MARGINS STATUS AFTER LLETZ IN HIV-POSITIVE PATIENTS AT A SOUTH AFRICAN TEACHING HOSPITAL: POST UNIVERSAL HAART PROGRAM REVIEW

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

by

Dr Christelle Mbangu Ngwey-Sompo

Student no**: 0210648G**

MMed (Obstetrics and Gynaecology)

Supervisors

Dr L Mbodi: MBChB (Medunsa), FCOG(SA), MMED O&G(Wits), MSc O&G(Wits), Cert Gyn Oncol (SA), PGD H Sc Ed (Wits)

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DECLARATION

I, **Christelle Mbangu Ngwey-Sompo,** declare that this work is an original research report, and it has not been submitted before for any degree or examination at any other University.

It is being submitted for the Degree of Master of Medicine in the branch of Obstetrics and Gynaecology at the University of the Witwatersrand, Johannesburg.

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17 December 2024 at Johannesburg.

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Thirdly, I am grateful to Ms Livhuwani Mphaphuli Nedzingahe, who assisted with statistical analysis, and the Anatomical Pathology Department, NHLS, at Charlotte Maxeke Johannesburg Academic Hospital for the availability of histology results.

DEDICATION

This research is dedicated to my dear parents for their love, endless support, and encouragement. My father, the late Professor Crispin Ngwey Ngond'A Ndenge who did not live to witness this achievement, was a constant source of inspiration, and taxed himself dearly for my education and intellectual development. My mother, Odette Makumi Asn'tu, is a source of motivation and strength. Her prayers have accompanied me throughout this journey.

I am also dedicating the research to my siblings, Suzie, Lauredanna, Steve, and Gina, for their continued support.

I will not forget my tender loving children Zion-wonder, Emmanuella and Esther for their patience, prayers, sacrifices, enthusiasm, support and encouragement throughout the process

I am thankful to my husband, Dr AS Sompo, for everything he did that brought me close to the finish line.

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LIST OF ABBREVIATIONS

ARV	Antiretroviral
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
HSIL	High-grade Squamous Intraepithelial Lesion
LLETZ	Large Loop Excision of Transformation Zone
Pap smear	Papanicolaou Smear (Cervical Cytology)
WHO	World Health Organization

SUBMISSIBLE PAPER

Margins status after LLETZ in HIV-positive patients at a South African teaching hospital: Post universal HAART program review

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Authors:

***Ngwey-Sompo CM**[1], MBChB FCOG(SA)

Mbodi L[1,2], MBChB, FCOG(SA), MMED O&G, MSc O&G, Cert Gyn Oncol (SA), PGD H Sc Ed

Affiliations:

[1]*Department of Obstetrics and Gynaecology, School of Clinical Medicine, University of the Witwatersrand, Johannesburg, South Africa*

[2]*Department of Obstetrics and Gynaecology, Chris Hani Baragwanath Academic Hospital, Soweto, Johannesburg, South Africa*

***Corresponding author:** Christelle Mbangu Ngwey-Sompo

Email: christelle_ngwey@yahoo.fr

Address: Charlotte Maxeke Johannesburg Academic Hospital

7 York Road, Parktown, Johannesburg, 2193, South Africa

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ABSTRACT

Introduction: Cervical cancer is one of the most preventable diseases, yet it remains a leading cause of death. Despite recent advances, cervical cancer still poses a challenge in South Africa. We saw the need to determine the margin status following large loop excision of the transformational zone in women living with HIV at a South African Tertiary hospital as part of a post-universal HAART program review.

Objective: Analysis of margins after LLETZ and Pap-smear results at six months as a test of cure.

Methods: This was a unicentric retrospective cohort of patients who were seen at the colposcopy clinic at Charlotte Maxeke Johannesburg Academic Hospital, South Africa, with high-grade squamous intraepithelial lesion on their Pap smear, managed with LLETZ, and followed up after six months for the test of cure. A data collection sheet was used on files sampled between January 1st January 2016 and 31st December 2020 at the colposcopy clinic. Descriptive statistics were used to summarize the data. Chi-square analysis was employed to determine the association between demographic characteristics, clinical factors, and outcomes. Cohen's kappa statistic was used to determine the agreement between margin status after LLETZ in HIV-positive patients.

Results: During the period under review, a total of 802 patients had LLETZ; and more than half, 454 (55%), were HIV-positive and on antiretroviral treatment. Of these, 98 (22%) patients were cured. No statistical difference in cure rate between positive and negative margins, but 31.21% of patients with negative margins, and 25.38% of patients with positive margins were cured.

Conclusion: Patients had positive margins despite antiretroviral drugs, and there was a significant association between margin status following.

Keywords: HSIL, dysplasia, LLETZ, HIV-positive, South Africa.

INTRODUCTION

The 2022 Globocan report indicated that cervical cancer is ranked eighth in incidence with a reported 661 021 cases, and ninth in mortalities with 348 189 deaths globally.[1] Cervical cancer, a preventable disease, claims a woman's life every two minutes, according to a United Nations Agency for Children (UNICEF) report.[2]

There is evidence of an increasing trend of a women dying of cervical cancer every two minutes, whereas postpartum hemorrhage is responsible for the death of women every seven minutes, as shown by a UNICEF report.[2] Cervical cancer remains one of the most preventable diseases. We cannot stop women from having unprotected intercourse and we cannot force girls to abstain from sex; therefore, it is essential to refocus the goal in treating this deadly epidemic as well its precursor forms.

Emphasis must be placed on increasing the rate and number of screenings and early detection of cervical cancer, and specifically its precursor form. This will aid in meeting the 2030 World Health Organization (WHO) Global eradication goal of vaccinating 90% of girls by age 15, screening 70% of the female population by the age of 35 and again at 45 years, as well as treating 90% of precancer detected cases and managing 90% of those with invasive disease.[3]

We assessed the effectiveness of large loop excision of the transformation zone (LLETZ) as a treatment for high-grade squamous intraepithelial lesions (HSIL) in human immunodeficiency virus (HIV) positive women at the coloscopy clinic at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH). This treatment is used for dysplasia namely HSIL, cervical intraepithelial neoplasia (CIN) 2 and above, identified through Papanicolaou smear (Pap-smear). CIN1 does not usually require LLETZ since it can resolve spontaneously. Other modalities of treatment include laser

vaporisation and cryotherapy; however, with these techniques there is limited specimen available for histology which could result in inappropriate treatment of an early stage carcinoma of the cervix.[4]

METHODS

This was a retrospective unicentric cohort study. Descriptive statistics and Chi-square tests were applied to analyse the data. Data were captured in Microsoft Excel and analysed using SPSS version 29.0.

During LLETZ, a speculum is inserted through the vagina, the colposcope is used to examine the cervix. A thin wire using diathermy is used to conically remove the abnormal area of the cervix and the specimen is sent for pathological assessment. The pathological interpretation in LLETZ involves examining the resected specimen for the grade and extent of abnormal cells, whether there is invasive cancer, and importantly, gives accounts of the surgical margins (edges), should these be free of disease or not.

Cohen's kappa statistic was used to determine the agreement between margin status after LLETZ in HIV-positive patients.

Our inclusion criteriae were patients with HSIL, see and treat patients, women living with HIV (WLHIV), patient on ART.

We excluded patients younger than 1 years of age, patients previously treated by LLETZ, patient who were not living with HIV as well as those not yet initiated on ART.

RESULTS

Sampling

From 1 January 2016 to 31 December 2020, 802 files were retrieved from those referred and treated at the coloscopy clinic at CMJAH. 454 met the inclusion criteria and constituted the sample size for the study. A total of 348 patients were excluded. We employed a convenient sampling method where we included patients that presented to colposcopy and followed them after LLETZ. Figure 1 presents the details.

