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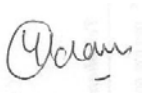
A RESEARCH REPORT SUBMITTED TO THE SCHOOL OF PUBLIC HEALTH, UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICINE FOR THE YEAR 2011

The incidence of cytological abnormality 12-24 months after a normal smear in a setting with a high prevalence of cervical abnormalities

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Declaration:

I, Yasmin Adam declare that this research report work is my own work. It is being submitted for the degree of Master of Science in Medicine in the field of Epidemiology and Biostatistics in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signature: 

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3 September 2010

Abstract:

Introduction: A screening program has been implemented in SA with intervals of 10 years after a normal cytological result. There are no studies which evaluate repeat screening at a shorter interval in SA.

Aim: This study aims to find the incidence of cytological abnormalities on a repeat test after a report of normal cytology or an inadequate pap smear. The factors associated with an abnormal cytology on repeat testing will also be explored.

Methods: This is a secondary data analysis of the information obtained in a trial investigating the use of the diaphragm in the prevention of HIV infection between 2004 and 2006. Women were offered a Papanicolaou (Pap) smear at the enrolment visit and again at the end of the trial. The incidence of abnormal cytology after a normal or inadequate cytology was obtained. Demographic factors, history, clinical findings and tests for STI were compared amongst women with a normal and abnormal Pap smear on repeat testing after a normal Pap smear.

Results: The incidence of cytological abnormalities was 6.48 % per annum in women with a previously normal Pap and 11.71% per annum in women with an inadequate smear result. (Log rank test for difference significant (p=0.03)). The incidence of cytological abnormalities in women with either an inadequate Pap smear or a normal Pap smear was 7.33%. However, the incidence of a high grade lesion was less than 0.5%. Factors associated with abnormal cytology in the multivariate analysis were a history of ectopic pregnancy [OR=9.25 (CI-1.78-48.03), p=0.01], number of male partners [0R=1.12 (CI-1.03-1.22),p=0.01], number of times

a women was treated for an STI [OR=6.59 (1.54-28.19), p=0.01], history of vaginal discharge [OR=13.95 (1.18-164.47), p=0.00], and HIV infection [OR=6.58 (1.14-38.16),p=0.04].

Conclusion: The incidence of significant cervical lesions is low, but it would be prudent to continue to repeat those Pap smears that are found to be inadequate with the present interval of 10 years. In women with a normal Pap smear, a repeat Pap smear after 1-2 years should only be performed if clinically indicated.

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List of Abbreviations:

ACOG	American College of Obstetricians and Gynaecologists
ACS	American Cancer Society
ASR	Age Standardised risk
CTFPHE	Canadian Task Force on the Periodic Health Examination
CIN	Cervical Intra-epithelial Neoplasia
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
HSV	Herpes Simplex Virus
IARC	International Agency for Research on Cancer
MIRA	Methods for Improving Reproductive Health in Africa
NHLS	National Health Laboratory Services
NZGG	New Zealand Guidelines Group
Pap	Papanicolaou
PHRU	Perinatal HIV Research Unit
SA	South Africa
SANAS	South African National Accreditation Society
STD	Sexually Transmitted Disease
STI	Sexually Transmitted Infection
UK	United Kingdom
US	United States

USPSTF	United States Preventive Task Services Task Force
WHO	World Health Organization

Definition of Terms:

Pap smears were reported using the 2001 Bethesda system(1).

Normal Pap smear:

The cytology report gives the result/ interpretation or general categorization as “negative for intraepithelial lesion or malignancy”, with a statement of adequacy.

Inadequate Pap smear:

The cytology report gives the result/interpretation or general categorization as “negative for intraepithelial lesion or malignancy”, adding a statement that the examination was “unsatisfactory” and the reason for this. A satisfactory Pap smear is one in which there are between 8000 and 12 000 well visualized squamous cells and the presence of ten well visualized endocervical or squamous metaplastic cells.

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Chapter 1: Background and Literature Review:

Background:

Cancer of the cervix remains a huge problem in South Africa (SA), it is the most common cancer in Black women with an age standardized risk (ASR) of 30.56 per 100 000 in 2001(2). The lifetime risk of a woman developing cancer is 1 in 8 (2001). Cancer of the breast (1 in 29) and cancer of the cervix (1 in 35) are the two leading cancers followed by uterine, colorectal and oesophageal cancer. There were 4817 cases of cervical cancer in 2001 in SA, with a quarter occurring in women between the ages of 40 and 50 years(2). Approximately 80% of the cervical malignancies now occur in the developing world(3).

Population based screening and treatment programs of pre-malignant lesions of the cervix has significantly reduced the morbidity and mortality associated with cervical cancer, with reductions in death of between 45% and 80%(4-6). Screening, using Papanicolaou smears (multichromatic staining of exfoliated cervical cells- hereinafter referred to as "Pap smear") was first shown to reduce the incidence rate and death rate from cervical cancer in British Columbia(7). Cervical cancer screening was advocated in several countries although comprehensive programs were only introduced in a few countries like Iceland, Finland and Northeast Scotland in the 1960's and 1970's.

Systematic screening was only implemented in the United Kingdom (UK) in 1988. This program involved every woman between the ages of 20 and 64 being called and recalled every 3 to 5 years. This has resulted in a reduction of the death rate by 50% since 1988(5). It

is estimated that 2000 lives a year are saved. Population coverage has been one of main reasons for the success of the UK screening program. The coverage in the UK had reached 80.6% by 2004.

The SA government introduced the National Screening program for the prevention of Cervical Cancer(8), using the WHO recommendation for middle income countries. The WHO recommendation, which was that when 80% of women have been screened, the interval should be between 5 and 10 years, as resources permit(9). In SA an interval of 10 years has been advocated for the present. The South African program aims at achieving a wide coverage and in earlier presentation. Screening intervals of more than 5 years have never been tested in any program.

The challenges in cervical cancer prevention are unlikely to be the same in highly resourced settings and in countries like SA which have a high disease burden(10, 11).

Prevention of cancer of the cervix is dependent on integration of screening, referral, diagnosis, treatment, and follow-up of women with pre-malignant lesions of the cervix. Every aspect of the prevention program has to be equally pursued to be successful.

It is important to know the incidence and prevalence of cervical abnormalities in order to provide treatment for screened women as well as for making decisions regarding repeat screening.

Problem Statement:

Even though the incidence of cervical cancer has been reduced in countries with both opportunistic and systematic screening, cervical cancer continues to be a health problem(5). Ten percent of cervical cancer cases occur in women who have been screened, but all of these were where the screening was at least 5 years ago and more than 50% occur in women who have never been screened(11). There have been no prospective studies comparing the outcomes of screening at different intervals in any population.

In SA, the cancer prevention program is aimed at getting wide coverage (screening 70% of women in 10 years). Women will be offered three pap smears in a lifetime starting at the age of 30, however if the first pap smear is after the age of 55 and is normal only one pap smear will be performed. Women in whom the cytology is reported as inadequate will be re-screened. Women with any gynaecological complaint should receive a smear as part of their gynaecological examination irrespective of their screening history(8).

It is predicted that the SA program will prevent 64-70% of cancers, if all aspects of the program work effectively(8, 11). The long interval between pap smears for screening has not been evaluated. It may be that shortening the interval in this setting with a high prevalence of disease may still be cost-effective. Repeat screening has also been advocated to address the poor sensitivity of the conventional Pap smear(12).

Justification for the Study:

With long screening intervals a test with a high sensitivity would be desirable, sensitivity of Pap smear screening is between 50% and 70% for detecting cervical

intraepithelial neoplasia 3 (CIN)(13). One of the reasons for such a big difference in sensitivity in different settings may be due to lack of clear definitions regarding disease thresholds. A screening test that misses CIN3 and above is, however, unacceptable. Repeat screening of negative and inadequate smears(12) and automated screening, may reduce false negatives. The majority of missed lesions are due to failure to sample adequately.

The International Agency for Research on Cancer (IARC) working group has reported that inter-screen intervals of up to 5 years(14) is protective; a case-control study in SA has also shown a protective effect with ever having had a pap smear of 60%(15). In this study the protection is increased with three Pap smears and decreased with length of time from last Pap smear. The OR was 0.3 (95%CI: 0.2-0.4) when the last smear was performed within the previous 5 years and 0.5 (95% CI: 0.4-0.7) when the last smear was approximately 15 years ago and the trend test was significant with a p=0.00.

Reports from other situations have shown that long intervals and missed screening is associated with the development of invasive malignancy(16, 17).

In 2011, the second round of cervical cancer screening should begin, it would be important to provide information that may aid policy-makers in making the inter-screen interval shorter or keeping it at 10 years.

It would also be useful to know which women are at a high risk of developing cervical abnormalities after a pap report of 'normal cytology'. One of the priorities for research identified by the WHO consultative process was to evaluate the need for repeat Pap smears by age(9).

This study will therefore determine the incidence of abnormal Papanicolaou (Pap) smears 12-24 months after a first normal Pap smear in an HIV negative population. Risk factors which predict an abnormal Pap smear 12-24 months after a normal Pap smear will also be explored.

Literature Review:

In this section, the role of HPV in cervical cancer is clarified. The natural history of CIN is discussed briefly and Pap smear screening in different countries is reviewed. Pap smear screening has not been able to eliminate cancer of the cervix, possible reasons for failure is explored. The high burden of disease in SA is also a cause for concern. Risk factors for Pap smear abnormalities are also discussed. The chapter ends with a description of the aims and specific objectives of the study.

