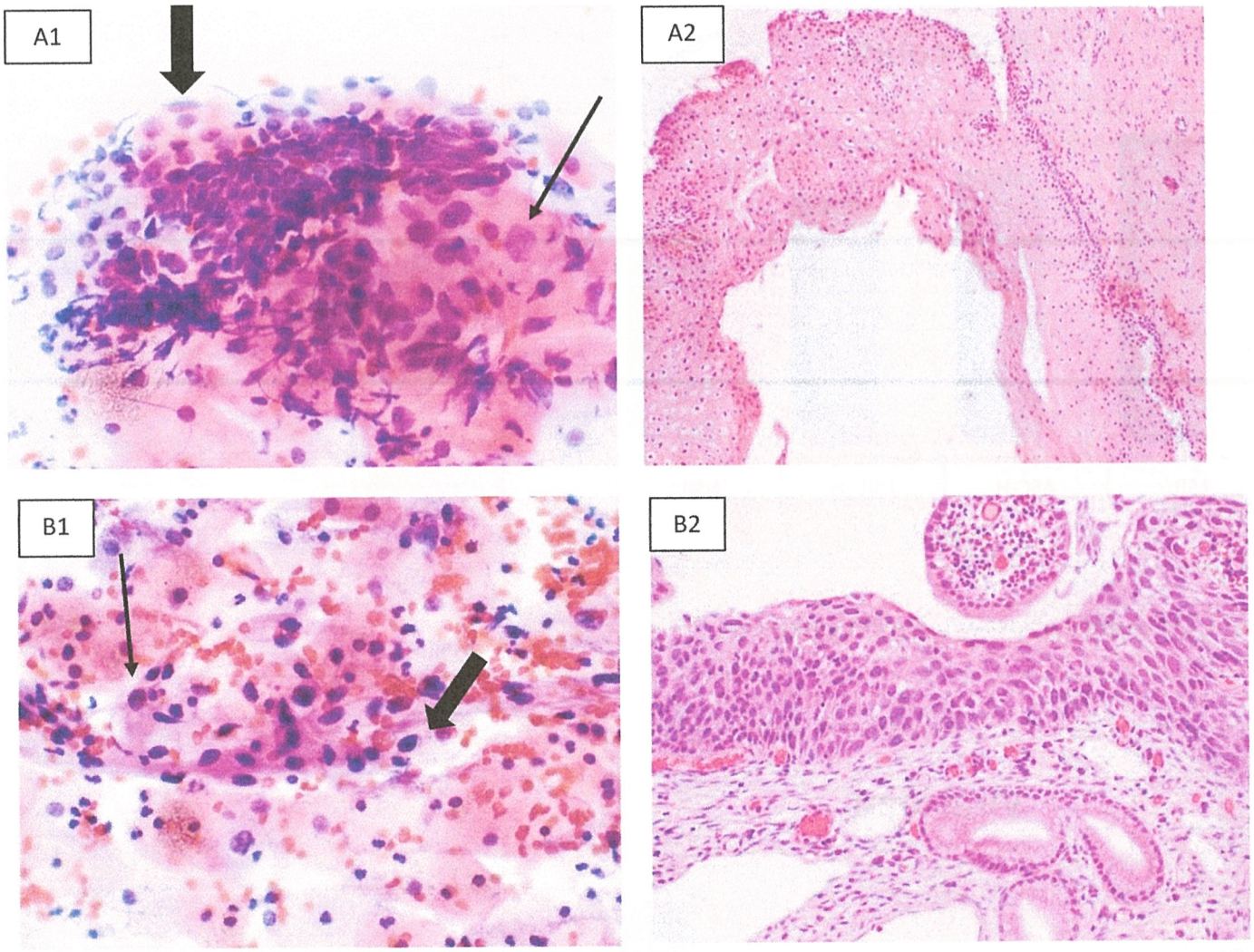

Fig 4.

A1: Conventional cervical smear showing LSIL (thin arrow). At the thick arrow, cells with a higher nuclear: cytoplasmic ratio are noted that may represent HSIL i.e. LSIL-H (Papanicolaou stain, x400). A2: Corresponding cervical biopsy showing LSIL (H&E, X100). B1: Conventional cervical smear showing LSIL (thin arrow). At the thick arrow, cells with a higher nuclear: cytoplasmic ratio are noted that may represent HSIL i.e. LSIL-H (Papanicolaou stain, x400). B2: Corresponding cervical biopsy showing HSIL (H&E, X200).

2.5. Tables

Table 1. Age distribution across abnormal Pap Categories

			Cytology diagnosis				
			LSIL	ASC-H	LSIL_H	HSIL	Total
Age grouped	20 - 29	Count	10	11	25	27	73
		% within Age grouped	13.7%	15.1%	34.2%	37.0%	100.0%
	30-39	Count	51	59	57	82	249
		% within Age grouped	20.5%	23.7%	22.9%	32.9%	100.0%
	40-49	Count	53	55	46	65	219
		% within Age grouped	24.2%	25.1%	21.0%	29.7%	100.0%
	50-59	Count	19	40	24	27	110
		% within Age grouped	17.3%	36.4%	21.8%	24.5%	100.0%
	60-69	Count	4	14	4	3	25
		% within Age grouped	16.0%	56.0%	16.0%	12.0%	100.0%
Total		Count	137	179	156	204	676
		% within Age grouped	20.3%	26.5%	23.1%	30.2%	100.0%

Table 2. CD4 counts across cytological categories

			Cytology diagnosis				Total
			LSIL	ASC-H	LSIL_H	HSIL	
CD4 grouped	up to 200	Count	13	19	27	48	107
		% within CD4 grouped	12.1%	17.8%	25.2%	44.9%	100.0%
	201-400	Count	26	36	42	49	153
		% within CD4 grouped	17.0%	23.5%	27.5%	32.0%	100.0%
	401-500	Count	9	21	19	25	74
		% within CD4 grouped	12.2%	28.4%	25.7%	33.8%	100.0%
	501-600	Count	14	20	20	16	70
		% within CD4 grouped	20.0%	28.6%	28.6%	22.9%	100.0%
	601-700	Count	11	22	11	11	55
		% within CD4 grouped	20.0%	40.0%	20.0%	20.0%	100.0%
	701-800	Count	17	8	6	9	40
		% within CD4 grouped	42.5%	20.0%	15.0%	22.5%	100.0%
	801-900	Count	9	19	9	3	40
		% within CD4 grouped	22.5%	47.5%	22.5%	7.5%	100.0%
	901-1000	Count	5	3	3	4	15
		% within CD4 grouped	33.3%	20.0%	20.0%	26.7%	100.0%
	>1000	Count	10	5	8	4	27
		% within CD4 grouped	37.0%	18.5%	29.6%	14.8%	100.0%
Total		Count	114	153	145	169	581
		% within CD4 grouped	19.6%	26.3%	25.0%	29.1%	100.0%

Table 3. Viral load across cytological categories

VL grouped * Cytology diagnosis Crosstabulation

			Cytology diagnosis				Total
			LSIL	ASC-H	LSIL_H	HSIL	
VL grouped	LDL	Count	37	43	45	45	170
		% within VL grouped	21.8%	25.3%	26.5%	26.5%	100.0%
	<1000	Count	84	116	92	117	409
		% within VL grouped	20.5%	28.4%	22.5%	28.6%	100.0%
	1001 - 10000	Count	7	7	7	13	34
		% within VL grouped	20.6%	20.6%	20.6%	38.2%	100.0%
	>10000	Count	3	5	7	14	29
		% within VL grouped	10.3%	17.2%	24.1%	48.3%	100.0%
Total		Count	131	171	151	189	642
		% within VL grouped	20.4%	26.6%	23.5%	29.4%	100.0%

Table 4. Biopsy results in relation to initial cytological diagnosis

Histology diagnosis

Cytology diagnosis			Frequency	Percent	Valid Percent	Cumulative Percent
LSIL	Valid	LSIL	103	75.2	75.2	75.2
		HSIL	18	13.1	13.1	88.3
		AIS	2	1.5	1.5	89.8
		NILM	14	10.2	10.2	100.0
		Total	137	100.0	100.0	
ASC-H	Valid	LSIL	104	57.8	57.8	57.8
		HSIL	48	26.7	26.7	84.4
		SCC	5	2.8	2.8	87.2
		NILM	23	12.8	12.8	100.0
		Total	180	100.0	100.0	
LSIL_H	Valid	LSIL	97	61.8	61.8	61.8
		HSIL	50	31.8	31.8	93.6
		NILM	10	6.4	6.4	100.0
		Total	157	100.0	100.0	
HSIL	Valid	LSIL	66	31.6	31.6	31.6
		HSIL	132	63.2	63.2	94.7
		SCC	2	1.0	1.0	95.7
		NILM	9	4.3	4.3	100.0
		Total	209	100.0	100.0	

Table 5. Summary of literature review of rate of high grade squamous intraepithelial lesions on histological follow up