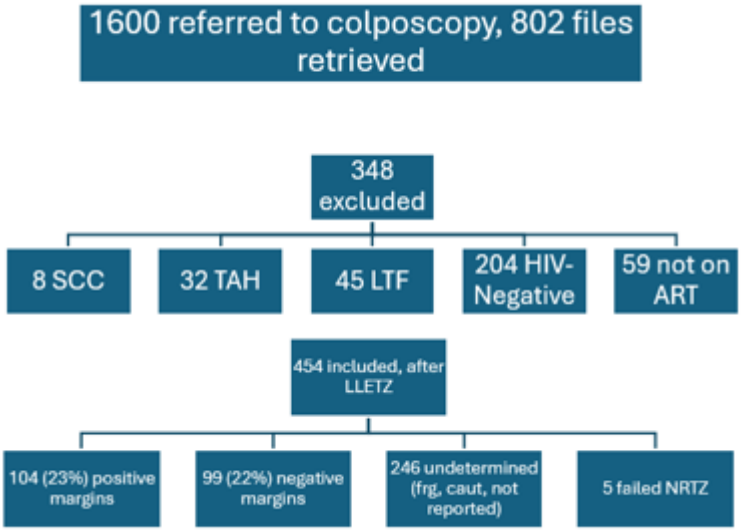

Figure 1: Sample realization

SCC: squamous cell carcinoma, TAH: total abdominal hysterectomy, LTF: lost to follow up, HIV: Human immune deficiency virus, LLETZ: large loop excision of the transformation zone, frg: fragmentation, NRTZ: non-representation of transformation zone.

Age statistics of patients

The mean age was 38 years ± 8, with the youngest being 20 and the eldest 67 years. Most of the patients were 36 years old.

Referring pap-smear results

Figure 2 presents referring pap smear results with 75% HSIL followed by ASC-H (atypical cells cannot exclude a high grade squamous intraepithelial lesion) in 9% of patients.

Figure 2: Pareto diagram indicating referring Pap smear results

HGSIL/HSIL/HGS: High-grade squamous intraepithelial lesion. LGSIL/LSIL: Low-grade squamous intraepithelial lesion. ASC-H: Atypical squamous cells, cannot exclude high grade squamous epithelial lesion. ASC-US: Atypical squamous cell of unknown significance. CIN1/2/3 Cervical intraepithelial neoplasia. NILM: Negative for intraepithelial lesion.

Patient profile by LLETZ outcome results

In this study, 98 (22%) patients were cured after LLETZ. Cure was determined by a combination of clear histological margins with no residual dysplasia at the edges and normal cytology. Menopause, CD4 level, Viral load, comorbidities, and type of HAART did not affect the outcome of LLETZ (p-value>0.05). 454 patient with abnormal pap smear underwent LLETZ during the study period. 104 (23%) had positive margins following LLETZ; 99 (22%) had negatie margins. 246 had undetermined margins, 98 (22%) were cured after LLETZ, 5 failed LLETZ due to non representation of the transitional zone.

- Age group: Most patients were 38 years or younger (53%), with those above 38 years comprising 47%. There was a significant association between the outcome results and age group (p-value=0.0003). Most patients that were cured after LLETZ were 38 years or younger. Comparing the two groups, 70 (71%) patients out of the 260 in the younger than 38 years old group and 25 (26%) out of the 189 older than 38 years old group were cured.

- Menopause: This study included 23 patients (5%) who were in menopause. Of these, only three (3%) were cured.
- CD4 count: In most patients that were cured, the CD4 count was not recorded. In the immunologically competent group, CD4>200 and VL<20, 6 patients were cured and 4 underwent hysterectomy. There was no statistical significance. 3 (3%) out of 29 patients with CD4>200 were cured, whereas 1 (1%) with CD4 count of less than 200 cured.
- Viral load: Of the 13 patients with a viral load of above 20, 1 (1.%) was cured, and 5 (5%) of patients with a viral load of less than 20 were cured.
- HPV: 14 patients had their HPV DNA tests done and recorded. Seven got cured and the other seven were lost to follow up.
- Comorbidities: Most of the patients' comorbidities status was not recorded; however, 1 (1.0%) patient with asthma and 1 (1.0%) with previous vulvectomy were all cured.
- Contraception use: This was only recorded in the minority of our patients, precluding proper evaluation.
- Type of HAART: Most patients who were cured were taking ART (antiretroviral therapy made of Tenofovir, Lamivudine/Emtricitabine and Efavirenz) as a type of HAART (98; 100%). Table 2 presents the patient profile.

Table 2: Patient profile by LLETZ outcome results

Of 454 patients, 85 (19%) had negative margins, with 35 (78%) being ectocervical margins, 48 (11%) endocervical, and two (0.44%) radial. Positive margins were found in 99 (22%), with 23 (5%) being ectocervical, 75 (17%) being endocervical and 1 (0.22%) radial. Four patients (0.88%) had repeat negative margins, with one (0.22%)

being ectocervical and three (0.66%) endocervical margins. Five patients (1%) had repeat positive margins, all endocervical. Figure 3 represents the margin status.

Figure 3: Margin status

Outcome

The study results indicate that 98 patients were cured (22%), as evidenced by negative intraepithelial lesion or malignancy or low-grade squamous intraepithelial lesion results after six months on Pap smear at test of cure. Patients with free-ectocervical margins were also cured (p-value<0.0001). patients with involvement of both the ecto and ectocervical margin were less likely to cure the disease. The Pap-smear results showed 17 with persistent HSIL, and eight patients progressed to cancer.

Level of association between referring Pap-smear results and the LLETZ outcome

Most referring Pap-smear results were HSIL 339 (75%). Of these, 79 (80.6%) patients were cured. There was no association between LLETZ outcomes and referring Pap-smear results (p-value>0.05).

Level of agreement between margin status and LLETZ outcome

There was no significant statistical difference in cure rate between patients who had negative 99 (22%) margins to those with positive margins 104 (23%).

There was a significant agreement between positive and negative margins: 25% of patients with positive and 31% of patients with negative margins were cured. Table 3 presents the level of agreement between margin status and LLETZ outcome.

Repeat LLETZ yielded little agreement at 3% for negative and 4.5% for negative margins.

Table 3: Level of agreement between margin status and LLETZ outcome.

DISCUSSION

LLETZ

In this study, 98 (22%) patients were cured. There was an agreement between the margin and the cured results. We found that patients with negative margins were more likely to be cured and that patients who were both endocervical and ectocervical margin-positive were less likely to be cured. Our cure rate is far less than that reported previously, compared to a randomised controlled trial by Andrej Cokan et al. that reported a cure of 92% post LLETZ vs 51% for patients treated with Imiquimod.[5] These differences could be due to the fact that we did not have a comparison group, our more recent roll-out in antiretroviral (ARV) drugs, and the prevalence of HIV in our population with South Africa continuing to grapple with the world's largest HIV epidemic. Our aim was margin determination of margins status in one study group according to the published literature and not to compare to a control group. This might weaken the reliability of cure finding, especially margin status on outcome, hence we employed a cohort and followed patients after test of cure. In our study, one colposcopist handled all specimens and only patients with abnormal cytology had LLETZ. We did not include patients with see-and-treat without a prior histological finding according to the South African guidelines.

It is crucial to know the ectocervical and endocervical margin status, lymphovascular invasion and crypt involvement, for the disease to be appropriately staged and modality of treatment planned thereafter. Julette found no difference between LLETZ and laser vaporisation in terms of cure rate;[6] however, at our colposcopy clinic we used LLETZ and not vaporisation.

LLETZ does not come without consequences, amongst which thermal destruction leading to difficulty in interpretation of margins results due to cauterisation of the margins leaving no specimen for histological assessment. This poses a great limitation, especially with an inexperienced surgeon.[7] As seen in our study, more than half (55%) of the margins could not be assessed due to fragmentation and cauterisation.

Margin status

In our study we found 104 (23%) patients with positive margins, 99 (22%) with negative margins, and 246 (55%) with undetermined margins due to cauterisation or fragmentation. In England, the National Health System recommends margins to be clear in 80% of specimens.[8] Flannelly et al. quoted positive margins of 37%,[9] whereas Adam et al. found 50% margins positivity in Soweto, South Africa, in 2008.[4] The American Association of Cervical Pathology and Colposcopy recommends that margins positivity after conisation should not exceed 20% and for endocervical margins not to exceed 15%.[10] To attain these international standards, we have to educate our patients and encourage proper adherence to ARV and increased screening for premalignant diseases. We achieved a 22% cure rate, almost similar to international levels. The 10th of May 2016 marked a milestone in the South African HIV history when the Minister of Health, Aaron Motsoaledi, announced in his budget speech the implementation of an evidence-based policy of offering HIV treatment to all people living with HIV by September 2016.[3] this implementation of the new policy influences margins by allowing for ART. This measure was an update from the old practice of measuring CD4 count prior to initiation of ARVs and is in line with the WHO guidelines on HIV treatment. This is a good initiative that should be encouraged.