The strength of association for HPV and cancer of the cervix, with an OR of more than 50(18, 19) provides important but not sufficient evidence for cause. Molecular epidemiologic evidence indicates that cervical cancer and pre-cancer are almost certainly caused by HPV. Persistent infection with high risk genotypes (called types) of HPV is necessary for the development of dysplastic change within the cervix (20, 21). The strength of the carcinogenicity amongst the different HPV types also varies. Most women will however clear the infection. HPV types classified as high risk in a large population based study showed a strong concordance between the newly diagnosed evolutionary groupings with the risk of

progression to CIN3 and cancer (high risk types - 16/18/26/31/33/35/39/45/51/52/53/56/58/59/66/68/73/82)(21)

There is a long period of between 10-20 years from HPV infection to dysplasia and to invasive cancer. A cohort of women in New Zealand between the years 1965 and 1974 were seen for cytological abnormalities and not treated. Pap smear data belonging to this cohort of women was linked to data from a cancer registry and has provided the most direct information regarding the natural history of CIN; with a cumulative incidence of cancer of the cervix of 31.3 % (95% CI=22.7-42.3) at 30 years and 50.3% (95% CI=37·3–64·9) at 50 years in women with CIN(22).

The options of preventing cervical cancer may be at a primary or secondary level. The association of HPV and cervical cancer(23) was first shown in 1980 and lead to the manufacture of vaccinations and the possibility of primary prevention. Vaccination using the bivalent HPV vaccine was associated with a reduction of CIN2 and CIN3 caused by HPV 16/18 of 93%, and a reduction of 53% when the HPV type was 31/33/45/52/58(24). It is unlikely that vaccination will eradicate invasive cervical cancer and the real effect on cervical cancer will only be known in 20-30 years. Other methods of preventing HPV infection like condom use and circumcision(25) have not been evaluated in populations, even though condom use has been shown to reduce HPV transmission(26)

Secondary prevention with pap smear screening introduced in the 1950's resulted in a reduction of cancer of the cervix in developed countries and this occurred prior to the knowledge of the association of HPV with cervical cancer.

Secondary prevention of cervical cancer is possible because of this long Natural History and the ability to treat the pre-malignant phase. Screening for pre-malignant cervical lesions is far from perfect. There are several controversial issues regarding when to screen, who to screen and how often to screen. Screening too often may lead to over-treatment, anxiety and can be expensive. The figure below provides a conceptual framework of where screening may fail. The focus of this study is shown in the dark black boxed area.

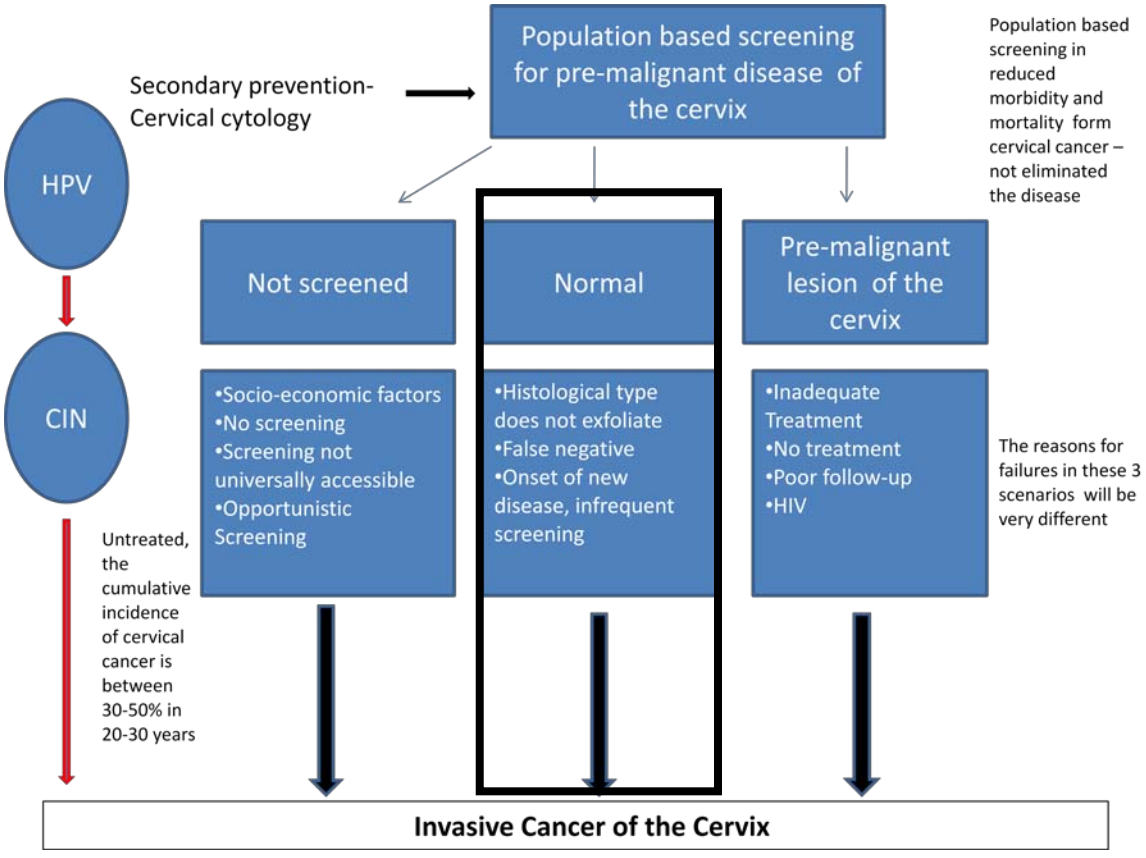


Figure 1: Conceptual Framework of Pap smear screening showing reasons for failures

Screening guidelines vary from country to country depending on the epidemiology of the disease and the cost. All the established screening protocols involve cytological screening. The table below summarizes some of the published guidelines.

Table 1: A Summary of cervical screening from published guidelines

Authority	Screening Initiation	Screening Intervals	Screening Cessation
South Africa, 2000 (8)	Age of 30	Every 10 years	60
Cancer Care Ontario, Canada, 2005(27)	Three years after the first intercourse	-Annually until 3 normal Pap and then after every 2-3 years	70
IARC, 2004(28)	25 years	Every 3 years for women 25-49 years Every 5 years for women of 50 and above	65 years
ACOG, 2003(29)	21 years (or 3 years after intercourse)	Annually for women under the age of 30. Every 2-3 years for women over 30 years	Not enough evidence to determine
ACS, 2002(29)	21 years (or 3 years after intercourse)	Annually for women under the age of 30. Every 2-3 years for women over 30 years	70 years
USPSTF, 2002(30)	21 years (or 3 years after intercourse)	At least every 3 years	65 years
NZGG, 1998(31)	20 years (for sexually active women)	Every 3 years	70 years
CTFPHE, 1994(32)	All sexually active women should be screened	Every 3 years and high risk women more frequently	No comment

The Dutch program began in 1980, inviting women between the ages of 35 and 53 every 3 years; this was revised in 1996 to include women between the ages of 30 and 60 every 5 years(33). The SA program targets women in the age category with the highest incidence of invasive cancers.

The prevalence of abnormal Pap smears in SA is high. The prevalence of abnormal pap smears in a multicentre study of over 20 000 women in 2002 was 4.69% (LSIL, HSIL, or invasion) (11). A study by medical students, looking at 5 local clinics within the Johannesburg Metropolitan area between July- 2004 and Dec 2004 found that 12.8% of these pap smears were abnormal(34). This is similar to the proportion of abnormalities of 13.7% in all clinics in this area in July- Dec 2006(35). It is not known whether this high prevalence of abnormal pap smears is due to the increase in the number of women who are HIV infected and who are also being screened with pap smears.

Screening in SA prior to 2001, had been mainly opportunistic and did not target women at high risk of cervical disease. Establishment of a project to screen women in Soweto in 1982 screened 32 365 women, but the number screened the next year was decreased by almost a third. The project was abandoned in 1986. The failure was attributed to failure in prioritizing screening in primary health clinics and failure in establishing a community education program(36). A study in 1994 showed that screening in the Western Cape over a 5 year period screened mainly pregnant women under the age of 30(37). Cronje et al, in 1985 showed that screening in the Free State target only 19% of women between the ages 35-44 and that there were only 16% in women over the age of 45. Furthermore 53% of the women were white even though they made up only 21% of the population(38). Screening in SA at present is erratic and has not reached the required coverage. There are still many challenges within the program. While attempting to achieve the 70% target for coverage some women will be due for their second round of screening so studies of interval length will aid in making decisions for individual women.

Cancers which occur within the time interval between prescribed screenings are termed "interval cancers". The figure below summarizes the factors which may lead to interval cancers in a screening program.

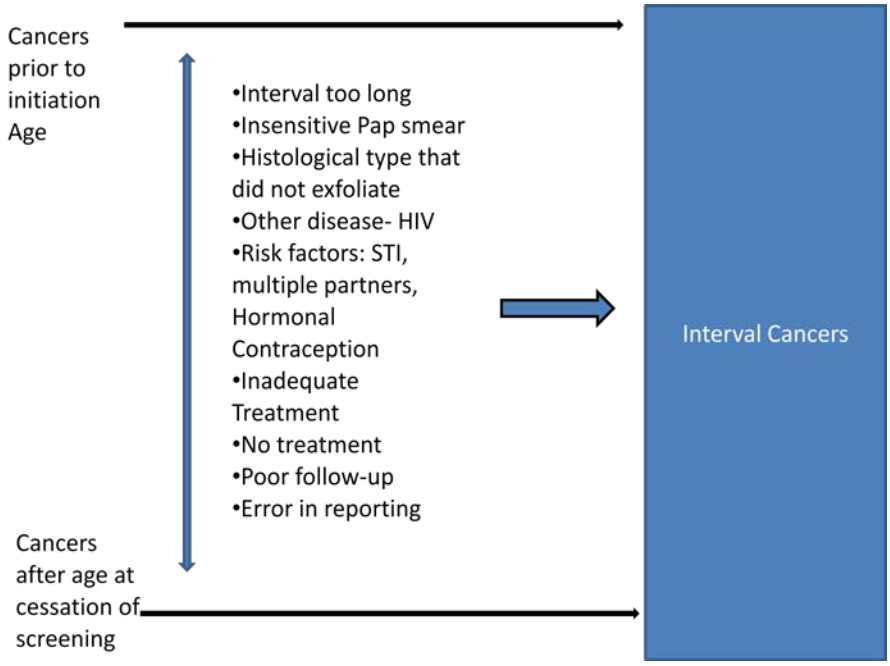


Figure 2: Schematic outlining causes of interval cancers

These may be new cancers or missed cancers due to sampling difficulties or due to errors in reporting. In a French study there were 148 cancers in women who had had some screening, 17% of whom had been adequately screened(16). Among women with interval cancers in an Australian study(17), review of the negative slides found that most were due to suboptimal sampling or to errors in reporting. Most reports of interval cancers occur in women under the age of 35, and whether this is due to greater participation of younger women in screening services or because cervical cytology is less effective in preventing cancers in younger women is not known.

