FACTORS PREDICTING SPONTANEOUS REGRESSION OF CERVICAL DYSPLASIA

A retrospective cohort study from a private gynaecological practice.

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A research report submitted to the Faculty of Health Sciences, in fulfilment of the requirements for the degree of Master of Science in Clinical Epidemiology

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DECLARATION

I, Lifetu Likanza, hereby declare that this research report is my own work. It has not been submitted before for any degree at any other university.

DEDICATION

I dedicate this work to the memory of my mother, Veronique Lifetu Makanisi.

ACKNOWLEDGEMENTS

My gratitude goes to my supervisor, Professor Paul Rheeder and to Mrs Loveness Dzikiti (statistician) for their guidance.

To my wife and my children, for helping me keep the momentum till the end, I am deeply thankful.

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LIST OF ABBREVIATIONS

ACOG	American College of Obstetricians and Gynecologists
ART	Anti-Retroviral Therapy
ASCUS	Atypical Squamous Cell of Undetermined Significance
CIN	Cervical Intraepithelial Neoplasia
DNA	Deoxyribonucleic Acid
HIV	Human Immunodeficiency Virus
HPV	Human Papilloma Virus
HSIL	High grade Squamous Intraepithelial Lesion
LR	Likelihood Ratio
LSIL	Low grade Squamous Intraepithelial Lesion
NPV	Negative Predictive Value
OR	Odds Ratio
PPV	Positive Predictive Value
ROC	Receiver Operative Characteristic
SA	South Africa
USA	United States of America
WHO	World Health Organization

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ABSTRACT

Background. HPV status correlates with spontaneous regression of cervical dysplasia. However, HPV testing is expensive and hence not readily available in low resource settings. This study aims to investigate whether demographic factors and clinical factors can correlate and hence predict spontaneous regression of cervical dysplasia.

Methods. A gynaecological practice cytology data base was used in which 142 eligible patients out of a total of 173 with positive cervical cytology tests were followed in a retrospective cohort study. The inception cohort was assessed against 7 predictor variables: age at initial positive cytology, age of onset of sexual activity, parity, smoking, oral contraceptive use, number of lifetime sexual partners and baseline grade of cervical lesion. Univariate and multivariate logistic regression for all predictor variables was performed. The Likelihood Ratio test was used to eliminate and select relevant variables. The model was validated by the use of bootstrapping.

Results. Cervical cytology was performed on 1 678 patients over the period June 2010 to April 2014. A total of 173 patients tested positive and 142 had a repeat test. Overall, 77 (54.2%) regressed and 65 (45.8%) had persistent lesions. The median age of the study was 30 years (range: 18-62). Most patients were younger than 30 (54.9%) and had fewer than 5 lifetime sexual partners (68.3%). Most had coitarche before age 18 (64.8%), did not prefer oral contraceptive use (71.1%) and did not smoke (91.6%). The majority had fewer than 2 children (86.6%), were not married (69.0%) and enjoyed tertiary education (79.6%). Age of onset of sexual activity was the only significant variable (odds ratio: 0.79; 95%CI 0.64-0.98, P=0.03). Women 30 years or older had less spontaneous regression, although non-significant (OR=0.98; 95%CI: 0.94-1.02, P=0.25). The number of lifetime sexual partners was non-significantly inversely related to spontaneous regression (OR= 0.90; 95%CI: 0.78-1.04, P=0.15).

Conclusion. Age of onset of sexual activity significantly predicts spontaneous regression of cervical dysplasia. Age at first abnormal test and number of sexual partners also predicted spontaneous regression although non-significantly.

1. INTRODUCTION

1.1. Global burden of disease

Cervical cancer is the second most common gynaecological malignancy in the world and a second-leading cause of gynaecological cancer deaths worldwide.(1) It is estimated that about 500 000 new cases and 240 000 deaths occur worldwide every year.(1) About 80% of these cases occur in the developing world. (2) Mortality however, is decreasing, mostly due to the widespread practice of screening with Papanicolaou smear.(3)

1.2. Developed world burden of disease

Approximately 12 800 cases of cervical cancer are diagnosed annually in the United States of America (USA) with 5000 people dying. The incidence seems to be proportional to the socioeconomic level, with death rates higher among poorer minorities in the USA. The same trend is noted in developing countries where cervical cancer is higher among poorer women.(3)

1.3. Developing world burden of disease

In Africa, it is estimated that 78 897 new cases are diagnosed every year with 78% dying.(4)

In South Africa (SA), cervical cancer is the most common cancer in black women, with an incidence rate of 40 per 100 000. It is less common in white women (4th most common).(5)

De Jonge found more aggressive tumours in black women as compared to white in SA.(6). About 5 000 new cases are reported every year while nearly 60% die from the disease every year.(7)

1.4. Human papilloma virus as biological risk factor

Epidemiological evidence have clearly supported that cervical dysplasia and carcinoma of the cervix are caused by various types of HPV, particularly with the cancer associated types of HPV. (8)HPV is an obligatory intranuclear virus that must infect mitotically active cells to initiate infection. The transformation zone in the cervix is the perfect target because of its high mitotic rate. More than 100 subtypes of HPV have been identified. HPV is divided into high- and low- risk types. The high- risk types are16, 18, 31, 33, 35, 45, 51, 52 and more. The low risk types are 6, 1, and more. Most HPV infections are cleared in about 18 months. Persistent infection with an oncogenic strain of HPV is a *sine qua non* condition for development of cervical cancer. (8) (9) There must be integration of certain HPV oncoproteins (E6 and E7) into the human genome to complete the process of transforming the human host into becoming HPV-DNA positive. That will put an individual at high risk for the development of pre-malignant and later invasive cancer.(9)That process is dependent on the host immune response. A competent immune system will usually lead to clearance of HPV infection in most cases. Only a minority will progress to persistent state. Recurrent infections will make persistence easier.(9) Cellular immunity appears critical in affecting viral clearance. T-cell suppression increases the risk of HPV infection and persistence, making an HIV-positive host more vulnerable to HPV infection. That explains as well why multiple strains have been usually found in this particular situation.(10)

