A retrospective study comparing the efficiency of recurrent LSIL cytology to high-grade cytology as predictors of high-grade cervical intraepithelial neoplasia or worse (CIN2+)

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Background: Cervical cancer (CC) is one of the most preventable cancers; however, it is the leading cause of cancer-related female deaths in South Africa. This study aimed to compare the efficiency of recurrent low-grade squamous intraepithelial lesion (LSIL) cytology as criteria to predict CIN2+ incidence, to a single initial high-grade squamous intraepithelial lesion (HSIL) cytology.

Methods: A retrospective cohort study comprising 344 women was conducted from January 2014 to December 2018 at the Colposcopy Clinic, Tygerberg Hospital. The women were categorised into two groups: (1) women with a recurrent LSIL cytology result, with recurrent cytology scheduled within 6–12 months; (2) women diagnosed with a single initial HSIL cytology result. The outcome was dichotomised into (1) normal or cervical intraepithelial neoplasia 1 (<CIN2) and (2) cervical intraepithelial neoplasia 2/3 or CC (CIN2+). Pearson’s chi-square test ($X^2$) and Fisher’s exact test were used to assess any association between the patient-related factors considered and CIN2+ incidence.

Results: The sensitivity, specificity, PPV and NPV for referral HSIL cytology was 72.73% (95% CI 65.96–78.80), 79.10% (95% CI 71.24–85.64), 83.72% (95% CI 78.54–87.85) and 66.25% (95% CI 60.61–71.46), respectively. HIV status ($p = 0.012$) and ARV treatment ($p = 0.015$) were found to have statistically significant associations with CIN2+ incidence.

Conclusions: A single initial HSIL result is a more efficient predictor of CIN2+ incidence compared with a recurrent LSIL cytology result. The HIV-negative women were more accurately identified as CIN2+, compared with HIV-positive women. Women not on ARV treatment were more accurately identified as <CIN2+, compared with women on ARV treatment.

Keywords: cervical cancer, cytology screening, human papillomavirus (HPV), high-grade lesions, low-grade lesions

Introduction
Cancer is predicted to be the leading cause of death in the twenty-first century.1,2 Globally, cervical cancer (CC) is the fourth most common cancer among women, whereby the incidence and mortality in 2018 were an estimated 570 000 and 311 000, respectively.3–5 Low- and middle-income countries (LMIC) experience 90% of the global CC mortality, with sub-Saharan Africa carrying the highest-burden of 76 387 deaths.1,2 Within South Africa the mortality and incidence rates have remained stagnant, if not increasing in LMIC countries.5

Cervical intraepithelial neoplasias (CINs) are precursor lesions for CC.6 Cytology results describe squamous intraepithelial lesions (SIL) as either low-grade (LSIL/CIN1) or high-grade (HSIL/CIN2/3). A causal relationship has been established between persistent high-risk human papillomavirus (hrHPV) infection and CC.7,8 The hrHPV genotypes common in South Africa include HPV 16, 18, 52 and 35.9 Approximately 70% of CC cases have been shown to be a result of HPV 16 and 18.6 Sexual transmission of HPV infection is most common; however, it often remains self-limiting in individuals with healthy immune systems.10,11 The infection can be persistent or reactivated in immunosuppressed environments, such as in women co-infected with human immunodeficiency virus (HIV).11

South Africa has the highest population infected with HIV.12,13 HIV-positive women had a seven-fold increased risk of hrHPVs persisting after one year, which indicates an increased risk of CIN and CC, in a study by Adler et al., which investigated the rate of hrHPV persistence among HIV-positive and HIV-negative women aged 17–21.11,12 After one year the hrHPV infections persisted in 19% of all the women, and a statistically significant difference between HIV-negative and HIV-positive women was found, i.e. 4% and 31%, respectively.12 The reduced immunocompetence as a consequence of HIV infection has shown CC to be an AIDS-defining cancer.12,14 Several patient-related factors considered, such as smoking, contraception use, multiple sexual partners, sexual activity from a young age and multiparity, have been shown to predispose women to HPV infection.10,16

Cytology screening has been the key driver in reducing CC rates in high-income countries (HICs), but success is dependent on the efficacy of the screening technique and population coverage. Although cytology is an essential component of secondary prevention, it requires several return visits.

In the South African context, this contributes to infrequent screening, through which women potentially at risk are left undetected thus allowing CIN progression. HICs that continue to conduct recurrent cytology do so in conjunction with HPV deoxyribonucleic acid (DNA) or HPV messenger ribonucleic acid (mRNA) testing. Women with positive HPV results are
referred for further colposcopy examination and biopsy collection. Women with negative HPV results are referred for a follow-up cytology after one year.16,17

Screening for hrHPV could provide 60–70% higher protection from CC than cytology screening.18 However, HPV DNA or mRNA testing is not yet readily available in the South African public sector setting. This could be indicative of a gap in detecting potential cases that could progress to CIN2+. This study aimed to investigate the efficiency of recurrent LSIL cytology to predict CIN2+, compared with a single initial HSIL cytology result. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for both referral cytology results. Furthermore, the association between CIN2+ progression and numerous patient-related considered factors was assessed.