Reasons may be that the lesion did not exfoliate or that a particular malignancy progressed too rapidly for detection by screening. These should however be minimized and diagnosed at an earlier stage in a setting where there is organized screening. Many studies have shown that women with cancer of the cervix had been less well screened than controls(14, 39). False negative pap smears occur more commonly in women with invasive cervical cancer(40) than with CIN2 and CIN3(41).

Protection conferred by a negative Pap smear:

Even though screening with Pap smears has limitations, some screening is better than none. A study using the Finnish Cancer registry showed that the risk of CIN3 was low immediately after a normal Pap smear and then increased over the following 5 year period(42). A case-control study in SA also showed that having ever had a Pap smear is protective, but that the protection wanes when the Pap smear was performed more than 5 years ago(15).

A meta-analysis of screening programs found no age difference in the protection conferred by a negative Pap smear in the 1960's and 1970's(14). Sasieni et al showed that the protective effect of a negative pap is less in younger women (20-39) than in women aged 40 or older. As a result they advocated shorter screening intervals for younger women(4).

Repeat testing within two years, has been used in many screening programs to increase the sensitivity of the Pap smear(29) however this has not been shown to be associated with a reduction in invasive disease in the British system(4).

Savings to the health system may derive from substantially increasing the duration of the interval between screens without losses in sensitivity for high-grade intraepithelial lesions by adding Human Papillomavirus (HPV) testing to the screening. HPV testing has a high negative predictive value(43, 44) and would therefore enable a longer interval of screening.

Unnecessarily screening too often may lead to an increase in false positives, increased cost and increased anxiety for women who are being screened.

Histological Type:

The protection offered against adenocarcinoma of the cervix with Pap smear screening is not clear. Cytological screening in the 1970's and 1980's(45) showed little protection while a study in the 1990's indicated that there was some protection from a negative Pap smear, although to a lesser extent than for squamous malignancy. An annual increase of Adenocarcinoma in situ of 12.25% between 1973 and 1995 was reported by the US National Cancer Institute Surveillance, Epidemiology and End Results database (from 0.02/100 000 to 0.61/100 000), was reported(46). It is not clear whether this is a real increase or whether it is a reflection of better reporting.

A series reported in Australia showed that of the adenocarcinomata detected on histology 88% were reported as a high grade abnormality on the Pap smear(47).

Risk factors associated with cancer of the cervix:

Several guidelines consider risk factors in the suggested intervals for cervical cancer screening, in particular HIV(27, 48). The South African guidelines make provision for Pap smear on the basis of a woman's medical history, that is that any woman with any

gynaecological complaint requiring a Pap smear should receive a Pap smear as part of the routine gynaecological examination.

Risk factors like multiparity(49) , smoking(50), sexually transmitted infections (STI) like Chlamydia and Herpes(51) and oral contraception (>5years) (46) have also been associated with cervical neoplasia.

Oral contraceptive use has been associated with cancer of the cervix both directly and indirectly, with long term users at 3-4 times increased risk for cervical cancer(52). However a subsequent study has shown that the risk of cervical cancer in HPV negative users of COC was not higher(53).

The availability of the oral contraceptive pill in the sixties made condom usage the method used in only 25% of sexually active individuals(54). At least 80% of unmarried females who use hormonal contraception have been shown to omit condom use(55). Increased Chlamydia, Mycoplasma and other STD's have been shown to be associated with hormonal contraception(56), probably as a result of the reduction in condom use.

Condom usage has been shown to be associated with lower transmission(26), persistence(26) and seropositivity(57) of HPV. Observational studies have found that the diaphragm has been associated with a reduction of some genital infections(58), a randomized trial however showed no difference in incidence of HPV between the diaphragm and condom(59).

HSV is a marker of previous unsafe sex, with seropositivity of up to 60 % in attendees at sexually transmitted disease clinics(60). Therefore the increased risk of cervical cancer

associated with HPV and HSV co-infection(61) may be related to other correlates of unsafe sex.

Higher frequency of intercourse has been shown to increase the risk of both HPV infection and cervical cancer(62) –suggesting a dose response . Safe sex practice appears to be beneficial in women who are HPV infected, longer durations of safe sex in women with HPV infection is associated with reduced incidence of cervical intra-epithelial neoplasia(63), and resumption of safe sex is associated with a regression of neoplasia(64).

Observational studies have shown that cervical cancer is reduced in communities which practice male circumcision(65). Recognizing the role of HPV in causing cervical a cancer and with better methods of detecting HPV, it has been shown that there is a reduction in the detection of HPV in women whose male partners had been circumcised(25). A similar protective effect was shown in a randomized control trial of the effect of circumcision on the presence of genital HPV in female partners. However the effect on CIN could not be quantified(66).

Aim of the study:

The purpose is to determine the proportion of women who develop an abnormal Pap smear 12-24 months after a normal pap smear. Risk factors associated with the Pap smear abnormalities may be important in determining the optimum screening interval for certain subgroups of women.

Specific Objectives:

- To determine the incidence of Pap smear abnormalities in a Cohort of women in Soweto, 12-24 months after a normal Pap smear.
- To determine the incidence of Pap smear abnormalities after an inadequate Pap smear.
- To determine the incidence of Pap smear abnormalities 12-24 months after a normal or inadequate Pap smear.
- To investigate factors associated with the development of an abnormal Pap 12-24 months after a previously normal or inadequate Pap smear.

Chapter 2: Methodology:

This is a secondary analysis of data collected during the "Methods for improving Reproductive Health in Africa" (MIRA) trial which was a randomized control trial on the use of the diaphragm, lubricant gel plus condoms versus condoms alone in preventing HIV infection in women(67). Women were offered a Pap smear at enrollment and again at exit as optional tests. The outcome of interest is the incidence of abnormal cytology 12 to 24 months after a normal Pap smear.

Primary data-set:

All women were screened for HIV infection and STI's prior to enrollment.

After signing a consent form for the screening procedures they were asked to do a questionnaire on demographics and condom use. They then were counseled on HIV and STI's. Urine was taken for PCR testing of Chlamydia trachomatis, Neisseria gonorrhea and Trichomonas vaginalis. A blood sample from a finger-prick or venous puncture was obtained

for rapid HIV testing (Oraquick® and Determine®). The women were notified about the HIV result and there was post test counseling for both positive and negative women. Women with equivocal results would have a confirmatory laboratory ELISA test.

At the enrollment visit, a pelvic examination was carried out on all women including a speculum examination to determine the presence of a healthy cervix. At this visit all women were also offered a Pap smear (the protocol requirements were that all women who had not had a Pap smear in the last 12 months be offered a Pap smear). The Pap smears were conventional smears that were performed by nurse clinicians that had all had primary health care training and had repeat training during the study period. All Pap smears were reported on by the NHLS cytopathology department. NHLS is accredited by South African National Accreditation Society (SANAS). The recommendations regarding management of Pap smear abnormalities were that any Pap smear where there was no endocervical or ectocervical component had to be repeated even when the descriptive diagnosis said "within normal limits". A pap report of LSIL or ASCUS – was repeated in 6 months, HSIL or AGS – was referred to Colposcopy.

All STI results were discussed and all positive results had to be treated prior to entry into the trial.

Women who met all eligibility criteria and joined the trial were provided an HIV prevention package which included male condoms, safe sex and risk reduction counseling and treatment of STI's detected at any visit. Women were randomized into an intervention group of diaphragm and lubricant jelly and a control group (condoms only). 1028 eligible

women were enrolled at the Perinatal HIV Research Unit in Soweto, Johannesburg. Women were followed up every 3 months for HIV and other STI's.

Information on socio-demographic factors, history of sexual behavior, a gynaecologic history, a medical history, clinical examination, and urine and blood tests for STI's were sought. The medical history and demographic information was obtained through interviewer administered questionnaires and Audio-Computer Assisted Self-Interview (ACASI).

Study Population:

The study population was recruited at family planning clinics, well baby clinics and from the community in Soweto. "Screening" should be of women who are asymptomatic and these women would be different from women presenting to a clinic with a complaint. However the women in this study are a decade younger than the prescribed age of screening in the SA guidelines. Recruitment began in March 2004. These women are drawn from a population in Soweto which is a high prevalence area for cervical abnormalities(34, 35). This data may therefore aid in evaluating the intervals at which Pap smears should be performed in an HIV negative population.

Women who had a Pap smear at both the beginning and the end of study form the study population.