STUDY REFERENCE	NUMBER OF CASES	LSIL (%)	ASC-H (%)	LSIL-H (%)	HSIL (%)
1. Nasser et al (2003)[8]	294 (150 LSIL; 144 LSIL-H)	23%	Not assessed	42%	Not assessed
2. Elsheik et al (2006)[19]	1033 (575 LSIL; 59 LSIL-H, 110 ASC-H, 289 HSIL)	13%	44.6%	40.7%	74%
3. Shidham et al (2007)[20]	792 (557 LSIL, 88 LSIL-H, 38 ASC-H, 109 HSIL)	10%	31%	33%	69%
4. Owens et al (2007)[3]	703 (426 LSIL, 86 ASC-H, 81 LSIL-H, 110 HSIL)	10.8%	23%	40%	65.5%
5. Nourhji et al (2008)[21]	378 (194 LSIL-H, 184 LSIL)	7%		24%	
6. Ince et al (2011)[12]	1713 (185 LSIL-H, 127 ASC-H, LSIL 1137, HSIL 264)	21%	43%	40%	81%
7. Thrall et al (2008)[9]	2053 (126 LSIL-H, 1828, LSIL, 99 ASC-H)	7.6%	35.3%	31.9%	
8. Chiaffarano et al (2017)[22]	1049 (394 ASCUS, 481 LSIL, 66 LSIL-H, 33 ASC-H, 75 HSIL)	8%	52%	30%	77%
9. Segura et al (2019)[10]	3272 (2412 LSIL, 257 LSIL-H, 224 ASC-H, 379 HSIL)	7.5%	33.5%	31.5%	64.4%
10. Current study (2020)	683 (157 LSIL-H , 209 HSIL, 180 ASC-H, 137 LSIL)	**13.1%**	**26.7%**	**31.8%**	**63.2%**

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SECTION 3: APPENDICES

3.1 Ethics Approval

R14/45 Dr LP Mekoa

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M190530

NAME: (Principal Investigator)	Dr LP Mekoa
DEPARTMENT:	School of Pathology Department of Anatomical Pathology Medical School University
PROJECT TITLE:	Cervical cytology with a diagnosis of at least low-grade squamous intraepithelial lesion cannot exclude high-grade squamous intraepithelial lesion (LSIL-H). What does this mean in HIV positive women? A cytologic-histologic correlation
DATE CONSIDERED:	2019/05/31
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Professor P Michelow
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2019/07/18

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Philip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. I **agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in May and will therefore require re-certification will be due early in the month of May each year. Unreported changes in the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

24/07/2019
Date

PLEASE QUOTE THE CLEARANCE CERTIFICATE NUMBER IN ALL ENQUIRIES

3.2. Head of Department Approval Letter

NATIONAL HEALTH LABORATORY SERVICE
UNIVERSITY OF THE WITWATERSRAND – JOHANNESBURG

SCHOOL OF PATHOLOGY
Division of Anatomical Pathology

P.O. Box 1038, Johannesburg 2000
Tel : +27-11-489-8479
+27-11- 489-8492
Fax::+27-11-489-8512

Division of Anatomical Pathology
Faculty of Health Sciences
7 York Road
Parktown

Practice Number: 5200296

04th April 2019

Human Research Ethics Committee (MEDICAL)
University of the Witwatersrand, Johannesburg

Dear Sir/ Madam

Mmed research

I hereby give permission for Mmed research to be carried out in the Department of Anatomical Pathology, School of Pathology, Faculty of Health Sciences by Lucretia Mekoa and Pam Michelow from the University of the Witwatersrand Anatomical Pathology Department.

The research will be retrospective in nature and consist of analyzing cytology and histology records retrieved from the NHLS trackcare database for patients following up at Right to Care clinic at Helen Joseph hospital, Johannesburg. This will include analysis of pap smears for the time period 1st July 2013 to 30 June 2015 and comparison of these specimens with the relative histology. The main objective will be to analyze the diagnosis of at least Low-grade squamous intraepithelial lesion cannot Exclude high-grade squamous intraepithelial Lesion (LSIL-H) and determine whether this condition should be a distinct cytological entity. There will be a particular focus of this entity in the HIV context.

Yours sincerely

Dr Yvonne Perner
HOD: Anatomical Pathology Division
School of Pathology
Faculty of Health Sciences
University of the Witwatersrand/ NHLS

3.3. Letter of Approval Right to Care

Right to care
Treating Health Seriously

15 April 2019

Human Research Ethics Committee (MEDICAL)
University of the Witwatersrand, Johannesburg

Dear HREC Committee:

I'm am writing this letter in support of the MMed research protocol entitled, "Cervical cytology with a diagnosis of at least low-grade squamous intraepithelial lesion cannot exclude high-grade squamous intraepithelial lesion (LSIL-H): what does this mean in HIV positive women?" submitted by Dr. Lucretia Mekoa.

The primary objective of the project proposed by Dr. Mekoa will be to determine whether this condition LSIL-H should be a distinct cytological entity. The research will be retrospective in nature, and will involve the analysis histology records retrieved from the NHLS trackcare database for HIV positive women receiving cervical screening at Themba Lethu Clinic, Helen Joseph Hospital. This will include analysis of Pap smears for the time period 01 July 2013 to 30 June 2015, and comparison of these specimens relative to cervical histology.

For her project, Dr. Mekoa will be supervised by Dr. Pamela Michelow in the Department Anatomical Pathology at University of the Witwatersrand. We look forward to Dr. Mekoa's important findings.

Sincerely,

Carla Chibwesha, MD, MSc
Associate Professor of ObGyn, UNC Global Women's Health
Co-Director, UNC-Wits-Right to Care Partnership for Cervical Cancer Prevention, Helen Joseph Hospital

3.4. Letter of Approval Helen Joseph Hospital

GAUTENG PROVINCE

REPUBLIC OF SOUTH AFRICA

Gauteng Department of Health
Helen Joseph Hospital
Enquiries: Dr M Mukansi
Research Committee Chairperson
Tel: (011) 489-0306/1087
Fax: (011) 489 1038
E-mail: Murunzi.mukansi@wits.ac.za

04 July 2019

To whom it may concern

Subject: HELEN JOSEPH HOSPITAL RESEARCH COMMITTEE APPLICATION

PROTOCOL TITLE: Cervical cytology with a diagnosis of at least low-grade squamous intraepithelial lesion cannot exclude high grade squamous intraepithelial lesion (LSIL-H) what does this means in HIV positive Women? A cytologic-histological Correlation.

Protocol Ref No: Lucretia Portia

Ethic Clearance: Pending
Principal investigator: Lucretia Portia

Department: Anatomical Pathology

Committee Recommendations
The Committee is giving you Conditional assess while awaiting the final ethical clearance certificate from the University of Witwatersrand HREC.

It is the duty of the researcher to collect the data to the relevant department after the Research Committee approved the study

Dr. M. Mukansi
Chairperson of HJH Ethic and Research Committee

3.5. Turnitin Originality report

ORIGINALITY REPORT

12%	3%	11%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Haijun Zhou, Mary R. Schwartz, Donna Coffey, Debora Smith, Dina R. Mody, Yimin Ge. "Should LSIL-H be a distinct cytology category?", Cancer Cytopathology, 2012 Publication	2%
2	Tarik M. Elsheikh. "The significance of "low-grade squamous intraepithelial lesion, cannot exclude high-grade squamous intraepithelial lesion" as a distinct squamous abnormality category in Papanicolaou tests", Cancer, 10/25/2006 Publication	1%
3	C. Hunter. "Cytology and outcome of LSIL: cannot exclude HSIL compared to ASC-H", Cytopathology, 02/2009 Publication	1%
4	www.science.gov Internet Source	1%
5	Sheila E. Segura, Gloria Ramos-Rivera, Laleh Hakima, Mark Suhriand, Samer Khader. "Low-	1%

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Word count:	3,399
Character count:	18,465
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3.6. Supervisor Letter: Turnitin Report

Turnitin report – Dr Lucretia Mekoa

Title: Cervical cytology with a diagnosis of Low-grade squamous intraepithelial lesion, cannot exclude high-grade squamous intraepithelial lesion [LSIL-H]. What does this mean for HIV positive women?

This research report was analyzed by the Turnitin programme on 13th of September 2020 and a similarity index of 12% was obtained. The highlighted areas in the report have been checked and I am satisfied that the report is the candidate's original work.

Yours Sincerely

Professor Pamela Michelow

3.7. Plagiarism Declaration

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

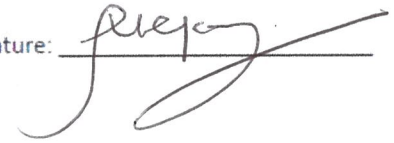
PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Lycretia Mekoa (Student number: 1808152) am a student registered for the degree of Mmed (Anatomical pathology) in the academic year 2020

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature:  Date: 04/10/2020

3.8. Protocol Application and Appointment of Supervisors

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG | FACULTY OF HEALTH SCIENCES

RECOMMENDATION FOR APPOINTMENT OF SUPERVISOR(S) OF RESEARCH REPORT, DISSERTATION OR THESIS

Motivation / Reason for Appointment:
- Wide knowledge and published in the field of cytopathology.
- Previous experience in supervising Mmeds.
- Involved in registrar teaching & training

Recommendation of Division / Department / School:

As above

Student Surname and Full name(s)	Lucretia Portia Mekoa
Student number	1808152
Degree	Mmed
Div / Dept / School	Anatomical Pathology
Title	cervical cytology in a diagnosis of LSIL-H, what does this mean in HIV +ve women?

