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ORIGINAL RESEARCH

The impact of positive margins and crypt involvement in excisional procedures of the cervix on recurrence rates of premalignant disease of the cervix

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Background: Recurrent disease after cervical excisional procedures has been linked to many factors. We aim to determine whether positive margins and crypt involvement increased the rate of recurrence of premalignant disease in patients who underwent excisional procedures.

Methods: This study is a retrospective review of the colposcopy database, patient records and pathology database. Women who underwent cervical excisional procedures at the Groote Schuur Hospital colposcopy clinic during 2010 were followed up until 2015. Treatment failure was based on high grade cytology or histology at follow-up. Chi-square tests were used to compare treatment failure rates.

Results: In total, 270 women were included in the final analysis. Of these women, 130 had CIN III and 94 had CIN II at the excisional procedure. Also, 85 (31.5%) had endocervical margin involvement, 46 (17%) had ectocervical margin involvement, and 24 (8.9%) had dual margin involvement, while 213 (79.2%) of the women had crypt involvement. Treatment failure occurred in 30 (19.4%) of the 155 women for whom follow-up data were available. Of those that failed, 19 ($p < 0.001$) had positive endocervical margin involvement, 10 ($p = 0.007$) had ectocervical margin involvement, 9 ($p < 0.001$) had dual margin involvement, and 28 ($p = 0.058$) had crypt involvement. Conversely, 155 women (43%) were lost to follow-up.

Conclusion: Positive margins in excisional procedures of the cervix have a statistically significant increased risk of treatment failure. There was a trend towards treatment failure in those women who had crypt involvement.

Keywords: margins, treatment failure, recurrence, crypt involvement

Introduction

According to the 2018 GLOBOCAN report, cervical cancer is the fourth most common cancer among women.[1] It is reported that there were 569 847 new cervical cancer diagnoses made during 2018.[1] There was a slight decrease in incidence at 13.1 per 100 000, compared to 14 per 100 000 in 2014.[1] The prevalence is higher in vulnerable populations such as those who are immunocompromised. This is a major contributing factor to rates in sub-Saharan Africa as there is a high prevalence of HIV.[2] Incidence of cervical cancer in southern Africa remains the highest worldwide at 43.1 per 100 000, which is an increase from 2014.[1] It is the second most commonly diagnosed cancer in South Africa at an incidence of 44.4 per 100 000, with 12 983 patients diagnosed in 2018.[3]

The GLOBOCAN report also indicated 311 365 deaths with a mortality rate of 6.1 per 100 000.[1] Mortality rates are the fourth highest in southern Africa at 20 per 100 000.[1] Cervical cancer is the leading cause of death from cancer in South Africa with 5 595 deaths reported in 2018.[3] Late presentation with advanced disease is thought to contribute to the higher mortality rates.[2] A South African review of cancer services in the public sector recognised that surgical skill is often limited to academic settings and radiation services may not be available due to lack of maintenance, contributing to poorer outcomes.[4]

Human papillomavirus (HPV), the causative agent of cervical cancer, is a double stranded DNA virus acquired through skin-to-skin contact. Most people will clear the infection within two years, but the persistence of HPV infection predisposes women to premalignant disease.[5] Oncogenic HPV strains (subtypes 16 and 18) are responsible for up to 70% of cervical cancer cases.[6] Those with compromised immune systems such as transplant patients or HIV-infected patients are at increased risk of persistent disease; while smoking is also a contributory factor.[5]

Cervical cytology is performed to screen for premalignant lesions and abnormalities found are reported on according to the 2014 Bethesda classification.[7] Women with high-grade cytological abnormalities, namely high-grade squamous intraepithelial lesions (HSIL) and atypical squamous cells-cannot exclude HSIL (ASC-H), require referral to colposcopy for review.[8] Women with evidence of persistent HPV infection, those with recurrent low-grade intraepithelial lesions (LSIL) and atypical squamous cells of undetermined significance (ASCUS) will also be referred to colposcopy according to South African guidelines.[9] Persistence of high-grade lesions, which are cervical intraepithelial neoplasm (CIN) II and III lesions, can progress to cervical cancer within 10 to 20 years.[10] If colposcopy is in keeping with a high-grade lesion, an excision procedure will be performed.[11] Excisional procedures are termed type 1, 2 and 3 depending on the type

of transformation zone and the findings at colposcopy.[12] Other treatment modalities include local destructive techniques, namely cryotherapy and laser vaporisation. While these modalities are effective in destroying the lesion, no specimen for histology is left and therefore one could potentially be inappropriately treating an early cervical cancer.[13]

The pathology report comments on the grade of CIN, the presence of microinvasion, crypt involvement and whether the ectocervical and/or endocervical margins are involved, and the relevant grade.

