CHAPTER

Arguments in favor of HPV testing for cervical screening and post-treatment CIN2+ monitoring

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ABSTRACT

Several studies have shown that the human papilloma virus (HPV) test is a more sensitive and objective primary cervical cancer screening tool than cytology. Therefore, conversion of cytology into HPV screening (as is planned in The Netherlands and some other European regions) will result in a better protection against cervical cancer and high-grade precursor lesions. Moreover, offering selfsampling for HPV testing will increase screening attendance by re-attracting former non-attendees. However, triage of HPV positive women is necessary because the specificity of HPV testing is 2–4% lower than of cytology. Several triage strategies have been evaluated, of which two, with cytology testing included, are feasible and were recently recommended. As an alternative for cytology triage, objective, non-morphological disease markers are upcoming and so far have shown promising results. Finally, HPV testing can also contribute to a more efficient monitoring of women treated for high-grade cervical precursor lesions, permitting fewer follow-up visits.
Arguments in favor of HPV testing for cervical screening

BACKGROUND

Cervical cancer is the third most common cause of cancer-related death in women worldwide.\textsuperscript{1} Nationwide, cytology-based cervical screening programs with a call and recall system have proven to be effective in decreasing the incidence of and mortality from cervical cancer.\textsuperscript{2,3} However, the decrease of incidence of cervical cancer has leveled off and apparently a plateau has been reached for cytology-based screening. The main causes of this stabilization are the marked number of women (~30%) that fail to participate in the screening programme, and the relatively low sensitivity of cytological testing.\textsuperscript{4}

Since it is known that an infection with high-risk human papillomavirus (hrHPV) is the main causative event for the multi-step process of cervical carcinogenesis, efforts to improve screening have focused on hrHPV testing as additive or primary screening tool. Unlike cytology, the hrHPV test is objective and has shown to be more sensitive for the detection of cervical intraepithelial neoplasia grade 2 or worse and grade 3 or worse (CIN2+/3+) compared with cytological testing (94% vs 65%).\textsuperscript{5-6} The success of cytology-based screening programs can be attributed to relatively frequent repeat testing (in many countries every 1 to 3 years) to compensate for the low sensitivity of cytology. A meta-analysis of four randomised controlled trials\textsuperscript{7-10} showed a 57% lower risk for CIN3+ amongst hrHPV test negative women compared with cytology test negative women.\textsuperscript{5} In addition, a pooled analysis of these four trials revealed a 60-70% lower risk for cervical cancer in hrHPV test negative women compared to cytology test negative women.\textsuperscript{11} Collectively, these data indicate that a hrHPV test is a more objective and more sensitive screening tool that better protects against cervical (pre)cancer than cytology. HPV testing permits extension of screening intervals resulting in less screening rounds and lower surveillance costs. Based on these data, the Ministry of Health in the Netherlands has decided to replace cytology testing by HPV testing as primary screening tool in 2016.\textsuperscript{12} Moreover, five regions in Italy will introduce primary HPV screening in the forthcoming year, and several other developed countries such as Denmark and Sweden are considering to change their primary screening test as well. When hrHPV testing is proposed as primary screening test, it is important that a clinically validated HPV test should be used. Only HPV tests which have been proven to detect HPV infections associated with cervical cancer and its precursors (CIN2+) with low false positivity rates should be used to prevent follow-up of women with transient HPV infections resulting in unnecessary repeat tests, colposcopy referrals and overtreatment.

Another limitation of present cervical screening programs is the suboptimal participation rate. Since more than half of cervical cancers arise in women not attending cervical screening, it is important to target these non-attendees.\textsuperscript{4} Offering self-sampling of cervico-vaginal material for hrHPV testing (HPV self-sampling) has shown to be effective to increase screening compliance: about one third of the non-attendees submit self-sampled material for HPV testing when HPV self-sampling is offered.\textsuperscript{13} Also for HPV self-sampling, it is important to use a validated combination of self-sampling device and hrHPV test to achieve clinical equivalence in terms of CIN2+ detection of HPV self-sampling compared to physician-taken HPV testing.\textsuperscript{14-15} Based on this statement, HPV-self-sampling could be introduced as an alternative for a physician-taken smear.
Triage of HPV positive women

Even when a clinically validated HPV test is used, the specificity of HPV testing for CIN2+ is lower than that of cytology and the majority of women who are positive with such an HPV test will not have clinically relevant disease.5–6 Therefore, a triage test is necessary to identify those HPV-positive women who have disease (CIN2+) and thus are in need for immediate colposcopy referral. It has been proposed that women with a > 10% risk of CIN2+ should be referred for colposcopy, whereas those with a risk between 2% and 10% benefit from more intense follow-up by repeat testing within a year.16 Women with a CIN2+ risk of < 2% can be dismissed from further follow-up until the next screening round after 3 to 5 years depending on the screening interval used. Moreover, to prevent overtreatment of HPV positive women, positive predictive values of 10% (as accepted in the US) to 20% (as accepted in the Netherlands) are used as a norm.16,18 Guided by the criteria listed above, several triage strategies for hrHPV positive women have been evaluated of which two were recently recommended. First, direct cytology triage at baseline with repeat cytology at 6 or 12 months, with or without HPV16/18 genotyping at baseline, was found to be the triage strategy of first choice in recent longitudinal studies.17–19 This strategy resulted in the optimal balance between the safety of a triage strategy and the screening burden for patients and clinicians, because the low CIN3+ risk in women with two subsequent negative cytology test results was acceptable to refer them back to the national screening programme, while the colposcopy referral rate remained modest. The second strategy which was preferred in another study was HPV 16/18 genotyping in combination with cytology.20 This strategy resulted in a relatively low CIN3+ risk (just beneath the CIN3+ threshold risk of 2%) for HPV-positive women who were tested negative for HPV16 and HPV18. However, retesting after 1 year was recommended in women with a negative triage test. Which of the above mentioned triage strategies will be used depends on the quality of cytology tests and the resources available in a specific country.21

