

CERVICAL CANCER SCREENING – A NEW VIRAL PARADIGM

Cervical cancer is the second most frequent cancer among women in South Africa and worldwide.

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An average of 6 000 women are diagnosed with cervical cancer and more than 3 000 die from this disease in South Africa (SA) every year.¹ Human papillomavirus (HPV) infection is now a well-established cause of cervical cancer. Most cases of cervical cancer could be prevented if the new improved screening techniques and HPV vaccination strategies are universally implemented.

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HPV vaccination of girls before their sexual debut is the best primary prevention method currently available. Incorporation of HPV vaccination in the public health sector is still to be seen in the developing world, mostly due to high vaccine cost. A significant HPV vaccination-induced reduction of cervical cancer burden is not likely to be evident for another 5 - 15 years. The best secondary prevention method is regular and adequate screening. New international and SA private sector guidelines propose the incorporation of molecular testing for HPV in screening and patient management, backed by good scientific evidence. It is transition time for screening programmes: a move from the annual Pap test to a new viral paradigm.

HPV types and disease association

HPV is found in 99.7% cervical cancer cases,² and is also aetiologically linked to a significant

proportion of anal, vulvar, vaginal, penile and oropharyngeal cancers. Not all HPV types have the same ability to cause cancer, therefore the 15 types with the highest risk have been named oncogenic or high-risk HPV (hrHPV) types. The hrHPV types 16 and 18 account for over 70% of cervical cancers worldwide.³ Low-risk HPV types (mainly HPV 6 and 11) cause genital warts, a proportion of low-grade cervical dysplasias, and oral, laryngeal or conjunctival papillomas.

Epidemiology and natural history of HPV infection and cervical cancer

HPV is the most common sexually transmitted infection (STI) worldwide. The virus is primarily spread by direct skin-to-skin contact. Sexually active women have a lifetime risk of up to 80% to be infected with one or more HPV types.⁴ HPV infection rises rapidly after the onset of sexual activity and then declines with age, resulting in the highest prevalence in women younger than 30 years of age (Fig. 1).⁵ At least 21% of women in the general South African population are estimated to harbour cervical HPV infection at a given time,¹ but the prevalence of hrHPV may be as high as 60% in certain populations (unpublished data) and 85% in women infected with HIV.⁶

As many as 4 out of every 5 genital HPV infections in immunocompetent women will be cleared by the host immune system within 24 - 36 months. These transient infections are usually asymptomatic and the virus may unknowingly be transmitted to sexual partners. In a small subset of women persistent infection

with hrHPV types may lead to integration of viral DNA into the host cell DNA, which could lead to the development of pre-malignant cervical lesions (low-grade squamous intra-epithelial lesions/LSIL, high-grade squamous intra-epithelial lesions/HSIL) and eventually to cervical cancer (Figs 2 and 3). Factors associated with an increased risk of persistence are immunosuppression associated with HIV/AIDS, immunosuppressive drugs (e.g. steroids), diabetes, renal failure and cigarette smoking. The prevalence of HPV infection in SA has increased dramatically over the last decade due to the HIV-associated immune compromise in an increasing subset of women.

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Cervical cancer incidence peaks in the third and fourth decade (Fig. 1),⁷ implying that it is the persistent infections occurring in women over age 30 years that are of most concern. Several studies have shown that invasive cervical cancer in HIV-positive women tends to present 10 - 15 years earlier than in their HIV-negative counterparts⁶ and this effect is not reversed by antiretroviral therapy.⁸