Age distribution

A striking observation was the 20-year-old patient with HSIL. This could be due to vertical transmission, increasing the risk of Human Papilloma Virus (HPV) acquisition (OR 2.86),[11] a history of genital warts (OR 20.46), smoking (OR 2.95), and HPV infection where persistent high-risk types are crucial in the development of HPV.[11,12]

The mean age of our patients was 38 years, which is in line with the age cited in the literature.[13] In the study by Ding et al., the highest prevalence was 41 to 50 years, like in our study, and the incidence was noted to be from 21 to 59 years.[13] In our study, eight menopausal patients met the inclusion criteria. Of these eight, two were cured. The first had HPV-cytopathy with HPV-16 and K67; the second one, who was asthmatic, had positive endocervical margins. However, two other menopausal patients were lost to follow-up, one with positive endocervical margins and the other with negative margins. We found that more premenopausal patients were cured of the disease as opposed to the menopausal group, with the literature showing that menopause affects cervical margins after LLETZ. It is associated with higher risk of incomplete margins, with menopausal women having an 81,2% likelihood of persistent lesion compared to 46.9% in non-menopausal patients after LLETZ.[14]

Importance of CD4 and viral load

It is known that patients with good immunity are more likely to clear the HSIL dysplasia as opposed to patients with decreased immunity. In our study, although most of this data was missing, six patients with CD4 counts above 200 and a viral load of less than 20 or suppressed, got cured, with four patients opting for hysterectomy. This is in line with the findings of Adam et al. in 2008 where a higher CD4 count was associated

with lower odds of persistent abnormalities, and involvement of both endocervical and ectocervical margins predicted an increased persistence.[4] Viral load is an equally critical indicator of treatment success. In women over 35 years and being HIV-positive, it was found that persistent cytological abnormalities occurred in 49% of patients, with margin involvement being a strong predictor.[4] In these patients, therefore, a proper follow-up plan becomes paramount for monitoring of recurrence.

Persistence of HSIL

Studies have shown that repeating LLETZ in patients with persistent HSIL after LLETZ reduces margin positivity status from 53% to about 37.3%.[15] We had 12 patients with persistent HSIL after LLETZ in our study, but whether this was a true reflection of persistence or patients presenting with progression of disease or treatment failure is not known. In this study we had many patients that were lost to follow-up, and HIV-positive patients with incomplete margins at LLETZ were found to be at higher risk of progression to precancer lesions.[16]

In closing, margin status is an important indicator of incomplete treatment after the first year of treatment, as shown in a meta-analysis by Rico et al.[17] Also, surveillance is not inferior to additional surgical modalities in patients with positive margins.[18]

Limitations of the Study

The limitations of the current study involved low return rate, as many patients were lost to follow-up. The study spanned from 2016 to 2021, with the last two years being during the COVID-19 pandemic where the imposed restrictions and lockdown forced patients to seclude themselves and only present in case of an emergency.

There were a considerable number of variables with missing information such as smoking history, comorbidities, viral loads, and CD4, mostly due to lack of proper

referring clinic record keeping. The missing information made it difficult to perform the analysis and draw relevant conclusions.

Progesterone-only containing contraception is known to cause atrophy and obstetrics complications; however, contraceptive use was only recorded in a minority of patients. As the study was conducted in a single centre; findings cannot be generalised.

CONCLUSION

Summary of main findings

In line with the literature, this study found that after LLETZ, margin-free status was observed.

Women living with HIV can be cured of HSIL with LLETZ, especially those aged 31–40 years. In this group, those with a viral load of less than 20 have an increased chance of being cured. LLETZ can therefore be applied in any patient with HSIL for her to be cleared of pre-cancer lesions after her six-month follow-up. Results show a significant association between LLETZ follow-up and margin status, including comorbidities and referring pap-smear results.

Recommendations and contribution

- Age group, comorbidities, referral Pap smear and viral load should be considered when treating HIV-positive patients with LLETZ.
- Computerisation of data.
- Based on new evidence, in a population of patients with HIV and HSIL, research suggests LLETZ to be the preferred treatment due to its reported effectiveness in treating HSIL. The findings of this study highlight the

importance of tailored approaches for HIV-positive patients with HSIL, especially those with low CD4 counts.

- Sills training on LLETZ procedures.
- Patient compliance alongside patient education remains key.
- The study allowed us to examine a population at risk and to examine the effectiveness of LLETZ post universal treatment.

Suggested future research

- This study suggests a follow-up study; a prospective study on patients on current antiretroviral therapy (FDC with Dolutegravir) and with all variables controlled. The outcome will help evaluate how to best help our patients using the two modes of treatment, namely ARVs and LLETZ.
- Margin status in women on progesterone-only contraceptives.

The key take-home message remains long term surveillance in HIV-HSIL patients, regardless of the margin excision status.

Conflicts of interest

The authors declare no conflict of interest.

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Authors' contribution

All authors participated in the elaboration of the study.

CM N-S: Conception, literature review, funding, data collection, statistical analysis, writing and editing.

L M: Conception, methodology, and reviewing and supervising the project.

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SUPPORTING INFORMATION

Figures

Tables

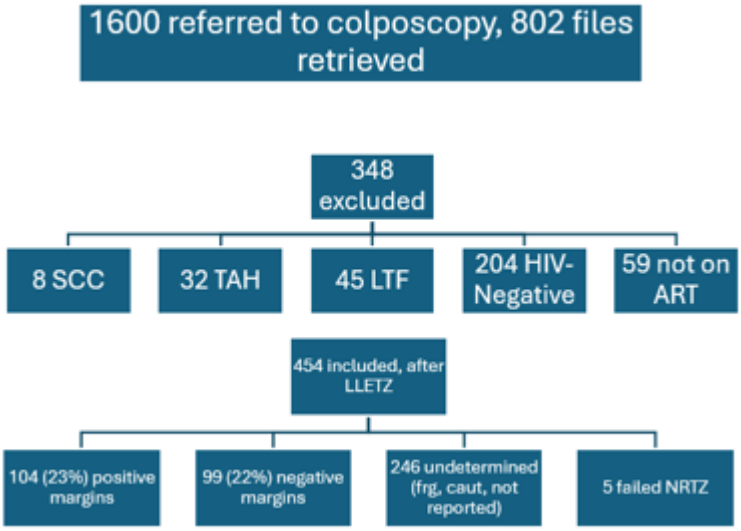

Figure 1: Sample realization

SCC: squamous cell carcinoma, TAH: total abdominal hysterectomy, LTF: lost to follow up, HIV: Human immune deficiency virus, LLETZ: large loop excision of the transformation zone, frg: fragmentation, NRTZ: non-representation of transformation zone.