Methods

Study design and selection of participants

A retrospective cohort study was proposed and initiated after approval by the Health Research Ethics Committee (HREC: reference no. S19/10/202), Stellenbosch University. A registry-based search was conducted using the colposcopy clinic registry in Tygerberg Hospital. The initial approval for the study was granted for the registry dated January 2018 to December 2018 (Appendix 2.6). However, upon lack of adequate patient reports matching the criteria, an amendment form was submitted and granted by HREC to expand the data collection period from January 2014 to December 2018 (Appendix 12.7). A non-probability convenience sampling method was used to select patient data. The study population included women referred with either a recurrent LSIL cytology result or a single HSIL cytology result during January 2014 and December 2018.

The inclusion criteria included (i) women above 21 years of age with either a recurrent LSIL cytology result or a single HSIL cytology result and (ii) the detection of recurrent LSIL cytology within 6–12 months of initial LSIL result. The exclusion criteria involved women who (i) were previously treated for CIN2+, (ii) had abnormal smear diagnoses prior to recurrent LSIL cytology or HSIL cytology and (iii) no biopsy procedure after an abnormal cytology result during follow-up.

Study procedures

A data capture sheet was structured to collect all the information for each patient individually. All the information was recorded onto a Microsoft Excel spreadsheet (Microsoft Corp, Redmond, WA, USA) to further code for statistical analysis. Women with a single LSIL cytology result during follow-up.

Women with negative HPV results are referred for a follow-up cytology after one year.16,17

The categorical variables were categorised into three modalities (Yes/No/Unknown). Contraception use was further detailed into seven categories, namely, oral, injectables, intrauterine contraceptive devices (IUCD), barrier methods (condoms, diaphragm and cervical cap), implants, tubal ligation or others. Biopsy types were categorised into cervical punch biopsy, cone biopsy (conization) or large loop excision of the transformation zone (LLETZ). The standard list of descriptive colposcopy observations listed were aceto-white lesions, metaplasia, leukoplasia, mosaicism, punctuation, abnormal blood vessels (ABN) and warty atypia. The outcome categories referred to the confirmed histopathology results, separated into four categories, namely, normal, CIN1 (LSIL), CIN2-3 (HSIL) or CC. The treatment types were separated into excisional (i.e. LLETZ, cold knife conization and hysterectomy) and ablative (i.e. cryotherapy and laser therapy).

Statistical analysis

Statistical analyses were performed using STATA® version 15.1 (Stata Corp, College Station, TX, USA) and MedCalc for Windows, version 19.4 (MedCalc Software, Ostend, Belgium). STATA® was used to calculate the Pearson’s chi-square test (χ²) and Fisher’s exact test to assess any association between the patient-related factors considered and CIN2+ incidence. Risk ratios (RR) and 95% confidence intervals (CI) for the retrospective cohort study were estimated by applying a log-binomial regression. Categorical variables were reported as numbers (frequencies) and percentages. The confounders that were adjusted in the multivariable model were HIV status, smoking status, multiparity and contraception use. Differences with a p-value ≤ 0.05 were considered statistically significant. MedCalc was used to calculate the clinical sensitivity, specificity, PPV and NPV in 2 × 2 tables.

Results

Overall, 344 conveniently sampled and de-identified women who met the study criteria were included. Referral cytology was dichotomised as either HSIL (n = 172) or recurrent LSIL cytology (n = 172). Comparisons were conducted between the two groups, regarding their sociodemographic and clinical factors.

In Table 1, the number of women diagnosed with CIN2+ and the corresponding PPV are presented for each referral cytology group. The PPV of recurrent LSIL cytology was 33.75% (95% CI 28.54–39.39) compared with 83.72% (95% CI 78.54–87.85) of a single HSIL cytology result.
In Tables 2 and 3, the sensitivity, specificity, PPV and NPV of a single HSIL cytology compared to recurrent LSIL cytology were found to be 72.73% (95% CI 65.96–78.80), 79.10% (95% CI 71.24–85.64), 83.72% (95% CI 78.54–87.85) and 66.25% (95% CI 60.61–71.46), respectively.