Current literature is ambivalent about positive margins alone as a predictor for recurrent or residual disease.[19-22] This study aims to determine the effects of positive margins and crypt involvement on treatment failure in patients attending the colposcopy clinic at Groote Schuur Hospital (GSH).

Materials and methods

This is a retrospective review where follow-up was done among women who had an excisional procedure of the cervix at the colposcopy clinic at GSH in Cape Town, South Africa during 2010. Women who attended the clinic in 2010 were followed up over a five-year period. Patients who had low-grade lesions and therefore did not have excisional procedures, were excluded from the study, as well as patients who were referred with vulval lesions. The clinic adopts the see-and-treat method. Patients with clinically high-grade lesions visualised on colposcopy were offered immediate treatment and all specimens were sent for histology. The standard follow-up is at four to six months for a repeat pap smear and colposcopy, then either six months or a year later depending on the pap smear results and findings at colposcopy. Thereafter, follow-up was done annually for 3–5 years before returning to normal screening.

The colposcopy database of the clinic was used to extract the folder numbers of the patients who had excisional procedures during 2010. Additional information was obtained from patient folders and the national health laboratory services (NHLS). Demographic data were collected. Pathology results were coded according to the highest level of abnormality. Patients were coded as having a treatment failure based on high-grade cytology (HSIL or ASC-H) or histology (CIN II or CIN III) at follow-up. Histology took precedence over cytology when both were available. No differentiation was made between recurrent and persistent disease. It was all coded as treatment failure. Some patients had missed their follow-up and re-presented again with an abnormal pap smear making it difficult to differentiate between recurrence and persistent disease. Loss-to-follow-up was defined as the year they missed their follow-up appointment. The colposcopy database as well as the study was approved by the University of Cape Town's Faculty of Health Science's Human Research Ethics committee and in keeping with the Declaration of Helsinki.[15]

Data were analysed using Microsoft® Excel and SPSS (Version 26; IBM Corp., USA). Descriptive data were presented as mean and standard deviation for continuous variables, and as proportions for categorical data. Chi-square tests were used to compare treatment failure and loss-to-follow-up rates between women with (a) endo-margin involvement and those without, (b) ecto-margin involvement and those without, (c) dual-margin involvement and those without, and (d) crypt-involvement and those without. Statistical significance was regarded as $p < 0.05$ as confidence intervals were set at 95%.

Results

There were 383 patients who had excisional procedures of the cervix during 2010, as recorded in the colposcopy database. Of these, 102 patients were excluded (Figure 1) and the demographics for the remaining 281 patients were collected (Table I).

Demographic data

Table I: Demographics of the cohort ($n = 281$)

	Number	Percentage
Age (mean)	37.6 (20–69)	
Parity	2.3 (0–9)	
Contraception ($n = 246$)		
1. Injection	120	48.8
2. COC	15	6.1
3. Implanon	1	0.4
4. IUCD	2	0.8
5. Tubal ligation	16	6.5
6. Condom	12	4.9
7. None	80	32.5
HIV status ($n = 281$)		
Unknown	22	7.8
Negative	83	29.6
Positive ($n = 176$)	176	62.6
• No treatment	65	36.9
• First line	100	56.8
• Second line	11	6.3
CD4 count ($n = 176$)		
Unknown	30	17
> 500	16	9
< 500	130	74
Viral load ($n = 176$)		
Unknown	138	78
Lower than detectable	27	15
> 1 000	6	4
< 1 000	5	3
Smoking ($n = 281$)		
Yes	53	19
No	211	75
Unknown	17	6
Relevant medical history ($n = 281$)		
None	214	76
Diabetes	9	3
Tuberculosis	16	6
Autoimmune disease	1	0.4
Other	41	14.6

IUCD – intra-uterine contraceptive device, HIV – human immunodeficiency virus, CD4 – cluster of differentiation 4

The mean age was 37.6 years (SD = 8.42; range = 20–69 years), 74% were between the ages of 30 and 49 years. On average the women had a parity of 2.3 (SD = 1.5; range = 0–9).