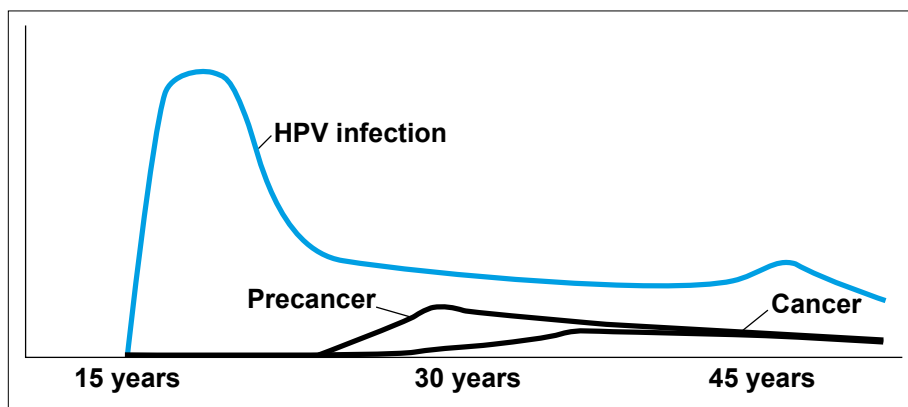


Fig. 1. Prevalence of HPV infection and cervical pre-cancerous and cancer lesions according to age (peaks of curves not drawn to scale). (Adapted from reference 5.)

HPV infections and cervical cancer

HPV infects the actively dividing squamous cells of the basal layer when introduced through microfissures of the skin, or in naturally accessible areas like the cervical or anal transformation zones. With the initial HPV infection the viral DNA is separate from the host DNA. With persistent infection integration may take place, where some viral genes become part of the host DNA. If these genes include the viral oncogenes E6 and E7 uncontrolled proliferation of the cell may lead to cervical cancer (Fig. 3).⁹

Cervical cancer screening

The goal of cervical cancer screening is to identify pre-invasive lesions and early-stage

invasive lesions, as treatment of these lesions may halt progression of the disease. Cervical cancer screening is a success because squamous cell cervical cancer follows a predictable course from initial HPV infection to premalignant cellular changes to cancer, which takes an average of 9 - 15 years (Figs 1 and 2). Thus, routine screening has a good chance of detecting most premalignant infection and/or cellular changes.

Conventional screening using cytology
Papanicolaou staining of exfoliated squamous cervical cells (commonly called 'Pap smear') detects cellular changes, including the effect of HPV infection (koilocytes, LSIL, HSIL or cervical cancer cells). Cervical cytology screening was introduced in 1943 and is

considered the most successful cancer screening programme to date.¹⁰ But annual screening requires a high level of medical infrastructure and patient compliance. Cervical cytology testing is far from perfect and, even with annual screening, some cancers may slip through the cracks. Human errors in sampling and interpretation contribute to the low sensitivity of conventional cytology (30 - 87%)¹⁰ and liquid-based cytology/LBC (70 - 80%).³ This translates to a large number of false-negative results and the need for frequent screening. Specificity is higher, ranging from 86% to 100%.¹⁰ Cytology also does not have an acceptable sensitivity for the detection of cervical adenocarcinoma and its precursors.