Table 2: Overall sensitivity, specificity, and diagnostic accuracy of HSIL cytology to detect CIN2+ incidence among 344 women

| HSIL cytology | Histopathology result | CIN2+ | <CIN2+ | Total |
|---------------|-----------------------|-------|--------|-------|
| Referral      | HSIL                  | 144   | 54     | 198   |
| LSIL          |                       | 54    | 106    | 160   |
| Total         |                       | 198   | 134    | 332   |

Validity of HSIL cytology to act as a predictor of CIN2+

|            | (95% CI) |
|------------|---------|
| Sensitivity| 72.73 (65.96–78.80) |
| Specificity| 79.10 (71.24–85.64) |
| PPV        | 83.72 (78.54–87.85) |
| NPV        | 66.25 (60.61–71.46) |

LSIL = low-grade squamous intraepithelial lesions. HSIL = high-grade squamous intraepithelial lesions. CIN2+ = women with CIN2+, CIN3 and cervical cancer (CC). PPV = positive predictive value. NPV = negative predictive value.

As shown in Table 4, there was a statistically significant (p < 0.05) association between CIN2+ incidence and two clinical factors, i.e. HIV status (p = 0.012) and ARV treatment (p = 0.015). However, age, CD4+ count, viral load, smoking status, multiparity and contraception use were not found to be associated with CIN2+ incidence.

According to our study, HIV-negative women with either referral cytology were more accurately identified with histologically confirmed CIN2+, compared to HIV-positive women. The distribution of HIV-positive women between <CIN2+ and CIN2+ were similar. However, the distribution of HIV-negative women depicts a higher proportion of women with CIN2+ compared to <CIN2+. The ability to differentiate between <CIN2+ and CIN2+ from referral cytology with a higher accuracy in HIV-negative women compared to HIV-positive women depicted the statistically significant association (p = 0.012).

According to our study, the women not on ARV treatment were more accurately identified with histologically confirmed <CIN2+, compared to women on ARV treatment, with either referral cytology. The women on ARV treatment were distributed almost equally between <CIN2+ and CIN2+. However, women not receiving ARV treatment had shown the distribution of histologically confirmed CIN2+ to be five times lower than women presenting with <CIN2+. The ability to differentiate between <CIN2+ and CIN2+ from referral cytology, with a higher accuracy in women not on ARV treatment compared to women on ARV treatment, depicted the statistically significant association (p = 0.015).

In univariate log-binomial regression analysis, we compared recurrent LSIL cytology to a single HSIL cytology as a predictor for CIN2+ incidence (Table 5). The risk of CIN2+ incidence was 2.67 times (95% CI 2.12–3.36) higher in patients who had a single HSIL cytology result compared to those who had recurrent LSIL cytology.

In the multivariable log-binomial regression, the adjusted risk ratio (RR) was 2.68 (95% CI 2.09–3.43) and similar conclusions were drawn in the presence of confounders (Table 5).

Discussion

Cervical cytology is the main screening test in the South African public healthcare sector. This retrospective study of 344 women
Further studies have shown that HPV mRNA was found to be 74%, and the highest prevalence was reached after receiving the HPV vaccine.20, 24

Comparing the efficiency of recurrent LSIL cytology to high-grade cytology as predictors of high-grade cervical intraepithelial neoplasia or worse was conducted to compare whether recurrent LSIL cytology or a single initial HSIL cytology result is a more efficient predictor of CIN2+ incidence. Overall, a single HSIL result was found to be a more efficient predictor of CIN2+ incidence compared to recurrent LSIL cytology. In summary, among all the women referred with recurrent LSIL cytology, 33.75% (95% CI 28.54–39.39) of the women were truly affected by CIN2+ incidence (PPV). Similarly, a study conducted by Sorbye et al. depicted the PPV of recurrent LSIL cytology as 35.1%.7 In a study conducted by Nygård et al., women with recurrent LSIL cytology had a 15-fold risk over the following three years of CIN2+ incidence.23 In contrast, a study by Ciavattini et al. had shown that over 90% of recurrent LSIL cytology had undergone complete regression at the one-year follow-up.26 In addition, HSIL progression occurred in 0.7% of the cases over the following two years.26

Clinical recommendations regarding the preferred method of triage for LSIL cytology have ranged from HPV DNA/mRNA testing to direct colposcopy referral.7,24 According to the landmark ASCUS-LSIL Triage Study (ALTS) (2003), it was concluded that colposcopy is the preferred initial management for LSIL and reflex HPV testing for ASCUS management.25,30 Further research has shown that HPV DNA testing increases the specificity of cytology, though independently it has a low specificity. An average specificity of Hybrid Capture 2 (HC2)—an HPV DNA test for LSIL triage—was 28.6% (95% CI 22.2–35.0). This has been explained as women with LSIL cytology commonly having a high hrHPV presence. HPV mRNA was found to be more sensitive at 94.2% (95% CI 88.7–99.7) and significantly more specific at 86% (95% CI 81.5–90.5) compared with recurrent LSIL cytology.17