Of the patients included, 35 women (12.5%) were menopausal and thus not included in the contraception group. The majority of the group (120; 48.8%) used injectable contraception, followed by a third ($n = 80$) not using any contraceptive method.

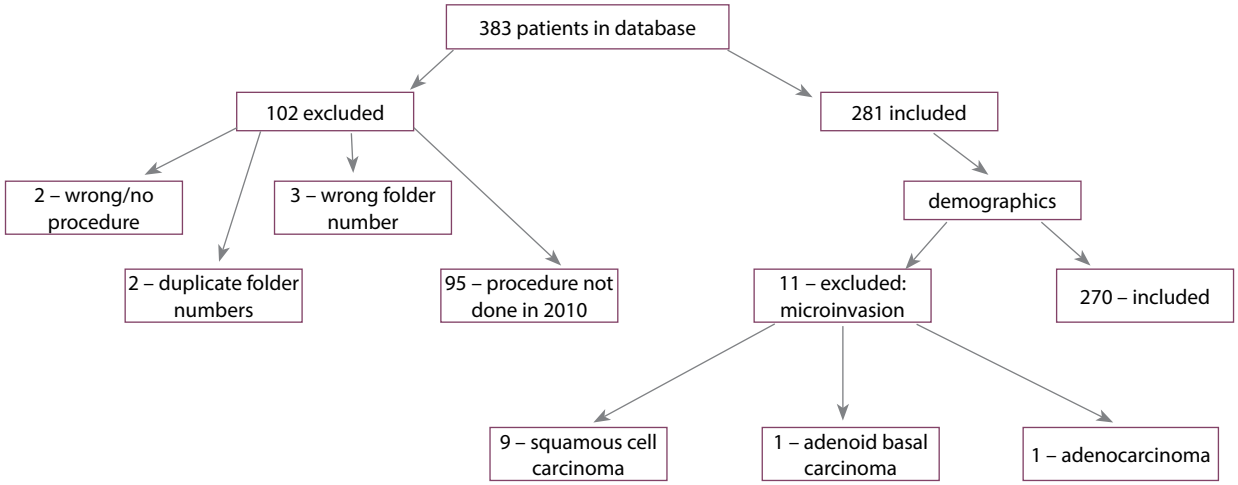

Figure 1: Flow chart detailing inclusion or exclusion of patients

In total, 83 women (29.6%) were self-reported HIV-negative and 22 (7.8%) did not know their HIV status. The remaining 176 (62.6%) were HIV-positive, of which 65 were not on treatment, 100 were on first line and 11 were on second line treatment. The majority (130; 74%) had a CD4 count below 500. Most (n = 138; 78%) of the HIV-positive patients had unknown viral loads while 27 patients (15%) had lower than detectable (LDL) viral loads.

Furthermore, 53 women (19%) smoked and 211 (75%) were non-smokers. The remaining 17 (6%) were unknown as it was not documented in either the patient files or the database.

The majority (n = 241; 86%) of women had no medical history of note if HIV was excluded. Those with significant histories included 9 (3%) diabetics, 16 (6%) who previously had TB or were on current treatment for TB and one (0.4%) who had an autoimmune disease.

Outcomes data

There were 248 large loop excision of the transformation zone (LLETZ) procedures done and 33 cone biopsies. Also, 11 patients were diagnosed with microinvasion and were excluded from the rest of the analysis. The majority (n = 224; 83%) of the patients had high-risk lesions (CIN II and CIN III) with only six patients (2%) having no pathology.

