Incidence of cytological abnormalities within 24 months of a normal cervical smear in Soweto, South Africa

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Background. A screening programme for cervical cancer has been implemented in South Africa (SA) with intervals of 10 years after a normal cytological result. There are no studies that evaluate repeat screening at a shorter interval in SA.

Objectives. (i) To find the incidence of cytological abnormalities on a repeat test after a report of normal cytology or an inadequate Pap smear; and (ii) to explore the factors associated with an abnormal cytology on repeat testing.

Methods. This was a secondary data analysis of a randomised controlled trial of diaphragm, lubricant gel and condoms v. condoms in the prevention of HIV infection. HIV-negative women were recruited between November 2003 and December 2005, with a normal Pap smear at entry. Observation time was from the first Pap smear to the date of the repeat Pap smear. Explanatory variables used were baseline, excepting any new HIV infection.

Results. The incidence of cytological abnormalities was 6.48% yearly in women with a previously normal Pap smear and 11.71% yearly in women with an inadequate smear result ($p=0.03$). The incidence of high-grade squamous intra-epithelial lesions (HSILs) was <0.5%. Factors associated with abnormal cytology were a history of ectopic pregnancy (odds ratio (OR) 9.25; confidence interval (CI) 1.78 - 48.02; $p=0.01$), number of male partners (OR 1.12; CI 1.03 - 1.22; $p=0.01$), history of vaginal discharge (OR 13.95; CI 1.18 - 164.47; $p=0.04$), and incident HIV infection (OR 6.56; CI 1.14 - 38.16; $p=0.04$).

Conclusion. The incidence of HSILs is low in the first 2 years after a normal or inadequate Pap smear, even in a setting with a high prevalence of cytological abnormalities.


Cytology has a false-negative rate of 15 - 30%. In a South African study, 10% of cervical cancer cases occurred in women who had been screened, but at least 5 years previously. More than 50% occurred in women who had never been screened. Prospective studies have not evaluated screening intervals >5 years between Pap smears.

Between 2004 and 2006 in Soweto, Johannesburg, a trial investigated the use of the diaphragm in preventing HIV infection, and also offered a Pap smear at the enrolment visit and again at the end of the trial. These data allowed us to assess the incidence of abnormal cytology after a normal or inadequate Pap smear in a setting with a high prevalence of cervical abnormalities. We aimed to determine the proportion of women who developed an abnormal Pap smear within 24 months of a normal Pap smear, in a cohort of women in Soweto. Risk factors associated with an incident abnormal Pap smear were explored.