Our study illustrates that of all the women referred with recurrent LSIL cytology, 33.75% (95% CI 28.54–39.39) of the women were truly affected by CIN2+ incidence (PPV). Similarly, a study conducted by Sorbye et al. depicted the PPV of recurrent LSIL cytology as 35.1%.7 In a study conducted by Nygård et al., women with recurrent LSIL cytology had a 15-fold risk over the following three years of CIN2+ incidence.23 In contrast, a study by Ciavattini et al. had shown that over 90% of recurrent LSIL cytology had undergone complete regression at the one-year follow-up.26 In addition, HSIL progression occurred in 0.7% of the cases over the following two years.26

### Table 4: Association between demographic factors and the outcome, CIN2+

| Predictor                     | <CIN2+ | CIN2+ | p-value |
|-------------------------------|--------|-------|---------|
| Age                           | n = 146| n = 198| 0.193   |
| 20–29                         | 21 (14.38) | 18 (9.09) |         |
| 30–39                         | 59 (40.41) | 94 (47.47) |         |
| 40–49                         | 54 (36.99) | 61 (30.81) |         |
| 50–59                         | 8 (5.48) | 20 (10.10) |         |
| 60–69                         | 2 (1.37) | 4 (2.02) |         |
| 70+                           | 2 (1.37) | 1 (0.51) |         |
| HIV status                    | n = 145| n = 196| 0.012   |
| Positive                      | 111 (76.55) | 125 (63.78) |         |
| Negative                      | 34 (23.45) | 71 (36.22) |         |
| CD4+ count                    | n = 96 | n = 96 | 0.461   |
| <100                          | 13 (13.54) | 10 (10.42) |         |
| 100–199                       | 8 (8.33) | 14 (14.58) |         |
| 200–349                       | 24 (25.00) | 27 (28.13) |         |
| 350+                          | 51 (53.13) | 45 (56.88) |         |
| ARV treatment                 | n = 110| n = 121| 0.015   |
| Yes                           | 100 (90.91) | 119 (98.35) |         |
| No                            | 10 (9.09) | 2 (1.65) |         |
| Viral load                    | n = 101| n = 112| 0.938   |
| <20                           | 64 (63.37) | 71 (63.39) |         |
| 20–99                         | 20 (19.80) | 25 (22.32) |         |
| 100–999                       | 8 (7.92) | 8 (7.14) |         |
| 1 000+                        | 9 (8.91) | 8 (7.14) |         |
| Smoking status                | n = 140| n = 183| 0.066   |
| Yes                           | 26 (18.57) | 50 (27.32) |         |
| No                            | 114 (81.43) | 133 (72.68) |         |
| Multiparity                   | n = 145| n = 197| 0.850   |
| Yes                           | 95 (65.52) | 131 (66.50) |         |
| No                            | 50 (34.48) | 66 (33.50) |         |
| Contraception use             | n = 141| n = 184| 0.296   |
| Yes                           | 96 (68.09) | 115 (62.50) |         |
| No                            | 45 (31.91) | 69 (37.50) |         |

< CIN2+ = women with cervical intraepithelial neoplasia (CIN) 1 or normal. CIN2+ = women with CIN2, CIN3 and cervical cancer (CC).

### Table 5: Log-binomial regression analysis to predict CIN2+

| Predictor | Crude RR (95% CI) | p-value | Adjusted RR (95% CI) | p-value |
|-----------|------------------|---------|----------------------|---------|
| Cytology (reference: LSIL): | | | | |
| HSIL | 2.67 (2.12–3.36) | < 0.001 | 2.68 (2.09–3.43) | < 0.001 |
| HIV Status (reference: negative): | | | | |
| Positive | – | – | 0.95 (0.82–1.10) | 0.490 |
| Smoking (reference: non-smoker): | | | | |
| Smoker | – | – | 0.98 (0.84–1.16) | 0.850 |
| Multiparity (reference: no): | | | | |
| Yes | – | – | 0.99 (0.80–1.16) | 0.903 |
| Contraception use (reference: no): | | | | |
| Yes | – | – | 1.01 (0.87–1.17) | 0.867 |

RR = risk ratio.
found among women aged 18–25 years (86.4%). A number (~30%) of incident HIV infections occur among women aged 15–24 years. A study conducted by Burger et al. found that 50% and 75% of the acquired causal hrHPV infection was by ages 21 and 31 respectively.

The overlap in age ranges between (i) highest HPV prevalence, (ii) incident HIV cases and (iii) hrHPV acquisition could explain the high CC incidence and mortality rate in South Africa. Makura et al. found that less than 50% of HIV-infected women receive cytology screening. This is indicative of the gap during which intervention could restrict potential CIN progression, further developing into CC.

Our study found a significant association between ARV treatment and CIN2+ detection. A study conducted by Soncini et al. showed a protective function against CIN incidence with ART treatment. Two studies depicted that ARV treatment duration had decreased the probability of CIN2 progression compared with ARV-naive women. Although ARV treatment is associated with CIN2+ progression in our study, numerous women had cited a lack of sustained adherence, accessibility, unknown ARV initiation and duration, and being misinformed regarding its requirement if their partners were also HIV-positive etc.