Table II: Histological findings of the excisional procedures

Pathology findings	n = 270	Percentage
CIN III	130	48.2
CIN II	94	34.8
CIN I/HPV	39 (36+3)	14.4
Cervicitis	1	0.4
No pathology	6	2.2

CIN – cervical intraepithelial neoplasm, HPV – human papillomavirus

Table II describes the overall diagnosis of the initial lesion on the excisional procedure of the cervix. The pathology findings at the margins of these procedures are illustrated in Figure 2. Margin involvement was defined as having CIN II or CIN III present at either the endocervix, ectocervix or both margins. Of the 270 women, 85 (31.5%) had endocervical margin involvement, 46

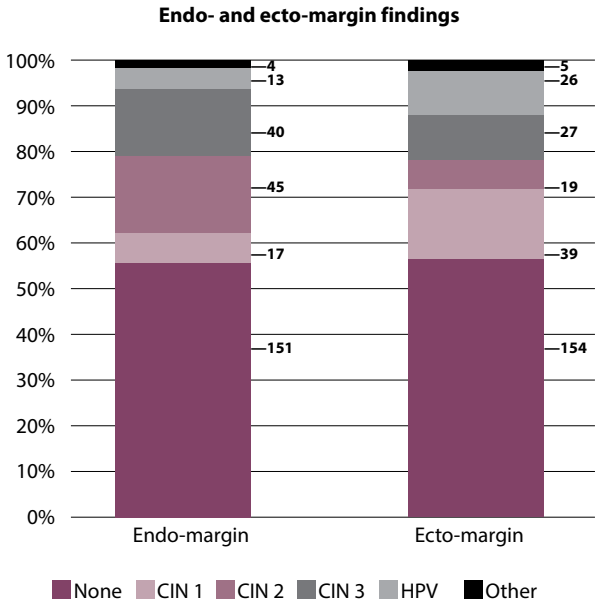

Figure 2: Histological involvement of the margins of the cervical excisional procedures

(17%) had ectocervical margin involvement and 24 (8.9%) had dual margin involvement. Margins were clear of CIN in 115 specimens.

Ectocervix mainly had CIN III involvement and in the endocervical margin there was mainly CIN II involvement. Of those who had dual involvement, the majority (54%) had CIN III at both margins.

Crypt involvement was present in 213 (78.9%) patients, while 56 (20.7%) patients had no crypt involvement. One pathology report, however, had no comment on the crypt involvement.

Treatment failure was found in 30 (19.4%) of the 155 patients who were followed up for the duration of the five years. However, 115 women (43%) were lost to follow-up. Women with endocervical, ectocervical and dual margin involvement were significantly more likely to have treatment failure compared to patients where the margins were clear of CIN ($p < 0.001$; $p < 0.007$ and $p < 0.001$, respectively). There was a trend towards treatment failure

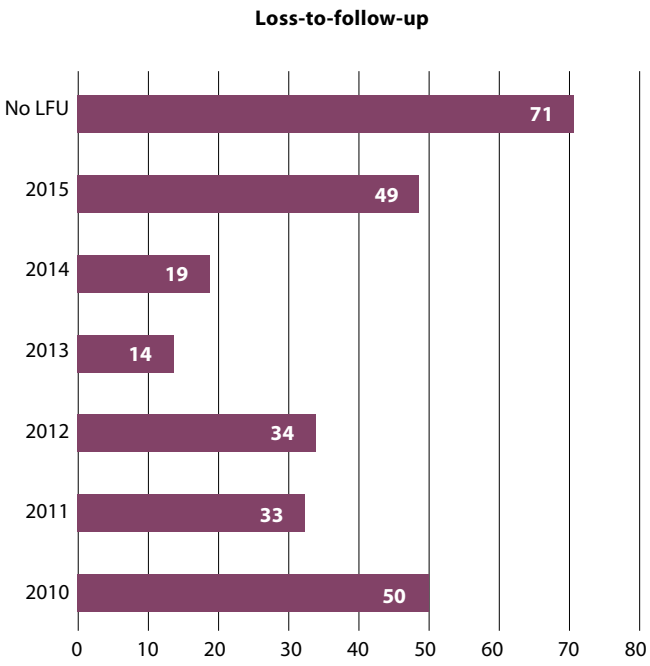

Figure 3: Loss-to-follow-up rates of women who had excisional procedures of the cervix in 2010

in patients with crypt involvement compared to those without crypt involvement ($p = 0.58$).

Patients were followed up for five years. Illustrated in Figure 3 are the loss-to-follow-up rates over the years.

Ten women had repeat LLETZ procedures, nine had a repeat cone biopsy and eight had a hysterectomy. The majority ($n = 243$) of patients had either no repeat procedure or were lost to follow-up

Discussion

There were 281 women who had excisional procedures of the cervix at our clinic during 2010. Of these, 11 (4%) had evidence of microinvasion. In the remaining 270 women, 46 (17%) were overtreated with no evidence of CIN II or CIN III, while 224 (83%) had premalignant disease.