Methods
Between November 2003 and December 2005, the Soweto site of the Methods for Improving Reproductive Health in Africa (MIRA) trial recruited 1 028 women from the surrounding community and other settings.
distributions of explanatory variables among women who continued to
the date from the first Pap smear (enrolment) to the date of the repeat
Pap smear results were coded as normal/inadequate or abnormal
Pap smears were conventional smears performed by nurse
clinicians, and reported on by the SA National Health Laboratory
Service (NHLS) cytopathology department. This accredited
laboratory has stringent internal and external quality control.
for any treatable STI and did not desire a pregnancy at the time.
Participants were monitored quarterly for a period of 12 - 24 months
for HIV infection and other STIs.
Women also underwent a pelvic examination at the first
visit and all were offered a Pap smear (protocol required that
all women had to have a Pap smear in the preceding 12
months be offered a Pap smear). Women were also offered a Pap
smear at exit. The Pap smears were conventional smears performed by nurse
clinicians, and reported on by the SA National Health Laboratory
Service (NHLS) cytopathology department. This accredited
laboratory has stringent internal and external quality control. The
recommendations for management of Pap smear abnormalities
stipulated that any Pap smear without an endo- or ectocervical
component was inadequate and had to be repeated, even when the
descriptive diagnosis was ‘within normal limits’. Any Pap report of
low-grade squamous intra-epithelial lesions or atypical squamous
cells of undetermined significance (ASCUS) was repeated in 6
months. Meanwhile, reports of high-grade squamous intra-epithelial
lesions (HSILs) or atypical glandular cells were referred to the
Colposcopy Clinic at Chris Hani Baragwanath Academic Hospital
for further management.
Only STI tests at enrolment (prevalence data) and incident
HIV test results were used. Polymerase chain reaction (PCR)
testing was performed for Chlamydia trachomatis, Neisseria
gonorrhoeae and Trichomonas vaginalis. A blood test was
provided at enrolment and at exit for herpes simplex virus 2
(Focus HSV2 enzyme-linked immunosorbent assay (ELISA))
and syphilis (rapid plasma reagin (RPR) and Treponema pallidum
haemagglutinin (TPHA)). A finger-prick blood sample or venous
puncture was obtained for rapid HIV-1/2 testing (Oraquick and
Determine tests).
Women with equivocal results underwent confirmatory laboratory
ELISA testing. Participants were notified about the HIV result
and post-test counselling was provided to both HIV-positive
and negative women.
We conducted a secondary analysis of the MIRA trial data.
Pap smear results were coded as normal/inadequate or abnormal
(ASCUS, LSIL, HSIL or more severe). We defined observation time as
the date from the first Pap smear (enrolment) to the date of the repeat
Pap smear. All explanatory variables used were baseline variables,
except new HIV infection.
We performed a bivariate analysis using a logistic regression to
determine factors associated with the outcome. We entered all variables
found to be significant in bivariate testing (p<0.2) and used a backward
selection procedure to arrive at a final multivariate model.
To compare the risk of an abnormal Pap smear in those who
had a normal Pap smear at baseline with those who had an
inadequate Pap smear at baseline, we used standard methods
for time-to-event data to determine time to abnormal cervical
cytology.
The Human Research Ethics Committee (Medical) of the University
of the Witwatersrand approved the study protocol for the MIRA trial
(M031111) and granted ethical clearance for the use of the data in
this secondary analysis (M090676).

Results
Among those eligible for the study, 850 women underwent a repeat
Pap smear; 97 (11.41%) had an abnormal Pap smear at entry and
were excluded (Fig. 1). These abnormalities comprised of
ASCUS (31 cases; 3.65%), LSIL (51 cases; 6%) and HSIL (15 cases;
1.76%).

The incidence of cytological abnormalities in this group of women
(baseline Pap normal or inadequate) was 7.33% per annum. The incidence
of cytological abnormalities was 6.88 % in women with a previously
normal Pap and 11.71% in women with an inadequate smear result. Fig. 2
depicts the risk of having an abnormal Pap smear. The median follow-up
time was 297 days (interquartile range (IQR) 182 - 455).
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 Pap smear when compared with an
inadequate one (p=0.03).
Of both the normal and inadequate Pap smears <0.5% had an
HSIL reported on their subsequent Pap smear and the rest of the
abnormalities were LSIL and ASCUS (Table 1).

Fig. 1. Flow of participants eligible for the study. ASCUS = atypical
squamous cells of undetermined significance; LSIL = low-grade squamous
intraepithelial neoplasia lesion; HSIL = high-grade squamous intraepithelial
lesions.
The subjects’ median age was 28 years (IQR 22 - 35) and the median number of years they had been educated was 12 (IQR 10 - 12). Participants were not asked about smoking or alcohol use.

Types of contraception being used at the time of enrolment were: combined oral contraception (n=58; 7.70%); injectable hormonal contraception; (n=223; 29.61%); progestogen-only pills (n=20; 2.66%); intra-uterine contraceptive device (n=7; 0.93%); and withdrawal or natural methods (17; 2.26%).

The association between condom usage and the incidence of an abnormal Pap smear was contradictory: while there were fewer women with cervical abnormalities in those randomised to the control group (condoms only), there was no association in women who said they used condoms at the last intercourse or were currently using them (Table 2).

The median parity in this group was 2 (IQR 1 - 3), with a range of 0 - 7. The difference in pregnancy history (live births, vaginal deliveries, miscarriages and abortions) in women who had a subsequent normal or abnormal Pap smear was not significant. An ectopic pregnancy in the past was associated with an abnormal Pap smear, but this association was not statistically significant (4 (4.82%) v. 10 (1.61%); p=0.7).