It is noted that although the most common 8 high-risk HPV types found in association with cervical cancer remain more or less constant (subtypes16, 18, 31, 33, 35, 45, 51 and 52), more and more isolated types are becoming more predominant, mostly in HIV-positive patients.(11) The distribution differs from one region to another.(10) (11) It is therefore important to have accurate knowledge of the HPV strains prevalent per region. Different regions may harbour different strains of HPV. Furthermore, HIV positive women have additional strains (35 and 41subtypes) apart from the usual 16 and 18 high-risk subtypes.(11)

The prevalence of abnormal cytology in HIV positive women is reported to be around 75% in Sub-Saharan Africa.(12)

Even after treatment, persistent disease detected on the repeat Pap smear was reported as 65, 03% in a study in Soweto, South Africa. The use of antiretroviral therapy did not improve the outcome. Authors argued that it could be due to low initial CD4 counts.(12) However the relationship between antiretroviral therapy and cervical neoplasia is not been agreed upon. There are still conflicting results as to whether treatment promotes regression of disease or not.(12) (13)

HPV is found in about 70% to 78% of CIN 1, 83% to 89% of CIN 2/3 and 95% of invasive cancer. The resolution rate is about 95% within 5 years. (14) High risk HPV is associated with 95% of squamous cell carcinoma. The HPV subtypes involved are the following: 16, 18, 31, 33, 35, 45, 52 and 58. HPV 18 is more associated with adenocarcinoma and HPV 16 with squamous cell carcinoma.

1.5. Spontaneous regression

Most low-grade lesions will regress spontaneously. A study in New Zealand found that, left untreated, 20%-30 % of CIN3 lesions will progress to cervical cancer over a period of 7 to 8 years.(15)

The prolonged latent phase gives an opportunity for detection and treatment of precursor lesions.

Most pre-invasive lesions will never develop to cancer. Studies have shown that 57%, 43% and 3.32% of CIN 1, 2 and 3 lesions respectively were likely to regress. Persistent lesions accounted for about 32%, 35%, and 56% for CIN 1, 2, and3 respectively.(16-19)

1.6. Behavioural and demographic risk factors

Some factors have been found to increase the risk persistence of HPV infection: smoking, early coitus before age 18, increased number of lifetime sexual partners (more than 2), sexual partners who have had multiple partners (high risk males), oral contraceptive use and immune suppression.(20, 21)

These risk factors have been tested in various studies to determine the association with cervical abnormalities: age, marital status, education level, parity, number of lifetime

sexual partners, age of onset of sexual intercourse, smoking and condom use, as well as male circumcision.(22- 27)

As experimental, clinical, and epidemiological evidence has strongly suggested that genital HPV infections are sexually transmitted, it was alleged that female sex workers mostly serve as reservoir of the infection, whereas male serve as carriers and vectors of oncogenic HPV.(22) So, acting as such, male partners can then be viewed as important contributors to the risk of cervical cancer in their female partners. Skegg hypothesised that societies where males are promiscuous have, interestingly, higher rates of cervical cancer than societies where males are not.(23) Another study on monogamous females showed that the risk of their contracting cervical cancer was related to the number of sexual partners their husbands had had.(24) Castellsague found that circumcised men were about 3 times less likely to have HPV in their penis than uncircumcised ones and that circumcision reduced the risk of both HPV infections and cervical cancer in female partners.(25)

Matsumoto et al found that tobacco smoking may interfere with regression of cervical precursor lesions. (26) The probability of regression within 2 years was significantly lower in smokers than in non-smokers (55, 0% vs., 68, 8%; P=0,004). The rest of LSIL persistence increased with smoking intensity and duration and with younger age at starting smoking (P=0,003, P<0, 001 and P=0, 03 respectively). Smoking had twice the risk of persistent HPV infection compared to non-smokers (OR= 2.50. 95% CI 1.30-4.81, P=0.006). (26) Moschicki found that probability of regression from LSIL was 61% (95% CI 53-70) at 12 months follow-up. (27) No associations were found between LSIL regression and HPV status at baseline, sexual behaviour, contraceptive use, substance or cigarette use, evidence of sexually transmitted infection or biopsy. Multivariate analysis showed that only HPV status at the current visit was associated with rate of regression. Negative HPV status was associated with regression.

Adolescents who smoke are more likely to have persistent cervical dysplasia than non-smoking adolescents.(27) Oral contraceptive pills and smoking have been shown to accelerate the cervical maturation process leading to an increased cell proliferation and increased susceptibility to HPV.(28) In a Chinese cohort followed over 8 years, it was

found that being single, having received primary education only, having had three or more sexual partners, onset of sexual activity at age 18 or younger, having reported bleeding after intercourse, and having more than 3 pregnancies are associated with cervical abnormalities.(29)

In South Africa, lower socioeconomic status, alcohol intake, and being single or black were linked to increased sexual activity in women. All that were supporting the finding that early sexual debut was associated with lower education, increased number of life time sexual partners and alcohol use.(30)

The widespread use of the Pap smear has long been accepted but has been, supported only by observational studies. The support from randomised control trials only came later on.(29) It was shown though that where it is applied consistently and persistently, there is a decrease in prevalence and mortality of cervical cancer. (9) There are success stories from many Nordic countries with success rate from a 34%to 80% decrease in incidence for more than 20 years of widespread cervical screening program.(31-33). Women who have never had screening as well as those who have defaulted for many years run about the same risk.(34)