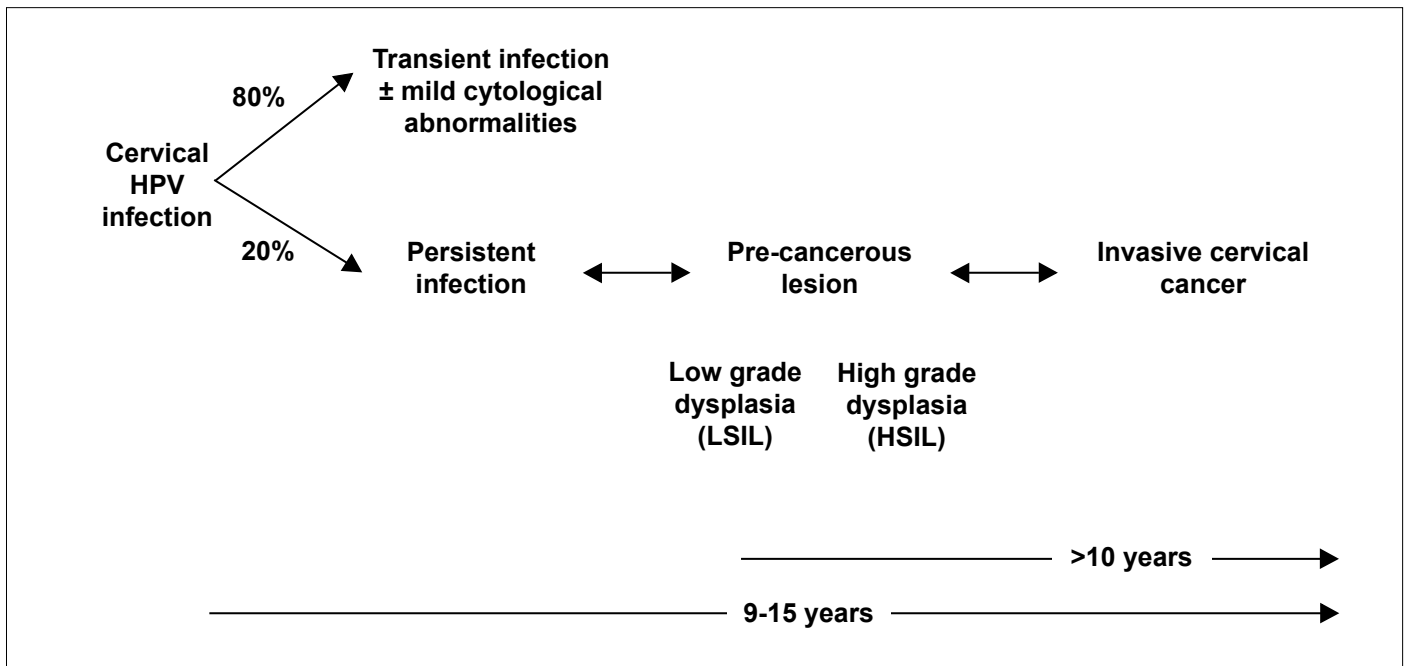


Fig. 2. The natural history of HPV infection and cervical cancer. (Adapted from reference 5.)