In our study, no association was found between age, CD4 count, viral load, smoking status or contraception use and CIN2+. A few studies have shown that a high CD4 count was associated with a reduced risk of 36–70% for hrHPV and 36–80% for CIN2+. A lack of up-to-date CD4 counts could have contributed to inaccurate data capturing, producing a result that did not show association with CIN2+ incidence.

Although no association was found between parity and CIN2+ in our study, the mechanism of association has been suggested to be the increased hormone levels during pregnancy and an impaired immune response. In addition, women who had Caesarean delivery had not shown increased risk of CC compared with nulliparous women. A study by Parazzini et al. found that women <45 years old with >3 births had an eight-fold increased risk of CC compared with nulliparous women.

Similar to our results, a lack of association between smoking and CIN2+ was found in the study by Parazzini et al. Other studies have further analysed smoking duration and intensity (number of cigarettes per day), which further contributed to associative outcomes for CIN2+. Our results might not have found an association due to recall bias, i.e. women were not correctly providing their smoking status. Unlike other studies, data regarding smoking duration and intensity were not available.

Again similar to our study, no association was found between hormonal contraception use (i.e. combined oral contraception, injectable Depo-Provera, implants etc.) and lesion progression. In contrast, oral contraception and other hormonal contraceptives have been shown to increase the risk of CIN progression.

The uptake of screening remains low in Africa. Women cite barriers such as fear of the procedure and outcomes, breach of modesty, cultural issues like stigmatisation, inaccessibility of screening services, and a perspective that screening is needless if one is feeling healthy. Lack of education has been found to be prevalent in urban and rural populations on aspects of HPV transmission mode, eligibility and age range of vaccination.

Apart from HPV DNA and mRNA testing, DNA methylation, which is aimed at detecting lesions that are a result of altering hrHPV infection (HSIL and CC), could be an efficient screening method for HIV-infected women. A study conducted by De Vuyst et al. concluded that DNA methylation in combination with HPV DNA testing is preferred for Kenyan HIV-infected women.

A study conducted by Van Zummeren et al. assessed hrHPV testing and methylation analysis separately and in combination in a South African HIV-infected population. The combined utilisation of these two screening methods resulted in a specificity of 81.5% and sensitivity of 73.8% for CIN3+. The combined tests’ specificity was higher in comparison with individual specificity.

**Strengths and limitations**

**Strengths**

The study sample was large, with 344 women, thus increasing the power of the study. The retrospective study design allowed for an inexpensive, rapid and multivariable analysis to be conducted. There was no loss of patients to follow up compared with prospective studies. Internal validity calculated through sensitivity 72.73% (95% CI 65.96–78.80) and specificity 79.10% (95% CI: 11.24–85.64) had a high precision of the estimates. The high precision could be attributed to the large sample size (n = 344). The Tygerberg Hospital laboratory conducted the histopathology assessments, ensuring uniform quality assurance.

**Limitations**

Inherent to retrospective studies are certain limitations, such as the inability to control for all confounders, e.g. the number of partners, age of sexual debut and sexually transmitted infection (STI). Although a thorough review of medical reports was conducted, a few patients with prior abnormal cytology results from other institutions may have been missed.

Selection bias (convenience sampling) could have been a source of systematic error. Selection bias with convenience sampling lacks generalizability to the population. The 6–12-month period to assess CIN2+ incidence in recurrent LSIL cytology patients could be considered narrow, as CIN2+ can develop over a long period of time, thus acting as a disadvantage.

**Conclusion**

A single HSIL cytology is a more efficient predictor of CIN2+ compared to recurrent LSIL cytology. HIV-negative women who underwent either type of referral cytology were identified with CIN2+ more accurately than HIV-positive women. Women who were not on ARV treatment were more accurately identified with <CIN2+ than CIN2+, from either referral cytology group. In clinical practice it may be necessary to add a different triage test other than repeat cytology, e.g. HPV DNA testing or identification of DNA methylation markers (of HPV), in HIV-positive women.