1.7. Testing interval

Different recommendations have been published when it comes to spacing the testing without running the risk of exposing women to the risk of cancer. The American College of Obstetricians and Gynecologists (ACOG) recommends a 3-year interval after 3 consecutive negative results.(35) The South African policy in the public sector, however, is more in line with the WHO recommendation for poor countries. The starting point is 30 years of age and thereafter a smear per decade up to age 50.The model aims to decrease the incidence of cervical cancer by 64%.(36)

1.8. The cytology test and its limitations

Test accuracy is determined by sensitivity and specificity. (30) Cervical cytology sensitivity in detecting CIN 2-3 lesions ranges from 47% to 62% and specificity from 60% to 95 %38. (37) In Zimbabwe the sensitivity and specificity were 44.3% (95% CI,

37.3-51.4%) and 90.6% (95% CI, 89.2-91.9%). In SA, the ranges for sensitivity and specificity were 48.9%-78.3% and 94.2%-96.3 %.(38) Test performance depends on the prevalence of the disease. It is perceived that the higher the disease prevalence in the general population, the higher the corresponding positive predictive value (PPV) no matter how high the sensitivity would be.(39) In another review comprising randomised or quasi-randomised trials, it was found that the Pap smear sensitivity medium rule was around 60 %(29%-80%).(37) The point is that with a sensitivity ranging around 60%, one can predict a lot of false positives results. It means more unnecessary procedures as well as higher cost. In actual terms, even HPV DNA testing with sensitivity as high as 91%, in the presence of a low prevalence setting, the PPV will not still be that great.(39). The most it can do will be to add a much higher negative predictive value. Repeating the test will be an attempt to improve the PPV.

Combined HPV and cytology testing has been shown to have high negative predictive value. Furthermore, a long term NPV, a main determinant of the safe interval to use, is a key factor for the cost of a screening programme. The long- term PPV is another major factor in evaluation of cost efficiency.

Newer technology in the form of liquid- based cytology portends to improve the sensitivity issue (although debatable).(35) Liquid-based cytology has the advantage of allowing HPV, gonorrhea, and Chlamydia testing with the same specimen.(4) Better ability to filter out most of the contaminating blood and inflammatory cells and debris has been reported.(11) Its use is widespread in most developed countries and has been associated with improved specimen adequacy rates as well as increased detection rate of high- grade lesions by the repeated cytology.(4)

2. RESEARCH QUESTION

Cervical cancer is a well-known problem worldwide. South Africa faces its own challenges. Firstly, only 20 percent Pap smear coverage is achieved so far in some of the biggest primary health care facilities in the country.(40) Secondly, there is high number of loss to follow up.(40) Thirdly, overtreatment is a recognised problem.(40) So for the few that we manage to see, how can we get the maximum out of them, without compromising them in the process and remaining cost effective? HPV screen is costly and South Africans are probably not yet ready to afford it for all the cases of abnormal cytology. The question is: which simple factors in the clinician's hands can help predict which one of the patients with a positive cervical cytology is likely to regress spontaneously? That will help to select those that should be dealt with more aggressively to avoid loss to follow up and unnecessary future obstetrical complications (cervical incompetence). More precisely mechanism will be put in place to be able to trace them more easily.

Our study considered demographic, socio- economic as well as biological factors involved in the progression to cervical cancer to depict those factors that would favour and consolidate spontaneous regression. The clinician who faces the patient with an abnormal cytology is as much interested as the patient to know what would happen next. It is about how likely this particular lesion would spontaneously regress or not. The question is even more relevant in a setting where HPV screening is not routinely available. It is a prognostic study. We chose liquid- based cytology as our medium of specimen collection. It has been used routinely in our practice since2009.

2.1. Aim

The aim of the study was to outline a model predicting changes from an abnormal cervical lesion to a lesser-grade lesion.

2.2. Objectives

The objectives were:

1. To describe the characteristics of patients with abnormal Pap smear in private hospital catering for mostly black patients in Pretoria.
2. To compare the characteristics of patients with abnormal Pap smear that eventually regresses from those that do not eventually regress.
3. To determine the factors that predict the spontaneous regression of the lesions.

3. METHODS

3.1. Study design

This was an observational, retrospective prognostic cohort study, conducted between 01/06/2010 and 30/04/2014. It was more precisely a prognostic study. Data were collected from the practice Pap smear data base as well as patients' clinical files.

3.2. Setting

Louis Pasteur Private Hospital is one of the independent network groups of private hospital, situated in the Pretoria CBD. It caters mostly for the black population in town as well as from the surrounding townships of Atteridgeville, Soshanguve, Mabopane, Ga-Rankuwa and Mamelodi. The researcher's practice provides general obstetrical and gynaecological care. It runs a cervical cytology clinic with patients selected from the practice clientele as well as referral from general practitioners.

3.3. Study population

The study population was all women attending the researcher's practice and who had an abnormal cytology. Those with low-grade lesions were usually reviewed after 3 to 6 months for a repeat cytology and further management. All those who had a repeat cytology following an abnormal one were included in the cohort. They were further divided into 2 groups: those who would remain abnormal as before and those who would have had lesion downgrading or reverting to normal altogether. Thus, all those with a lesser grade of disease were seen as cases.

3.4. Sampling

A census method was used, meaning all patients with abnormal cervical cytology were considered.

The sample size was evaluated by selecting 20 patients for every explanatory variable. Therefore, for seven explanatory variables, we needed at least 140 women.

3.5. Data collection

The researcher selected all the patients with a positive cytology who repeated the procedure. The laboratory report was attached to the patients 'files where the researcher recorded other demographic and clinical parameters needed.

