Primary Screening for Human Papillomavirus in Cervical Cancer Prevention

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Abstract

Acquisition of a high risk Human Papillomavirus (hr HPV) infection is a necessary step in the pathogenesis of cervical cancer. Hr HPV detection has a significantly higher sensitivity in the early detection of cervical precancerous lesions than cytology, but a lower specificity. For primary screening tests higher sensitivity is preferential to a higher specificity, since the goal is to prevent as much as possible. The large majority of cervical cancer screening programs in Europe are still cytology based. A switch from cytology based to hr HPV DNA based screening is being implemented in several pilot settings.

Since the specificity of hr HPV-testing in primary screening settings is lower compared to cytology, triage of hr HPV positive women before referral to colposcopy is necessary. Triage with cytology, proliferation proteins, detection of methylated DNA regions and viral load slope plot is being investigated.

Different subgroups of patients would benefit from different screening programs. Hr HPV based screening is preferential in women aged 30 years and older. In younger women, cytology has a better performance, since the high prevalence of hr HPV infection makes the specificity of HPV-testing even worse. In vaccinated cohorts hr HV-testing is preferred, since lesions are thought to be smaller and more easily missed by cytology. In low resources setting, hr HPV-testing significantly reduces cervical cancer incidence and cancer mortality, in comparison with cytology and visual inspection based screening.

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Introduction

Cervical cancer is the third most common cancer in women worldwide.(1) Cervical cancer and pre-cancer arise at the transformation zone where the squamous and the columnar epithelium of the endocervix meet. HPV infection at this site is the primary step in the pathogenesis of this disease. HPV infection induces changes in the cell cycle, by changing methylation of cell DNA, inducing transcription of viral oncogens and thereby formation of proliferation proteins.(2)

HPV infection is common as 80% of all women are infected at some point in their lives,(3) but most of these infections are transient. HPV infections occur most in young women after initial sexual contact. Less than 3-5% of woman infected with a high risk HPV type acquire cervical cancer.(4)

Screening used to focus on the detection of precancerous or early invasive lesions by cytology. New screening methods arise, since more about the pathogenesis is known.

The first screening test was based on the cytological detection of abnormal cells in a smear that was taken at the cervical transformation zone. This test was invented by and named after Georgios Papanikolaou in the beginning of last century, and therefore was called PAP-smear. Screening by cytology was

introduced for screening programs in Northern America and Europe 50 years ago. Organised screening programs are responsible for an important reduction in the incidence of cervical cancer in some parts of the developed world.[5]

Liquid based monolayer cytology has replaced the conventional PAP-smear in large parts of the world. In the last decade, automated computer image analysis systems for screening by cytology have been introduced. Unfortunately these systems are expensive and still need some human supervision. Therefore cytology screening is not available in low resource countries.

Cytology based screening still is the most commonly used primary screening test, despite some important disadvantages. It has a low sensitivity to find precancerous lesions ranging from 38%-87% and specificity ranging from 86%-98%,[6-8] leaving a part of the cervical cancer precursors undiagnosed. A short screening interval is therefore necessary. Sensitivity and specificity depend on cut-off values of cytology. When the cut-off for cytology is low (ASCUS: Atypical Squamous Cells of Unknown Significance), sensitivity improves at the cost of lower specificity.

Hr HPV-testing as triage after equivocal PAP-smear

ASCUS is associated with a risk for CIN 2+ (CIN 2, CIN 3, adenocarcinoma in situ) of 9.7% (95% Confidence Interval: 7.7-11.7%), and a very low risk for cervical cancer (0.1-0.2%). Hr HPV-testing as a triage test in ASCUS-cytology showed a high sensitivity in predicting high grade CIN (92.5% (95% CI: 90.1-94.9%).[9] It is at least as sensitive as an immediate colposcopy.[10]