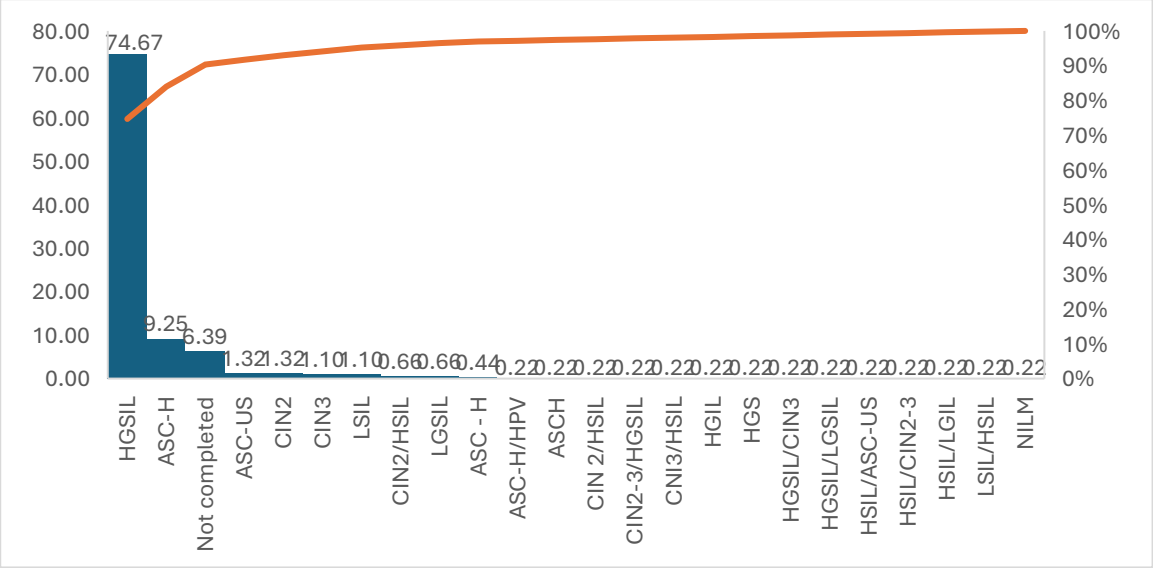

Figure 2: Pareto diagram indicating referring Pap-smear results

HGSIL/HSIL/HGS: High-grade squamous intraepithelial lesion. LGSIL/LSIL: Low-grade squamous intraepithelial lesion. ASC-H: Atypical squamous cells, cannot exclude high grade squamous epithelial lesion. ASC-US: Atypical squamous cell of unknown significance. CIN1/2/3 Cervical intraepithelial neoplasia. NILM: Negative for intraepithelial lesion.

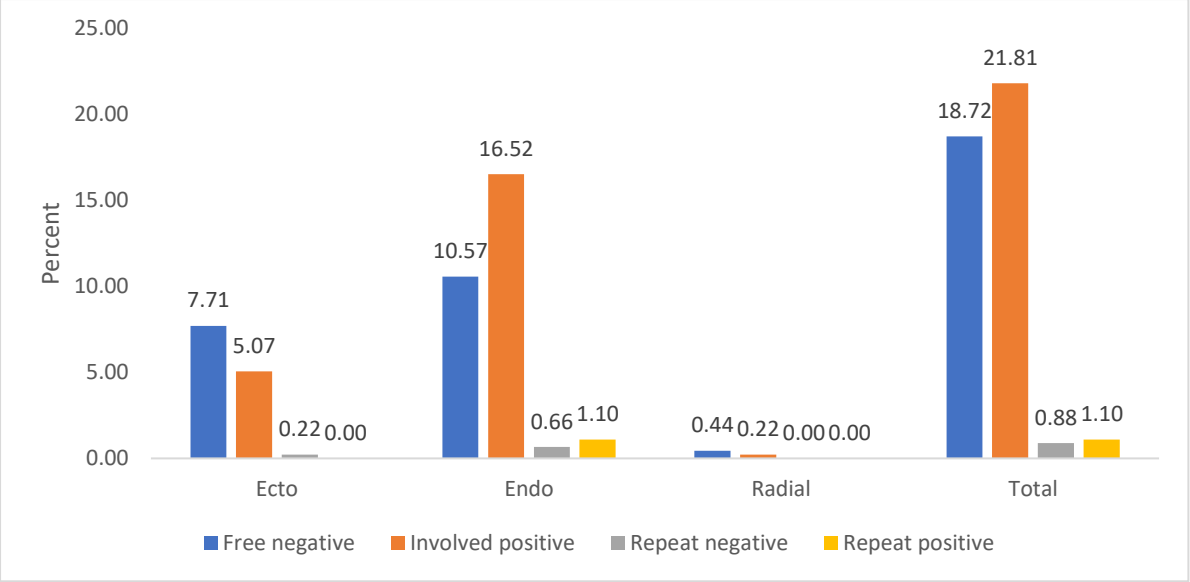

Figure 3: Margin status

Ecto: ectocervical. Endo: endocervical. Free: negative margin. Involved: positive margin. Repeat: Repeat Large loop excision of transformation zone.

Table 1: Patient profile by LLETZ outcome results Age

Variable	Description	Yes	%	No	%	Total	%
Age group	38 years or less	70	71.4%	190	53.4%	260	57
	Above 38 years	25	25.5%	164	46.1%	189	41
	Not completed	3	3.1%	2	0.6%	5	1
	Total	98	100%	356	100%	454	10
Menopause	No	6	6.1%	28	7.9%	34	7
	Yes	3	3.1%	20	5.6%	23	5
	Not completed	89	90.8%	307	86.2%	396	87
	Total	98	100%	356	100%	454	10
CD4 count	Greater than 200	3	3.1%	26	7.3%	29	6
	Less than 200	1	1.0%	8	2.2%	9	2
	Not done	94	95.9%	322	90.4%	416	91
	Total	98	100%	356	100%	454	10
Viral Load	Above 20	1	1.0%	12	3.4%	13	2
	Less than 20	5	5.1%	16	4.5%	21	4
	Not done	92	93.9%	328	92.1%	420	92
	Total	98	100%	356	100%	454	10
Comorbidities	Diabetic	0	0.0%	1	0.3%	1	0
	MF2/Ovarian Cancer/ FP2	0	0.0%	1	0.3%	1	0
	Previous vulvectomy	1	1.0%	0	0.0%	1	0
	Pulmonary TB	0	0.0%	1	0.3%	1	0
	Not completed	95	96.9%	352	98.9%	447	98
	Total	98	100%	356	100%	454	10
If HIV-Positive, Type of HAART	ART	98	100.0%	350	98.3%	448	98
	Total	98	100%	356	100%	454	10

Table 1: Level of agreement between margin status and LLETZ outcome

Variable	**Descrip.**	**Cured**	**Not cured**	**Total**	**Value**	**Asymptotic Std Erro**
Free-Ecto	No	73	346	419	0.2959531	0
		74%	97%	92%		
	Yes	25	10	35		
		26%	3%	8%		
	Total	98	356	454		
		100%	100%	100%		
Free-Endo	No	67	339	406	0.3294887	0
		68%	95%	89%		
	Yes	31	17	48		
		32%	5%	11%		
	Total	98	356	454		
		100%	100%	100%		
Free-Radial	No	97	355	452	0.0114646	0
		99%	100%	100%		
	Yes	1	1	2		
		1%	0%	0%		
	Total	98	356	454		
		100%	100%	100%		
Involved-ecto	No	87	344	431	0.1086741	0
		89%	97%	95%		

	Yes	11 11%	12 3%	23 5%		
	Total	98 100%	356 100%	454 100%		
Involved-endo	No	66 67%	313 88%	379 83%	0.2248676	0
	Yes	32 33%	43 12%	75 17%		
	Total	98 100%	356 100%	454 100%		
Involved-radial	No	97 99%	356 100%	453 100%	0.0159106	0
	Yes	1 1%	0 0%	1 0%		
	Total	98 1	356 1	454 1		
Repeat free-ecto	No	97 99%	356 100%	453 100%	0.0159106	0
	Yes	1 1%	0 0%	1 0%		
	Total	98 1	356 1	454 1		
Repeat free-Endo	No	96 98%	355 100%	451 100%	0.0316202	0
	Yes	2 2%	0 0%	2 0%		
	Total	98 100%	355 100%	453 100%		
Repeat free-Radial	No	98 100%	356 100%	454 100%		
	Total	98 100%	356 100%	454 100%		
Repeat- involved-ecto	No	98 100%	356 100%	454 100%		
	Total	98 100%	356 100%	454 100%		
Repeat involved-endo	No	94 96%	355 100%	449 99%	0.0579267	0
	Yes	4 4%	1 0%	5 1%		
	Total	98 100%	356 100%	454 100%		
Repeat involved-radial	No	98 100%	356 100%	454 100%		
	Total	98 100%	356 100%	454 100%		

APPENDICES

Appendix A: Author instructions - International Journal of Obstetrics and Gynaecology (IJGO)

- Authors

o Primary research studies carried out in low-/middle-income countries must have at least one local co-author.

- Author affiliations

o Please list department, institution, city, country only.