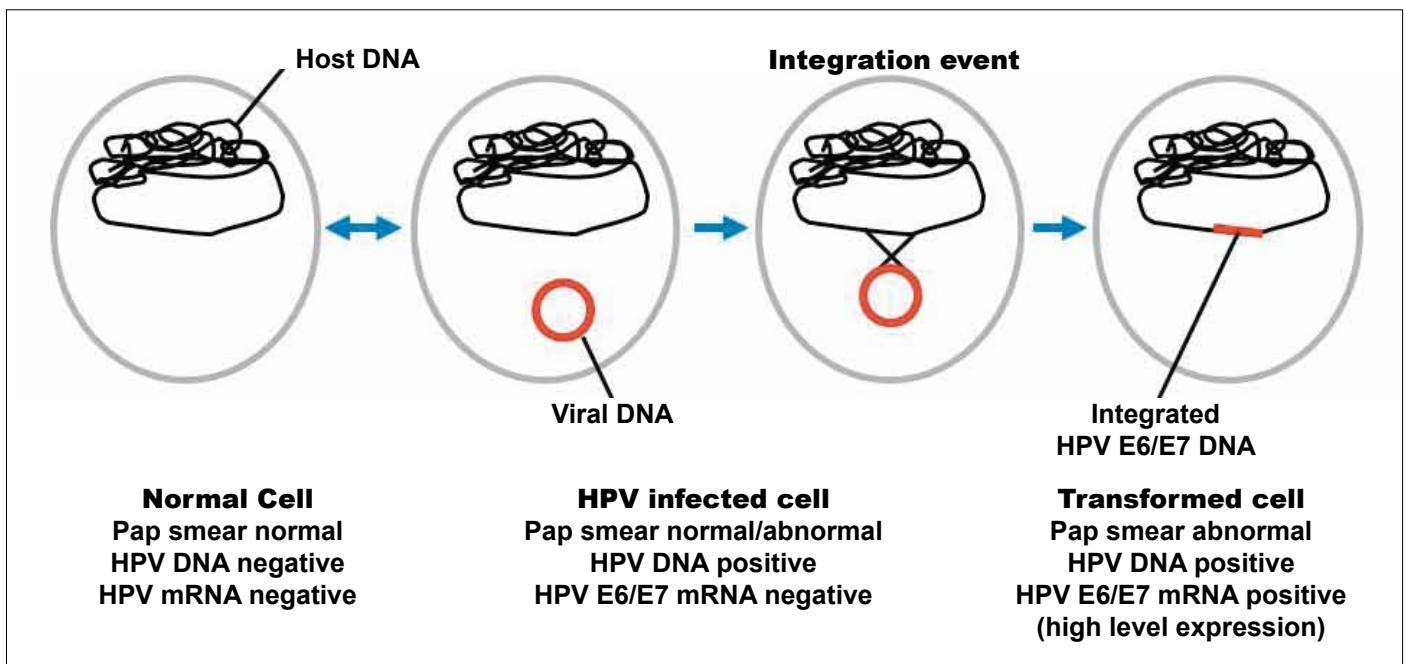


Fig. 3. The steps in HPV carcinogenesis and screening tests for cervical cancer. (Adapted from reference 9.)

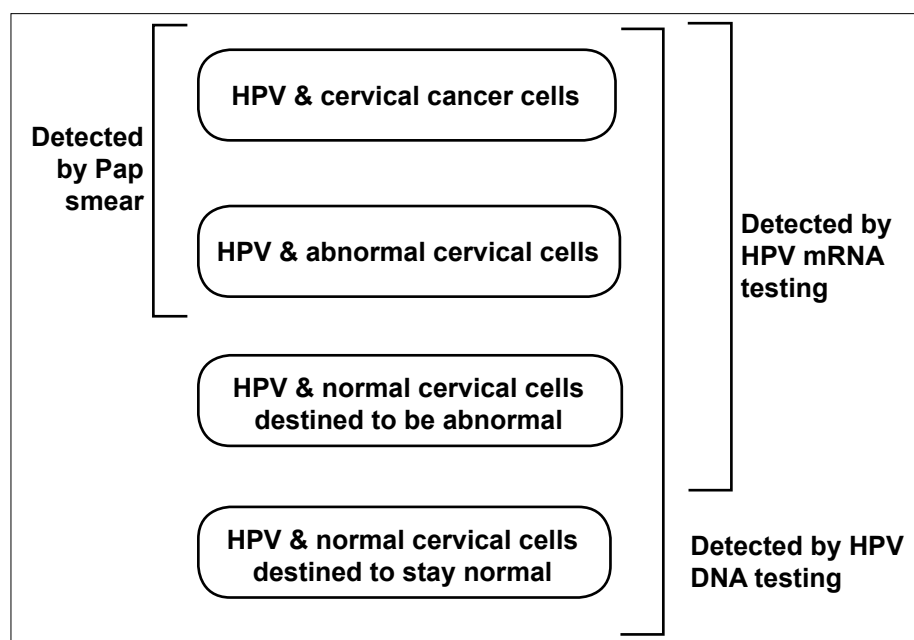


Fig. 4. The limitations of Pap smears and HPV DNA testing versus HPV E6/E7 mRNA testing. (Adapted from reference 9.)

HPV DNA testing

Testing for HPV DNA focuses on detection of the cause of cervical cancer and can detect HPV-infected cells before they become cytologically abnormal. Like a Pap smear, HPV DNA testing is performed on a sample of exfoliated cervical cells, which can be obtained from LBC specimens or via a cervical swab/brush specimen which can be placed in manufacturer-specific transport mediums. Different types of HPV DNA tests are available. Qualitative tests detect 13 - 15 hrHPV types, but do not specify which type(s) tested positive (i.e. give only a positive or negative result). Newer versions of these tests specify if HPV types 16 and/or 18 were present, which allows further risk stratification. HPV genotyping assays specify 15 hrHPV types, 3 probable hrHPV types and 19 low-risk HPV types.