It was found that 31.5% of the patients with premalignant disease had endocervical-margin involvement, 17% had ectocervical-margin involvement and 8.9% had dual-margin involvement. Crypt involvement was found in 78.9% of the patients. Treatment failure occurred in 19.4% of the patients who were followed up, while 43% were lost to follow-up.

The mean age of our population was 37.8 with a range of 20–60 years old. Almost half (48%) of these women was using an injectable form of contraception. Implants were only introduced in the state sector in 2014.[16] In the 2016 South African demographic and health survey,[17] women were most aware of male condoms followed by injectable contraception as forms of contraception. In this survey of sexually active women between the ages of 15 and 49, injectables were the most commonly used form of contraception at 24.8% among the 60% that were using contraception.

The abovementioned study also found that 40% of women were not using any form of contraception which is in keeping with our study which revealed a rate of 32%. These studies emphasise the need for education regarding contraception in younger women in South Africa.[17]

By the end of 2018, internationally there were 37.9 million people living with HIV. Of those, 20.6 million were living in eastern and southern Africa.[18] According to Statistics South Africa (Stats SA), the prevalence of HIV in South Africa around the same time was 7.52 million people.[19] Our colposcopy clinic saw a disproportionately large number of HIV-positive patients during this period (59.7%).[20] Colposcopy clinics are likely to see disproportionately higher numbers of HIV-infected patients as it is shown that HIV-positive patients are more likely to have higher incidences of abnormal pap smears even though this might not translate to a higher prevalence of invasive cancer of the cervix.[21]

Adam et al. indicated a self-reported HIV-positive rate of 22.4% (266 positive cases out of 1 186) in their study done at the Chris Hani Baragwanath Academic Hospital (CHBAH) colposcopy clinic.[30] Our high prevalence of HIV is also consistent with a study done by Batra et al.[22] at the GSH from 2007 to 2009, where the prevalence of HIV-positive women in the colposcopy clinic was approximately 50% (1 022/2 031). The Western Cape Department of Health at the time instructed that all HIV-positive women should undergo annual pap smears from the time of diagnosis, which was different from the national screening guideline that offered all women three free pap smears done at ten-year intervals from the age of 30.[22,23] This could explain the high prevalence of HIV in our study as more HIV-positive women were being screened more frequently at that time. The current national guidelines, however, state that HIV-positive patients should have cervical cytology from the time of diagnosis and repeated every three years if normal.[24]

In 2010, new HIV guidelines were released,[25] stating that viral load testing was to be done six months after initiation, six months later and thereafter every year. These guidelines were updated in 2010 when our study was initiated, therefore very few patients had viral load results done and available. The burden of HIV in South Africa meant that the number of patients that needed testing was more than the NHLS could accommodate.

It has been well documented that cigarette smoking as well as passive smoking have been associated with an increased prevalence of cervical cancer[26] and thus are considered risk factors for cervical cancer. We looked at the prevalence of cigarette smoking in our study population. At 19%, the prevalence was in keeping with the Western Cape province, for which the 2012 national health and nutrition examination survey found a prevalence of smoking in women of 26.9%.

Patients seen at the GSH colposcopy clinic are managed by the see-and-treat protocol. Patients referred with an abnormal pap smear and a colposcopy that is in keeping with a high-grade lesion are offered immediate treatment with an excisional procedure. The positive predictive value of the colposcopy should be at least 65% for CIN II and above. Overtreating is the biggest concern with the see-and-treat method.[27]

Overtreating has been quoted to be between 13% and 72% for normal pap smears and LSIL, and said to be reduced when

see-and-treat is limited to HSIL and high-grade colposcopy findings only.[28] Overtreatment can be justified when see-and-treat is done in a low-resource setting with a high prevalence of cancer and a low treatment complication.[28] The alternative is to do a colposcopy-guided biopsy, followed by an excisional procedure if necessary. Colposcopy guidelines[27,29] recommend that overtreatment is acceptable in 10–15% of cases. In our study the overtreatment rate was 17% which is slightly higher than recommended. Comparable studies of see-and-treat patients done at CHBAH and GSH had overtreatment rates of 9.7% and 16.7%, respectively.[30]