The mean age of first intercourse was 17.58 years (standard deviation (SD) ±2.12; range 11 - 27). There was no statistical difference in having a subsequent abnormal Pap smear in women who had an earlier sexual debut (p = 0.24) or in women who had sex in exchange for money (p = 0.11). Women with an increased number of sexual partners were more likely to have an abnormal Pap smear on repeat testing (mean 4.85 (SD ±3.40) v. mean 3.48 (SD ±2.73); p=0.00). The trial excluded women who performed <3 coital acts in the month; therefore, it was not meaningful to assess difference by coital frequency. Age at first intercourse was not reflected in any statistical difference in the outcome (Table 3).

Table 4 shows the association between the risk of developing an abnormal cytology on Pap smear, and whether subjects had a history of cervical intra-epithelial neoplasia (CIN)

The trial excluded women who performed <3 coital acts in the month; therefore, it was not meaningful to assess difference by coital frequency. Age at first intercourse was not reflected in any statistical difference in the outcome (Table 3).

Table 4 shows the association between the risk of developing an abnormal cytology on Pap smear, and whether subjects had a history of cervical intra-epithelial neoplasia (CIN)
suggestive of an STI in the preceding 3 months or alternatively confirmatory tests of STIs. A history of a vaginal discharge in the preceding 3 months, or symptoms of an STI, were important differences between those who did and those who did not develop cytological abnormalities. Clinical findings for STIs were discovered on examination, rather than following complaints from the women, as could be expected in a research setting.

There were no statistical differences in incident cervical cytological abnormalities among women with objective evidence of an STI at baseline and no infection (Table 4). Incident STIs such as chlamydia \((n=32; 4.25\%)\), gonorrhoea \((n=13; 1.73\%)\) and trichomonas \((n=38; 5.05\%)\) were not associated with an increase in cytological abnormalities.

Incident HIV infection was associated with a 6-fold increase in the risk of an abnormal Pap smear. An abnormal discharge in the last 3 months and having had an ectopic pregnancy at any time also increased the risk. Having used a condom after the last intercourse had no effect, but being allocated to the control (condom) arm of the study reduced the risk by approximately 58% (Table 5).

**Discussion**

The SA cervical screening guidelines predict a reduction of cervical cancer of between 64 - 70% with a screening interval of 10 years. An evaluation of screening intervals shows that screening is associated with a reduced incidence of cervical cancer, ranging from 84% with 5-year intervals to 94% when the screening was performed every 2 years, in both cases in women aged 35 - 64 years.

We used the presence of any cytological abnormality as an outcome measure, because this would mean that the particular woman is at risk of cervical cancer, albeit with a different degree of risk. Of note was a low incidence of HSILs, which is the threshold for treatment in our setting: 0.46% and 0.37% in the inadequate and adequate Pap smear groups, respectively. The prevalence of HSILs in women who were recruited for the study was 1.76%.

### Table 2. A description and comparison of barrier contraception \((N=753)\)

<table>
<thead>
<tr>
<th>Abnormal cytology (N=84 (11.16%)</th>
<th>Normal cytology (N=669 (88.84%)</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomised to the intervention arm (diaphragm) (n=388 (51.53%))</td>
<td>53 (63.10)</td>
<td>335 (50.07)</td>
</tr>
<tr>
<td>Randomised to the control arm (n=365 (48.47%))</td>
<td>31 (36.90)</td>
<td>334 (49.93)</td>
</tr>
<tr>
<td>Condoms used at last vaginal sex (n=438 (71.57%))</td>
<td>46 (64.79)</td>
<td>392 (72.46)</td>
</tr>
<tr>
<td>Ever used condoms (n=586 (77.82%))</td>
<td>64 (76.19)</td>
<td>522 (78.03)</td>
</tr>
<tr>
<td>Current condom use (at baseline) (n=506 (67.20%))</td>
<td>53 (63.10)</td>
<td>453 (67.71)</td>
</tr>
<tr>
<td>Female condoms used at last intercourse (n=27 (4.41%))</td>
<td>4 (5.63)</td>
<td>23 (4.25)</td>
</tr>
</tbody>
</table>

\(^*\)Fisher’s exact used when there were <5 observations in a cell.