The data were collected from the practice Pap smear register. All patients undergoing cervical cytology were considered. All patients with a positive cytology, 173 from a total of 1 678 were included in the cohort members. These patients were then followed up as required by standard management of abnormal cervical cytology where a repeat cytology is usually required between 3 to 6 months after the initial low-grade lesion. High-grade lesions were treated more aggressively by means of biopsy and possible excision. Those patients whose cytology regressed to negative were considered as cases, whereas those with persistent lesions were non-cases. We included high-grade lesions if for any reason there was a delay in performing the biopsy, in which case a repeat cytology was performed.

All patients were assessed according to a uniform set of criteria (prognostic factors). Those factors were demographic and clinical criteria routinely obtained clinical notes.

All specimens were collected in liquid- based medium using a cytobrush and were reported on by the Ampath Laboratory.

3.5.1. Explanatory variables.

The explanatory variables were demographic, clinical and histopathological. They were:-1) age at last birthday at first positive smear, -2) parity, -3) use of oral contraception, -4) number of lifetime sexual partners, -5) smoking, -6) type of lesion, and -7) age of onset of sexual activities.

The information set out immediately above was obtained as part of routine interrogation during a gynaecological consultation, taking into consideration the risk factors for cervical cancer.

A question about number of sexual partners was asked after a positive pap smear with the patient having the option of not answering if not comfortable.

Patients were given information about cervical cancer and the need and usefulness of a pap test.

After interrogation, the researcher then proceeded to the general and gynaecological physical examination. The cervical specimen was collected by use of cytobrush which was then placed into the Sure Path vial. (Figure 1). The vial contained a liquid medium. The specimen was then sent to the laboratory for further evaluation and cytology.

Results were usually available within a week and were attached in individual patients' files.

Confounders were never an issue from the outset as the researcher was aware that adjustment for confounders was only essential to prove causality. Causality, as in etiologic research has a mission of assessing whether there is causal link between an outcome variable and particular explanatory variables. In contrast, the goal in this researcher's study was prognostic research, "to predict as accurately as possible the probability or the risk of future occurrence of a certain outcome as a function of multiple predictors.''(40)

3.5.2. Outcome variable.

The cytology results from the laboratory contained the answer to the question whether or not there was regression to a lesser grade of lesion than previously.

The researcher then decided on further management. Those whose lesions regressed were treated expectantly, whereas those with persistent lesion were treated more aggressively.

3.6. Data analysis

Comparison between groups with or without persistent lesions was performed using descriptive statistics and chi square or Fisher's exact tests where appropriate. The same applied when comparing the sample patients with those lost to follow-up.

All continuous variables were subjected to a Box-Tidwell test to assess a linear relationship between the continuous variable and the logit. That was necessary in order to determine whether to use the continuous variables as such in the logistic model or alternatively to recode the continuous variables as categorical or dichotomous. Age, parity, number of lifetime sexual partners and age of onset of sexual activity were subjected to the test. The cut-off value was p-value of 0.1. All were significant and therefore were categorised as dichotomous. The cut-off values were the 50th centiles.

Logistic regression analysis was used to assess the association between prognostic factors and the outcome. A multivariate logistic analysis was done starting with the elimination of variables and the covariables.The Likelihood Ratio (LR) test and a stepwise backwards hierarchical approach were used. The final model was reached until a point where we could no longer drop any variable without a resulting statistically significant LR test. The evaluation of the accuracy of the model was assessed by means of calibration and discrimination using Pearson's goodness of fit test and Receiver Operating Characteristic curve respectively. The external validation of the model was assessed by means of bootstrapping.

All data were analysed using Stata 12 statistical software.

3.7. Ethics

The study protocol was approved by the Ethics Committee of the University of Pretoria (378-2013).

A waiver of informed consent from individual patients was obtained from the Ethics Committee, considering that the data were coming from the practice's clinical data with no input from the patients.

Figure 1. Cytobrush and vial containing liquid medium

4. RESULTS

Cervical cytology testing was performed on 1678 patients over a period June 2010 and April 2014. A total of 173 tested positive and 142 had a repeat test. Overall, 77 (54.2%) regressed and 65 (45.8%) had persistent lesions (Figure 1).

Table 1 shows the demographic- and clinical characteristics of the study population. The median age of the study population was 30 years, ranging between 18 and 62 years. Most of them were younger than 30 years old (54.9%), had fewer than 5 sexual partners (68.3%). Most of them had started sexual activities before age 18 (64.8%), did not prefer oral contraceptive use (71.1%) and did not smoke (91.6). The majority had fewer than 2 children (86.6%), were not married (69.0%) and had enjoyed tertiary education (79.6%).

More than two-third had low-grade lesions (LSIL 66.9% and ASCUS 19.7%).

Patients older than 30 years had more persistent lesions (51.6%), as well as those with more children (54.75). Patients who had started sexual activity before 18 years had lesions that regressed as compared to those who started later (Table 2).

The results of univariate and multivariate analyses are shown in tables 3 and 4, respectively.

Age of onset of sexual activity was the only significant variable, with an odds ratio of 0.79 (95% CI 0.64-0.98) and a p-value of 0.03. Nevertheless age and number of sexual partners could still make the final model owing to the fact that the LR tests were significant for both, implying that their removal from the model made a statistically significant difference. In logistic regression the Wald test power is low and there is no adjusted R2 as in linear regression, so the LR test is used instead for deciding whether to drop a variable or not.(41)

Post-regression analyses are shown in Table 6. The Pearson's goodness of fit testing was non-significant at a P-value of 0.23; so there was agreement between the model prediction and observed diseases states. The area under the receiver operating

characteristic (ROC) curve was 0.66 (Figure 2). This result revealed that our model was adequate.