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References
1. Ferlay J, Colombet M, Soerjomataram I, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019;144(8):1941–53.
2. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
3. Lin L, Yan L, Liu Y, et al. Incidence and death in 29 cancer groups in 2017 and trend analysis from 1990 to 2017 from the Global burden of disease study. J Hematol Oncol. 2019;12(1):96.
4. South Africa: Human Papillomavirus and Related Diseases Report. ICoT/ARC HPV Information Centre; 2019 Jun.
5. Catarino R, Petignat P, Dongui G, et al. Cervical cancer screening in developing countries at a crossroad: emerging technologies and policy choices. World J Clin Oncol. 2015;6(6):281–90.
6. Lowy DR, Schiller JT. Reducing HPV-associated cancer globally. Cancer Prev Res (Phila Pa). 2012;5(1):18–23.
7. Meisels A, Fortin R. Condylomatous lesions of the cervix and vagina. I. Cytologic Patterns. Acta Cytol. 1976;20(6):505–9.
8. Purola E, Savia E. Cytology of gynecologic condyloma acuminatum. Acta Cytol. 1977;21(1):26–31.
9. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–386.
10. Denny L, Adewole I, Anorlu R, et al. Human papillomavirus prevalence and type distribution in invasive cervical cancer in sub-Saharan Africa. Int J Cancer. 2014;134(6):1389–98.
11. Strickler HD, Burk RD, Fazzari M, et al. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women. J Natl Cancer Inst. 2005;97(8):57–76.
12. Adler D, Wallace M, Bennie T, et al. High risk human papillomavirus persistence Among HIV-infected young women in South Africa. Int J Infect Dis. 2015;33:219–21.
13. Moscicki A-B, Ellenberg JH, Vermund SH, et al. Prevalence of and risks for cervical human papillomavirus infection and squamous intraepithelial lesions in adolescent girls: impact of infection With human immunodeficiency virus. Arch Pediatr Adolesc Med. 2000;154(2):127–34.
14. Serraino D, Dal Maso L, La Vecchia C, et al. Invasive cervical cancer as an AIDS-defining illness in Europe. AIDS Lond Engl. 2002;16(5):781–6.
15. Ezeh OC, Pettersson KO, Okolo CA, et al. The association between HIV infection, antiretroviral therapy and cervical squamous intraepithelial lesions in South western Nigerian women. PLoS ONE. 2014;9(5):e97150.
16. Alves RRF, Rabelo-Santos SH, Ribeiro AA, et al. Usefulness of repeat cytology at the time of first colposcopy. Diagn Cytopathol. 2009;37(1):68–73.
17. Serbye SW, Arbyn M, Fisman S, et al. Triage of women with low-grade cervical lesions - HPV mRNA testing versus repeat cytology. PLoS ONE (Internet). 2011 Aug 30 (cited 2020 Nov 18);6(8). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3168878/.
18. Tshomo U, Franceschi S, Tshokey T, et al. Evaluation of cytology versus human papillomavirus-based cervical screening algorithms in Bhutan. Oncotarget. 2017;8(42):72438–46.
19. Karia N, Van Loon A, Simoes C, et al. The positive predictive value of high-grade squamous intraepithelial lesion on cytology for the histological diagnosis of cervical intraepithelial neoplasia 2 or higher: a systematic review. Acta Cytol. 2019;63(3):206–14.
20. Sultana F, Winch K, Saville M, et al. Is the positive predictive value of high-grade cytology in predicting high-grade cervical disease falling due to HPV vaccination? Int J Cancer. 2019;144(12):2964–71.
21. Boonlikit S. Prevalence of high-grade cervical lesion in women with LSIL and HSIL cytology and prevalence of invasive cancer in women cytologically positive for malignancy. Asia Pac J Cancer Prev. 2008;9(4):715–8.
22. Benedet JL, Matisic JP, Bertrand MA. An analysis of 84244 patients from the British Columbia cytology-colposcopy program. Gynecol Oncol. 2004;92(1):127–34.
23. Alvarado RD, Wright TC. Optical Detection Group. Effective cervical neoplasia detection with a novel optical detection system: a randomized trial. Gynecol Oncol. 2007;104(2):281–9.
24. Palmer TJ, McFadden M, Pollock KJG, et al. HPV immunisation and cervical screening—confirmation of changed performance of cytology as a screening test in immunised women: a retrospective population-based cohort study. Br J Cancer. 2016;114(5):582–9.