Eligibility Criteria for women in the MIRA trial:

Table 2: Eligibility and Exclusion criteria used in primary study and how it would affect this present study

Eligibility criteria for this study	How this is expected to affect the study
Age: 18-49 years	Weakness, Pap smear screening:30-60 years
HIV- Negative within 14 days of enrollment	Strength, because the participant's status is known
Chlamydia trachomatis -Negative Tested within 30 days of enrollment or treated	Weakness, No indication of symptomatic or not. In clinical scenario Chlamydia testing would only be done if woman was symptomatic
Neisseria gonorrhea- Negative Tested within 30 days of enrollment or treated	Weakness, No indication of symptomatic or not. In clinical scenario Gonorrhea testing/treatment would only be done if woman was symptomatic
Cervix on speculum examination- Normal	Strength, Pap smear can only be done if cervix normal
Planning to live in area for next 24 months	Strength, reduces loss to follow-up
Willing to be randomized and follow protocol	Strength
Willing to consent	Neither strength nor weakness
History of Toxic Shock syndrome	Neither strength nor weakness, number of women very small
Allergy to latex	Neither strength nor weakness
Currently pregnant or desiring to be pregnant	Neither strength nor weakness
No cervix	Strength, the risk of disease in women who have had a hysterectomy is different and very low if there has never been cervical disease.
Refuses treatment for an STI	Weakness, now not able to assess risk in these women, but small number of women
No pelvic surgery or pregnancy in last 6 months	Neither strength or weakness
Presence of clinically apparent lesion	Strength, these women would be screened anyway if presented to a clinic
Injected illicit drugs in last 12 months	Neither strength nor a weakness
Unable to use diaphragm	Neither strength nor a weakness
Participation in any other clinical trial	Strength, the effect of any other vaginal product may affect the Pap smear.
Unable to speak English, Zulu, Shona or Sotho	Weakness, may exclude women from rural areas who may have a different risk.
Other conditions which in the opinion of the investigator would compromise the integrity of the trial	Strength, need good follow-up
Does not meet eligibility criteria within 5 visits	Neither strength nor a weakness

Study Design:

Secondary data analysis of a Randomized control trial. This is a longitudinal cohort of all participants who were randomized to a diaphragm, lubricant gel and condoms or to condoms only. They had to have had a normal or inadequate Pap smear at enrollment and then had a repeat Pap smear.

Study variables:

Variables that were extracted to be studied were those that were known risk factors for cancer of the cervix, socio-demographic variables and clinical factors that would make a woman interact with a health care worker. Such an interaction may allow opportunistic screening and may be a time when a repeat Pap smear may be performed.

Socio-demographic Variables:

Age, years of education, number of rooms in the home, number of housemates, married, living with husband/ partner.

Pregnancy History:

Parity, live births, abortions, miscarriages, mode of delivery.

Sexual history:

Age at first intercourse, number of sexual partners

Medical history & examination:

Current and past contraceptive use, history of TB, hypertension, diabetes, admission to hospital, blood transfusion, Hepatitis. History and clinical findings suggestive of sexually transmitted disease.

Gynaecological history and examination:

History of abnormal vaginal bleeding over the last 3 months, vaginal discharge, vaginal odor, any vaginal lesions

Laboratory Tests:

Pap smear results at enrollment and any repeat during the trial.

Chlamydia trachomatis (Urine PCR), Neisseria gonorrhea (Urine PCR), Trichomonas vaginalis (urine PCR) testing at all visits.

Herpes (Focus HSV-2) and Syphilis (RPR and TPHA in those women with a positive RPR) results at enrollment and exit. Only those women who tested negative for Herpes had a repeat test at exit.

HIV infection at all visits ((Oraquick HIV=1/2 Rapid Test and Determine-HIV-1 Rapid test) and HIV DNA for confirmation, HIV ELISA discordant initial testing)

Data Analysis:

Any variable that was thought to be associated with cancer of the cervix or dysplasia was explored for association. The tables with the socio-demographic data was merged with tables containing Pap smear results, medical history, gynaecological history, clinical findings of an STI and with every table that contained results for every STI- thereby creating a "master dataset". The tables were already in a dta. format. There were 5 duplicates, which were then deleted. All analysis was done using STATA 10 statistical software (Stata Corp, Texas, USA).

A new variable: “Pap smear abnormality” was made which was the outcome of interest. The Pap smear findings at exit were coded, with “0” representing normal or inadequate, and “1” LSIL, HSIL, ASCUS, ASC-H. There were no Pap smear results of invasion or AGUS. Another variable “follow-up time” was made and this was the date from the first Pap smear (enrollment) to the date of the repeat Pap smear. This was useful in modeling time to abnormal cytology (incidence) using the Cox proportional hazards model.

The explanatory variables were mainly baseline variables. HIV infection, Chlamydia PCR, Gonorrhea PCR, RPR, HSV Elisa, were also exit variables.

All the explanatory variables were compared in those individuals who continued to have a normal Pap smear versus those who developed abnormal cytology. The Cross Tabulation tables were performed with every explanatory variable and the outcome. For the numeric (continuous) variables– the Wilcoxon rank sum test was used to test the equality of the median values where the variables were not normally distributed. Normality was assessed both graphically (using box and whisker plots and histogram plots) and statistically (using the Shapiro-Wilk test). For normally distributed numeric variables the ttest was used. All categorical variables were compared using the chi2 test or the Fisher exact test for those that had fewer than 5 observations in a cell.

To compare the risk of an abnormal Pap smear in those with a normal Pap smear at baseline with those with an inadequate Pap smear at Baseline Kaplan – Meier curves for both cohorts were drawn and the equality of both curves tested using the log rank test.

Logistic regression models were used to find the risk factors associated with the outcome. Univariate analysis was first performed and crude odds ratios for the association of these variables with an abnormal Pap smear was noted. Multivariate models were built using variables where the p-value was less than 0.2 and interaction issues assessed for. All variables of interest were included in the multivariate model and backward selection procedure was performed. The assumption of linearity in the logit scale was also assessed and the Hosmer – Lemeshow goodness of fit test was used for model diagnostics. Also the sensitivity of the model was obtained and graphs of the receiver operator characteristic (ROC) curves for both models were plotted.

Ethical Considerations:

Ethical clearance was obtained from the Human Research Ethics Committee (Medical) in June 2009. The Clearance number was M090676 (Appendix 1). Permission for the use of the data was obtained from Liz Montgomery and Heidy Fancher for the MIRA Investigators (Appendix 2).

Chapter 3: Results

This chapter begins with an outline of the flow of participants through the trial, who form the study population. The Pap smear results for participants who had a Pap smear at entry and exit is then tabulated. The Incidence of abnormal Pap smears in the whole group and in women with a normal Pap smear results is shown. The tables that follow are a description and comparison of every variable that was included in the study. Finally, the results of a

univariate and multivariate analysis is shown. This analysis was done to find risk factors which may predict an abnormal Pap smear on a repeat test between 12 and 24 months.

There were 850 women amongst those eligible for the study that had a repeat pap smear. Ninety seven women (11.41%) had an abnormal Pap smear at entry and would be excluded. Figure 3 shows the flow of participants eligible for the study and Table 3 shows the type of cytological abnormalities that were detected at enrollment and when repeated.

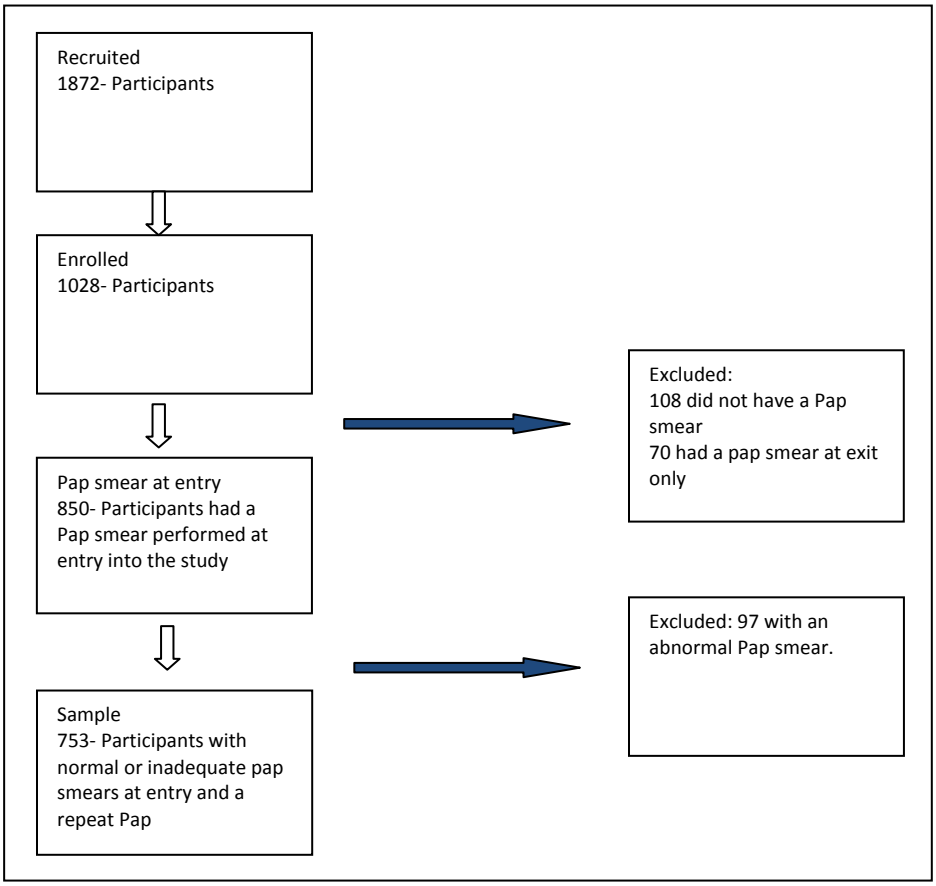


Figure 3: Flow chart of participants

Table 3: Cytological results on Pap smear at baseline

	Categories of Pap smears at enrollment →						
Categories of Pap smears at repeat Testing (Bold and in italics) ↓		**Normal and Satisfactory for evaluation**	**LSIL**	**ASCUS**	**HSIL**	**Inadequate Pap smear**	**Total**
	Normal	**476 (88.97%)**	31 (60.78%)	21 (67.74%)	8 (53.33 %)	**193 (88.53%)**	729 (88.53%)
	Any abnormality (LSIL, ASCUS, HSIL)	**59 (11.03%)**	20 (39.22%)	10 (32.26%)	7 (46.67 %)	**25 (11.47%)**	121 (14.24%)
	Totals of pap at enrollment	**535 (62.94%)**	51 (6%)	31 (3.65%)	15 (1.76%)	**218 (25.65%)**	850 (100%)

The incidence of cytological abnormalities in this group of women (Baseline Pap normal or inadequate) was 7.33% per annum. The median follow-up time was 297 days (IQR182-455). The incidence of cytological abnormalities is 6.48 % in women with a previously normal Pap and 11.71% in women with an inadequate smear result. The graphs depict the risk of having an abnormal Pap smear.