Bootstrapping was done as method of validation of the model (Table 5). There is similarity between both results.

Figure 2. Study schema

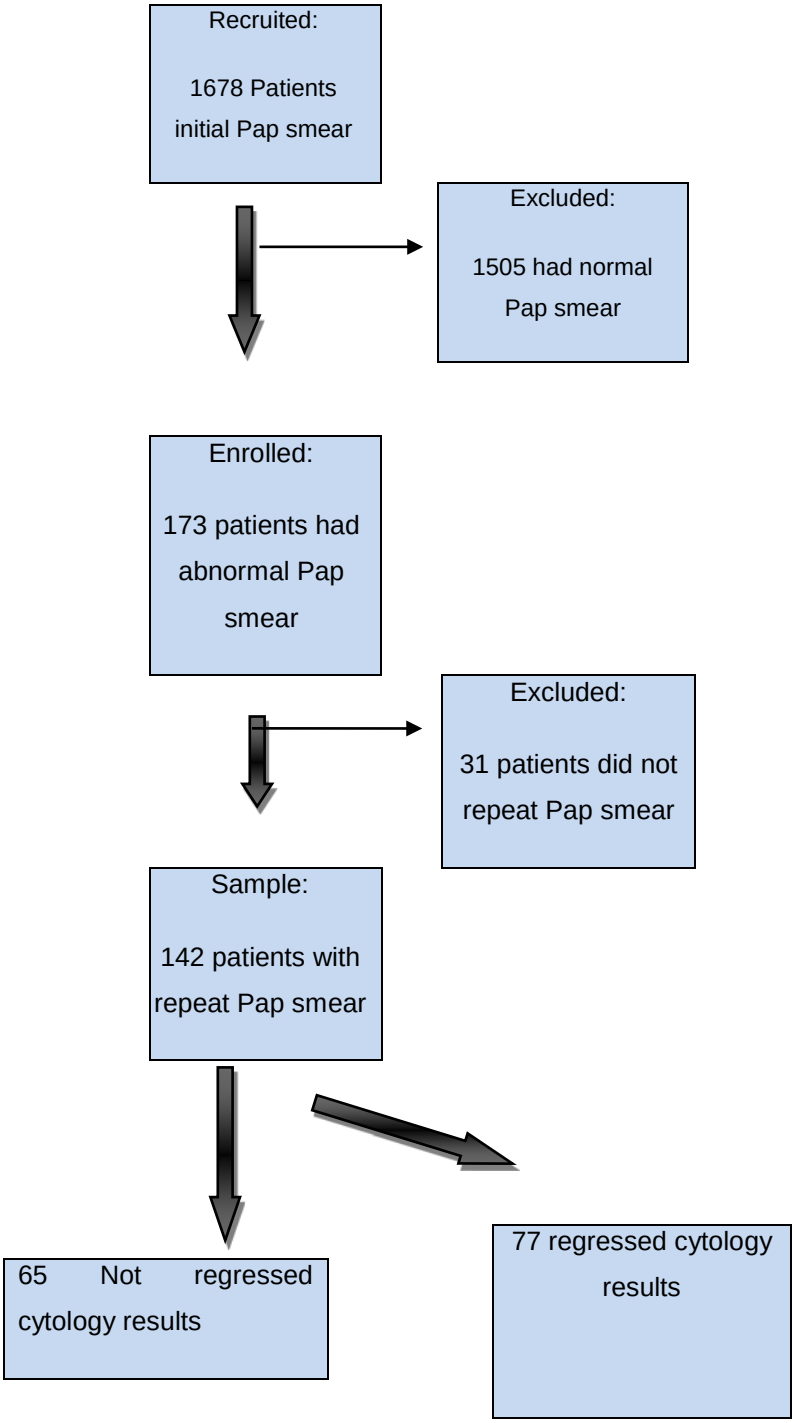


Table 1. Demographic- and Clinical Characteristics

Demographic information	**N=142** **n (%)**
1. Age at diagnosis of lesion (years)	
Median	30
Interquartile range	24 – 34
<30	78 (54.9)
= >30	64 (45.1)
2. Parity	
Median	1
Interquartile range	0-4
<2	123(86.6)
=>2	19(13.4)
3. Number sexual partners	
Median	5
Interquartile range	3 – 6
<5	97 (68.3)
= >5	45 (31.7)
4. Age of onset sexual activities	
Median	18
Interquartile range	17 – 19
<18	92 (64.8)
= >18	50 (35.2)
5. Oral contraceptive use	
No	101 (71.2)
Yes	41 (28.8)
6. Smoking	
No	130 (91.6)
Yes	12 (8.4)
7. Regression of lesions	
No	65 (45.8)
Yes	77 (54.2)
8. Baseline cytological results	
1.ASCUS	28 (19.7)
2.LSIL (CIN I)	95 (66.9)
3.HSIL (CIN II)	13 (9.5)
4.HSIL (CIN III)	6 (4.2)
9. Level of education	
Secondary	29 (20.4)
Tertiary	113 (79.6)
10. Marital status	
Married	44 (31.0)
Single	98 (69.0)

ASCUS=atypical squamous cell on undetermined origin; LSIL=low-grade squamous intraepithelial lesion; HSIL=high- grade intraepithelial lesion; CIN=cervical intraepithelial neoplasia.