- Corresponding author info

o Only one corresponding author should be listed. The corresponding author listed in the manuscript file must match the corresponding author listed in Editorial Manager.

o Full postal address and email address should be listed.

- Structured abstract

o Headings: Objective; Methods; Results; and Conclusion.

o Guideline word count: Up to 250 words.

- Keywords

o Guideline: Up to 8 keywords. At least 3 keywords must be included.

- Synopsis

o Brief synopsis of up to 25 words describing the key findings of the study.

- Main text

o Guideline word count: Up to 2500 words

o Continuous line numbering used throughout

o Citations: in-text references listed using Arabic numerals in square brackets (e.g. [1,2]) OR using superscript reference indicators (e.g. 1,2). Do not use parentheses (curved brackets).

o No footnotes

o Main headings:

 Introduction

 Present the background to the study briefly, supported by a limited number of references. State the rationale for the study and include the study objectives and hypothesis at the end of this section.

 Materials and methods

 State the type of study at the beginning of this section (e.g., retrospective cohort study).

 Describe concisely the study setting, dates (day, month and year), participants, methods and procedures, and statistical methods. Where appropriate, state the program used for statistical analysis (including model and version number), and the cut-off for statistical significance. This section should include sufficient detail to allow others to replicate the study.

 For studies of patients, patient records, or volunteers, include a statement of prospective local Ethics Committee approval (including full name of Committee).

 For studies with human participants, include a statement about informed consent. If consent was not needed/obtained, include an explanation. Authors must provide copies of the appropriate documentation if requested.

 Results

 Include the outcome of the study and statistical significance, if appropriate.

 Tables and figures should be cited here in order, to illustrate the outcomes and supplement the text.

 Discussion

 Discuss the relevance of the results. Compare with previously published studies; discuss strengths and limitations of the study; address any proposals for future research where relevant.

 Conclusions

 Discussion and Conclusions sections may be formatted as one section as preferred.

 Briefly state the key findings of the study, and major implications for clinical practice/research where relevant.

 Author contributions: List each author’s role in the design, planning, conduct, data analysis, and manuscript writing. Ensure that all authors meet the ICMJE criteria for authorship (anyone who does not fulfil all four criteria should be moved to an Acknowledgments section).

- Funding: All sources of funding received for the research, authorship, and/or publication of the article must be listed here. This should match any funding sources listed in Editorial Manager. If no funding was received for the research, state ‘None’.
- Conflict of interest: List any relationships that may be deemed COIs, or state ‘The authors have no conflicts of interest’.
- References: See 6.3. References for reference style. Guideline: up to 25 references.
- Figures and tables: See Figures and Tables sections below for further information.

6. Editorial style

6.1. Language

Papers are published in English, using US spelling. Authors are strongly encouraged to use neutral, unbiased language that respects women’s bodies and agency. The editors reserve the right to make any necessary editorial changes.

Authors whose first language is not English are encouraged to have their manuscripts reviewed by a native English speaker or a professional editing service before submission, e.g. http://wileyeditingservices.com/en/. It is important for all submissions to be clear and coherent for the editors and reviewers.

6.2. File types

Papers should be submitted as Word documents, formatted in Arial 12pt with double line spacing. For information on accepted file types for figures and tables, please see 6.4. Tables and 6.5. Figures.

6.3. References

IJGO uses a modified Vancouver style of referencing, i.e. references must be numbered and listed as they are cited in the article, using Index Medicus abbreviations for journal titles (e.g. Int J Gynecol Obstet). Most references should be dated within the past 10 years.

Cite the names of all authors when there are six or fewer; when there are seven or more, list the first three authors followed by “et al.” Include the volume number.

Journal article

[1] Vellacott ID, Cooke EJ, James CE. Nausea and vomiting in early pregnancy. Int J Gynecol Obstet 1988; 27:57–59.

Book

[2] Speroff L, Glass BH, Kase NG. Clinical Gynecologic Endocrinology and Infertility. Baltimore: Williams and Wilkins; 1982.

Chapter in a book

[3] Disaia PJ, Creasman WT. Invasive Cancer of the Vulva. In: Disaia PJ, Creasman WT, eds. Clinical Gynecologic Oncology. St Louis: C.V. Mosby; 1984:214–219.

Web reference

[4] World Health Organization. WHO Recommended Surveillance Standards, Second Edition [WHO website]. 1999. http://www.who.int/csr/resources/publications/surveillance/whocdscsrisr992.pdf. Accessed January 15, 2016.

Text references should be indicated by Arabic numerals in square brackets on the line; for example, [1–4] and [1,5,11,17]. Alternatively, they may be indicated using

superscript numbering. Please ensure whichever method is used, that it is used consistently throughout. To avoid any delays in the editing process, authors must make every effort to ensure that each reference is correct and complete.

All references must be in English. Citation information of documents originally in other languages must be translated into English in the reference list.

Numbered references to personal communications, unpublished data, statistical software, or manuscripts that have not been accepted for publication (i.e. "submitted" or "under consideration") must not be included. Reference to such material, if required, can be incorporated at the relevant location in the text.

If bibliographic software has been used for managing the reference list (e.g. EndNote or Reference Manager), the reference list and citations must be unlinked before submission.

When citing articles available as preprints, which have not yet been published, the designation “[preprint]” should be included in the reference.

6.4. Tables

- Tables must be titled, numbered consecutively, and cited in the text as ‘Table 1’, ‘Table 2’, etc. All tables must be created and submitted in editable Word format.
- Use the Word table function (not the "enter" key, spaces, or the "tab" function) to create a separate cell for each table entry.
- Footnotes to tables should be listed as a, b, c etc., rather than *,†,‡ etc.
- If tables are deemed to be too large or there are too many, they may be published as online-only supporting information.

6.5. Figures

- Upload figures individually, in separate files (one figure per file). Do not embed the figure into the main manuscript file.
- Preferred image formats are TIFF, JPEG, or EPS (at least 300 dpi).
- Upload CONSORT flow charts as editable Word/PowerPoint files.
- For every figure, provide a short figure title (numbered consecutively in the order of citation, using Arabic numerals) in the manuscript file, following the reference list.
- Cite all figures in numeric order in the main text as "Figure 1" etc.
- There are no charges for colour figures.
- If photographs of identifiable people are used, authors must obtain and submit a signed statement of informed consent from the identifiable person(s) or their next of kin. Authors should not try to conceal identity with black bars over eyes etc.
- If any figures have previously been published elsewhere, excluding those published open access under a CC-BY licence, authors must first obtain the permission of the copyright holder, in addition to giving precise reference to the original work. Confirmation should be included in the cover letter (the actual permission correspondence from the copyright holder does not need to be submitted).

6.6. Supporting information (Supplementary files)

- Authors may submit supporting information such as additional tables and figures, presentations, and videos.

- These should be uploaded as file type ‘Supplementary file’, and cited in the main manuscript file as “Table S1”, “Figure S1”, “Video S1”, etc.
- Supporting information will be hosted online only.
- Supporting information will not be edited or formatted, but the editors and reviewers may suggest changes.

6.7. Statistics and reporting of numbers

6.7.1. Statistics

The statistical tests used, and the significance level set should be listed in the methods for all studies that employ statistical analysis. Include information regarding the statistical software programs used in the Methods section; for example, “SPSS version 20 (IBM, Armonk, NY, USA).”

P values should be provided where calculated. The largest P value that should be expressed is $P > 0.99$. The smallest P value that should be expressed is $P < 0.001$.

For measures of effect (e.g. relative risks, risk ratios, odds ratios), authors should also report confidence intervals (e.g. 95% CI) so that the precision of the effect estimate can be assessed.

6.7.2. Numbers

Use Arabic numerals for weights, measures, percentages, and degrees of temperature. Weights and measures should be abbreviated according to the International System of Units (SI), or non-SI units mentioned in the SI, followed by conventional units in brackets on first mention. Provide percentages after numerals when reporting results.

6.8. Drugs

Give generic names of all pharmaceutical preparations and, where appropriate, include the trade name and manufacturer's name and address in parentheses. Review drug names and dosages with care. The author is responsible for all recommended dosages.

6.9. Manufacturer information

Give the manufacturer's name and address in parentheses following the name of any instruments or equipment cited by brand name. Do not include the trademark or registered trademark symbol.