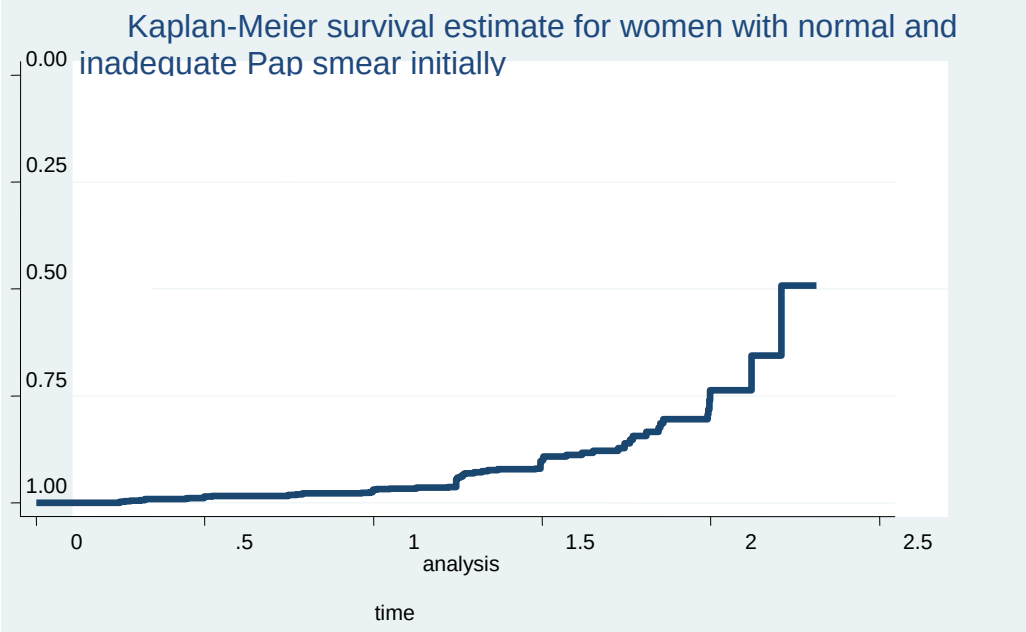


Figure 4: Abnormal cytology on repeat testing in the whole sample.

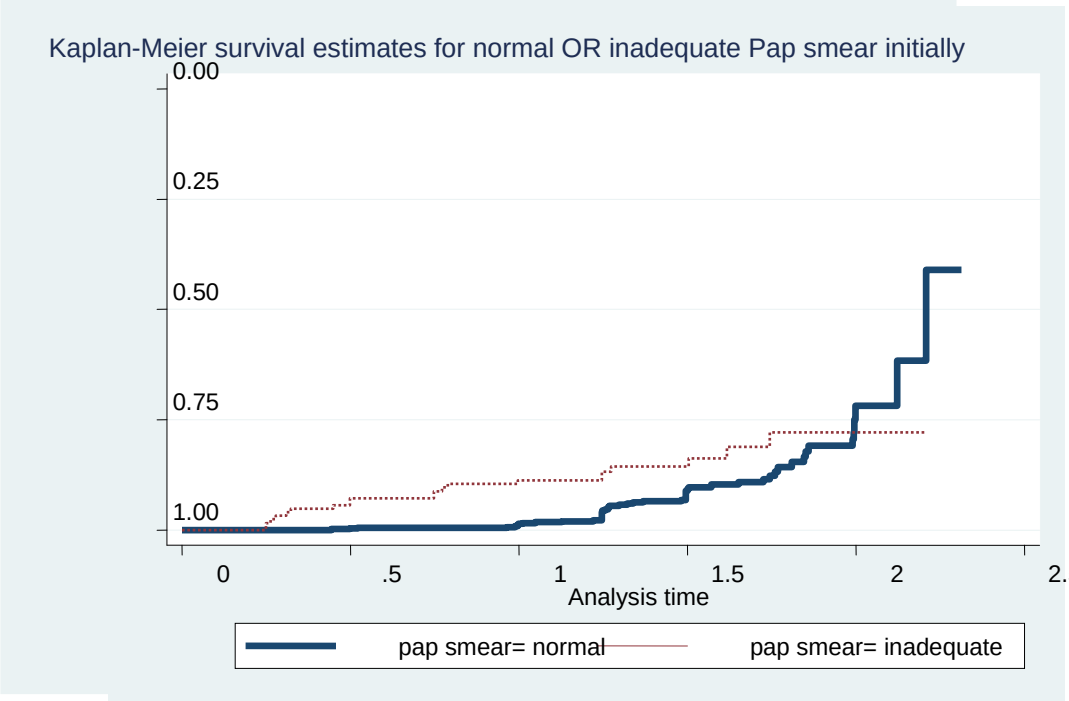


Figure 5: Abnormal cytology on repeat testing after a normal or inadequate Pap smear

The log-rank test for equality of survivor function indicates that there is a statistically significant difference in the risk of a cytological abnormality after a normal when compared to an inadequate Pap smear (p=0.03).

Less than half a percent of both the normal and inadequate Pap smears had a HSIL reported on their subsequent Pap smear and the rest of the abnormalities were LSIL and ASCUS (table 4). However the presence of any cytological abnormality would mean that that woman is at risk of cervical cancer albeit with a different degree of risk. The analysis will therefore combine all cytological abnormalities as the outcome of interest.

Table 4: Cytological findings on the second Pap smear

First Pap	Normal	Inadequate	Total
Normal	460 (85.98%)	177 (81.19%)	637 (84.59%)
LSIL	39 (7.29%)	13 (5.96%)	52 (6.91%)
ASCUS	18 (3.36%)	11 (5.05%)	29 (3.85%)
HSIL	2 (0.37%)	1 (0.46%)	3 (0.40%)
Inadequate	16 (2.99%)	16 (7.34%)	32 (4.2%)
	535	218	753

(p=0.00)

Demographic characteristics:

Comparing socio-demographic factors with having an abnormal repeat pap smear is shown in the table 5 below. There were no differences of statistical importance in the 2 groups.

Table 5: Description and comparison of socio-demographic factors in women with normal and abnormal cytology at repeat Pap smear.

	Totals n=753	**Abnormal Cytology n= 84 (11.16%)**	**Normal Cytology n=669 (88.84%)**	**p-value**
Age:	**Mean=29.19(sd-8.11) Median 28 (iqr-22-35) Range= 18-48**	Mean=28.04 (sd-8.18) Median= 25 (iqr13.5) Range=18-48	Mean=29.33 (sd-8.09) Median=28 (iqr-14), Range 18-48	0.17* 0.13**
Education in number of years	**Mean=10.94 (sd-2.25) Median=12 (iqr-10-12) Range=0-17**	Mean=10.85 (sd-2.34) Median=12 (iqr-2)	Mean=11.02 (sd-2.20) Median=12 (12 (iqr-2)	0.51* 0.49**
Married	**n=165 (21.91%)**	12(14.29%)	153 (22.27%)	0.07 (Chi2)
Live with husband/partner	**N=319 (42.36%)**	35 (41.67%)	284 (42.45)	0.89 (Chi2)
Number of rooms at home:	**Mean =3.44 (sd-1.73) Range 1-10**	Mean=3.39 (1.74) Median=4(2)	Mean=3.45(1.73) Median=4(2)	0.79* 0.87**
Number of Housemates	**Mean=4.85 (sd-2.20) Median=4(iqr-3) Range=8-15**	Mean=4.54 (sd-1.92) Median= 4(iqr-2)	Mean=4.89 (sd-2.23) Median =4 (iqr-3)	0.17* 0.12*** 0.14**
Children currently being care for	**Mean= 1.66 (sd-1.46) Median 1(iqr-1) Range=0-7**	Mean=1.60 (sd-1.65) Median 1(iqr-1)	Mean 1.67 (sd-1.44) Median = 1(iqr-2)	0.60* 0.29**

***ttest with equal variances **Wilcoxon rank-sum ***ttest with unequal variance**

Participants were not asked about smoking or alcohol use. The use of vitamins, medical supplements or herbs was not associated with having an increased risk of an abnormal pap smear (p=0.91)

Contraceptive Use:

Women were asked about contraceptive use and their current method. They were unfortunately not asked about the length of time that they had used the method. None of the women had used the diaphragm, spermicides or were with a partner who had had a vasectomy. There was no statistical difference in the use of non-barrier methods in these women (table 6).

