Diagnosis for Early Detection


Human Papillomavirus Testing in Primary Screening for Cervical Cancer

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Abstract

There is a high level of evidence coming from large randomized controlled trials that primary human papillomavirus (HPV) screening is better than cytology in detecting cervical intraepithelial neoplasia type 3 and cervical cancer as well as in reducing the incidence of and the mortality from cervical cancer. However, HPV screening will only be beneficial and cost-efficient in women above 30 years and only in well-structured programs with longer screening intervals. Pilot projects in the USA and Germany show that HPV screening is feasible even on local or regional levels with similar efficiency as observed in randomized controlled trials. The single remaining serious argument against screening with HPV testing, i.e. the missing algorithm for women with normal cytology who tested positive for HPV, may be solved by a triage using p16/Ki67-stained cytology.

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During the last decades, Papanicolaou (Pap) smear screening programs significantly reduced the incidence of cervical cancer in most industrialized countries. Screening for cervical cancer is a so-called secondary prevention method because it does not prevent the full cycle of carcinogenesis. The central concept of this exceptionally successful cancer prevention is the identification and treatment of women with high-grade intraepithelial lesions [cervical intraepithelial neoplasia (CIN) 2 and CIN3]. Excision of these lesions interrupts the genesis of cervical cancer at a preinvasive stage.

Although future human papillomavirus (HPV) vaccines may offer complete protection from all diseases caused by HPV [1], screening will be needed to prevent cervical cancer in nonvaccinated women and not per protocol vaccinated individuals for the next decades. Even women who were vaccinated per protocol will need to participate in screening programs as the current vaccines will prevent only 70–80% of all high-grade CIN and invasive cancers. This reduction is equivalent to the effect of the established Pap smear programs. Combining vaccination and screening will offer best protection from cervical cancer.
What Is the Best Screening Test?

The full process of carcinogenesis from HPV infection to malignant growth is typically extended over 15–30 years; intervals shorter than 10 years are extremely rare, and the minimum latency period seems to be in the range of 7–8 years [2]. As CIN3 lesions persist typically for years before progressing to malignancy, chances of an early detection within the screening program are high [3–6].

The implementation of a Pap smear-based primary screening for cervical cancer and its precursors in the second half of the 20th century was one of the greatest success stories in cancer prevention. While in countries without such screening programs 3–6.5% of the female population will develop cervical cancer, the corresponding risk is 1% in areas with Pap smear screening [7, 8]. Although the Pap smear is still the undisputed screening test in most programs to prevent cervical cancer, numerous studies could demonstrate that the sensitivity of a single Pap smear for CIN2/3 is much lower than conceived previously. In a meta-analysis that included 6 different controlled studies with more than 60,000 women attending for primary cancer screening, only 53% of high-grade lesions were detected by cytology compared to a sensitivity of 96% of HPV DNA testing [9].

Based on several large randomized controlled trials, there is now a high level of evidence that under certain conditions HPV testing is a better screening strategy than cytology in women aged above 30 years. In organized screening programs HPV testing will

- detect CIN3 and cancer with significantly higher sensitivity than cytology;
- be more efficient in preventing invasive cervical cancer than cytology;
- be better in reducing mortality from cervical cancer than cytology, and
- have a better negative predictive value and allow for longer screening intervals than cytology.

Randomized population-based studies in Italy, Canada, the UK, Finland, Sweden and the Netherlands confirmed that HPV DNA testing detects CIN3 lesions with significantly better sensitivity than Pap smear screening [10–15]. Earlier arguments that CIN3 detected by HPV testing would be irrelevant and regress spontaneously over time could be cleared by the Swedish and the Dutch trials. During a 6-year follow-up, the number of CIN3 and invasive cancers was identical in the intervention group with HPV testing and the control group with conventional Pap smear screening. However, in the intervention group, significantly more cases were found at study entry, while most cases in the Pap smear cohort were detected in the second screening round 5–6 years later. This improvement in earlier detection of CIN3/cancer was achieved although referral rates to colposcopy were only slightly higher in the intervention group (3.6 vs. 3.2% within 6 years).

The Italian screening trial could demonstrate that the better sensitivity of HPV testing for CIN3+ finally results in a lower incidence of cervical cancer. While no decline in cervical cancer incidence was observed in the cytology arm (9 cases of
cancer in each screening round), no case of invasive cancer was diagnosed in the HPV screening cohort in the second screening round [13].