HPV DNA testing is far more sensitive than cytology and able to detect small numbers of HPV genomes. Unfortunately this excellent analytical sensitivity of HPV DNA testing makes it much less clinically specific. HPV DNA testing will also pick up those women who are infected with HPV but do not have severe dysplasia and thus have an 80% chance to clear the infection without treatment. The same positive signal is generated from infected cells that are destined to be cleared without symptoms, to be cleared after mild dysplasia or to develop into cancer (Fig. 4). HrHPV DNA-positive women will need follow-up and substantially more women will be referred for colposcopy and biopsy, resulting in increased costs as well as unnecessary anxiety among these women. This potential for over-diagnosis of pre-invasive lesions that would have regressed spontaneously complicates the proper place of DNA testing in cervical cancer screening algorithms.

The biggest advantages of HPV DNA testing are the superior sensitivity for high-grade lesions over that of cytology alone and its very good negative predictive value.¹¹ A woman who tests negative for hrHPV will probably not need cervical cancer screening for the next 6 years (range 3 - 10 years).¹²

The SA national guideline allows for 3 Pap smears in a woman's lifetime taken at 10-year intervals from 30 years of age.

HPV mRNA testing

E6 and E7 are the oncogenes of HPV and expression of these genes is required for malignant transformation. E6 and E7 mediate degradation of the tumour suppressors p53 and retinoblastoma protein (pRb) and interfere with cell-cycle regulation. E6/E7 proteins from low-risk types are less competent in interfering with p53 and pRb functions than E6/E7 proteins from high-risk types. Therefore, low-risk HPV infections are associated with benign proliferations, such as genital warts and low-grade intraepithelial lesions prone to regress.

Several assays have been designed to detect messenger RNA (mRNA) of the E6/E7 genes of hrHPV. Expression of E6/E7 mRNA increases with the severity of the lesion. In HSIL and cervical carcinoma high-level expression of E6/E7 mRNA is present due to the associated integration of E6 and E7 genes into the host's cellular DNA. Expression in LSIL lesions is low. These tests have high

clinical specificity for detecting disease and may also predict which women with LSIL or atypical squamous cells of unknown significance (ASCUS) have the potential to progress to cervical cancer (Fig. 4). The lower number of women testing positive with the mRNA test compared with DNA test results in fewer women who will need further follow-up.

South African cervical cancer screening guidelines

There are several approaches for the prevention of cervical cancer. The SA national guideline allows for 3 Pap smears in a woman's lifetime taken at 10-year intervals from 30 years of age.¹³ Currently there is no population-wide screening programme, with partial or no screening in several areas. In the private sector opportunistic screening is commonly practised.⁴ The update to the SA HPV Advisory Board 2010 guidelines advocate Pap or HPV testing as primary screening, with three different algorithms for different resourced settings.⁸

International cervical cancer screening guidelines

In developing countries cytology may not be viable with the current infrastructure and vaccination is too costly. The main prevention strategy in lower-resourced settings involves screening with HPV testing or visual inspection of the cervix.¹⁴

In developed countries there is a transition towards combining vaccination with screening. In Canada and the USA Pap smears are often combined with HPV tests in women older than 30 years. Screening in Europe is more cytology based, but primary HPV screening with cytology triage of HPV-positive women is considered.

Summary of optimal screening practices in well-resourced settings

When to start screening

The first Pap smear should be taken at age 21 years or within 3 years of onset of sexual activity.¹⁵

Women 21 - 29 years old

This age group should have a Pap test every 1 - 2 years. Primary screening with HPV DNA testing is not recommended in women under the age of 30. HPV DNA testing should be used only to triage Pap readings determined to be ASCUS.¹⁵

Women 30 years and older

Cytology testing every 3 years is safe and cost-effective for low-risk women 30 and older who have had three consecutive negative Pap test results.¹⁵ HPV testing can be used in women 30 years and older with or without cytology. Low-risk women who have negative cytology and HPV DNA results can be reassured that their risk for cervical cancer is extremely low and that protection lasts several years. Recent studies support the safety of extending screening intervals up to 6 years for these women.¹²

Women who have risk factors

Women who are immunocompromised, who were exposed to diethylstilboestrol (DES) *in utero*, and who have previously been treated for HSIL or cancer should have more frequent screening.¹⁵ The SA Department of Health 2010 HIV treatment guidelines advise cervical cancer screening for all HIV-positive women on diagnosis and, if normal, every 3 years.¹⁶