Because cytology is a subjective test with a large inter- and intra-observer variability5, there is a need for more objective and robust triage markers. Cytology can be made more objective when cells are dual stained for p16 and Ki-67 by immunohistochemistry. HPV infected cells in high-grade lesions show high p16 expression in proliferating (i.e. Ki-67 positive) cells due to viral oncoprotein E7 overexpression in these cells. Thus, p16/Ki-67 double positive cells are indicative for the presence of CIN2+, because this combination of markers is not present in normal or reactive cervical cells. p16/Ki-67 dual-staining of cervical smears has shown promising, more objective results as triage test for hrHPV positive women.22

Non-morphological candidate markers include markers that detect DNA methylation of promoter regions of tumor suppressor genes involved in cervical carcinogenesis.23 Recently, it has been shown that a molecular marker panel, targeting CADM1 and MAL genes, on physician-taken cervical smears, was equally discriminatory for the detection of CIN3+ as cytology, or cytology in combination with HPV 16/18 genotyping.24 Moreover, methylation markers could also play an important role in triaging women with hrHPV positive self-sampled material.25–26 Currently, a visit to the clinician is necessary for triage testing in women with a positive HPV self-sample, because cytology is not feasible on self-sampled material.27 Since methylation marker testing is directly applicable on self-samples, this will open the possibility for full molecular screening, by offering a
combination of primary HPV screening with methylation marker testing as a triage tool. This could optimize screening especially among current non-attendees.

**HPV testing in women treated for high-grade cervical disease**

Women treated for high-grade disease retain an elevated post-treatment risk of CIN2+ for at least ten years.\(^{28-29}\) Therefore, it is important to identify these women in order to repeat conservative treatment to reduce the risk of future invasive disease. Post-treatment surveillance should identify both women with residual disease as well as women with a persistent hrHPV infection who have an increased risk of progressive incident lesions.\(^{10}\) In most European countries, treated women are followed-up by cervical cytology 6, 12 and 24 months after treatment. After three consecutive negative test results, women return to the screening programme where screening takes place at intervals of at maximum 5 years.

Current post-treatment protocols have several drawbacks, being the low compliance rate and the limited sensitivity of cytological screening. The current guidelines can be improved by implementing HPV testing since it is significantly more sensitive than cytology and has equal specificity compared to follow-up cytology in the detection of post-treatment CIN2+/3+.\(^{6,28}\) Thus, a positive HPV test may better identify women with an increased risk of post-treatment disease. The best results of detecting post-treatment disease have been reached by performing co-testing (both hrHPV and cytology). Women who test negative for both cytology and HPV 6 months after treatment have a low risk of developing post-treatment disease and may omit the 12-month visit. The 5-year CIN2+ risk in women with negative co-testing at 6 and 24 months post-treatment is 1.0%. This risk is similar to that of women with normal cytology in population based screening, which indicates that these double negative tested women could safely return to regular screening.\(^{31}\)

**HPV testing in low and middle resource countries**

In low- and middle- resource countries cervical cancer remains largely uncontrolled because of ineffective or no screening possibilities. These countries have limited resources to provide for the costs and organisation of a screening program and, if present, cytology is of poor quality. As a result, a high incidence of and mortality from cervical cancer can be found. In addition, women in these countries are at best screened a few times per lifetime.\(^{32}\) Therefore, a clinically validated and easy to perform test should be used.\(^{33}\) Indeed, an Indian study showed a significant decrease of mortality from cervical cancer after one screening episode with HPV testing by HC2 compared to cytology and visual inspection with acetic acid.\(^{34}\) Even more success, particularly with regard to the participation rate, might be expected when offering HPV self-sampling.\(^{14,35}\) In the Mexican Appraisal of Routine Cytology versus vaginal HPV screening (MARCH) study,\(^{36}\) HPV self-sampling revealed a higher sensitivity for CIN2+ compared with cytology testing. Moreover, a pooled analysis of HPV self-sampling in China concluded that ‘Self-HPV testing may complement current screening programs by increasing population coverage in settings that do not have easy access to comprehensive cytology-based screening.’\(^{33}\) Therefore, the combination of the higher CIN2+ sensitivity in comparison with cytology and the expected higher participation rate make HPV
self-sampling a good alternative to cytology in low and medium resource countries. However, implementation of HPV self-sampling in these countries should be preceded by education programs about the validity of self-collection that target general population to achieve maximum benefit.37

In conclusion, several studies have shown that HPV testing can replace cytology as primary screening tool in the detection of CIN2+/CIN3+ lesions. This replacement will increase the efficacy of current cervical screening programs by improving the sensitivity of regular screening. Moreover, HPV self-sampling could be used in cervical screening by increasing participation rate in developed countries and in low- and middle- resource countries lacking medical services. By the introduction of non-morphological markers, for example, methylation markers, as triage test in hrHPV positive women, full molecular screening might be feasible in the near future. Finally, the addition of HPV testing to cytology in women post-treatment, monitoring of high-grade cervical disease will result in a more efficient post-treatment follow-up, permitting fewer follow-up visits.
REFERENCES