Table 6: A description and comparison of non-barrier contraception

n=753	Abnormal Cytology n= 84 (11.16%)	Normal Cytology n=669 (88.84%)	p-value
Combined oral contraception- ever n= 235 (31.21%)	24 (28.57)	211 (31.54)	0.58 (chi2)
Combined oral contraception- current n=58 (7.70%)	9 (10.71)	49 (7.32)	0.27 (chi2)
Injectable Hormones-ever n=564 (74.90%)	62 (73.81)	502 (75.04)	0.81 (chi2)
Injectable hormones-current n=223 (29.61%)	26 (30.95)	197 (29.45)	0.78 (chi2)
POP -ever n=91 (12.08%)	9 (10.71)	82 (12.26)	0.86****
POP -current n=20 (2.66%)	1 (1.19)	19 (2.84)	0.72****
Ever used hormonal contraception n=619 (82.20%)	65 (77.38)	554 (82.81)	0.22 (chi2)
Tubal Ligation 51 (6.77%)	4 (4.76)	47 (7.03)	0.64****
Ever used an IUD n=86 (11.42%)	7 (8.33)	79 (11.81)	0.35 (chi2)
Current use of an IUD n=7 (0.93%)	0 (00)	7 (1.05)	1****
Ever used Natural Methods n=8 (1.06%)	2 (2.38)	6 (0.90)	0.22****
Currently use Natural Methods n=2 (0.27%)	1 (1.19)	1 (0.15)	0.21****
Ever used Withdrawal n=17 (2.26%)	2 (2.38)	15 (2.24)	1****
Current use of withdrawal n=6 (0.80)	0(00)	6 (0.90)	1****
Ever used Traditional Methods n=3 (0.40)	0 (00)	3 (0.45)	1****

******Fisher's exact for any cell with less than 5 observations**

The use of Barrier Methods of contraception:

There were fewer women with cervical abnormalities in those who were randomized to the control group. "Condom use at last intercourse" as a probe of condom use is often

thought to be best way of assessing use because it reduces re-call bias, however in this analysis it was not different in the 2 groups, even on stratifying by randomization. Interestingly there were more women in the diaphragm group with abnormal cytology. The prevalence of female condom use is low in this group of women and not different in the 2 groups. The use of "female condoms" was discouraged owing to probable problems with fitting for women in the intervention arm.

Table 7: A description and comparison of Barrier contraception

n=753	**Abnormal Cytology n= 84 (11.16%)**	**Normal Cytology n=669 (88.84%)**	**p-value**
Randomized to the intervention arm n=388 (51.53)	53 (63.10)	335 (50.07)	0.02 (chi2)
Randomized to the control arm 365 (48.47)	31(36.90)	334 (49.93)	
Condoms used at last vaginal sex 438 (71.57)	46 (64.79)	392 (72.46)	0.18 (chi2)
Ever used condoms 586 (77.82)	64 (76.19)	522 (78.03)	0.70 (chi2)
Current condom use (at baseline) 506 (67.20%)	53 (63.10)	453 (67.71)	0.40 (chi2)
Female condoms used at last intercourse 27 (4.41%)	4 (5.63)	23 (4.25)	0.54 ****

******Fisher's exact used when there were fewer than 5 observations in a cell.**

Pregnancy History:

Increase parity has been associated with an increase in risk of cancer of the cervix; the table below shows no significant association with parity. The history of ever having had an ectopic pregnancy has the most impact on the outcome.

Table 8: A description and comparison of pregnancy history.

	Totals n=753	**Abnormal Cytology n=84 (11.16%)**	**Normal Cytology n= 669 (88.84%)**	**p-value**
Pregnancies	**Mean=1.8 (sd-1.48) Median=2 (1-3) Range=0-7**	Mean=1.77 (sd-1.62) Median =1(1-3)	Mean =1.81 (sd-1.47) Median=2 (1-3)	*0.84 **0.54
Number of live-births	**Mean=1.64 (1.38) Median =1 (0-2) Range 0-7**	Mean=1.54 (sd-1.50) Median= 1(0-2)	Mean=1.65(sd-1.37) Median=1 (0-2)	*0.46 **0.24
Vaginal births	**Mean=1.46(sd-1.39) Median=1 (iqr-0-2) Range=0-6**	Mean=1.44 (sd-1.54) Median=1(0-2)	Mean=1.46 (sd-1.37) Median= 1(0-2)	*0.91 **0.54
Vaginal Births if ever pregnant	**n=686 mean =1.59 (sd-1.37)**	Mean= 1.52 (sd-1.54)	Mean=1.60 (sd-1.35)	*0.62
Number of women who have ever had a caesarian section	**Mean 0.22 (sd-0.56) No of Caesars= 120**	Mean= 0.19 (sd-0.51)	Mean= 0.22 (sd-0.57)	*0.62
Termination of pregnancy	**n=14 (1.87%) Mean=0.02 (sd-0.15)**	Mean=0.02 (sd-0.14)	Mean 0.02 (sd-0.15)	*0.80
Termination of pregnancy if ever pregnant	**14 (1.99%)**	2(2.41%)	12(1.94%)	0.68 ****
Miscarriage if ever pregnant	**n=42 (5.97%)**	5 (6.02%)	37 (5.97%)	1 ****
Ectopic pregnancy if pregnant		4 (4.82%)	10 (1.61%)	0.07 ****
Any poor pregnancy outcome	**n=64 (8.58%)**	11 (13.25%)	53 (7.99)	0.14****

***ttest ** Wilcoxon ranksum ****Fishers exact**

Medical History and Other Medical Conditions:

Women who attend Community health care facilities may be offered a Pap smear as part of the opportunistic screening service. A comparison of medical conditions that women present with may be helpful in determining which women should be offered a repeat Pap smear more frequently than is suggested by the National guidelines. Below is a comparison of the medical history in these women. Women who were admitted to hospital for any reason (not birth related) were statistically more likely to have an abnormal Pap smear.

Table 9: A description and comparison of symtoms that a woman may present with.

n=753	Abnormal Cytology n=84 (11.16%)	Normal Cytology n= 669 (88.84%)	p-value
History of TB N=23 (3.08%)	3 (3.61%)	20 (3.02%)	0.73****
History of Urinary tract infection 24 (3.22%)	2 (2.41%)	22 (3.32%)	1****
History of Hypertension 67 (8.98%)	7 (8.43%)	60 (9.05%)	0.85 (chi2)
History of Hepatitis 1 (0.13%)	0 (00%)	1 (0.15%)	1****
History of Diabetes 2 (0.27%)	0 (00%)	2 (0.30%)	1****
Non- Gynae visit to doctor 113 (15.01%)	11 (13.10%)	102 (15.25%)	0.60 (chi2)
History of cervical biopsy/ cancer 1 (0.13%)	0 (00%)	1 (0.15%)	1****
History of admission to hospital- not birth related 118 (15.82%)	20 (24.10%)	98 (14.78%)	0.03 (chi2)
History of Blood Transfusion 26 (3.49%)	2 (2.41%)	24 (3.62%)	0.76****

******Fisher's exact**

Sexual History and Sexually transmitted disease:

Exploring the association with sexual history and the incidence of an abnormal Pap smear is shown in the table below. Women with an increased number of sexual partners were more likely to have an abnormal Pap smear on repeat testing. The trial excluded women with less than 3 coital acts in the month and therefore it would not be meaningful to assess difference by coital frequency. Categorizing "age at first intercourse" to less than 17, 18 or 19 did not show ant statistical difference- data not shown.

Table 10: A description and comparison of sexual history

Variable	n=753	Abnormal Cytology n=84 (11.16%)	Normal Cytology n= 669 (88.84%)	p-value
Age at first intercourse	**Mean= 17.58 (sd-2.12) Median=18 (iqr-16-19) Range=11-27**	Mean=17.32 (sd-1.78) Median=17 (16-19)	Mean=17.61 (sd-2.15) Median=17 (16-19)	0.24* 0.18*** 0.26**
Less than 20years at 1st intercourse	**n=647 (85.92%)**	77 (91.67%)	570(85.20%)	0.11 (chi2)
Sex in exchange	**Sex in exchange N=17(2.26%)**	4 (4.76%)	13 (1.94%)	0.11 ****
Number of male partners	**Mean=4.02 (sd-2.90) Median=3 (iqr2-5) Range 1-26**	Mean= 4.85 (sd-3.40) Median=4 (2-5)	Mean= 3.84 (sd-2.73) Median=3 (2-5)	0.00* 0.00**
Average sex in the last 3 months:	**Mean= 9.46 (sd-5.12) Median= 8(iqr6-12) Range= 3-40**	Mean= 9.46 (sd-4.96) Median=8 (-4-12)	Mean=9.46 (sd-4.96) Median=7 (5-12)	1* 0.39**

ttest **Wilcoxon ranksum ***ttest (unequal variances) * Fisher's exact**

Sexually transmitted disease is associated with cancer of the cervix. The tables below shows the association between history (Table 11) and clinical findings (Table 12) suggestive of an STI and confirmatory tests (Table 13) of STI's with the risk of developing an abnormal cytology on Pap smear in these women. Some clinical findings (variables) were excluded from the analysis as there were very few women with such an abnormality. The clinical findings were really incidental findings, rather than complaints from the women as would be the case in a research setting. Women were asked about symptoms suggestive of a sexually transmitted disease in the last 3 months and whether these symptoms were ongoing. A history of a vaginal discharge in the last 3 months, or symptoms of an STI were important difference in the 2 groups of women.