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IJGO is a ‘hybrid’ journal – i.e. it is a subscription-based journal, but some authors may choose to publish their article Open Access, whereby it will be immediately and permanently free for everyone to read and download. There is no charge to authors or their institutions for subscription-based publication.

- To cover the cost of publishing Open Access, authors or their institutions pay an article publication charge (APC). The APC for IJGO can be found on Wiley’s Open Access Pricing page.

- Payment must be received before the article can be made open access. Some organizations pay APCs for their authors via a Wiley Open Access Account.

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- Articles will be automatically submitted to PubMed Central and PMC mirror sites, when appropriate
- Authors retain copyright with a Creative Commons license
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If your manuscript is not accepted for publication in IJGO, your manuscript may be suitable for submission to the Wiley-published journal, Reproductive, Female and Child Health (RFCH). RFCH is a peer-reviewed, multi-disciplinary, Gold Open Access

journal, which aims to improve clinical practice in reproductive, female, and child health.

If your manuscript is considered suitable for submission to RFCH, you will receive a decision email from the IJGO Editor with the opportunity to complete a form to transfer your manuscript to RFCH's submission site. Please note that submission to RFCH does not guarantee acceptance of your manuscript, and please note that, as a fully Gold Open Access journal, an Article Publication Charge is required for publication in RFCH.

8. Policies

8.1. Data-sharing policy

This journal expects data sharing for Clinical Articles, and authors must include a data availability statement. Review Wiley's Data Sharing policy where you will be able to see and select the data availability statement that is right for your submission. Brief Communications, Review Articles and Editorials do not need a data availability statement but may include one if relevant.

8.2. Appeals

- An author may appeal a Reject decision within 30 days of receipt of the decision email.
- Appeals will be considered only if factual errors are believed to have been made during the review process.
- Only one appeal is permitted per manuscript, and decisions on appeals are final.

- The basis for an appeal must be outlined clearly; the corresponding author should email the IJGO Editorial Office at ijgo@figo.org.

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Plagiarism entails the "use or close imitation of the language and thoughts of another author and the representation of them as one's own original work." Self-plagiarism, a form of misconduct in which an author reuses his/her previously written text, data, or ideas, wholly or in part, without indicating previous dissemination, will also be considered plagiarism. Verbatim copying of sentences, even if a citation is provided (unless the sentence appears in quotation marks), is plagiarism. Papers are checked for plagiarism (including self-plagiarism); if plagiarism is detected, action will be taken following the Committee on Publication Ethics guidelines.

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Wiley believes that journals publishing for communities with established preprint servers should allow authors to submit manuscripts that have already been made

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This journal will consider for review articles previously available as preprints on non-commercial servers such as ArXiv, bioRxiv, psyArXiv, SocArXiv, engrXiv, etc. Authors are requested to update any pre-publication versions with a link to the final published article. You can find Wiley’s full preprint policy here.

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Authors may choose to publish under the terms of the journal's standard copyright transfer agreement (CTA), or under open access terms made available via Wiley Open Access.

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Once the paper has been typeset, the corresponding author will receive an email containing instructions about how to access and mark corrections to the proof online. The corresponding author must also respond to any queries listed on this proof.

Corrections must be returned to the publisher within 48 hours of receipt. The author is responsible for detecting typesetting errors; no major changes in–or additions to–the edited manuscript will be allowed at this stage.

9.4. Publication as an Early View article

Final, proofed articles will appear as Early View articles within 1 week of completing the proof-checking stage; the link to the article in PubMed will automatically be updated.

Early View articles are complete full-text articles published online in advance of their publication in an issue. Articles are therefore available as soon as they are ready. Early View articles are complete and final. They have been fully reviewed, revised, and edited for publication, and the authors' final corrections have been incorporated.

Because they are in final form, no changes can be made after online publication, other than in cases of serious error (in which case, please contact the Editorial Office at ijgo@figo.org to request an erratum or corrigendum). The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the traditional way. They are therefore given a DOI, which allows the article to be cited and tracked before it is allocated to an issue. After issue publication, the DOI remains valid and can be used to cite and access the article.

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Free access to the final PDF offprint of your article will be available via Wiley's Author Services only. Please therefore sign up for Author Services if you would like to access your article PDF offprint and enjoy the many other benefits the service offers.

Appendix B: Study Protocol

1.1. Introduction

Cervical cancer is the fourth most diagnosed cancer globally and the fourth leading cause of morbidity in women populations, despite being one of the most preventable cancers.[1] This is due to primary prevention with the Human Papilloma Virus (HPV) vaccine, and secondary prevention with screening measures. Despite these prevention measures, cervical cancer is still the most common cancer; causing a high burden of disease among women worldwide.[1] There are demographic differences in the prevalence of cervical cancer due to cultural and socioeconomic factors.[2] Therefore, the most vulnerable areas are the most affected.

1.2. Colposcopy

Colposcopy is an indispensable tool in the assessment of women with abnormal Pap smears. The main goals of colposcopy are in the assessment of pathologies detected in the initial Pap smear, confirming the diagnosis by collecting a biopsy sample, to exclude invasive disease, to help in outpatient management and for review after the surgical treatment.2 Colposcopy uses microscopic light and low power examination of the epithelium of the lower genital tract. The colposcope was first used in Hamburg, Germany in the 1920s, due to the collaboration between the University of Hamburg and Leitz, the German microscope manufacturer.[3\

Classically, colposcopic examination eases the identification and localization of abnormality within the “at-risk” epithelium, in other words, the transformation zone (TZ), enabling confirmatory biopsy or excision/ablation of the TZ, or both. Of these specimens, from these areas, it has been reported that up to 14.9% would be normal on histology reports. This is overtreatment, defined as cervical intraepithelial neoplasia

(CIN) I or less at final histopathology analysis. The availability of the HPV test used in combination with cytology reduces the overtreatment rate.[3]

Large Loop Excision of Transformation Zone (LLETZ) is the term coined in the early 1980s to describe the excision of the TZ using a low-voltage diathermy loop with a thin wire under local anaesthesia. The term is to be differentiated from René Cartier's small samples that he used for taking biopsies in his practice in Paris. From his technique and LLETZ and loop electro-excision procedure (LEEP) was developed in the early 1980s in Bristol, United Kingdom.[3]

Since 2006, the American Society for Colposcopy and Cervical Pathology has recognized the "see-and-treat" approach using an immediate loop electrosurgical excisional procedure after the first evaluation as an acceptable method to manage and treat women with high grade squamous intraepithelial lesions (HSIL).[2]

1.3. Definitions of margin status

"Treatment should conduct complete eradication of the TZ and not only the lesion. It should ablate the TZ, the whole TZ, and, ideally, nothing but the TZ. Whether the TZ was excised or destroyed, ablation to a depth of seven mm is considered optimal"- Shafi, 2006.[2] The rationale for the depth is that the deepest gland crypt can contain CIN of four millimetres. Destroying the TZ to seven mm therefore gives enough degree of safety and reduces the risk for residual disease.[3]

1.4. Margins positivity, residual lesions, and recurrent lesions

Chen defined a residual lesions HSIL lesions that are found. Patients who had lesions at a repeat LLETZ.[4] If the HSIL diagnosis is three months after surgery, it was considered a recurrent lesion. In their study CIN I, which is part of Low-grade squamous intraepithelial lesion (LSIL) was not considered as residual or recurrent as

it required no destructive treatment. Patients who had two or more consecutive negative postoperative biopsies were deemed free of residual lesions, regardless of the HPV test results. When HSIL lesions are found in the resection margins (be it in the ectocervical margin, in the endocervical margin, or in both), of about 100 µm or less, it is considered as a positive margin.[5] Histopathological finding of CIN along the specimen margin, regardless of the CIN grade is considered positive.[5]

A Chris Hani Baragwanath Academic Hospital study in 2008 reported persistent cytological abnormalities in 49% of their patients following the LLETZ procedure. HIV infection and positive margins (both ecto- and endo-) were predictors of persistence. In HIV positive women, they found that the CD4 count level also predicted the persistence of abnormalities on follow-up smear.[6]