(Supervisor 1): Pamela Michelow
(Name &Surname)

Supervision %: 100

Supervisor Qualifications: MBBCh MSc PODip(HSE)

Supervisor Department: Anatomical Pathology

Supervisor Telephone: 0114899402 E-mail: pamela.michelow@nhls.ac.z

Supervisor 2:
(Name &Surname)

Supervision %:

Supervisor Qualifications:

Supervisor Department:

Supervisor Telephone: E-mail:

Student Signature:

Supervisor 3:
(Name &Surname)

Supervision %:

Supervisor Qualifications:

Title of the Research Project:

Cervical cytology with a diagnosis of at least Low-grade Squamous intraepithelial lesion, cannot exclude high-grade squamous intraepithelial lesion, LSIL-H. What does this mean for HIV positive women?

Date: 28/4/2019 Applicant's Signature:

WHO WILL SUPERVISE THE PROJECT? (Where applicable)

Name Prof Pam Michelow Department: Anatomical Pathology

Telephone No: 011 4899402 Email: Pam. Michelow@nhls.ac.za

Signature: Date: 28/4/2019

HEAD / RESEARCH COORDINATOR OF DEPARTMENT / ENTITY IN WHICH STUDY WILL BE CONDUCTED (Where applicable) (Wits Students Academic HOD must sign)

Name: Dr Y. Perner Department / Entity: Department of Anatomical Pathology

Tel No: 011 4898479 Email: Yvonne.Perner@nhls.ac.za

Signature: Date: 28/4/2019

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

CANDIDATE'S SURNAME: [Please print] Mekoa	FIRST NAME/S. Lucretia	STUDENT NUMBER: 1802152
CURRENT QUALIFICATIONS: MBChB [UCT] ; DA(SA)		
TEL: 011 489 8468 CELL: 073 997 2639	E-MAIL: Lpmekoa@gmail.com	FAX: N/A
DEGREE FOR WHICH PROTOCOL IS BEING SUBMITTED: MMed, Anatomical Pathology		
PART-TIME OR FULL-TIME: Full time		
FIRST REGISTERED FOR THIS DEGREE:	TERM: January (First term)	YEAR: 2017
DEPARTMENT: Anatomical Pathology		
TITLE OF PROPOSED RESEARCH: Cervical cytology with a diagnosis of at least Low grade squamous intraepithelial lesion, LSIL-H; what does this mean for HIV +ve women?		
CANDIDATE'S SIGNATURE:		DATE: 11/4/2019
SUPERVISOR 1 (NAME & SURNAME): P Michelow		% Supervision 100%
SUPERVISOR'S QUALIFICATIONS: MBBCh MSc PGDip(HSE)		
SUPERVISOR'S DEPARTMENT: Anatomical Pathology		
SUPERVISOR'S ADDRESS / TEL / E-MAIL: Cytology Unit, NHLS, Bloemfontein.	pamela.michelow@nhls.ac.za	
SUPERVISOR 2 (NAME & SURNAME):		% Supervision
SUPERVISOR'S QUALIFICATIONS		
SUPERVISOR'S ADDRESS / TEL / E-MAIL:		
SUPERVISOR 3 (NAME & SURNAME):		% Supervision
SUPERVISOR'S QUALIFICATIONS		
SUPERVISOR'S ADDRESS / TEL / E-MAIL:		

SYNOPSIS OF RESEARCH: (Brief summary of proposed research project; between 200-300 words only; with sub-headings: an introduction and justification for study, aim/s, proposed methodology and expected outcome/s)
[Use reverse side of this page if more space is required]

Introduction:

South Africa has one of the highest rates of HIV in the world. The prevalence of Atypical squamous cells (ASCUS) and squamous intraepithelial lesion (SIL) have been found to be upto twice as higher compared to HIV negative women. Current reporting of cervical lesions is based on a two-tiered classification system/scheme. The lesions are thought to have two different natural histories, with LSIL representing transient lesions and HSIL precancerous lesions. The current management guidelines for cervical lesions all use the LSIL and HSIL nomenclature.

Justification for study:

Occassionally in cervical cytology specimens [illegible]

WITS ETHICS NOT REQUIRED: WITS ETHICS PENDING: WITS ETHICS APPROVED: (circle appropriate symbol)*	Yes No Yes No es No	IF SUPPLY ETHICS CLEARANCE CERTIFICATE AS ATTACHMENT AND INCLUDE ETHICS NUMBER HERE:
*Please note the final human ethics clearance certificate or animal ethics certificate must be available prior to starting research		
As supervisor/s, I/we confirm that I have read the protocol which has been submitted for assessment.		
SIGNATURE OF SUPERVISOR/S:		
SIGNATURE PC OFFICE STAFF	REGISTERED YES ✓ NO.....	STAMP 17 APR 2019

SYNOPSIS OF RESEARCH CONTINUED

there is currently no consensus method for reporting these cases across laboratories and terms such as 'LSIL cannot exclude HSIL' or 'LSIL-H' have been proposed. Furthermore current management guidelines all use LSIL and HSIL nomenclature without an intermediate category, leading to confusion among clinicians regarding the patient's appropriate management when 'LSIL-H' is reported. In addition, the significance of this category in the HIV context has never been interrogated in the literature.

AIMS AND OBJECTIVES.

1. Determine the histological outcomes in HIV positive patients with pap tests interpreted as LSIL, LSIL-H, ASCUS, HSIL and ASCH with particular emphasis on LSIL-H in an HIV infected population
2. To determine if LSIL-H represents a distinct morphological category associated with different histological outcomes when compared to both LSIL and ASC-H in an HIV infected population.
3. Determine the prevalence of LSIL, ASCUS, ASC-H and LSIL-H at "Right to care" clinic Helen Joseph hospital.
4. Determine the histological findings of LSIL-H.
5. Compare histological follow up of LSIL-H with histological follow up of ASCUS, LSIL, ASC-H and HSIL.
6. Determine the best management of women with LSIL-H in the South African public health sector.

Methodology

A search of the laboratory information database at the NHLS Anatomical pathology department at Charlotte Maxeke hospital will be undertaken. The search will include cases of all pap smears performed on the first of July 2013 to 30th June 2015. INFORMATION regarding the patient's CD4 count, viral load and whether or not they are on ARV's at the time of pap smear will

11 March 2019/MP

Expected outcomes:

1. Increased prevalence of HSIL lesions in the HIV population.
2. LSIL-H represents a distinct cytological category with different histological outcomes as compared to LSIL and ASC-H.
3. optimal management of HIV positive women with a diagnosis of ASC-H is colposcopy and directed biopsy.
4. Prevalence of the term ASC-H is between 1 and 5% of total pap smear diagnosis.
5. Histological follow up of women with a diagnosis of 'LSIL-H' will include both CIN1/LSIL and CIN2-3/HSIL.

3.9. Letter to the Editor Acta Cytologica

16 September 2020

Dear Editor

Cervical cytology with a diagnosis of Low-grade squamous intraepithelial lesion, cannot exclude high-grade squamous intraepithelial lesion (LSIL-H). What does this mean for HIV positive women?

Cervical carcinoma is currently the leading killer of women in Sub-Saharan Africa. Furthermore, South Africa has one of the highest rates of HIV and HPV infection rates in the world. HIV positive women are 5 times more likely to harbor high-risk HPV infection compared to HIV negative women and with more women gaining access to combined antiretroviral treatment they are living relatively longer lives and are thus at higher risk for progression to Cervical intraepithelial neoplasia (CIN) 2/3 and to invasive cervical carcinoma. Early screening for cervical carcinoma upon diagnosis and placement of effective and reproducible management protocols is thus paramount.

In 2014, the Bethesda committee declined to add the category LSIL-H in the reporting and recommended management of cervical smears creating inconsistencies with regards to the manner in which cytopathologists report these lesions. Previous studies in the literature are of the opinion that these lesions show different histological outcomes and rates of HPV prevalence when compared to LSIL and HSIL.

The intent of this current study is to investigate the category LSIL-H in an HIV-positive Population. To the best of our knowledge, this has not as yet been interrogated in the literature. The aim is to follow up the outcome of these cases on histology biopsies in order to better understand their natural history. This is relevant in the context of the third world country where allocation of resources needs to be appropriated in the most economically feasible manner.

We believe that this article will be of interest to readers of Acta Cytologica as it is pertinent to those who live in under-developed nations who may experience similar problems in addition to all readers of this esteemed journal who report out cervical cytology.

This manuscript has not been submitted elsewhere and the authors have no financial disclosures.

Yours sincerely

Dr Lucretia Mekoa

Dept of Anatomical Pathology National Health Laboratory Service and University of the Witwatersrand, Johannesburg, South Africa
Tel: 011 489 8468/8465
Email: Lucretia.Mekoa@nhls.ac.za or Lpmekoa@gmail.com

3.10. Manuscript Submission Confirmation

Acta Cytologica <acy@manuscriptmanager.net> Sep 16, 2020, 8:34 PM (8 days ago)

to me

Submission: ACY-2020-9-7 - Cervical cytology with a diagnosis of Low-grade squamous intraepithelial lesion, cannot exclude high-grade squamous intraepithelial lesion [LSIL-H]. What does this mean for HIV positive women?

Submitting author: Dr. Lucretia Mekoa

Attention: Dr. Mekoa

Dear Author

Thank you very much for submitting the above manuscript. Please use the manuscript number as listed above on all correspondence about the manuscript.