Table 2. Comparison between group with persistent lesions and group with regressed lesions (N=142)

Demographic information	No regression N=65(45.77%) n (%)	Regression present N=77(54.23%) n (%)	P-value
1. **Age at diagnosis of lesion (years)**			
<30	32 (41.03)	46 (58.97)	
= >30	33 (51.56)	31 (48.44)	0.238
2. Parity			
<2	57 (46.34)	66 (53.66)	
= >2	8 (42.11)	11 (57.89)	0.81
3. Number sexual partners			
<5			
= >5	39 (40.21)	58 (59.79)	
	26 (57.70)	19 (42.22)	0.07
4. Age onset sexual activity			
<18			
= >18	38 (41.30)	54 (58.70)	
	27 (54.00)	23 (46.00)	0.16*
5. Oral contraceptive use			
No			
Yes	48 (47.52)	53 (52.48)	
	17 (41.46)	24 (58.54)	0.51
6. Smoking			
No	60 (46.15)	70 (53.85)	
Yes	5 (41.67)	7 (58.33)	0.77
7. Baseline Cytology			
1.ASCUS	10 (35.71)	18 (64.29)	
2.LSIL(CIN I)	47 (49.47)	48 (50.53)	
3.HSIL(CIN II)	4 (30.77)	9 (69.23)	
4.HSIL(CIN III)	4 (66.67)	2 (33.33)	0.27

*Fisher's exact used when there were <5 observations in a cell

Table 3. Crude univariate odds ratio.

Regression of lesion	OR (95% CI)	P-value
Age at diagnosis (years)		
<30		
= >30	0.65(0.34 - 1.27)	0.21
Age onset sexual activities		
<18	0.60 (0.30 – 1.20)	0.15
=>18		
Number sexual partners		
<5		
=>5	0.49 (0.24 – 1.01)	0.05
Parity		
<2		
=>2	1.01 (0.77 – 1.34)	0.72
O/contraceptive		
No		
Yes	1.28 (0.61 – 2.67)	0.51
Smoking		
No		
Yes	1.20 (0.36 – 3.98)	0.77
Type of lesions		
2 LSIL	0.57 (0.24 – 1.36)	0.20
3 HSIL (CIN II)	1.25 (0.30 – 5.11)	0.76
4 HSIL (CIN III)	0.28 (0.83 – 3.90)	0.14

OR=odds ratio

Table 4. Final Model

Regression of lesion	Adjusted OR (95% CI)	P-value
Age onset sexual activities		
<18		
=>18	0.79 (0.64 – 0.98)	0.03
Number sexual partners		
<5		
=>5	0.90 (0.78 – 1.04)	0.15
Age at diagnosis		
<30		
=>30	0.98 (0.94 – 1.02)	0.25

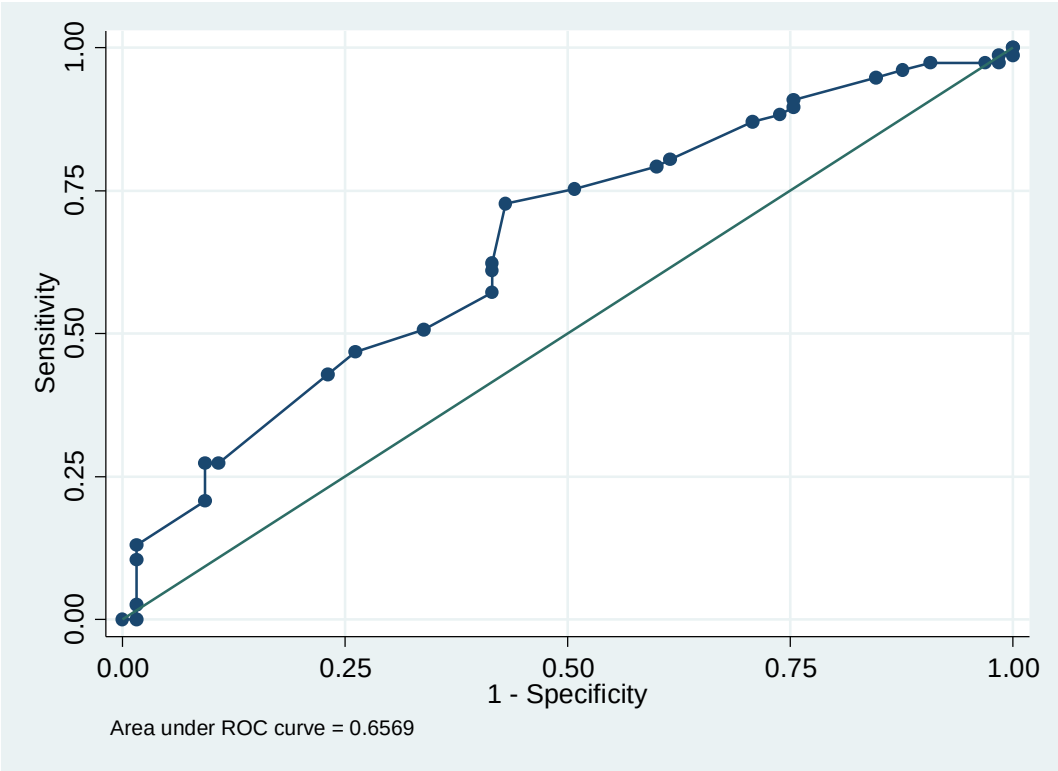
Table 5. Bootstrapping

Regression of lesion	Adjusted OR (95% CI)	P-value
Age onset sexual activities		
<18		
=>18	0.79 (0.65 –0.97)	0.03
Number sexual partners		
<5		
=>5	0.90 (0.78 –1.05)	0.17
Age at diagnosis		
<30		
=>30	0.98 (0.93 - 1.02)	0.32

Table 6. Post regression analysis

Pearson's goodness-of-fit test :
P-value=0.23.
Area under ROC curve=0.66.