25. Nygaard M, Raysland K, Campbell S, et al. Comparative effectiveness study on human papillomavirus detection methods used in the cervical cancer screening programme. BJM Open. 2014;4(1):e003460.
26. Ciavattini A, Clemente N, Tisroglou D, et al. Follow up in women with biopsy diagnosis of cervical low grade squamous intraepithelial lesion (LSIL): how long should it be? Arch Gynecol Obstet. 2017;295(4):997–1003.
27. Wright TC, Massad LS, Dunton CJ, et al. 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. Am J Obstet Gynecol. 2007;197(4):346–55.
28. Boardman LA, Kennedy CM. Management of atypical squamous cells, low-grade squamous intraepithelial lesions, and cervical intraepithelial neoplasia 1. Obstet Gynecol Clin North Am. 2008;35(4):599–614. ix.
29. ASCUS-LSIL Triage Study (ALTS) Group. Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. Am J Obstet Gynecol. 2003;188(6):1383–92.
30. ASCUS-LSIL Triage Study (ALTS) Group. A randomized trial on the management of low-grade squamous intraepithelial lesion cytology interpretations. Am J Obstet Gynecol. 2003;188(6):1393–400.
31. Winer RL, Feng Q, Hughes JP, et al. Risk of female human papillomavirus acquisition associated with first male sex partner. J Infect Dis. 2008;197(2):279–82.
32. Richter L, Mabaso M, Ramjith J, et al. Early sexual debut: voluntary or coerced? evidence from longitudinal data in South Africa – the birth to twenty plus study. S Afr Med J. 2015;105(4):204–307.
33. Mbulawa ZZA, Coetzee D, Williamson A-L. Human papillomavirus prevalence in South African women and men according to age and human immunodeficiency virus status. BMC Infect Dis (Internet). 2015 Oct 26 (cited 2020 Nov 24);15. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4624185/.
34. Shisana O, Rehle T, Simbayi LC, et al. South African National HIV Prevalence, Incidence and Behaviour Survey, 2012 (Internet). HSRC Press; 2014 (cited 2020 Nov 25). Available from: http://repository.hsrc.ac.za/handle/20.500.11910/2490.
35. Burger EA, Kim JJ, Sy S, Castle PE. Age of acquiring causal human papillomavirus (HPV) infections: leveraging simulation models to explore the natural history of HPV-induced cervical cancer. Clin Infect Dis. 2017;65(6):893–9.
36. Makura CBT, Schnippel K, Michellop P, et al. Choropleth mapping of cervical cancer screening in South Africa using healthcare facility level data from the National Laboratory Network. AIDS Public Health. 2016;3(4):849–62.
37. Soncini E, Zoncada A, Condemi V, et al. Reduction of the risk of cervical intraepithelial neoplasia in HIV-infected women treated with highly active antiretroviral therapy. Acta Bio-Medica Atenei Parm. 2007;78(1):36–40.
38. De Vuyst H, Mugo NR, Chung MH, et al. Prevalence and determinants of human papillomavirus infection and cervical lesions in HIV-positive women in Kenya. Br J Cancer. 2012;107(9):1624–30.
39. Kelly HA, Sawadogo B, Chikandiwa A, et al. Epidemiology of high-risk human papillomavirus and cervical lesions in African women living with HIV/AIDS: effect of anti-retroviral therapy. AIDS Lond Engl. 2017;31(12):273–85.
40. McIntyre-Seltman K, Castle PE, Guido R, et al. Smoking is a risk factor for cervical intraepithelial neoplasia grade 3 among oncogenic human papillomavirus DNA–positive women with equivocal or mildly abnormal cytology. Cancer Epidemiol Prev Biomark. 2005;14(5):1165–70.
41. Westreich D, Jamal N, Smith JS, et al. Injectable and oral contraceptives and the incidence and progression of cervical disease in HIV-infected women in South Africa. Contraception. 2014;89(4):286–91.
42. International Collaboration of Epidemiological Studies of Cervical Cancer. Cervical carcinoma and reproductive factors: collaborative reanalysis of individual data on 16,563 women with cervical
carcinoma and 33,542 women without cervical carcinoma from 25 epidemiological studies. Int J Cancer. 2006;119(5):1108–24.