The manuscript will now be forwarded to our Editors and reviewers and we shall inform you as soon as a decision has been made by the editorial board.

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3.11. Certificate of submission for examination of masters research report signed by higher degrees candidates

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

CERTIFICATE OF SUBMISSION FOR EXAMINATION OF MASTERS RESEARCH REPORT / DISSERTATION OR PHD THESIS SIGNED BY HIGHER DEGREES CANDIDATES

Full name	Lucretia Portia Mekoa		
Student number	1808152		
Title of submitted Research Project: Cervical cytology with a diagnosis of Low-grade squamous intraepithelial lesion, cannot exclude high-grade squamous intraepithelial lesion [LSIL-H]. What does this mean for HIV positive women? *NB: If this title is different to your previously approved title, no further action can be taken by the Faculty Office until a change of title has been approved.*			
Contact no	072 997 8639	E-mail	Lpmekoa@gmail.com

1. If you are likely to move in the next 6-12 months, please give the anticipated date of move: Not applicable.
2. I hereby submit my **Masters (research report) for examination**
3. I have checked all copies of my research report and declare that no pages are missing or poorly reproduced.
4. I have submitted **two** bound copies and **one** electronic copy on CD
5. **I confirm that I have:**

   a) A signed declaration indicating my understanding of the concept of plagiarism and a denial of plagiarism in my research document.

b) A report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document included as an appendix.

6. **I confirm that I have:**
   a) Not used either human or animal tissue or records: Yes
   b) If yes: I have included the ethics waiver letter pertinent to my research as an appendix Yes
   c) Done research using animals: **No**
      If yes: I have included a copy of the animal ethics committee clearance certificate as an appendix in this document: N/A
   d) Done research using human subjects, human tissue or patient records **Yes**
      If yes: I have included a copy of the human ethics clearance certificate as an appendix to the research
      document **Yes**
7. I understand that I may not graduate unless my University fees have been paid in full.
8. My Supervisor(s) names, departments, telephone numbers and email addresses are as follows:

Name	Professor Pamela Michelow		
Department	Anatomical Pathology		
Telephone	082 469 6170/011 489 9402	E-mail	Pamela.Michelow@nhls.ac.za
Name			
Department			
Telephone		E-mail	

List all publications, which you have published in peer-reviewed journals from your postgraduate research report/dissertation/thesis during the course of your studies in the Faculty of Health Sciences (Include authors, year, title of paper, name of journal, volume number and page numbers). This information is mandatory.

This research report was submitted to Acta Cytologica on 16 September 2020 and is currently undergoing peer review.

Signature of candidate: _____ Date: 04/10/2020

CERTIFICATE OF SUBMISSION FOR EXAMINATION SIGNED BY SUPERVISORS OF HIGHER DEGREES CANDIDATES

Full name	Lucretia Mekoa
Student number	1808152

Candidate for the degree of: _____MMed (Anatomical Pathology)_____
has submitted his/her thesis/dissertation/research report

Entitled: _____Cervical cytology with a diagnosis of at least low grade squamous epithelial lesion cannot exclude high grade squamous intraepithelial lesion (LSIL-H): What does this mean in an HIV positive women? A cytologic-histologic correlation.

Contact no	0114898479/69	E-mail	lpmekoa@gmail.com

Mark with an X on appropriate box	**Yes**	**No**
Has this thesis/dissertation/research report been submitted with the acquiescence of the supervisor?	X	
To the best of your knowledge are you able to verify that this is the candidate's work, except as otherwise stated by the candidate?	X	
The substance (nor any part of it) has not been submitted in the past nor is being submitted for a degree in any other university?	X	
The candidate has acknowledged wherever any information used in the thesis, dissertation or other work has been obtained by him/her while employed by, or working under the aegis of, any person or organization other than the University or its associated institutions?	X	
Have examiners been nominated and approved?	X	

I certify that this thesis/dissertation/research report has the approval of the Animal Ethics Committee / Committee for Research on Human Subjects and the Number of the Certificate of Approval is:

_____M190530_____

List all publications, which your student has published in peer-reviewed journals from his/her postgraduate research report/dissertation/thesis during the course of his/her studies in the Faculty of Health Sciences (Include authors, year, title of paper, name of journal, volume number and page numbers). This information is mandatory.

One manuscript was submitted to Acta Cytologica in mid-September 2020 and is currently undergoing peer review. __

Lucretia Mekoa, Carla J. Chibwesha, Pamela Michelow. Cervical cytology with a diagnosis of Low-grade squamous intraepithelial lesion, cannot exclude high-grade squamous intraepithelial lesion [LSIL-H]. What does this mean for HIV positive women?

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Name of Supervisor 3:

Telephone: Email:

Signature: Date:

==========

IMPORTANT NOTICE WITH REGARD TO THE SENATE STANDING ORDERS:

A.22 Submission against advice of Supervisor

If the Supervisor is not prepared to agree to the submission of a thesis, the candidate shall still be entitled, if he or she wishes, to submit it for examination. When a thesis is submitted against the advice of the Supervisor, this should be recorded in the minutes of the Faculty Graduate Studies Committee. In such a case, no internal examiners are appointed but a Supervisor's report will still be required. After the examination process, the external examiner(s) will be advised by the Chairperson of the Faculty Graduate Studies Committee that the thesis was submitted against the advice of the Supervisor.

A.24 *Nomination of Examiners*:

Nomination of examiners should take place at least six weeks before submission of the thesis or dissertation. *(The Postgraduate Office will not accept any submission for examination without the confirmed appointment of the nominated examiners.)*

A.25 Confidentiality of names of examiners (both external and internal)

The names of the examiners should be confidential during the examination process and may only be revealed to the candidate with the acquiescence of the examiner once the final version of the thesis has been submitted to the Faculty and the process has been completed.

3.13. Final submission checklist

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

FINAL SUBMISSION CHECKLIST

Full name of candidate	Lucretia Portia Meko g		
Student number	1808152		
Name of Degree	Mmed', Anatomical pathology	Date submitted	

Submission requirements Tick list √	
Student enrolled for current year (the research unit must be part of this enrolment, as a record must be active to capture the PASS result)	
Research Title checked and correct on system	
2 unbound copies of the research	
Declaration signed by the student, with current date (not 1st submission date)	
Copy of ethics clearance certificate (as an annexure in the research output)	
CD of copy of research (must be in PDF format)	
Signed supervisor submission form (if more than one supervisor ALL supervisors' signatures must appear)	
ETD form (signed by supervisor(s) and candidate)	
ETD payment receipt (R180.00)	
HOD/HOS approval letter	
Student's list of corrections (only if corrections had been recommended – in the case where no further corrections is the outcome, this is not needed)	
Research report passed with distinction	
Qualified with distinction	
Qualified without distinction	

MMed and MDent students: Final exams passed, prior to final submission of research report? If not, when will final exams be written?

_____

_____

Full name of PG Staff:

_____

SECTION 4: RESEARCH PROPOSAL

TITLE OF STUDY	**Cervical cytology with a diagnosis of at least Low-grade squamous intraepithelial lesion cannot exclude high-grade squamous intraepithelial lesion (LSIL-H) what does this mean in HIV positive women?** **A cytologic-histological correlation**
STUDENT NAME	Dr Lucretia Portia Mekoa
QUALIFICATIONS	MBBCh (UCT); DA (SA)
STUDENT NUMBER	1808152
DEPARTMENT	MMED (Anatomical Pathology) National Health Services Laboratory (NHLS) / University of the Witwatersrand
CONTACT DETAILS	Contact details: 072 997 8639 lpmekoa@gmail.com
SUPERVISOR	**Professor P. Michelow** **MBBCh Msc (Med Sci) PGDip (HSE) MIAC** Department of Cytology, Anatomical Pathology National Health Services Laboratory (NHLS) / University of the Witwatersrand

4.1. Introduction

Globally, cervical carcinoma is the fourth most frequent cancer affecting women and accounts for 12% of cancers in resource-constrained settings. It is currently the leading killer of women in Sub-Saharan Africa. Furthermore, South Africa has one of the highest rates of HIV and HPV infection rates in the world[1]. Human papilloma virus infection (HPV) is a significant risk factor for the development of cervical carcinoma, more specifically chronic persistent HPV infections. HIV is a well-recognized cofactor for persistent HPV infections. It not only augments the virulence of the virus but also increases its aggressiveness thus accelerating the progression to malignant transformation[2]. As women are accessing combined antiretroviral treatment they are living relatively longer lives and are thus at higher risk for progression to Cervical intraepithelial neoplasia (CIN) 2/3 and to invasive cervical carcinoma[2]. The effect of antiretroviral therapy (ART) on the progression of HPV related disease is controversial[3]. Early screening for cervical cancer using Pap smears has had a major impact in detecting lesions early and preventing cervical cancer related deaths[1,2].

Bethesda system for reporting cervical cytology was introduced in 1988 and divided HPV-related disease into low-grade SIL (LSIL) and high-grade SIL (HSIL), which correspond to histologic lesions of CIN 1 and CIN 2 and/or precancerous CIN 3 (CIN 2/3), respectively[4,5]. It was further modified in 1991, 2001 and 2014. This classification system was put into place to clearly and effectively communicate diagnosis to clinicians in order to facilitate patient management. In addition, this classification system mirrors the biologic differences and behaviors between these two lesions. That is, LSIL lesions result from transient infections with high-risk HPV that regress spontaneously and HSIL represents pre-cancerous lesions[4].