Figure 3. ROC curve



4. DISCUSSION

We followed a cohort of 173 patients with abnormal cervical cytology from a private gynaecological practice. The aim was to depict factors that could predict spontaneous regression of cervical dysplasia. More than half of the cases of mild dysplasia returned to normal and about almost the same proportion of moderate lesions had the same fate. Only 3 factors could fit the model: age of onset of sexual activity, age at diagnosis of the lesion and number of lifetime sexual partners.

This study noted that women whose age of sexual onset was more than 18 years old were less likely to regress than those with younger age of onset (OR=0.79; 95% CI 0.64 – 0.98). Furthermore, women 30 years or older had less chance of spontaneous regression of lesion as compared to the younger women (OR=0.98; 95% CI: 0.94-1.02. P=0.25). The association was however non-significant. It was again shown that the number of lifetime sexual partners is inversely related to spontaneous regression of lesions (OR=0.90; 95% CI: 0.78-1.04. P=0.15), although non- significantly.

A cohort study in Brazil also concluded that women under 30 years at diagnosis of LSIL were more likely to regress than older women, and women 50 years or older with HSIL were at higher risk of persistence and progression, when compared to younger women. (43)

The same study from Brazil found that women whose age of onset was under18 years old were more likely to progress instead, although not significantly.(43) This finding appears to contradict our study.

It is commonly accepted that early onset of sexual activity puts women at higher risk of cervical cancer, with an odds ratio of 2.70 (95%CI: 1.178-4.11) and 2.01 (95%CI: 1.23-3.30) for both squamous cell carcinoma and adenocarcinoma respectively. (44) Could we speculate that acute infection of HPV at the time when the transformation zone is less susceptible to cellular regeneration and hence higher chances of persistence and progression of lesions? Probably one should not look at it in isolation. A possible interaction with other risk factors could have a possible role? An increased number of sexual partners at that older age would increase the chances of persistence of lesions. Cytological regression usually occurs five to six months before HPV clearance, given the speed of epithelium regeneration.(45) An increased re-infection rate brought on by an increased number of sexual partners would, in a setting of decreased epithelium regeneration, possibly lead to delayed viral clearance with subsequent persistence of lesions.

Furthermore, although CIN-1 cytology is accepted as representative of acute infection, a study of the incidence of HPV infection in teenagers found that the risk of CIN-2/3 lesions is possibly high in the first six months after detection of HPV, with a rapid

decline thereafter. (46) The scenario could possibly change should the rapid decline not happen.

There is a positive correlation between having four or more vaginal sex partners in the previous six months and detection of incident HPV infection.(47) Other studies found that infection with high-risk types of HPV and older age were risk factors for persistent HPV infection.(48) Persistent infection would increase the occurrence and persistence of cervical dysplasia.(49) (50) One can also speculate that there is an increased probability of infection with a high- risk type HPV considering the increase number of opportunities to get infected during multiple sexual encounters.

HPV and its natural history have been the backbone of recent guidelines for the prevention of cervical cancer and management of cervical dysplasia. There is a certain demarcation between two groups of women, the younger and the older. At the centre of it is the persistence of HPV infection in older women with subsequent manifestation and rapid progression to higher grades of cervical dysplasia and eventually cervical cancer. The age of demarcation varies depending on different study references and may differ from country to country. In South Africa, 30 years of age is the cut-off. The South African screening programme recommends screening from age 30 and thereafter once every 10 years until age 50.(36)

Age was also prominent in the recent ACOG guidelines and knowledge of HPV status was highlighted because of the propensity to persistence with subsequent triggering of cervical dysplasia.(35)

The high negative predictive value that comes with the combination of the two tests (HPV-DNA testing and cervical cytology) justifies the 5-year interval between testing. Adam found two years to be acceptable enough to proceed. It all goes according to how much sacrifice one is prepared to take. The South African guideline of starting at age 30 and thereafter every 10 years is done with the understanding that about 40% of the eligible population can be missed. Others do not feel comfortable with that 40% and go on to increase the frequency of testing despite the high cost involved.

The Japanese guidelines for cervical cancer screening also point towards targeting age 30 and above for screening as the impact on reducing mortality is more substantial among that age group.(51) A population-based case control study in the United Kingdom shows that there is little or no impact in the reduction of mortality and the rate of invasive cancer up to age 30 years.(51)

There seems to be consensus of opinion that HPV is the causal factor of most cervical dysplasia and persistence of the infection is the trigger factor of the disease process. (52, 8, 53, 54) The high-risk strains are the ones mainly implicated.

Different demographic, socio-economic and biological factors have been identified as risk factors related to the epidemiology of HPV.(55, 44, 45, 56) That knowledge was consolidated through a variety of cohort studies concerning the natural history of cervical dysplasia and cervical cancer risk. But modern medical research history has learned to become cautious in assuming that if particulars risk factors have been proven to cause cervical dysplasia or cancer, the reverse might not necessarily be true. (57-58)

A few cohort studies have suggested that most HPV infections are transient in young women and that persistent infections are more common in older women. All is not yet known about the determinants of persistent HPV infection.(59)

A prospective study found that cervical cytology (Pap smear) is more important than HPV screening in women under the age of 35 than it is for older women. However, HPV high-risk subtypes positivity is used in predicting the risk of progression of the lesion.(60) HPV is the main factor determining regression or progression of the lesion. Regression of LGSIL was significantly detected in 23% of high risk HPV-positive women as compared to 50% high-risk negative women. On the other hand, progression was detected in 29% of high-risk HPV women and 11% of high-risk HPV women. However the relation was significantly borderline. (P=0.055)

HPV-related risk factors can be used as surrogate variables.(61)