Hr HPV-testing in secondary screening after treatment

Despite treatment for CIN, the risk of (recurrent) disease after treatment is higher than in the general population. A continued surveillance and follow up is required. Hr HPV persistency is recorded in 20% of these cases and it is strongly correlated with residual/recurrent CIN.[10,11]

The best way to monitor this high risk population is a combined cytology and HPV test at six months (sensitivity of 96% (95% CI 89-99), specificity 81% (95% CI 77-84)). A single hr HPV-test is also highly sensitive (OR: 1.27 (95%CI 1.06-1.51), with only a slight loss in specificity (OR: 0.94 (95%CI 0.87-1.01)). In women with a negative co-test (cytology+hr HPV DNA), testing at 12 months after treatment can safely be skipped, but should be repeated at 24 months.[10]

HPV testing in primary screening

Since HPV infection is a necessary step in the pathogenesis of cervical cancer and pre-cancer, methods based on the detection of high risk HPV DNA can identify women at risk for cervical cancer.

Several HPV DNA tests are available. Hybrid Capture 2 (HC2) was developed in 1997. This test recognizes thirteen high-risk HPV genotypes, but the test cannot determine the specific HPV genotype present. It is the most frequently used diagnostic HPV test worldwide. HC2 is FDA approved for ASCUS triage and for primary screening in conjunction with cytology in women over age 30.[12] The sensitivity is 23% higher than that of cytology, the specificity is -7% lower in comparison to cytology based screening.[9] The pooled specificity of HC2 in excluding high-grade cervical pre cancer is 88.2% (95% CI: 86.2-90.1%).[4]

For detecting a specific hr HPV genotype, real time PCR HPV testing was introduced. Using that test in primary screening resulted in a pooled sensitivity that was lower than HC2 84.2% (95% CI 77-91.5%), but the pooled specificity was higher 95.1%, (95% CI: 93.4%- 96.8%).[4]

Primary screening with HPV DNA testing results in a higher sensitivity, but lower specificity in detecting high grade CIN in comparison to cytology based screening. A higher sensitivity gives a higher negative predictive value and screening intervals can be extended. Screening intervals of at least 6 years are as safe as a screening interval of 3 years with cytology.[13]

Furthermore HPV testing is more effective in detecting adenocarcinoma and its precursors than cytology.[14]

A lower specificity could be the result of the detection of transient infections that do not cause cytologic changes.[15] This is the main reason not to use HPV DNA testing in primary screening since more women are referred to colposcopy[16] leading to a greater cost and psychological burden for these women.

Transient HPV infections are more common in women aged 30 and younger. So primary HPV-screening in this category is not cost-effective. Ronco *et al* stated that HPV screening leads to overdiagnosis of regressive CIN 2 in women aged 35 years and younger.[17] Rijkaart *et al*, found that HPV testing in women aged 29-33 years does not result in an overdiagnosis of regressive CIN and HPV based screening can be implemented in screening programs starting at the age of 30 years.[18]

The data of Ronco *et al* support the use of stand-alone hr HPV-testing as the primary screening test and HPV-positive women older than 35 should be triaged with cytology or molecular markers such as P16 before referral to colposcopy.

Combined cytological and HPV screening is thought not to be cost-effective, since there is a greater cost, without significantly increasing sensitivity.[17] HPV as an initial test triaged with cytology could be cost effective since it can extend screening intervals based on longer-term protection from HPV negative tests.[19]

The management of HPV positive women is still unclear. Different triage algorithms are described.[20]

Primary HPV testing with triage cytology

HPV DNA testing provides an automated, objective and very sensitive primary test. Cytology can be reserved for the 5-15% of women who are hr HPV positive. HPV-based screening of women older than 30 years followed by cytology triage of hr HPV positive women, does not increases diagnostic work-up and over-treatment and therefore appears to be the most feasible cervical screening strategy.[21,22]