In spite of these guidelines, diagnostic dilemmas occur in the interpretation of cervical cytology specimens which show intermediate morphological patterns in between LSIL and HSIL which has led to some authors calling this category "at least LSIL cannot exclude HSIL"[6]. Other proposed definitions include (i) LSIL with rare atypical cells suggestive but not diagnostic of HSIL, (ii) Atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H) without any reference to the LSIL, (iii) LSIL with ASC-H, (iv) HSIL or LSIL, and (v)

LSIL: cannot exclude HSIL (LSIL-H)[7,8,9]. A number of studies in the literature are of the opinion that these lesions show different histological outcomes and rates of HPV prevalence and have gone as far as investigating the frequency of malignancy in LSIL-H compared to LSIL, ASC-H and HSIL. A common theme in these studies is that LSIL-H is associated with higher rates of high-grade lesions as compared to LSIL, similar rates to ASC-H and lower rates when compared to HSIL[8,10].

Despite the similarities in outcome, there is currently an undefined relationship between ASC-H and LSIL-H and some researchers have suggested including LSIL-H in the same reporting category as ASC-H. One study however went to show that LSIL-H was associated with an increased frequency of high-risk HPV status as compared to ASC-H[6].

Even though the cytology literature has had heated disputes regarding LSIL-H as a distinct entity, this category was still excluded in the latest (2014) Bethesda revision. This has been done to maintain the two-tiered classification system. Furthermore, this category is not recognized by current management guidelines [5,11]. This can lead to confusion among clinicians regarding the appropriate management of these patients when LSIL-H is reported. In addition, the significance of this category in the HIV context has never been interrogated in the literature.

4.2.Aims and objectives

4.2.1. Aims:

a) Determine the histological outcome in HIV positive patients with Pap tests interpreted as LSIL, LSIL-H, HSIL and ASC-H with particular emphasis on LSIL-H in an HIV infected population.
b) To determine if LSIL-H represents a distinct morphologic category associated with different histological outcomes when compared to both LSIL AND ASC-H in an HIV infected population.

4.2.2. Specific objectives:

a) Determine the prevalence of LSIL, ASC-H, HSIL and LSIL-H at "Right to Care" clinic, Helen Joseph Hospital.
b) Determine the histological findings of LSIL-H.

c) Compare histological follow up of LSIL-H with histological follow up of LSIL, ASC-H AND HSIL.
d) To make recommendations for the best management of women with LSIL-H in the South African public health sector.

4.3. Study design:

This is a cross sectional retrospective study which will be performed on cytology and histology records retrieved from the NHLS track care database for patients following up at the Right to Care clinic at Helen Joseph Hospital, Johannesburg, South Africa for the time period 1st of July 2013-30 June 2015[5].

4.4. Study population and place of study:

The study population comprises all HIV positive adult female patients over the age of 18 who have undergone screening pap smears at Themba Lethu Clinic at Right to Care, located at Helen Joseph Hospital for the time period mentioned. These smears were performed routinely as part of established patient management and were not performed for study purposes. The laboratory receives Pap smears from public health care facilities of the central Gauteng region. This clinic receives approximately 2000 smears pap smears per year.

4.5.Inclusion criteria:

1. All HIV positive female patients over the age of 18.
2. Patients with a cytological diagnosis of LSIL, LSIL-H and HSIL with corresponding histology records retrieved by a computer based search.

4.6. Exclusion criteria:

1. HIV negative patients.

2. Female patients less than 18 years of age.

3. Patients with cervical cytology only without corresponding histopathology will be excluded.

4. Patients who underwent cervical cytology assessed at NHLS Braamfontein but have histology results from other departments other than those from the University of the Witwatersrand.

5. Patients who have not undergone a pap smear at Helen Joseph Hospital

6. Women who have Pap tests with ASCUS and atypical glandular abnormalities.

7. Patients with negative Pap smear results.

8. Inadequate specimens.

4.7. Data collection: methods and tools:

A search of the laboratory information system database at the National Health Laboratory Service (NHLS) Anatomical Pathology Department at the Johannesburg Hospital laboratory will be undertaken. The search will include cases of all pap smears performed on the 1st of July 2013 to the 30th of June 2015. Information regarding the patients CD4 count and viral load will be collected. All cases identified will be subjected to a second computer-based search for appropriate histology. This search will involve a cross-check of the patient's name and date of birth in the NHLS laboratory information system. Those cases with cytology and no corresponding histology will be excluded.

4.8. Data collection sheet:

Please refer to Appendix "B".

4.9 Sample size:

Following ethics approval, a data search will be conducted to determine how many women had pap smears performed for the aforementioned time period. Advice will be sought from a statistician regarding sample size required for statistical significance.

4.10. **Statistical planning:**

Statistical advice will be sought from postgraduate students in Epidemiology and Biostatistics from the School of Public Health, University of the Witwatersrand. The data will be captured

onto a spreadsheet using Microsoft Excel® 2010. Descriptive statistics (median, mean) will be used to describe the data. This data will be represented in the form of tables and histograms.

4.11. Ethical considerations:

Application to the Human Research Ethics Committee (Medical) and Postgraduate Office of the Faculty of Health Science, University of the Witwatersrand will be submitted for the clearance of this study. Patient anonymity should be ensured by allocating a study number to each patient. Only the primary researcher will have access to the patient's details. The patients to be included in the study are not known to the investigator, neither will it be possible to communicate directly with these patients or get any form of written or verbal consent. No personal details will be utilized. The information will be discussed with the statisticians in a completely anonymous form. Patient names will not be divulged at any time during or after the study. The information will only be accessible to the primary researcher and supervisor and the documents will be locked in cupboard. The data will be stored in a password protected computer. There will be no financial implications for patients, Helen Joseph Right to care clinic and the NHLS. This is a retrospective study and thus, no new procedures will be performed. In addition, this study will not influence patient care nor will it hold any financial implications for the patient. Permission to undertake this study will be obtained from Helen Joseph Right to Care Clinic as well as the department of Anatomical pathology, University of the Witswatersrand. Information acquired from this study will be disseminated to Right to Care, presented at a pathology conference and submitted to a cytopathology journal for publication.

4.12. **Limitations of the study:**

We may not be able to find histology results on all patients. More than one histology result may be available for a single patient with a risk of discrepancy between those results. In this case, the most serious lesion will be utilized as the final histological "gold standard" diagnosis. Where there is more than one cytology result for any patient, and there is a discrepancy in the diagnoses rendered, the first ("index") pap smear will be used. We are currently unable to assess HPV infection and subtypes as it is not available in the South African public health sector.

4.13. Budgeting and funding:

No additional funding will be necessary for the purposes of this study.

4.14. Timeline of events:

Activity	JAN '19	FEB '19	MAR '13	APR '19	MAY '19	JUN '19	JUL '19	AUG '19	SEP '19	OCT '19	NOV; '19	DEC' 19	JAN '20
Proposal	■	■	■	■									
Lit. review			■	■	■	■	■						
Submit propopsal				■									
Ethics					■								
Prop Corrections					■								
Data collection						■	■	■	■				
Data analysis										■	■	■	
Write up													■
Submission													

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4.16. Appendices

4.16.1. APPENDIX A: DEFINITION OF TERMS

Human Immunodeficiency Virus (HIV): A particular family of retroviruses that cause immunodeficiency.

Highly Active Antiretroviral Therapy (HAART): Cocktail of antiviral drugs used to treat HIV.

Papanicolaou smear: A screening process whereby epithelial cells are scraped from the cervix and placed on a slide to be stained and examined in order to detect epithelial abnormalities.

High grade Squamous Intraepithelial Lesion (HSIL): Moderate or severe dysplasia of cervical epithelium as determined on Pap smear.

Low grade Squamous Intraepithelial Lesion (LSIL): Mild dysplasia of cervical epithelium as determined on Pap smear.

Atypical squamous intraepithelial lesion cannot exclude High-grade squamous intraepithelial lesion (ASC-H): Defines a cytological category between atypical squamous cells of undetermined significance (ASCUS) and high-grade squamous intraepithelial lesion (HSIL)

LSIL-H: Defines a cytological category between LSIL and HSIL.

4.16.2. APPENDIX B: DATA COLLECTION SHEET

Basic Data Collection Sheet:

PATIENT NO.	AGE	CD4	VIRAL LOAD	TREATMENT (Y/N)	CYTOLOGY RESULTS	HISTO RESULT 1	HISTO RESULT 2	TIME PERIOD BETWEEN PAP SMEAR AND HISTOLOGY

Detailed data collection sheets are included below.

NILM: negative for intraepithelial lesion; ASCUS: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesions; LSIL-H: Low-grade squamous intraepithelial lesion cannot exclude high-grade squamous intraepithelial lesion. SCC: Squamous cell carcinoma. CIN1: cervical intraepithelial neoplasia 1; CIN2: cervical intraepithelial neoplasia 2; CIN3: cervical intraepithelial neoplasia 3.

TABLE 1: RELATIONSHIP BETWEEN CYTOLOGICAL DIAGNOSIS AND HISTOLOGY DIAGNOSIS.