When to stop screening

Women who have had a hysterectomy with removal of the cervix for benign conditions, such as uterine fibroids, and who have no history of HSIL, should discontinue routine cervical cancer screening. Consider discontinuation of screening in women between 65 and 70 years of age who have had three consecutive normal smears and no abnormal test results in the past 10 years.¹⁵

Screening after treatment for HSIL lesions

Follow-up will be determined by economic factors and estimated likelihood of recurrence. Generally, a patient can be referred back to the population screening programme when both cytology and hrHPV tests are negative, when she has had two consecutive negative cytology tests or when the initial HPV type is cleared and the cytology negative.⁴

Vaccinated women will still need screening

Due to the very high efficacy of the HPV vaccines, pre-cancerous lesions due to HPV 16 and 18 will virtually disappear. Cervical cancer screening will still be necessary to

prevent the 30% of cervical cancers caused by non-vaccine HPV types, and for women who were vaccinated after their sexual debut who may have been exposed to HPV before vaccination. Screening with HPV markers at prolonged intervals will probably be the most efficient and cost effective.

The future of cervical cancer screening programmes

Most guidelines on HPV screening recommend a co-testing approach utilising HPV DNA testing and cytology in women aged ≥ 30 years. However, there is now overwhelming scientific evidence that HPV testing as the primary screening test for women aged over 30 is better than screening with cytology. The vast majority of studies found that cytology rarely detects HSIL that are not also HPV positive. This provides the basis for reserving cytology to triage HPV-positive women, thereby reducing the number of unnecessary referrals for transient lesions. HPV mRNA testing is also now proposed as a better triage test when compared with cytology. Because HPV testing leads to a longer period of low risk than after a negative smear, adding cytology testing adds very little added benefit in terms of negative predictive value.¹²

Detection of hrHPV DNA and/or mRNA is considered to be potentially useful in three clinical applications:

- as primary screening
- in triage to select women showing minor cytological lesions (LSIL and ASCUS) needing referral for diagnosis and treatment
- in follow-up of women treated for high-grade lesions to predict persistent or recurrent disease.

Unfortunately, transition from conventional programmes to HPV-based screening protocols has proven to be difficult due to high cost, limited resources in developing countries, logistical issues (e.g. lack of education of health care workers and uncertainty regarding new HPV-related management protocols), organisational challenges (e.g. training of laboratory staff and reallocation of cytotechnologists) and education of the general public (e.g. the simplification of screening demands is often negatively perceived as a reduction of acquired social rights).

Conclusion

There is now enough scientific evidence available to predict that conventional cervical cancer screening with cytology will eventually be replaced by testing for markers of HPV infection associated with disease progression. It is vital that clinicians understand the benefits, limitations and harms of testing. False-positives can be reduced by adhering to the guidelines for age-related testing and the recommended longer screening intervals. Patients must be

well informed regarding the role of HPV infection testing in cervical cancer screening. Some critical issues regarding HPV-based screening also still need to be more clearly defined, e.g. optimal screening intervals, the best age to start screening, improved methods for management of HPV-positive patients and new biomarkers of disease progression.

References available at www.cmej.org.za

IN A NUTSHELL

- HPV infection is the primary cause of cervical cancer and plays a central role in cervical carcinogenesis.
- More than 80% of HPV infections are transient, especially in young immunocompetent women.
- Progression to invasive carcinomas is associated with persistent hrHPV infection with subsequent integration of parts of the HPV genome into the host chromosomes.
- Cytology is still recommended for cervical cancer screening in women younger than 30 years.
- Screening for cervical cancer in women 30 years and older needs to move toward primary HPV testing, with cytology or other biomarker of disease progression reserved as a triage test for HPV-positive women.
- HrHPV E6/E7 mRNA testing correlates better with severity of the lesion compared with HPV DNA testing, and seems to be a predictive marker to identify women at risk of developing cervical carcinoma.
- HIV-positive women will need more regular screening.
- Vaccinated women will still need screening.