Triage with HPV typing information

HPV type 16 is more persistent and more often associated with high grade disease. HPV 18 is more often associated with cytology negative endocervical or glandular lesions, that remain hidden for colposcopy.[4] These two HPV genotypes account for 70% of the cervical cancers. Khan et al suggested a less aggressive management of HC2 positive, but HPV 16 and 18 negative women, since only 3% of these infections lead to high grade CIN in the next 10 years, whereas for HPV 16 positive women a cumulative incidence rate of 17.2% and for HPV 18 13.6% was seen.[23] HPV 16/18 positive and cytology-negative; women should be referred to colposcopy, since there is a short-term risk for CIN3.[24]

Triage with p16 INK4A (9)

P16 is a cycline dependent kinase inhibitor which is downregulated by the retinoblastoma (RB) gene, it is overexpressed in cervical cancer cell lines where RB is inactivated by HPV E7 oncoproteins. It is therefore a marker for activated expression of viral oncogenes.

HPV testing (HC2) with p16 INK4A triage has a sensitivity of 88% (95% CI: 80-94) and specificity of 61% (95% CI: 57-64%). The sensitivity is much higher than cytology. The specificity is comparable with cytology, therefore an equal number of women are referred to colposcopy. In women with CIN, the proportion of women that shows p16 overexpression, ranges from 53% in CIN 1 to 91% in CIN 3 or invasive cancer.[25] Part of the p16 positive lesions regress, especially when the percentage of cells overexpressing p16 is low.

Methylation markers

Promoter methylation of tumor suppressor genes has been reported to be an early event in carcinogenesis. Various methylated gen promoters for cervical neoplasia have been tested, but there are no large population based studies. Eijsink *et al* identified a set of new methylation markers with a higher identification of CIN3 and cervical cancer and higher percentage of correct referrals for colposcopy compared to hr HPV-testing in combination with conventional cytology.[26]

HPV load slope curves

Depuydt *et al* discovered a new strategy to differentiate between transient and persistent infection. The profile of viral load evolution over time could distinguish HPV infections with carcinogenic potential form infections that regress.[27] Transient infections generated similar increasing and decreasing slope curves. In persistent infections, the viral load slope was less steep but linear. In this study only single type infections were analyzed, suggesting that combining viral load at two time points could identify women that have a persistent infection and therefore are at risk of developing precancerous and invasive lesions.

Screening in a HPV vaccinated cohort

In a vaccinated cohort, the risk of cervical cancer and pre-cancer is significantly reduced by preventing HPV16 and 18 infection. The lesions are expected to be smaller with a higher risk of missing these by cytology or colposcopy directed biopsies. Therefore HPV typing is thought to be a better primary screening test in a vaccinated population. It is thought that in this population the start of screening could be delayed and screening intervals could be longer.[24]

Lower resources settings

In developing countries, a cost effective program with immediate treatment of screening positive women is the most important goal. HPV testing based on fast-HPV technology with immediate cervical cryotherapy of HPV positive women would be a practical approach.

Sankaranarayanan *et al* performed a study in rural India, where the effect of a single round of screening with HPV-test, cytology and a visual inspection test was compared. HPV-test was the only test that was related to a significant reduction in the numbers of advanced cervical cancers and cancer deaths.[28]

Conclusion

In unvaccinated women under the age of 30, current evidence supports primary screening starting at 25 years with cytology and hr HPV-DNA testing in cases of equivocal cytology.[24] In women above the age of 30, primary screening with hr HPV-DNA testing with cytology triage is currently the most feasible option. For the moment cytology screening intervals are kept relatively short (3 years). As follow-up trials are published, HPV screening intervals could be extended to 6 years for HPV-negative women without loss of sensitivity.

In vaccinated women, screening should start at age 25 years and HR-HPV-DNA testing with cytology triage every 5 years is currently recommended.

In the future, other triage tests for HPV positive women will become available, giving an even better prediction of persistent HPV infection and progressive CIN. These triage tests are at the moment available but they are not validated in clinical settings in large population based trials. Furthermore, the organisation of cervical cancer screening in large populations remains the mainstay of cost-effective cancer prevention.

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