TABLE 1	HISTOLOGICAL	DIAGNOSIS					
CYTOLOGICAL DIAGNOSIS							
NILM							
ASCUS							
LSIL							
LSIL-H							
ASC-H							
HSIL							
SCC							

TABLE 2: RELATIONSHIP OF AGE AND CYTOLOGICAL DIAGNOSIS

TABLE 2 AGE	NUMBER OF CASES	#NILM (%)	#LSIL(%)	#LSIL-H(%)	#ASC-H (%)	HSIL	SCC	#TOTAL NO. OF CASES (%)
<19								
20-24								
25-29								
30-34								
35-40								
41-45								

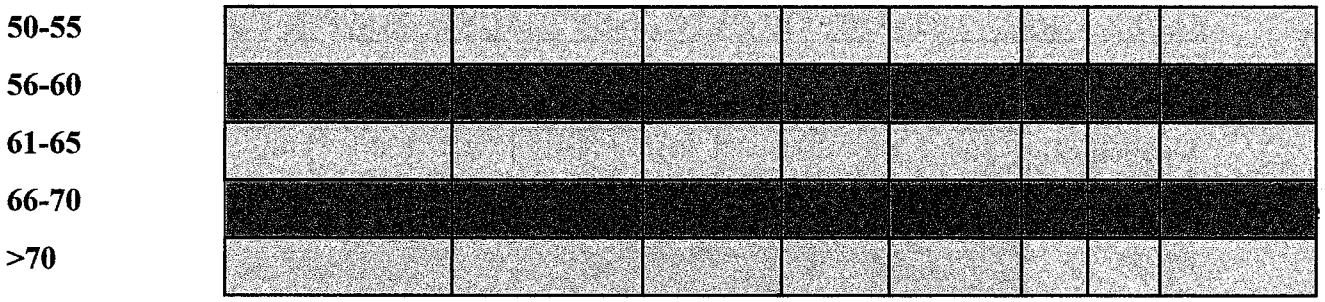
50-55
56-60
61-65
66-70
>70

TABLE 3: RELATIONSHIP OF CYTOLOGY DIAGNOSIS WITH CD4 COUNT.

	CD4 COUNT (Latest CD4 count)									
Cytological diagnosis	<200	201-400	401-500	501-600	601-700	701-800	801-900	901-1000	>1000	TOTAL
NILM										
ASCUS										
LSIL										
LSIL-H										
ASC-H										
HSIL										
SCC										

TABLE 4: RELATIONSHIP OF CYTOLOGY DIAGNOSIS WITH VIRAL LOAD. .

	VIRAL LOAD			
Cytological diagnosis	Below detection limit	<1.000 copies/mm^3	>10.000 copies/mm^3	TOTAL
NILM				
ASCUS				
LSIL				
LSIL-H				
ASC-H				
HSIL				
SCC				

SECTION 5: EXTENDED LITERATURE REVIEW

5.1 Disease burden of Cervical Cancer:

Cervical carcinoma is the fourth most frequent cancer affecting women globally(1). The number of deaths due to cervical cancer were estimated to be over 300 000 in 2018(1). There are broad disparities in the incidence and mortality rates of cervical cancer between first and third world countries. The latest statistics from Globocon quote cervical carcinoma as the leading killer in African women with more than 80 000 deaths arising in 2018 alone (1). A significant fraction of these cases derive from East and Sub-Saharan Africa(1). Cervical carcinoma is the second most frequent cancer in South Africa, second to breast carcinoma(1). Despite this, it supersedes breast cancer as the leading cause of cancer related mortality(1).

5.2. Risk factors for Cervical Carcinoma:

The most important risk factor for the development of cervical carcinoma is infection with Human Papillomavirus (HPV) (3). The risk of genital HPV infection is directly coupled to sexual practices and thus supportive cofactors include the age of the patient, early sexual debut, multiple sexual partners, cigarette smoking, long term use of oral contraceptives and infection with human immunodeficiency virus (HIV)(1–3). Age has a particular influence on the metaplastic changes that occur within the transformation zone which is the site of oncogenic transformation of the HPV virus(4). Increased metaplastic activity seen in younger women provides a good nidus and foundation for the establishment of HPV infection(1,5).

There are over 150 subtypes of HPV with more than 40 of these reported within the anogenital tract(3). HPV is subclassified into high and low-risk oncogenic subgroups(3). Low-risk HPV types include types 6, 11, 42, 43, and 44 and high-risk HPV include types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70(2,3,6,7). Low-risk subtypes are associated with development of genital warts while high risk types are linked to malignancy(3,6). While most HPV infections are cleared spontaneously, the presence of persistent infection by high-risk oncogenic subtypes consequently leads to the development of cervical carcinoma(3,6).

Altered immune response, such as in the setting of HIV infection leaves the body's defence system vulnerable and allows the virus to effortlessly escape immune surveillance(8). Clearance of HPV infection is dependent on intact cell-mediated immunity which is diminished in HIV positive patients thus increasing progression of disease (8).

5.3. Pathogenesis of Cervical Cancer:

HPV is a double stranded DNA virus(9). "The HPV genome consists of between 6800 - 8000 base pairs organized in eight open reading frames: The E6 and E7 genes are responsible for the viral genome whereas E1, E2, E4 E5 and E8 are involved in DNA replication. L1 and L2 are responsible for the assembly of viral particles" (7,9). Infection of the genital tract by HPV is acquired through direct skin to skin contact(9) and 10-15% of the cases occurring in women establish persistent infection. (9).

Following a breach to the cervical epithelium, HPV gains entry into the basal cells(7,9). Viral replication takes places a few weeks thereafter. (2,7,9). The replication continues in the upper parts of epithelium (2,7) and following a period of prolonged and persistent infection, there is integration of the HPV genome into the host DNA (2,7). Integration of the virus into the genome leads to genetic rearrangements including deletions, translocations, activation of proto-oncogenes and the upregulation of E6 and E7 oncogene expression(2,7). Only 0.8% of these cases progress to cancer and thus HPV on its own is not sufficient to produce malignancy(7). Additional epigenetic events, including inflammation, altered immune response and exposure to environmental factors also play a role(3,9).

5.4. HIV and Cervical Cancer

As of 2018, the number of people living with HIV infection was 37.9 million(10). Women comprise 18.8 million of these cases globally(10). East and Southern Africa is home to 54% of the Worlds people living with HIV(10).

HIV is a well-recognized co-factor for persistent HPV infection. It not only augments the virulence of the virus but also increases its aggressiveness thus accelerating the progression to malignant transformation(11,12). HPV and HIV share similar risk factors pertaining to the individual's sexual practices. HIV infected patients serve as a unique population infected with

HPV as they are seen to have a higher likelihood of infection with multiple HPV sub-types with a greater prevalence of high-risk oncogenic subgroups which leads to a higher prevalence of cervical cancer precursors and a faster progression to more severe lesions(13). In addition, HIV-positive women are five times more likely to be infected with high-risk HPV infection compared to HIV-negative women(11,14). This occurs as a result of the altered viral-immune host interactions elicited by HIV thus modifying the natural history of HPV infection (15). Cervical cancer related mortality can be twice as high for HIV-positive women when compared to their HIV-negative counterparts(16). In addition, HIV-positive women in South Africa present with cervical lesions a decade earlier than HIV-negative women and usually experience increased risk of recurrences, advanced clinical stage and are more prone to develop treatment related complications(17).

Studies conducted in HIV-seropositive women in South Africa have shown that lower CD4 counts are consistently associated with a higher risk of cervical lesions(6). Consistent data also indicates that lower CD4 counts (< 500 cells/mm3) are associated with higher prevalence of HPV 16 and are also implicated in more severe cervical disease(6). In contrast, higher CD4 counts are associated with more quiescent HPV infections (15).

Women who have access to combined antiretroviral treatment are living longer lives and are thus at higher risk for progression to cervical neoplasia and invasive cervical carcinoma. The effect of antiretroviral therapy [ART] on the progression of HPV related disease is controversial with data regarding the impact of ART on HPV showing inconsistent and inconclusive findings(16). This is understandable seen how the studies differ with respect to study design, outcomes, timing of ART initiation and effectiveness of ART use, making it difficult to estimate the true effect of ART(14). Some studies have shown no association of Pap smear abnormalities among HIV-positive women with their immune status [CD4 count] and duration of Antiretroviral treatment (ART) (18,19). One study acknowledged that despite the lack of differences in the prevalence and persistence of high risk HPV in treated and untreated women or the natural history of the related cervical lesions, a significant reduction in the incidence of new HPV-16 and -18 infections were observed in the ART-treated women(20). Firnhaber et al [2012] reported increased clearance rates of SIL and oncogenic HPV with consistent adherence to HAART(21). In their study, they showed that HAART had a significant effect on any SIL with CD4 counts of

<350 cells/mm3. There was no significant difference seen in the effect of HAART in women with CD4 counts >350 cells/mm3(21). A meta-analysis reviewing the impact of ART on the prevalence of high-risk HPV and incidence of high grade cervical lesions showed supporting evidence that treatment was associated with lower rates of high-risk HPV and a reduction in HSIL-CIN2+ lesions(14). This was the first meta-analysis of its kind. A useful discovery deriving from this analysis was that studies conducted in Africa showed encouraging data demonstrating that timely and effective ART over a prolonged duration can prevent the incidence and progression of cervical lesions(14). Two South African based studies reinforce this hypothesis. An additional study showed that ART independently protected against progression of cervical lesions and this protective effect was seen even with brief periods of ART therapy(14).