43. Parazzini F, Chatenoud L, La Vecchia C, et al. Determinants of risk of invasive cervical cancer in young women. Br J Cancer. 1998;77(5):838–41.

44. Samir R, Asplund A, Tot T, et al. Oral contraceptive and progestin-only use correlates to tissue tumor marker expression in women with cervical intraepithelial neoplasia. Contraception. 2012;85(3):288–93.

45. Francis SA, Battle-Fisher M, Liverpool J, et al. A qualitative analysis of South African women’s knowledge, attitudes, and beliefs about HPV and cervical cancer prevention, vaccine awareness and acceptance, and maternal-child communication about sexual health. Vaccine. 2011;29(47):8760–5.

46. De Vuyst H, Franceschi S, Plummer M, et al. Methyltion levels of CADM1, MAL, and MIR124-2 in cervical scrapes for triage of HIV-infected, high-risk HPV-positive women in Kenya. J Acquir Immune Defic Syndr. 1999;20(1):311–8.

47. De Vuyst H, Franceschi S, Plummer M, et al. Methylation levels of CADM1, MAL, and MIR124-2 in cervical scrapes for triage of HIV-infected, high-risk HPV-positive women in Kenya. J Acquir Immune Defic Syndr 1999;2011;29(4):8760.

Appendices

Table A1: Baseline demographic and clinical characteristics of patients by cytology group

| Characteristic                  | LSIL (% n) | HSIL (% n) | Total (% n) |
|--------------------------------|------------|------------|-------------|
| **Age:**<br>20–29              | 24 (61.5)  | 15 (38.5)  | 39          |
| 30–39                          | 80 (52.3)  | 73 (47.7)  | 153         |
| 40–49                          | 56 (48.7)  | 59 (51.3)  | 115         |
| 50–59                          | 9 (32.1)   | 19 (67.9)  | 28          |
| 60–69                          | 1 (16.7)   | 5 (83.3)   | 6           |
| 70+                            | 2 (66.7)   | 1 (33.3)   | 3           |
| **Type of biopsy:**            |            |            |             |
| Punch biopsy                   | 107 (89.9) | 12 (10.1)  | 119         |
| Cone biopsy                    | 6 (66.7)   | 3 (33.3)   | 9           |
| LLETZ/LEEP                     | 47 (23.0)  | 157 (77.0) | 204         |
| None                           | 12 (100)   | 0 (0)      | 12          |
| **Biopsy result:**             |            |            |             |
| Positive                       | 146 (47.1) | 164 (52.9) | 310         |
| Negative                       | 26 (76.5)  | 8 (23.5)   | 34          |
| **Lesion type:**               |            |            |             |
| Normal                         | 30 (75.0)  | 10 (25.0)  | 40          |
| CIN 1                          | 88 (83.0)  | 18 (17.0)  | 106         |
| CIN 2/3                        | 53 (27.5)  | 140 (72.5) | 193         |
| CC                             | 1 (20.0)   | 4 (80.0)   | 5           |
| **Prescribed treatment:**      |            |            |             |
| None                           | 120 (90.2) | 13 (9.8)   | 133         |
| LLETZ/LEEP                     | 51 (25.0)  | 153 (75.0) | 204         |
| Cold knife                     | 0 (0)      | 2 (100)    | 2           |
| Further Ca treatment           | 1 (20.0)   | 4 (80.0)   | 5           |
| HIV status:                    |            |            |             |
| n = 172                        | n = 169    | n = 341    |
| Positive                       | 125 (53.0) | 111 (47.0) | 236         |
| Negative                       | 47 (44.8)  | 58 (55.2)  | 105         |
| Smoking status:                | n = 166    | n = 157    | n = 323     |
| Smoker                         | 30 (39.5)  | 46 (60.5)  | 76          |
| Non-smoker                     | 136 (55.1) | 111 (44.9) | 247         |

(Continued)
### Table A3: Continued.

| Predictor          | Crude RR          | Adjusted RR        | p-value (95% CI) | p-value (95% CI) |
|--------------------|-------------------|--------------------|------------------|------------------|
| Age (reference: 20–29): |                   |                    |                  |                  |
| 30–39              | 1.33 (0.90–1.91)  | 0.121              | –                | –                |
| 40–49              | 1.15 (0.79–1.68)  | 0.473              | –                | –                |
| 50–59              | 1.55 (1.02–2.34)  | 0.038              | –                | –                |
| 60–69              | 1.44 (0.75–2.79)  | 0.275              | –                | –                |
| 70+                | 0.72 (0.14–3.71)  | 0.697              | –                | –                |

RR = risk ratio.

### Table A4: Continued.

| Colposcopy diagnosis | Cytology, n (%) | LSIL | HSIL | Total |
|----------------------|-----------------|------|------|-------|
| Ace/Leuk/Punc/ABN    | 3 (100)         | 0 (0) | 3    |
| Ace/Leuko           | 1 (33)          | 2 (67) | 3    |
| Ace/Mos/ABN         | 0 (0)           | 3 (100) | 3    |
| Ace/Met/Pun         | 1 (50)          | 1 (50) | 2    |
| Ace/Leuko/ABN       | 0 (0)           | 2 (100) | 2    |
| Mos/Pun/ABN         | 0 (0)           | 2 (100) | 2    |
| Ace/Mos/Pun/ABN     | 0 (0)           | 2 (100) | 2    |
| Ace/Pun/Warty       | 1 (100)         | 0 (0) | 1    |
| Ace/Pun/ABN/Warty   | 0 (0)           | 1 (100) | 1    |
| Ace/Met/ABN         | 0 (0)           | 1 (100) | 1    |
| Ace/Met             | 0 (0)           | 1 (100) | 1    |
| Mos/Pun             | 0 (0)           | 1 (100) | 1    |
| O Warty             | 0 (0)           | 1 (100) | 1    |
| O Punctuation       | 0 (0)           | 1 (100) | 1    |
| O Mosaicism         | 0 (0)           | 1 (100) | 1    |
| Leuk/Mos/Pun/ABN    | 0 (0)           | 1 (100) | 1    |
| Ace/Pun/ABN/Warty   | 0 (0)           | 1 (100) | 1    |

Ace = Aceto White  
Mos = Mosaicism  
Punc/Punc = Punctuation  
Met = Metaplasia  
ABN = Abnormal Blood Vessels  
Warty = Warty Atypia  
Leuk/Leuko = Leukoplakia  
O Warty = Only Warty Atypia  
O Punctuation = Only Punctuation  
O Mosaicism = Only Mosaicism

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