The 2011 Limpopo Province study reported an overall rate of persistent or recurrent disease to be 78%. There were false negative margins in three, and false positive margins in fourteen cases and HIV status played a role.[7]

Chen reported predominance of LSIL lesions in HIV-positive patients compared with HIV-negative patients. A multivariate analysis showed that HIV infection, positive margins, and the involvement of glands were independently associated with recurrence of lesions. Other factors that predisposed to residual diseases were age, HPV infection and menopause status. The rate of residual lesions was unrelated to the surgical modality.[4]

The latter study conducted in 2014 in Limpopo, Polokwane, reported an unexpected higher rate of positive margins in the HIV-negative group as opposed to the HIV-positive group. However, there was an observation of a higher regression rate in HIV-negative patients. The progression rate was higher in the HIV-positive group.[7] There

was a similar persistence rate in both HIV-positive and HIV-negative groups. The persistence rate was higher in those not receiving treatment [30 (57.7%) out of 52 on HAART and 47 (69.3%) out of 60 untreated (P = 0.20)][8]

In a cohort study conducted in Rio De Janeiro, Fábio Russomano et al; found that following LLETZ, there was a higher rate of recurrence of CIN II and CIN III in HIV-positive patients as opposed to those who were HIV-negative. This was unrelated to the margin status or to the grade of the cervical pathology treated.[9] The use of HAART may lower this trend. A similar study in Nigeria showed recurrence of visual inspection with acetic acid (VIA) or visual inspection with Lugol's Iodine (VILI) positive lesions after thermo-coagulation in HIV-positive women with low CD4 count. It was related to immunosuppression.[10]

Palmer suggested that there should be a change in the way of reporting margin status in the absence of invasion for treated CIN, as the overall cure rate at test of cure (TOC) at six months was 98%.[11] However, contrary to our study, this was not limited to HIV-positive patients. Recurrence rate of CIN lesions in HIV-positive patients was previously reported as higher than in HIV-negative patients. The factors associated with recurrence in HIV-positive patients were amongst others, glandular involvement, and positive margins.[12]

The treatment failure in patients with CIN lesions was not different in relation to surgical treatment strategy (cryotherapy, loop electrocautery), in a systemic review report.[13] However, the significant treatment failure in HIV-positive women that was observed in this study warrants further investigations.

1.5. Summary

Positive margins seem to be a determining factor for treatment failure in HIV-positive groups.[13] The presence of an ongoing comorbidity such as human immunodeficiency viral infection, autoimmune disease, hepatitis B and/or C, diabetes, malignancy, genetic disorder, and/or organ transplant, high-risk human papillomavirus positivity, as well as incomplete excision is also a significant independent predictor for residual/recurrent high-grade CIN or worse.[14]

II. Justification of the study

In our setting, the available literature on the margins' status after LLETZ on HIV-positive patients comprised a study conducted at the Chris Hani Baragwanath Hospital (CHBAH) by Adam et al. in 2008. There were few patients who were on HAART during that study period. Their report showed that HIV-positive patients had involved margins and had a substantial risk of recurrence of the dysplasia. Currently, almost all patients who are treated at the colposcopy clinic for cervical dysplasia are on HAART due to the policy change that resulted in universal treatment of patients. However, it is not known whether the findings on margins status, in this era of universal treatment, has changed or not.

III. Study aim

We anticipate that due to universal HAART treatment, improved compliance and good immune response, the margin status in HIV-positive patients will not be different from the literature, when confounders such as experience of the colposcopist, type of cervix and quality of specimen including non-charring of the margins are excluded.

Objectives of the study

1.Primary study objective

o To assess the margin status report from the histology assessment of the LLETZ tissue specimen.

2.Secondary study objectives

o To get the cytology report (Pap smear) 6 months after LLETZ

o To consider the clinical status of the patient: CD4 count, Viral load (VL)

o To deduce the procedure (LLETZ) complication rate including acute (bleeding, perforations) and intermediate and long term (infections, persistent bleeding)

o To consider the follow-up default rate

IV. Research Methods

IV 1. Study type

This will be a retrospective, quantitative, descriptive study in one academic hospital, namely Charlotte Maxeke Johannesburg Academic Hospital colposcopy unit over a six year-period from 01 January 2016 to 31 December 2021.

IV 2. Study site

The study will take place at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) colposcopy clinic located at area 176 at the Department of Obstetrics and Gynaecology.

IV 3. Data collection

Since its inception, the CMJAH colposcopy clinic stores patient's files/clinic records separate from other hospital records. The patients' records saved in line with the prescribed standards of hospital records at the colposcopy clinic. The clinic performs colposcopy and LLETZ in two ways:

- The One-step (see and treat) as well as
- The Two-step (biopsy followed by LLETZ if HSIL or as indicated).

The following variables will be extracted from the files: the sociodemographic data including age, smoking, menopausal status, contraceptive use and clinical information such as HIV, CD4, Viral load and HAART, co-morbid diseases other than HIV, LLETZ procedure, injuries recorded, complications (delayed), histology report on margins, follow-up Pap-smear report, compliance to follow-up etc.

The LLETZ report will be that of the first LLETZ done and will include the margins completeness, margins integrity, representation of both endo and ectocervix and the final pathology.

Data will be collected by the primary investigator using the data collection sheet attached as Annexure 1. Data will then be extracted and entered onto an Excel spread sheet, coded, cleaned and transferred into the data statistical analysis program.

IV 4. Participant's entry

Pre-evaluation

The database available as hardcopy patient files at the colposcopy clinic will be screened to exclude all patients who were HIV-negative at the time of the colposcopy and LLETZ for the study period.

Inclusion criteria

- Confirmed HSIL at the time of referral to colposcopy clinic.
- Patients managed through the “See and Treat” colposcopy protocol.
- HIV positive results as reported and recorded and on HAART.

Exclusion criteria

- Pregnant patients.
- Patients less than 18 years of age.
- Patients not yet on HAART and HIV-Negative patients.
- Patients with previous LLETZ.

IV 5. Sample size

We will retrospectively include all the women who were referred to CMJAH for colposcopy and LLETZ from 01 January 2016 until 31 December 2021, and the LLETZ was done with histology results available. It is estimated that ten patients come to the clinic twice a week, which gives us approximately eighty patients per month. This is extrapolated to 800 a year and considering the Coronavirus Disease (Covid-19) pandemic and the Fire of 2021in CMJAH, where the Hospital had to close; we are looking at plus or minus four thousand patients. Of these, 40% would be HIV positive which gives us a sample size of 1600.

IV 6. Data analysis

Data will be managed and analysed in IBM SPSS Statistics.

Simple general descriptive analysis: Demographic characteristics and clinical factors will be analysed as follows. Categorical variables will be summarised using frequencies and percentages, whilst continuous variables will be described using

means (with standard deviations) for normally distributed data and medians (with inter-quartile range [IQR]) for non-normally distributed data.

Primary outcome: We will determine margins of positivity as per histology report and such will be defined as HSIL dysplasia at the margin. We will calculate the agreement between colposcopy diagnostic findings of HSIL (as determined by need for LLETZ) and histology in detecting HSIL in patients who are HIV-positive, had immediate treatment with LLETZ and compared to those who are HIV-negative from available literature. Agreement between cytology and histology will be assessed by the percentage of crude agreement, defined as p=(a+d)/N, where a= number positive by both investigations, d=number negative by both investigations and N=total sample and the Kappa statistic. We will calculate percentage positive and negative agreements, defined as a/ [(N+ a-d)] and d/[(N-a+d)], respectively. We will also describe the complications reported by the women on their follow up or at acute gynaecology consultation.

Secondary outcomes:

We will define the cure as any results of normal cytology (Pap smear) after the LLETZ or lesser dysplasia such as LSIL or ASCUS done at least six months after the procedure.

I. Ethical Issues

The study will only commence after approval by University of the Witwatersrand Human Research Ethics Committee. Permission to conduct the study will be sought from the Charlotte Maxeke Johannesburg Academic Hospital CEO and the Clinical HOD of the Obstetrics and Gynaecology Department. The study will also be registered with the National Health Research Database online. The files will be accessed and

screened at the colposcopy clinic together with histology reports without being removed and no patient's identifiers will be extracted from the files.