5.6. Cervical Cancer Screening:

The purpose of screening is to detect and allow early treatment of precursor lesions(22). Cervical intraepithelial neoplasia (CIN) is a premalignant lesion that may exist at any one of three stages: CIN1, CIN2, or CIN3(22). CIN2+ lesions can progress to invasive cervical carcinoma unless treatment of lesions ensues(22). Cervical cancer is one of the only diseases which is amenable to both primary and secondary prevention methods(4,22). The implementation of screening in health policies in developed countries has resulted in a significant reduction in cervical cancer related mortality(4,22). This has also been observed in some low and middle income countries where the initiation of cervical cytology programmes has resulted in a substantial decline in cervical cancer incidence and mortality despite the presence of limiting factors(23). A few examples of these limiting factors include limited public health education, barrier in access to health services, lack of laboratory infrastructure, skilled staff and well-regulated quality assurance protocols. In addition, where staff exist they are predominantly under resourced resulting in long waiting periods for cytology results (24,25).

"The current standard practice is to screen women using cytology (Pap test), and when cytology results are positive the diagnosis of CIN is based on subsequent colposcopy, biopsy of suspicious lesions, and then treatment only when CIN2+ has been histologically confirmed"(22). This established screening method has its limitations depending on the setting and resources (22). A direct measure of an effective screening protocol is reflected by a reduced incidence and

mortality of the disease it is trying to prevent. A study conducted in South Africa showed that despite the existence of a national cervical cancer screening programme since 2002, cervical cancer incidence has remained unchanged(13). An honest reflection on this statement would be acknowledging that screening methods need to be continuously re-evaluated with quality control measures put in place to ensure that they are implemented on the premise of the most up-to-date evidence-based methods(4,13).

Primary prevention of cervical carcinoma is a fairly recent addition to cervical cancer preventative protocols and entails the provision of two HPV vaccines to young girls prior to their sexual debut(25). These vaccines prevent infection with HPV 16 and 18 which are responsible for approximately three quarters of cervical cancer infections(4,25).

Secondary prevention methods include; cytology based screening, single visit approach (SVA) and HPV DNA testing(13,22).

5.6.1. Cytology based screening:

Exfoliative cytology has been around for over a century (9) and cytology-based screening programmes have been in existence for over 50 years(13). The preparation of conventional cytology slides was traditionally used with the introduction of liquid based-cytology (LBC) methods in the 1990s(26). LBC is associated with a higher unit cost but has the advantage of allowing reduced contamination, increased detection of high-grade cervical intraepithelial neoplasia and reducing the rate of unsatisfactory specimens(9,26). It provides the additional benefit of residual cellular material which could be utilized for triage and molecular evaluation, more specifically, the testing of high-risk subtypes of HPV(26).

The current screening policy in the South African public sector provides three free cervical cancer screening tests in ten year intervals for all HIV negative, asymptomatic women over the age of 30(13). HIV-infected women represent a unique risk population and have unique guidelines of their own (13). Screening of HIV positive women occurs at diagnosis and follow up occurs every three years or yearly depending on the outcome of the screening test(13).

5.6.2. Single visit approach (SVA):

The single visit approach is based on a 'screen-and-treat' modus operandi which is well suited for certain resource limited settings(13). It assists in combatting barriers such as follow up and access to repeat follow up visitations experienced in low income communities. The test utilises visual inspection of the transformation zone with acetic acid (VIA) and depending on the findings of a precursor lesion it works on the premise of providing treatment immediately following a positive screening testing without awaiting a histologically confirmed diagnosis of CIN2+(22). Despite this being an imperfect approach that is provider-dependent and variable it has been shown to be capable of detecting over 70% of true positive and over 80% of true negative cases making it a viable primary screening tool according to the WHO(22). It is a cost effective approach as it can be offered at a primary health care facility by nursing personnel and does not need laboratory infrastructure(13,27). The drawback of this test is the slightly higher false positive rates (13) and lower sensitivity and specificity(28). It is thus a less reliable screening method when compared to cervical cytology(28).

5.6.3. HPV DNA testing:

Testing for high-risk HPV as a primary mode of screening has been recently introduced and the WHO has recommended this as a preferred method of screening where resources permit(22). This is in view of the known genesis of cervical cancer and the direct role that HPV plays in its aetiopathogenesis (22). Randomised controlled trials have shown that this mode of testing may further assist in the reduction of new cancer cases by 70-80% and simplifies the screening process by having less screening examinations and interventions(4). These studies comprised a huge population size of a quarter million participants with a follow up of 8 years and found that HPV screening results led to a significantly better detection rate of high-grade precursors(4,9). It leads to a substantial decrease in the number of rounds of screening and a 70 per cent reduction in subsequent invasive cancer(4).

HPV based primary screening has been found to be more sensitive in the detection of pre-cancer and has a better negative predictive value when compared to cytology(29). However, combination of HPV testing and cytology is the best approach with a reported sensitivity approximating 100%(4). It is predicted that this method may replace cervical cytology testing in

the future(30). It is becoming an increasingly popular option as it introduces innovative methods of testing such as patient self-sampling(22). This may help to alleviate some of the barriers to screening (27,29,30). In addition, it may also serve a purpose of reducing the costs of screening in the long term. A study In Uganda showed that HPV self-testing followed by cryotherapy for eligible HPV positive women would be cost effective in that population(27). A study in India showed that testing for HPV as the initial method of screening for cervical cancer showed that a single round of HPV testing was associated with a significant decline in the rate of advanced cervical cancers, as compared with cytology, visual inspection or an unscreened control group(9,23). Studies have demonstrated a sensitivity of 96.1% and specificity of 90.7% for HPV testing versus cytology which had a sensitivity of 53% and specificity of 96.3%(9). A study conducted in South Africa, compared the current conventional method of cytological screening with HPV testing and showed that implementation of HPV testing would prevent approximately 650 to 1000 new cases and up to 600 deaths per 100 000 women(31). This argues for HPV testing as a cost effective option in the South African setting(31).

The limitations of a positive HPV test include that it is incapable of differentiation transient from persistent infections [39]. In addition, the provided tests do not differentiate between all relevant carcinogenic genotypes. The low specificity associated with HPV testing is a drawback which may be associated with an increased rate of false positive tests(32). Some authors have raised concerns that if invasive diagnostic procedures are entirely based on HPV testing this may lead to inadvertent overtreatment of cases and increased referral to services such as colposcopy which may burden health services(4). Cytological screening and concurrent HPV analysis is the current preferred method of cervical cancer screening in women over the age of 30 years in countries such as the United States(33). A recent study in the United States found that HPV testing used as a sole method of screening failed to detect cervical carcinoma 10.8% of the time(34). Findings within this study concluded that screening provided best results when co-testing using both cervical cytology and HPV testing was performed(34).

5.7. Bethesda System

The Bethesda system for reporting cervical cytology was introduced in 1988 and modified in 1991, 2001 and 2014. The fundamental principle of this system is to provide terminology that is uniform, clear and succinct with the diagnostic entities linked to standardised treatment (35) .

Moreover, the implementation of this system should be reproducible across different laboratory settings and this uniform terminology system improves patient management(35). A two-tiered reporting system for squamous intraepithelial lesions (SILs): low-grade SIL (LSIL) and high-grade SIL (HSIL) was selected as the most appropriate reporting method of reporting cervical lesions to permit effective and unambiguous clinical interpretation of lesions(35). This system divides HPV-related disease into low-grade SIL [LSIL] and high-grade SIL [HSIL], which correspond to histologic lesions of Cervical intraepithelial neoplasia [CIN] 1, and CIN 2 and/or precancerous CIN 3 [CIN 2/3], respectively(35). In addition, this classification system divides HPV-related disease based on morphology, biologic differences and behaviours between these two lesions. The vast majority of LSIL lesions result from transient infections with high-risk HPV that regress spontaneously while HSIL represents pre-cancerous lesions, a third of which progress to invasive squamous cell carcinoma(35). Table 1 demonstrates the current standardised reporting protocol for cervical cytology(35).

Table 1. The 2014 Bethesda System

SPECIMEN TYPE:
Indicate conventional smear (Pap smear) vs. liquid-based preparation vs. other

SPECIMEN ADEQUACY

- ❑ Satisfactory for evaluation *(describe presence or absence of endocervical/transformation zone component and any other quality indicators, e.g., partially obscuring blood, inflammation, etc.)*
- ❑ Unsatisfactory for evaluation . . . *(specify reason)*
  - ❑ Specimen rejected/not processed *(specify reason)*
  - ❑ Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of *(specify reason)*

GENERAL CATEGORIZATION *(optional)*

- ❑ Negative for Intraepithelial Lesion or Malignancy
- ❑ Other: See Interpretation/Result *(e.g., endometrial cells in a woman ≥45 years of age)*
- ❑ Epithelial Cell Abnormality: See Interpretation/Result *(specify 'squamous' or 'glandular' as appropriate)*

INTERPRETATION/RESULT

NEGATIVE FOR INTRAEPITHELIAL LESION OR MALIGNANCY
(When there is no cellular evidence of neoplasia, state this in the General Categorization above and/or in the Interpretation/Result section of the report--whether or not there are organisms or other non-neoplastic findings)
Non-Neoplastic Findings (optional to report)

- ❑ Non-neoplastic cellular variations
  - Squamous metaplasia
  - Keratotic changes
  - Tubal metaplasia
  - Atrophy
  - Pregnancy-associated changes
- ❑ Reactive cellular changes associated with:
  - Inflammation (includes typical repair)
    - Lymphocytic (follicular) cervicitis
  - Radiation
  - Intrauterine contraceptive device (IUD)
- ❑ Glandular cells status post hysterectomy

Organisms

- ❑ *Trichomonas vaginalis*
- ❑ Fungal organisms morphologically consistent with *Candida* spp.
- ❑ Shift in flora suggestive of bacterial vaginosis
- ❑ Bacteria morphologically consistent with *Actinomyces* spp.
- ❑ Cellular changes consistent with herpes simplex virus
- ❑ Cellular changes consistent with cytomegalovirus

OTHER

- Endometrial cells *(in a woman ≥45 years of age)*

(Specify if "negative for squamous intraepithelial lesion")

Table 1: Reporting guidelines for cervical cytology, Bethesda 2014(35).