The National Health System (NHS) in England recommend that margins should be clear in 80% of specimens.[27] Flannelly et al.[14] followed up women who had LLETZ procedures and quoted positive margin percentages of 37%. Lubrano et al.[31] also followed up patients post LLeTZ procedures and found 20.1% of the patients had incompletely excised lesions. Similarly, Treacy et al.[32] reported that 53.4% of patients had positive margins on LLETZ specimens. Adam et al.[33] found a positive margin rate of 50% in their cohort. By a comparison of these, our study had a relatively higher number of patients with positive margins at 57.4%. The Spanish Association of Cervical Pathology and Colposcopy (AEPCC) recommends that positive margins at conisation should not exceed 20% and positive endocervical margins should not exceed 15%.[29] Recurrence of premalignant lesions was found in those with positive margins, whether ecto- ($p = 0.007$), endo- ($p < 0.001$) or dual-margin ($p < 0.001$) involvement.

In addition, the United Kingdom NHS guidelines of 2016 suggest that the size of the specimen taken needs to be 7–25 mm depending on the type of transformation zone visible. Ideally this should be less for women of reproductive age to avoid complications such as cervical stenosis and cervical incompetence.[11] We would have to look at the size of specimens that we excised to review if the specimens are too small or if the population of patients have large lesions. Although residual disease at the margins is associated with an increase in recurrence of disease, it is currently not an indication of a repeat excisional procedure; instead, it warrants closer surveillance. The exception is women over the age of 50 as they are at higher risk of persistent or recurrent disease.[27]

Our study as well as the study by Adam et al.[30] has found a far higher rate of margin involvement than suggested by international literature. We are unable to comment on the size of our excisional procedures as this was not looked at in this study. Progesterone-based contraception may cause atrophy of the cervix.[34] Another hypothesis that we considered is whether the use of injectable contraception in the South African population has an impact on atrophy of the cervix, with potentially smaller cervices and, hence, smaller specimens. This hypothesis would require further studies.

Residual disease in the cervical crypts has also been shown to be an independent risk factor for recurrence of premalignant disease of the cervix.[35] Documented numbers of crypt involvement quoted in the literature range between 15% and 58%,[35] which means that our numbers are much higher in comparison at 78.9%. As crypt involvement reflects the extent of the disease, the higher numbers may be explained by the high prevalence of HIV-positive patients in our clinic population. HIV-positive patients with cervical dysplasia are prone to more severe disease. They have a higher incidence of disease, more rapid progression and higher treatment failure rates.[36] In addition, it may also reflect the long periods for screening in our HIV-negative population who are only screened every 10 years. We found a trend in the patients with crypt involvement having treatment failure compared to those without crypt involvement ($p = 0.058$), but this trend was not statistically significant.

At our clinic the standard of care was to follow-up all women 4–6 months after the initial visit and, thereafter, yearly for a total of five years regardless of margin status. At the five-year mark there was a 26% follow-up rate. In the study conducted at CHBAH by Adams et al.,[30] they had a follow-up rate of only 57% at 4–6 months. HPV DNA testing has allowed for the shortening of the follow-up period when used in conjunction with cytology six months after excision. Those screening negative continue with routine screening.[27] At the time of this study, high-risk HPV testing was not yet available to the public sector in South Africa.

Research has shown that treatment of premalignant lesions of the cervix reduces the rate of cervical cancer by 90–94%.[36] Studies, including our own, have also demonstrated that positive margins increase the chances of treatment failure. As demonstrated in the research, patients with positive margins are at risk and should therefore be followed up closely with a high index of suspicion.

Study limitations

The retrospective nature of the research as well as the very high loss-to-follow-up rate over the five years impacted on the ability to get the treatment failure rates that are a true reflection of the population.

Conclusion

Women who have had excisional procedures of the cervix with margin involvement have a significantly higher risk of treatment failure.

Suggested further research

1. HPV DNA testing in women without margin and crypt involvement versus those with involvement.
2. Comparing size of the histological specimens with and without margin involvement.
3. Specifically looking at the size of the specimens of women using injectable contraception versus those using other methods.

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Conflict of interest

The authors declare no conflict of interest.

Ethical approval

This study was approved by the University of Cape Town's Faculty of Health Science's Human Research Ethics committee and in keeping with the Declaration of Helsinki.

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