### Table 3. Description and comparison of symptoms and tests which are suggestive of a sexually transmitted disease \((N=753)\)

<table>
<thead>
<tr>
<th>Abnormal cytology (N=84 (11.16%)</th>
<th>Normal cytology (N=669 (88.84%)</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at first intercourse</td>
<td>Mean 17.32 (SD ±1.78)</td>
<td>Mean 17.61 (SD ±2.15)</td>
</tr>
<tr>
<td>Mean 11 - 27</td>
<td>Range 11 - 27</td>
<td></td>
</tr>
<tr>
<td>Sex in exchange for money (n=17 (2.26%))</td>
<td>4 (4.76%)</td>
<td>13 (1.94%)</td>
</tr>
<tr>
<td>Number of male partners Mean 4.02 (SD ±2.90)</td>
<td>4.85 (SD ±3.40)</td>
<td>3.84 (SD ±2.73)</td>
</tr>
<tr>
<td>Mean 1 - 26</td>
<td>Range 1 - 26</td>
<td></td>
</tr>
<tr>
<td>Average sex in the preceding 3 months: Mean 9.46 (SD ±5.12)</td>
<td>9.46 (SD ±4.96)</td>
<td>9.46 (SD ±4.96)</td>
</tr>
<tr>
<td>Mean 3 - 40</td>
<td>Median 8 (IQR 4 - 12)</td>
<td>Median 7 (IQR 5 - 12)</td>
</tr>
</tbody>
</table>

\(^*\)t-test; Wilcoxon ranksum; \(^*\)t-test (unequal variances); Fisher’s exact.
The Australian National Health and Medical Research Council Guidelines recommend that all pathologists report normal Pap smear results 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.11

There was no testing for human papillomavirus (HPV) in our study; if we use abnormal cytology as a proxy for HPV infection then its incidence is much higher than that of herpes or HIV. In women with a normal Pap smear at recruitment, the incidence of herpes was 3.32%, HIV 1.33% and HPV (using abnormal Pap smears as a marker) at least 7.33%. An HIV infection incidence of 1.33% is much lower than in the MIRA study, where it was 4%8 (as explained in Fig. 1, we excluded all women with an abnormal Pap smear at baseline).

HPV infection was not shown to be a risk factor for HIV infection in a Zimbabwean study,12 but because women with dysplasia on their entry Pap were excluded from this study it may be that dysplastic cervical epithelium accounted for the higher rate of HIV infection in the parent study.8

Condom use reduces HPV transmission,13 persistence14 and seropositivity.15 A history of male condom usage was protective against acquiring an abnormal Pap smear in this study, but this was not statistically significant. Assignment to the condoms-only arm of the MIRA trial, however, was associated with a 50% lower risk of an abnormal Pap smear than assignment to the diaphragm arm (p=0.01).

Asking subjects about ‘condom use at last intercourse’ is considered a good way to assess use because it reduces recall bias. However, in this analysis this criterion did not differ between the two groups, even on stratifying by randomisation. The prevalence of female condom use was low in this group of women and was discouraged by the researchers owing to probable problems with fitting of the diaphragm for women in the intervention arm (diaphragm, lubricant and condoms).

A limitation of the study was that only women who agreed to be treated for an STI that had been detected by a laboratory test were eligible for inclusion. The the rate of cytological abnormality may also differ in women symptomatic for STI presenting at a gynaecological clinic. The study sample was too small for stratifying into different age categories, and to fully evaluate risk factors. Pap smears have a poor sensitivity and therefore we were not able to evaluate sensitivity. Although Pap smear screening has a high specificity, only colposcopically directed biopsies can confirm the exact grade of the lesion. The study numbers were also too small for us to use the presence of HSILs as an outcome measure.

The study’s strengths included that its population comprised sexually active women from Soweto who had a high prevalence of cervical cancer, and loss to follow-up was approximately 7%.