The relation between persistent high-risk HPV infection and CIN2+ is well established. Persistence or clearance of high-risk HPV has been postulated as an early prognostic marker of failure or cure after CIN2+ as well as being more accurate than cytology or section margin status at the time of conisation. Older women were more likely to have recurrence. (62) Yet another cohort study found that persistence of HPV for more than 6 months, together with older age and, type pf HPV were associated with cervical cancer.(47)

Older women have a lower risk of acquiring HPV than younger women. There was a speculation that this lower risk could be due to acquired immunity to HPV from past exposure.(47, 63)

HIV infection with resultant deficient immune response to HPV might favour a situation of multiple-type infection predisposing to persistent infection. (50,64)

Our study showed a regression rate of CIN2 lesions of about 69%, depicting a similar behaviour with lower grade lesions. That percentage seems quite high compared to the trend expressed in the literature. A selection of cohort studies on natural history of cervical intraepithelial neoplasia grade showed a range between 26% to 63%.(65) One can speculate about misclassification bias as well as the impact of the loss to follow-up. But one thing that is obvious is that, in line with the literature, the natural history of CIN2

is closer to the natural history of CIN1.(66) As such and, considering the adverse obstetrical effects of the ablative procedure, perhaps a careful follow up could be justified. There is value in adding HPV serotyping to the scenario as studies have shown that none of the regressing CIN 2-3 lesions contained HPV-16.(67-68)

Our model presents 3 variables, with only one significant relation vis-a-vis the outcome. The two others (age and number of sexual partners), although not significant, are still relevant in the model. The answer to this subtle question comes from considering the likelihood ratio test against the Wald test in the context of logistic regression. The P-value in logistic regression corresponds to the Wald test statistic which does not weigh much in logistic regression.(41, 69, 70) The likelihood ratio approximate chi square if the sample size is large enough. However, there is no precise clarity about how large enough the sample size should be.(69-70)

The Wald test statistic has an approximately normal distribution in large samples. It represents Z which square is approximately chi square with one degree of freedom. In large samples, the Likelihood Ratio statistic gives approximately the same value as its corresponding square Wald statistic. So, if the sample size is large enough, it does not matter which statistic is used. However in small samples, the two statistics give different results. Statisticians have shown that the likelihood statistic is better in those situations.(69-70)

In our case, we used the Likelihood Ratio test to decide about which variable to keep in the model, irrespective of the Wald test P-value.(41, 69, 70)

We considered that the variables should not be considered in isolation, but rather collectively.

The Pearson's goodness of fit is average, as well as the area under the ROC curve. So, it is a fairly good model.

As a technique of repeated sampling from the same population, bootstrapping would give a real idea about a population. It is a validation strategy like different researchers do the same study in different setting. So bootstrapping help give us an estimate of the entire Pretoria region in a way that we can extrapolate our results beyond our own practice.

Limitations.

Our study had several limitations.

First, we considered mostly surrogate variables knowing that HPV play a bigger role in the natural history of cervical dysplasia.

Second, not knowing the HIV status was a hindrance to more accurate results, as we know that immune deficient patients have higher incidence of cervical dysplasia. The interaction between the two could have explained the fact that women with late onset of sexual activity or late encounter with HPV were prone to persistent infection; hence persistent dysplasia.

Third, misclassification was a limitation because the study was retrospective with data coming from medical records, with a possibility of non-uniformity in the recording of information.

Fourth, there was a high rate of loss to follow up. The same trend was present in a study in Soweto where a HIV cohort was followed up. It was interesting to notice that even knowledge of the HIV status could not be motivation enough to lead to a change of behaviour. Indeed, those on ART were more likely to be lost to follow up than those who were not on medication (49.08% v. 40.96%; P=0.04).(71)The same trend was noticed in general at Baragwanath hospital in Soweto, not only in HIV-positive patients.(40) A multicentre cross-sectional study finding the prevalence of precancerous lesions and cervical cancer in south Africa also found a similar trend.(72) Our study population did not differ much from the trend noted above, although one could have assumed that those who could afford private care would have been more compliant.

It is still to be determined what could be done to generate an appropriate response to the available guidelines. The media is one aspect of the solution. (73)

Fifth, the sample size seemed to be not sufficiently large enough to fit the likelihood ratio statistic and its Wald test statistic equivalent together. A larger size would have most probably given significant p-values.

Conclusion.

In conclusion, our study showed that clinical and demographic factors can be considered in predicting spontaneous regression of cervical dysplasia.

Only age of onset of sexual activity showed to be significant, although the model have still considered age at first diagnosis and number of sexual partners as components of the model.

Our model centred on age at diagnosis of the lesion, as well as the onset of sexual activity and the number of lifetime sexual partners. There was a conspicuous missing entity represented presumably by the absence of testing for HPV-DNA as well as HIV infection. It turned out most probably that all was about the natural history of HPV

infection in a setting with a high prevalence of HIV infection. Knowledge of these two parameters would certainly help identify those not at risk of progression of lesions and, therefore, reduce costs, as well as morbidity associated with ablative therapy. Our model is an attempt to help the clinician in the prognostication in the absence of HPV-DNA and HIV testing.

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The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
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Faculty of Health Sciences Research Ethics Committee

25/10/2013

Approval Certificate
New Application

Ethics Reference No.: 378/2013

Title: Factors predicting spontaneous regression of cervical dysplasia. A retrospective cohort study from a private gynaecological practice.

Dear Dr L Likanza

The **New Application** as supported by documents specified in your cover letter for your research received on the 09/09/2013, was approved by the Faculty of Health Sciences Research Ethics Committee on the 23/10/2013.

Please note the following about your ethics approval:
- Ethics Approval is valid for 1 years.
- Please remember to use your protocol number (378/2013) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of 6 monthly written Progress Reports, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

DR R SOMMERS; MBChB; MMed(Int); MPharmMed.
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee
University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

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