II. Timelines

	Dec '21	Jan '22	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov -
Protocol preparation												
Submission to Committee												
Corrections												
Ethics and Hospital application												
Data collection												
Data analysis												
Write-up and submission												

III. Project Funding

No external funding for the project is applied for, obtained, or needed. All expenses related to printing, data collection, data analysis, publication, and travel, will be the responsibility of the principal researcher. The estimated budget for the study is approximately as follows.

	Quantity	Cost per unit	Subtotal
Travel			R3000
Printing and photocopy	900	0.50	R450
Data analysis	1	R5000	R5000
Professional editing and proof reading	1	R4000	R4000
Publication	1	R6000	R6000
Total Cost			R 18500

IV. REFERENCES

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Data collection sheet

Demographics at Time of Diagnosis

We will assign codes and identifiers to each patient.

Age		
Date of presentation		
Date of Pap smear		
Date of LLETZ		
Date of repeat Pap smear		
Smoking	Yes	No
Menopause	Yes	No

Clinical information

HIV status	Negative	
	Positive	CD4
		VL
		HAART
		TYPE OF HAART
Comorbidities		
Referring Pap Smear results		

LLETZ complications

Acute complications	Bleeding	
	Perforation	
Late complications	Infection	
	Persistent bleeding	

Margin status following LLETZ

LLETZ	Margin free	Ecto	Endo	Radial
	Margin involved	Ecto	Endo	Radial
REPEAT LLETZ	Margin free	Ecto	Endo	Radial
	Margin involved	Ecto	Endo	Radial

Follow up at six months

	Cured	Persistent HSIL	Progression to cancer
LLETZ			
6 MONTHS PAP SMEAR			

Appendix C: Ethics clearance

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Dr CM Ngwey-Sompo

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M220549

NAME: (Principal Investigator)	Dr CM Ngwey-Sompo
DEPARTMENT:	School of Clinical Medicine Department of Obstetrics and Gynaecology Medical School University
PROJECT TITLE:	*Margins status after LLETZ in HIV-positive patients at a South African teaching hospital: post-universal HAART programme review*
DATE CONSIDERED:	2022/05/27
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Dr L Mbodj
APPROVED BY:	Dr CB Penry, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2023/02/15

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip 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 therefore reports and re-certification will be due in the month of **May** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator Date

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Innovation)

TO: Dr CM Ngwey-Sompo
School of Clinical Medicine
Department of Obstetrics and Gynaecology
Medical School
University

E-mail: christelle_ngwey@yahoo.fr

CC: Supervisor: Dr L Mbodi
<mlang2006@yahoo.co.uk>
and <HREC-Medical Research Office@wits.ac.za>

FROM: Mr Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2023/02/15

REF: R14/49

PROTOCOL NO: **M220549** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)

PROJECT TITLE: *Margins status after LLETZ in HIV-positive patients at a South African teaching hospital: post-universal HAART programme review*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.

M:\Works2003\Iain0007\Clearscan.wps

Appendix D: CMJAH CEO permission to conduct research

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL (CMJAH)
OFFICE OF THE SENIOR CLINICAL MANAGER

Enquiries: Ms N. Mzilu
Email: Nolwazi.Mzilu@gauteng.gov.za
Tel: 011 488 3365
Ref: 1/7/2
Date: 11 September 2022

GP_2022_07_036

Dear Dr Christelle Mbangu Ngwey-Sompo

RE: PROVISIONAL APPROVAL OF STUDY

TITLE: MARGINS STATUS AFTER LLETZ IN HIV-POSITIVE PATIENTS AT A SOUTH AFRICAN TEACHING HOSPITAL: POST UNIVERSAL HAART PROGRAM REVIEW

Permission to conduct the above-mentioned study is provisionally granted subject to:

1. Submitting the research protocol
2. Submitting an Ethics Clearance Certificate from the University of Witwatersrand Human Research and Ethics Committee (HREC).

Please submit the above documents at your earliest convenient within 3 months from the date of this letter. This will prompt the Chief Executive Officer (CEO) of Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) to grant you a final approval. It is important to note that your research can only commence once a final approval is granted. Failure to comply with the above-stated requirements may result in the withdrawal of your application.

Supported / ~~Not Supported~~

Dr J. Punwasi
Senior Clinical Manager

Approved / ~~Not Approved~~

Ms. G Bogoshi
Chief Executive Officer: CMJAH

Appendix E: Approval of Title

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

30 January 2024
Person No: 0210648G
PAG

Dr CM Ngwey-Sompo
1250 Prima Vista
24 Dereham Drive
Mulbarton
2059
South Africa

Dear Dr Christelle Ngwey-Sompo

Master of Medicine in Obstetrics and Gynaecology: Approval of Title

We have pleasure in advising that your proposal entitled *Margins status after LLETZ in HIV-positive patients at a South African teaching hospital: Post Universal HAART program review* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Appendix F: Acceptance for Women Health Oral presentation

Date: 25-07-2024

Acceptance Letter

To,
Dr. Christelle Mbangu Ngwey-Sompo,
University of The Witwatersrand,
South Africa.

We are pleased to inform you that your abstract titled "**MARGINS STATUS AFTER LLETZ IN HIV-POSITIVE PATIENTS AT A SOUTH AFRICAN TEACHING HOSPITAL: POST UNIVERSAL HAART PROGRAM REVIEW**" has been accepted for **Oral Presentation** by the Program Committee of **International Conference on Women's Health and Breast Cancer** scheduled on **November 18-19, 2024** in **Dubai, UAE** organized by **Prezentis Ltd**.

We extend our cordial invitation for your participation in the conference kindly grace us with your presence.

We would greatly appreciate it if you could accept our invitation.

For more information, please go through: https://prezentis.com/womens-health/ Write us back for any further queries.

With Thanks

Christy Morris

Christy Morris
Program Manager
Women's Health2024
Email: womenshealth@presentresearch.org

Appendix G: Anatomical/ Pathological sciences (NHLS) approval

NATIONAL HEALTH LABORATORY SERVICE
UNIVERSITY OF THE WITWATERSRAND – JOHANNESBURG

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

SCHOOL OF PATHOLOGY
Division of Anatomical Pathology

NATIONAL HEALTH LABORATORY SERVICE

P.O. Box 1038, Johannesburg 2000
Tel : +27-11-489-8477
+27-11- 489-8479
Fax::+27-11-489-8512

Division of Anatomical Pathology
Faculty of Health Sciences
York Road
Parktown e-mail : Yvonne.perner@nhls.ac.za

Dr Y Perner MBBCh (Witwatersrand) FCPath (SA); MMed (Witwatersrand)

Human Research Ethics Committee (Medical)
University of the Witwatersrand
Johannesburg
20000

February 2, 2023

Re: Consent for access to NHLS database

This letter serves to confirm that the Department of Anatomical Pathology at the University of the Witwatersrand and NHLS is happy to grant Dr Ngwey-Sompo access to the Histology reports for the purposes of this study entitled "Margins status after LLETZ in HIV-positive patients at a South African teaching hospital: Post Universal HAART program review".

Publication of such work is encouraged and in the event that the information used comprises the diagnosis only then joint authorship from a member of staff in the Department of Anatomical Pathology would not be expected. However, should additional information be extracted from the report for purposes of further interpretation such as morphological details and immunohistochemical profiles, it would be expected that this would be done in conjunction with a member of staff in the Department of Anatomical Pathology and that joint authorship would follow in resulting publications. Dr Ngwey-Sompo will be in contact with the Department of Anatomical Pathology in respect of this. She will ensure that she registers on the NHLS AARMS system. Professor Reubina Wadee from the Anatomical Pathology Department will be a collaborator on this study.

Assuring you of the Department of Anatomical Pathology's co-operation in this and future research projects.

With best wishes.

Yours sincerely,

Dr Yvonne Perner
Head: Department of Anatomical Pathology

Appendix H: Plagiarism form

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 Dr Christelle Mbangu NGWEY-SOMPO (Student number: 0210648G) am a student registered for the degree of Mmed (Obstetrics and Gynaecology) in the academic year 2019.

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: 20th September 2024

Appendix I: Turnitin report