Table 1 (continued)

EPITHELIAL CELL ABNORMALITIES

SQUAMOUS CELL

- Atypical squamous cells
  - of undetermined significance (ASC-US)
  - cannot exclude HSIL (ASC-H)
- Low-grade squamous intraepithelial lesion (LSIL)
  (*encompassing: HPV/mild dysplasia/CIN 1*)
- High-grade squamous intraepithelial lesion (HSIL)
  (*encompassing: moderate and severe dysplasia, CIS; CIN 2 and CIN 3*)
  - with features suspicious for invasion (*if invasion is suspected*)
- Squamous cell carcinoma

GLANDULAR CELL

- Atypical
  - endocervical cells (NOS *or specify in comments*)
  - endometrial cells (NOS *or specify in comments*)
  - glandular cells (NOS *or specify in comments*)
- Atypical
  - endocervical cells, favor neoplastic
  - glandular cells, favor neoplastic
- Endocervical adenocarcinoma in situ
- Adenocarcinoma
  - endocervical
  - endometrial
  - extrauterine
  - not otherwise specified (NOS)

OTHER MALIGNANT NEOPLASMS: *(specify)*

ADJUNCTIVE TESTING

Provide a brief description of the test method(s) and report the result so that it is easily understood by the clinician.

COMPUTER-ASSISTED INTERPRETATION OF CERVICAL CYTOLOGY

If case examined by an automated device, specify device and result.

EDUCATIONAL NOTES AND COMMENTS APPENDED TO CYTOLOGY REPORTS (*optional*)

Suggestions should be concise and consistent with clinical follow-up guidelines published by professional organizations (references to relevant publications may be included).

Table 1 (continued): : Reporting guidelines for cervical cytology, Bethesda 2014(35).

5.8. LSIL-H:

Diagnostic dilemmas occur in the interpretation of cervical cytology specimens which show intermediate morphological patterns in between LSIL and HSIL which has led to some authors calling this category "at least LSIL cannot exclude HSIL"(36). Other proposed definitions include [i] LSIL with rare atypical cells suggestive but not diagnostic of HSIL, [ii] Atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion [ASC-H] without any reference to the LSIL, [iii] LSIL with ASC-H, [iv] HSIL or LSIL, and [v] LSIL: cannot exclude HSIL [LSIL-H](37). This potentially leads to inconsistencies in the reporting of these lesions across laboratories and individual cytopathologists (38). Fortunately, this diagnosis is fairly infrequent across studies that have evaluated its incidence(38).

Data in the literature has demonstrated the disparate natural history of LSIL-H when compared to LSIL and HSIL. It has been shown to have different histological outcomes and rates of HPV prevalence(38–40). A consistent features is that LSIL-H is associated with higher rates of high-grade lesions as compared to LSIL, similar rates to ASC-H and lower rates when compared to HSIL(38–40). Table 1 summarises data from the literature regarding the frequency of high-grade lesions on histological follow-up for various cytological categories. The risk of developing high-grade dysplasia in LSIL-H lesions ranges from 24% to 42%(38–41). The risk for high-grade dysplasia is lowest in the LSIL category. A study by Owens et al (2007) looked at 703 cases of which 81 had a cytological diagnosis of LSIL-H(36). In this study, they concluded that LSIL-H had an intermediate risk for high-grade dysplasia (40%) that was significantly greater than LSIL (10.8%) but less than that of HSIL (65.5%)(36). Similar findings were found in a study by Nourhji et al (2008)(42) and Ince et al (2011) (43). The positive and negative predictive value of LSIL-H compared with ASC-H, LSIL and HSIL showed that the rate of high-grade dysplasia differed from LSIL and HSIL but was similar to ASC-H(43). In light of this data, all these studies rationalize the introduction of LSIL-H as a distinct cytological category(36,43,44). Many of the authors are in agreement that LSIL-H does not necessarily indicate a distinct biological entity but its addition as a separate diagnostic category may aid in ensuring earlier detection of high-grade lesions(38).

Table 1: Summary of literature regarding frequency of high-grade dysplasia across various cytological categories.

STUDY REFERENCE	NUMBER OF CASES	LSIL (%)	ASC-H (%)	LSIL-H (%)	HSIL (%)
11. Nasser et al (2003)	294 (150 LSIL; 144 LSIL-H)	23%	Not assessed	42%	Not assessed
12. Elsheik et al (2006)	1033 (575 LSIL; 59 LSIL-H, 110 ASC-H, 289 HSIL)	13%	44.6%	40.7%	74%
13. Shidham et al (2007)	792 (557 LSIL, 88 LSIL-H, 38 ASC-H, 109 HSIL)	10%	31%	33%	69%
14. Owens et al (2007)	703 (426 LSIL, 86 ASC-H, 81 LSIL-H, 110 HSIL)	10.8 %	23%	40%	65.5%
15. Nourhji et al (2008)	378 (194 LSIL-H, 184 LSIL)	7%		24%	
16. Ince et al (2010)	1713 (185 LSIL-H, 127 ASC-H, LSIL 1137, HSIL 264)	21%	43%	40%	81%
17. Thrall et al (2013)	2053 (126 LSIL-H, 1828, LSIL, 99 ASC-H)	7.6%	35.3%	31.9%	
18. Chiaffarano et al (2017)	1049 (394 ASCUS, 481 LSIL, 66 LSIL-H, 33 ASC-H, 75 HSIL)	8%	52%	30%	77%

Nayar et al (2015) advocates against the addition of intermediate morphological patterns such as LSIL-H as it goes against the benefits of a two tiered reporting system(35). A major drawback to LSIL-H not been recognized as a distinct entity is that there are no consistent and well-established guidelines on the management of these patients and further treatment is entirely based on clinical judgment(5). A lack of consistent management guidelines poses a diagnostic problem for a developing country such as South Africa where many primary health care facilities are either run by junior medical doctors or nursing staff. An alternative solution provided by some includes rendering two separate diagnosis In the same patient (i.e. LSIL and ASC-H) to reiterate the difference of this diagnosis to that of LSIL or HSIL(38)). This provides the advantage of accommodating current terminology but has the potential to create even more confusion with clinicians(38).

Several researchers have investigated the prevalence of high-risk HPV in both ASC-H and LSIL-H cases. Many of these studies have brought data to the forefront that LSIL-H is indeed associated with an increased risk of high risk HPV [HR-HPV](36,37,41). A study by Owens et al [2007] showed a significant difference in the prevalence of HR-HPV when comparing LSIL-H and ASC-H categories(36). Owen's study showed that all LSIL-H cases were associated with HR-HPV and only 59% of the ASC-H cases showed this phenotype(36). Zhou et al [2012] demonstrated similar findings with LSIL-H showing a higher frequency of HR-HPV [92%] when compared to ASC-H [78%], LSIL [74%] or LSIL and ASC-H combined [74%](45). A striking similarity was found regarding the HPV infection pattern and infection rates of HR-HPV between LSIL-H and HSIL(45). A later study corroborated these findings and reported the prevalence of high-risk HPV in LSIL-H [86.9%] to be closer to the high risk HPV prevalence in HSIL [92.6%] when compared to ASC-H[68.8%] and LSIL [78.8%]((46)). This has subsequently steered some authors proposing similar management algorithms for LSIL-H and HSIL patients(45). In light of the potential risk for high grade dysplasia, ancillary investigations such as HPV DNA testing and p16 have a potential role in the triage of these patients(47). p16 is considered a surrogate marker for persistent HR-HPV infection and shows high sensitivity and specificity for identifying high risk HPV infection, high-grade lesions as well as detecting carcinoma(47). The use of p16 in cytology has currently not been optimized and may be difficult to interpret in conventional smears(47).

5.9. Conclusion:

Cervical carcinoma is one of the leading causes of cancer-related morbidity and mortality in Sub-Saharan Africa. In addition, HIV infection rates are substantially high within this region serving as an excellent co-factor for the establishment of persistent HPV infection. In view of the increased risk of cancer development in these patients, clear communication with the treating clinician and well-structured management protocols are essential. Intermediate cytological categories such as LSIL-H still pose a diagnostic dilemma as the natural history of these lesions is not fully understood. Moreover, the histological outcome of these cases has not been assessed in the HIV context. The indicates a shortfall in the available data on this topic which requires further interrogation.

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