The following table summarizes the symptoms and tests which are suggestive of an STI:

<table>
<thead>
<tr>
<th>Description</th>
<th>Abnormal cytology</th>
<th>Normal cytology</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you ever been treated for an STI?</td>
<td>22 (26.51%)</td>
<td>131 (19.76%)</td>
<td>0.15 (χ²)</td>
</tr>
<tr>
<td>Number of times treated</td>
<td>Mean 1.91 (SD ±1.95)</td>
<td>Mean 1.15 (SD ±0.49)</td>
<td>0.00*</td>
</tr>
<tr>
<td>Symptoms suggestive of an STI</td>
<td>15 (18.07)</td>
<td>53 (7.99)</td>
<td>0.00 (χ²)</td>
</tr>
<tr>
<td>Abnormal bleeding in the last 3 months</td>
<td>1 (1.20)</td>
<td>21 (3.17)</td>
<td>0.288</td>
</tr>
<tr>
<td>Vaginal discharge on history in the last 3 months</td>
<td>11 (13.25)</td>
<td>26 (3.92)</td>
<td>0.00 (χ²)</td>
</tr>
<tr>
<td>Chlamydia at entry</td>
<td>5 (5.95)</td>
<td>36 (5.38)</td>
<td>0.84†</td>
</tr>
<tr>
<td>Gonorrhoea at entry</td>
<td>1 (1.19)</td>
<td>6 (0.90)</td>
<td>0.57†</td>
</tr>
<tr>
<td>Trichomonas</td>
<td>1 (1.19)</td>
<td>11 (1.64)</td>
<td>1†</td>
</tr>
<tr>
<td>WR positive</td>
<td>1 (5)</td>
<td>9(4.5)</td>
<td>1†</td>
</tr>
<tr>
<td>HSV at entry</td>
<td>56 (66.67)</td>
<td>426 (63.68)</td>
<td>0.81 (χ²)</td>
</tr>
<tr>
<td>Incident HIV</td>
<td>2 (2.38)</td>
<td>8 (1.20)</td>
<td>0.200†</td>
</tr>
</tbody>
</table>

*χ²-test †Fisher’s exact
The results of this study suggest that it would be safe for the next Pap to be delayed for at least 1 year even in women with an inadequate Pap smear result.

The lower incidence of HIV warrants further study on whether cervical dysplasia, and not just HPV infection, is an independent risk factor for HIV infection.

Disclosure. This paper was part of a dissertation submitted for an MSc degree (Epidemiology & Biostatistics) by Y Adam to the University of the Witwatersrand.

Table 5. Univariate and multivariate analysis

<table>
<thead>
<tr>
<th></th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age</td>
<td>0.98</td>
<td>0.95 - 1.01</td>
</tr>
<tr>
<td>Contraception-barrier</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Used a male condom at the last vaginal sexual intercourse</td>
<td>0.70</td>
<td>0.41 - 1.18</td>
</tr>
<tr>
<td>Condoms as opposed to diaphragm</td>
<td>0.59</td>
<td>0.37 - 0.94</td>
</tr>
<tr>
<td>Pregnancy history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Had an ectopic pregnancy</td>
<td>3.31</td>
<td>1.01 - 10.79</td>
</tr>
<tr>
<td>Sexual history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coitarche at age ≤19 years v. &gt;19 years</td>
<td>0.52</td>
<td>0.23 - 1.17</td>
</tr>
<tr>
<td>Number of male partners</td>
<td>1.10</td>
<td>1.03 - 1.18</td>
</tr>
<tr>
<td>STI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated for a STI</td>
<td>1.46</td>
<td>0.87 - 2.47</td>
</tr>
<tr>
<td>Number of times treated</td>
<td>2.43</td>
<td>1.21 - 4.89</td>
</tr>
<tr>
<td>History of vaginal discharge</td>
<td>3.74</td>
<td>1.78 - 7.89</td>
</tr>
<tr>
<td>HIV</td>
<td>2.08</td>
<td>0.43 - 10.00</td>
</tr>
</tbody>
</table>

References

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