Table 11: A description and comparison of symptoms which are suggestive of a sexually transmitted disease

n=753	Abnormal Cytology n=84 (11.16%)	Normal Cytology n= 669 (88.84%)	p-value
Have you ever been treated for an STI?	22 (26.51%)	131 (19.76%)	0.15 (chi2)

153 (20.51%) N=746			
Number of times treated Mean=1.26 (0.89)	Mean=1.91 (1.95)	Mean= 1.15 (0.49)	0.00* 0.08***
Symptoms suggestive of an STI n=68(9.12%)	15(18.07)	53 (7.99%)	0.00 (chi2)
Abnormal bleeding in the last 3 months n=22 (2.95%)	1 (1.20)	21 (3.17)	0.28****
Vaginal discharge on history in the last 3 months n=37 (4.96%)	11 (13.25)	26 (3.92)	0.00 (chi2)
Ongoing vaginal discharge on history n=11 (4.69%)	3 (3.61%)	8 (1.21%)	0.11****
History of vaginal odor in the last 3 months n=10 (4.69%)	1 (5%)	9 (4.66%)	1****
Ongoing vaginal odor 5 (2.35%)	1 (5%)	4(2.07%)	0.39****
History of genital irritation in the last 3 months 12 (1.61%)	1 (1.20%)	11 (1.66%)	1****
History of ongoing irritation 5 (0.67%)	1 (0.67%)	4 (0.60%)	0.45 ****
History of ongoing genital sores 1 (0.13)	0 (0.00)	1 (0.15)	1****
History of ongoing low abd pain not associated with periods 4 (0.54)	1 (1.20)	3 (0.45)	0.38****
History of ongoing genital symptoms 23 (3.08)	5 (6.02)	18 (2.71)	0.17****

***ttest ***ttest (unequal variances) ****Fisher's exact**

Clinical findings suggestive of Sexually transmitted diseases:

All participants were examined and had a pelvic examination. There was only 1 (0.13%) with inguinal lymphadenopathy, 1(0.13%) of women with a perineal/perianal abnormality, 7(0.94%) with a vulval abnormality, 3(0.40%) with a vaginal epithelium abnormality. The quantity of vaginal discharge was graded into small, moderate and abundant and an increased quantity was not associated with the outcome.

The vaginal discharge was characterized as normal (2(0.27%)), thin flows easily (18(2.41%)) and thick, does not flow easily (16 (2.14%)). There was no statistical association demonstrated with this finding. The consistency of the discharge was described as non homogenous, normal (4(0.54%)); non homogenous abnormal (10(1.34%)), homogenous smooth (15(2.01%)) and homogenous frothy (7 (0.94%)).

The cervix was examined for epithelial disruptions (0 women), mucus (0 women), quantity of mucus, and ectopy (0 women) and for contact bleeding. Table 10 shows those clinical findings that were more common. The pH of the vagina is normally less than 4.5. An analysis done with a pH which is greater than 5 did not show any statistical significance.

Table 12: A description and comparison of signs suggestive of sexually transmitted disease.

n=753	**Abnormal Cytology n=84 (11.16%)**	**Normal Cytology n= 669 (88.84%)**	**p-value**
Vaginal discharge on clinical examination n=36 (4.83%)	5 (6.02%)	31 (4.68%)	0.59****
Cervicitis n=30 (4.02%) same as PID	5 (6.02%)	25 (3.77%)	0.37****
Clinician identified RTI 43 (5.76)	7 (8.43%)	36 (5.43%)	0.27 (chi2)
Vaginal ph Mean= 4.35 (0.64) Median= 4.4 (4-4.7) Range=3-6.1	Mean=4.29 (sd=0.62)	Mean=4.34 (sd=0.57)	0.52*
Vaginal ph >4.5 n=231 (30.68)	32 (38.10%)	199 (29.75%)	0.12 (chi2)
Vaginal ph >5 =50 (6.64%)	6 (7.14%)	44 (6.58%)	0.84 (chi2)

***ttest ****Fisher's exact**

Tests suggestive of reproductive tract and Sexually transmitted disease.

At least 15 mls of first catch urine was sent for PCR testing of Chlamydia, Gonorrhea and Trichomonas at every visit. The women were asked to not clean the vulva and not to urinate at least 2 hours prior to collection of the sample. Blood tests for HIV were performed at every visit. Tests for Syphilis were done at entry and exit. TPHA was only done in 215 women who were RPR positive (not included in this analysis- only 5 were positive). A test for Herpes was done at entry and only repeated at the end of the trial in those women who tested negative. There were no statistical differences in incident cervical abnormalities amongst women with objective evidence of a STI and no infection.

Table 13: A description and comparison of test results of STI's

n=753	Abnormal Cytology n=84 (11.16%)	Normal Cytology n= 669 (88.84%)	p-value
Chlamydia at entry n=41 (3.44%)	5 (5.95%)	36 (5.38%)	0.84****
Chlamydia at repeat testing n=32 (4.25%)	2 (2.38%)	30 (4.48%)	0.54****
Gonorrhea at entry n=7 (0.93)	1 (1.19%)	6 (0.90%)	0.57****
Gonorrhea at repeat testing n=13 (1.73%)	2 (2.38%)	11 (1.64%)	0.52****
Trichomonas n=12 (1.59%)	1 (1.19%)	11 (1.64%)	1****
Trichomonas on repeat testing n=38 (5.05)	4 (4.76%)	34 (5.08%)	0.67****
Syphilis RPR at entry n=220 (29.22%)	20 (23.22%)	200 (29.90%)	0.39****
WR titre using <1/8 as negative and ≥ 1/8 as positive- n= 10 (4.55%) if RPR positive	1 (5%)	9(4.5%)	1 ****
RPR at exit n= 17 (2.26%)	1 (1.19%)	16 (2.39%)	0.13****
HSV at entry 482 (64.01%)	56 (66.67%)	426 (63.68%)	0.81 (chi2)
HSV was only repeated in those women where herpes was negative n=25 (3.32%)	3 (3.57%)	22 (3.29%)	0.84****
Pregnancy n=22 (2.92%)	2 (2.38%)	20 (2.99%)	0.35****
HIV N=10 (1.33%)	2 (2.38%)	8 (1.20%)	0.20****

******Fisher's exact**

Univariate & Multivariate Analysis:

Condoms compared to the diaphragm in this trial remained a significant factor in preventing incident cytological abnormalities. History of an ectopic pregnancy, vaginal discharge and every increase in the number of sexual partners were important risk factors.

The incidence of HIV was significantly associated with the outcome in the multivariate analysis. The multivariate analysis in Table 14 was using every variable (including those variable that were not significant in the Univariate analysis). Table 15 is the model only considering those factors that were significant in the Univariate analysis.

Table 14: Univariate and Multivariate analysis

	Univariate Analysis Odds Ratio (95%CI), p-value	**Multivariate Analysis Odds Ratio (95%CI), p-value**
Age	0.98 (0.95-1.01), 0.17	0.97 (0.89- 1.05), 0.42
Age more than 30 compared to under 30 years	0.84 (0.53-1.34), 0.46	1.81(0.56-5.83), 0.32
Unmarried women compared to being married	1.78 (0.94-3.36), 0.08	1.58(0.68-3.68), 0.29
Number of Housemates	0.92 (0.83-1.03), 0.17	0.90 (0.78-1.04),0.14
Contraception-Barrier:		
Have you used a male condom at the vaginal last sexual intercourse?	0.70 (0.41-1.18), 0.18	0.75 (0.40-1.39), 0.36
Ever used male condoms	0.90 (0.53-1.54), 0.70	1.34 (0.53-3.37), 0.53
Condoms as compared to diaphragm	0.59 (0.37-0.94), 0.03	0.51 (0.27-0.97), 0.04
Pregnancy History:		
Number of Vaginal births if pregnant	0.62 (0.38-1.01), 0.06	0.76 (0.38-1.52), 0.44
History of ever having had an ectopic pregnancy	3.31 (1.01-10.79), 0.05	9.25 (1.78-48.02), 0.01
History of any poor pregnancy outcome	1.76 (0.88-3.52), 0.11	1.08 (0.38-3.06),0.89
Sexual History:		
Coitarche at the age of 19 or less compared to older than 19	0.52 (0.23-1.17), 0.11	0.83 0.32-2.14(0.70)
Sex in exchange	2.52 (0.80-7.92), 0.11	3.60 (0.88-14.67),0.07
Number of male partners	1.10 (1.03-1.18), 0.00	1.12 (1.03-1.22), 0.01
Medical History:		
History of admission to hospital	1.83 (1.06-3.16), 0.03	1.50 (0.75-3.10), 0.25
Sexually Transmitted Disease:		
History of having been treated for a Sexually transmitted disease	1.46 (0.87-2.47), 0.15	0.98 (0.50- 1.90), 0.95
Number of times treated	2.43 (1.21-4.89), 0.01	6.59 (1.54-28.19), 0.01
History of symptoms suggestive of an STI compared to no such symptoms	2.54 (1.36-4.75), 0.00	0.37 (0.04-3.51), 0.39
History of Vaginal Discharge	3.74 (1.78-7.89), 0.00	13.95 (1.18-164.47), 0.04
History of ongoing vaginal discharge	3.07 (0.80-11.81), 0.10	0.11 (0.00-3.84), 0.22

Any ongoing genital symptoms	2.29 (0.83-6.36), 0.11	4.47 (0.25-78.78), 0.53
Vaginal ph> 4.5 as compared to <4.5	1.45 (0.91-2.33), 0.12	1.11 (0.53-3.37), 0.53
RPR at exit	0.56 (0.07-4.26), 0.57	
HIV	2.08 (0.43-10.00), 0.36	6.58 (1.14-38.16), 0.04

Model for predicting an abnormal Pap smear after a normal or inadequate Pap smear:

Incident HIV infection was associated with 6 fold increase in the risk of an abnormal pap smear. An abnormal discharge in the last 3 months increases the risk by 5 fold, and ever having had an ectopic pregnancy increases the risk 8 fold. Having used a condom after the last intercourse had no effect, but being allocated to the control (condom) arm reduced the risk by approximately 58%. The Model correctly classifies 89.88% of the abnormal Pap smears, with a low sensitivity of 9.38% and a specificity of 99.81%. There were no interaction issues in the model and model diagnostics were performed. The assumption of linearity on the logit scale was assessed for and ensured that it was not violated. The fit of the model was adjudged to be good by the Hosmer – Lemeshow goodness of fit test.

Table 15: Logistic regression model with predictors for an abnormal Pap smear

Predictor	**OR**	**p-value (CI)**
HIV infection	6.26	0.04 (1.14-34.50)
Ongoing abnormal vaginal discharge	0.09	0.07 (0.01-1.26)
Number of housemates	0.89	0.11 (0.78-1.03)
Abnormal Discharge in the last 3 months	5.14	0.00 (1.79-14.78)
Any ongoing genital symptoms	4.78	0.07 (0.89-25.78)
Having been treated for a sexually transmitted disease?	0.67	0.28 (0.32-1.39)
Number of male partners	1.12	0.00 (1.04-1.21)
History of ever having had an ectopic pregnancy	8.70	0.00 (2.07-36.45)
Sex in exchange for food, Herring or Money	3.44	0.07 (0.90-13.11)
Having had a Vaginal birth.	0.70	0.25 (0.38-1.28)
Having used a male condom at last intercourse	0.80	0.47 (0.44-1.47)
Being randomized to the	0.42	0.01 (0.22-0.77)

condom arm rather than to the diaphragm arm		

Chapter 4: Discussion, limitations and Conclusions.

Discussion:

The aim of this study was to find the incidence of Pap smear abnormalities when the Pap smear was repeated between 1-2 years and it was found that there is a significantly higher incidence of cytological abnormalities in women where the Pap smear was reported as inadequate as compared to where the Pap smear was adequate (and otherwise normal). The incidence of any abnormality was 7.33% per year; however the incidence of a HSIL was low, 0.46% and 0.37% in the inadequate versus the adequate group.

Repeating the Pap smear within two years in SA may increase the cost without adding any significant increase in finding disease. Although there are no prospective studies that have directly compared screening intervals in any given population, there is information from eight screening programs evaluating screening intervals. Screening every 5, 3, 2 or annually was estimated to reduce the incidence of cancer by 84%, 91%, 93% and 94% respectively among women 35-64 years of age(68). The SA guidelines predict a reduction of cervical cancer by between 64%-70% with an interval of 10 year(8, 15).

It is notable that the National Health and medical Research Council Guidelines of Australia recommend that all pathologists report normal Pap smear with a recommendation that the next Pap smear is due in 2 years irrespective of the presence or absence of endocervical component or of reactive change(69).

One of the priorities for research set by the WHO consultative process was to evaluate the need for repeat pap smears by age(9). In this study there was no difference in incidence with increased age. The median age was 28 and the odds of having an abnormal Pap smear was 1.80 (NS) for women older than 30years in the multivariate analysis. Sasieni et al showed that the protective effect of a negative pap is less in younger women (20-39 years) than in women aged 40 or older(4).

71.57% of women reported that they had used condoms with their last intercourse. The use of male condoms was protective against acquiring an abnormal Pap smear, but this was not statistically significant. Assignment to the condoms only arm was associated with a 50% less risk of an abnormal Pap smear than the diaphragm arm (p=0.01). This was however for the duration of the study, long term studies with consistent condom use is necessary. Condom use has been previously shown to reduce HPV transmission(70), persistence(71) and seropositivity(57).

A history of an ectopic pregnancy increased the risk of an abnormal Pap smear by 8 times as compared to women who have never had a Pap smear. This would suggest either long standing infection or some factor which allowed upper genital tract infection. Other variables which are indicative of Sexually transmitted disease were conflicting. A history of vaginal discharge in the last 3 months was associated with a 5 fold increased risk, whereas clinician detected infection, or objective tests for sexually transmitted disease showed no association.

There was no testing for HPV in this group of women; if we use abnormal cytology as a proxy for HPV infection then the incidence of infection with this virus is much higher than the

other two. The incidence of Herpes was 3.32%, HIV 1.33% and HPV (using Abnormal Pap smears as a marker) was at least 7.33%. HIV infection of 1.33% is much lower than in the parent study which was 4%(67). HPV infection was not shown to be a risk factor for HIV infection in the a Zimbabwean study, but because women with dysplasia on their entry Pap were excluded from this study it may be that dysplastic cervical epithelium may account for the higher HIV infection in the parent study. However we did not set out to examine the risk of HIV infection in this group of women - It is merely speculative at this time. The association between HIV and HPV is well known(72). However interventions such as circumcision which reduce the incidence HIV are also associated reduction in HPV related disease like cervical cancer(73).

The model that was used to find predictors of abnormal Pap smears had a low sensitivity of 9.38%, but the interval of less than 2 years which was used in this study is probably too frequent.

Limitations and strengths:

The study population used in this study was chosen post randomization and this may lead to bias. The study had limited power to determine the risk factors associated with an abnormal pap smear.

All women with an STI detected in the study were treated, and those who refused to be treated were excluded. The participants in this study were asymptomatic and recruited to participate in this study. The results may be completely different in symptomatic women presenting at a Gynaecological clinic or in asymptomatic women with an STI.

Cancer of the cervix is a disease of women in the 5th decade and only 25% of this group of women were older than 35 (Median age=28 (iqr-22-35). The sample size was too small to stratify women into different age categories. Variables that are traditionally associated with cervical cancer like “smoking” were not explored(50). The effect of hormonal contraception is dependent on duration of use and women were not asked about length of use(54, 55).

The association of HPV and cancer of the cervix , with an OR of more than 50(18, 19), makes this the most important predictor of cervical dysplasia and HPV testing was not performed. However in SA HPV testing is expensive and would not be clinically useful in the immediate future.

Although Pap smear screening has a high specificity, only colposcopically directed biopsies can confirm the exact grade of the lesion. Women with cervical abnormalities were managed according to clinical protocols and the results of colposcopy and histology were not part of this study.

The strengths of the study is that the study population comprised sexually active women from Soweto, an area with a high prevalence of cervical cancer and the completeness of the data and the low loss to follow-up.

Conclusions and Recommendations:

The incidence of cervical abnormalities is high, and higher in women with unsatisfactory Pap smears than in women with satisfactory Pap smears. The incidence of HSIL is less than 0,5% per annum. The situation is complicated by the fact that most women will clear a LSIL(74, 75) Repeat screening in women with a normal Pap smear with two years may

therefore increase the cost, increase the number of women referred to colposcopy services and increase levels of anxiety experienced by these women.

The SA guidelines recommend repeat Pap smears when the cytology report states that the examination of the slide was unsatisfactory. This is important in a service where the screening interval is 10 years and where many women may only have one Pap smear in a life-time. However this study suggests that it would be safe for the next Pap to be delayed for at least one year.

The Increased odds of having an abnormal Pap smear in women with incident HIV infection is further evidence that women who are HIV infected have a Pap smear at the time of diagnosis of the HIV and more frequently than the general population. The increased association with Vaginal discharge, ectopic pregnancy, and increased number of sexual partners would also warrant more frequent testing.

The ideal time of repeat testing cannot be determined, but further studies with increased numbers of women should be performed, and these should ideally be nested within a screening service at a primary health care level.

Intervals of screening are only one aspect in the prevention program and increased coverage with as many women as possible being screened should still be a priority.

The lower incidence of HIV infection in this sub-group of women in the MIRA trial does suggest that HPV or cervical dysplasia may increase the risk of HIV infection, so women with

a cervical cytological abnormality should be advised to use condoms and educated on safe sex.

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Appendices:

Appendix 1: Ethics approval for study.

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Yasmin Adam

CLEARANCE CERTIFICATE	**M090676**
PROJECT	The Incidence of Cytological Abnormality 12-24 Months after a Normal Smear in a Setting with a High Prevalence of Cervical Abnormalities
INVESTIGATORS	Dr Yasmin Adam.
DEPARTMENT	School of Public Health
DATE CONSIDERED	09.06.26
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 09.06.26 **CHAIRPERSON** .....
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr G de Bruyn

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

.....

Appendix 2:

Permission to use Dataset at PHRU:

Dear Yasmin,

Thank you for submitting a concept proposal to write a manuscript for MIRA. We received almost 30 concepts from all sites and institutions involved in the study. A group of Investigators had the chance to discuss the concepts on April 18.

The primary MIRA manuscript, corresponding to the main HIV outcome, is targeted for submission to a journal by the end of May. Once the main paper is accepted for publication, analysis on other papers can begin. The priority for the second tier of papers is: papers pertaining to the secondary objectives of the study and Investigator-led papers. Currently we anticipate that analyses for these second tier papers will continue through August. Thereafter, analyses for the remaining papers may begin.

Copies of the MIRA dataset will be made available to each site after the main paper is accepted for publication. If you, or someone from your organization, has the capacity to do your own statistical analyses, this will assist you in getting your analysis completed sooner.

However, there is still progress that can be made now. Several concept proposals in fact do not require statistical help, or do not rely on the main, CIDEA dataset, and can proceed now. Several concept proposals require more detail, which should be developed in this interim period.

The specific feedback on your concept was as follows:

Your concept was approved.

- Guy has indicated you should be able to complete this analysis in-house when the PHRU dataset is received (after the main paper is accepted).
- Jennifer Tuveson at UCSF may have some data on PAPs at PHRU that you could begin looking at, as she monitored this closely during the trial.

Should you have any other questions, please feel free to write us back.

Sincerely,

Liz Montgomery & Heidi Fancher

on behalf of the MIRA Investigators

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