In an Indian primary screening trial that compared the efficacy of HPV testing, cytology and VIA (visual inspection of the cervix with acetic acid) performed once in a lifetime, only primary HPV screening showed a significant drop in mortality from cervical cancer within an 8-year follow-up [16].

Unlike for cytology, there was no indication of heterogeneity of HPV testing between studies. Also, sensitivity was unaffected by age. Surprisingly, the best clinical HPV DNA test is not the most sensitive one. Studies that used very sensitive tests based on polymerase chain reaction (PCR) found very high HPV prevalence rates in healthy individuals but failed to detect HPV DNA in some cancers and CIN3 [17]. The latter can be explained by DNA deletions in malignant cells that might disable PCR primers to hybridize with the target DNA. The clinical state of the art in the year 2011 was either Hybrid Capture 2 (HC2, Qiagen, Hilden, Germany) or PCR with GP5+/GP6+ primers. However, standardized commercial HPV PCR tests like Cobas (Roche, Basel, Switzerland) showed very promising results in large trials and seem to be as good as HC2.

Almost all HPV DNA screening trials examined women aged 30 years or older. Due to the high prevalence of HPV in younger populations, the specificity of HPV testing is too low and referral rates to colposcopy would be too high in the younger age groups to make HPV testing a cost-efficient alternative to Pap smear screening. This might change in HPV-vaccinated populations within the next 10 years, but for the near future, primary HPV screening will have no place in this age group.

Besides the high sensitivity for CIN3+, the extraordinarily high negative predictive value is another advantage of HPV testing. HPV-negative women cannot develop cervical cancer within the next 5–7 years even if they get infected the next day because the minimum latency from infection to cancer is in the range of 7–8 years [2]. HPV testing will identify the minority of women at risk of having or developing CIN3+ with very high precision and exclude any risk for the majority of participants with a reliability that is unmatched in oncology.

Because of the very high sensitivity of HPV testing, cotesting with Pap smear was not better than HPV screening alone in detecting CIN2+ lesions [18]. Primary HPV testing followed by cytology in all HPV-positive cases will be an attractive concept in the future. However, at least in most European countries, primary HPV screening will be cost-efficient only in organized screening programs with an extension of screening intervals to 5–7 years.

**Why Has Human Papillomavirus Screening Not Yet Replaced Pap Smear Screening?**

In 2011, half of all Italian regions shifted from Pap smear to HPV testing as a primary screening method, Turkey proclaimed to start a program to prevent cervical cancer
with primary HPV screening, while Merck and Qiagen announced a partnership to set up an HPV vaccination and screening program in Rwanda. Furthermore, two pilot projects in Northern California (Kaiser Permanente, start 2003) and Northern Germany (Wolfsburg, start 2006) successfully introduced primary HPV screening into clinical routine.

But despite all benefits of primary HPV screening, in most health systems a number of obstacles need to be overcome before the shift from Pap smear to HPV screening can be accomplished. Apart from political resistance from various groups with a financial interest in the established Pap smear screening, there are at least three serious arguments against primary HPV screening.

1 Opportunistic screening programs. There is no evidence that primary HPV screening is efficient in such programs without defined patient pathways, quality assurance and invitation concept. Countries with opportunistic screening will need to build up the infrastructure for organized screening first.

2 Women below the age of 30 years. Because cervical cancer is very rare before the age of 25 years, screening below this age does not seem to be useful. Harm from overtreatment may outweigh benefits in this young population. However, most experts feel uncomfortable to leave 25- to 29-year-old women without screening. As HPV screening is not useful below the age of 30, two different, age-dependent screening programs would be needed.

3 There is no satisfying clinical management algorithm for women testing Pap negative/HPV positive. In primary screening programs based on a combination of cytology and HPV testing, women with normal Pap cytology who tested positive for high-risk (HR) HPV may carry a risk of 3–7% for underlying high-grade CIN.

USA and Germany are countries with opportunistic screening. However, the two pilot projects show that it is possible to organize screening on a regional or local level with centralized data collection, defined patient pathways and quality assessment [19, 20]. Furthermore, both projects confirm that it is feasible to run different screening concepts in women below and above age 30. The strongest argument against HPV screening seems to be the missing optimal algorithm for HPV-positive women. Theoretically, there are a number of options: immediate colposcopy of all HPV-positive women, repeat Pap smear and/or repeat HPV testing after 6–12 months, HPV genotyping and triage with immunocytometry or detection of mRNA E6/E7.

Advantages of colposcopy are a high compliance and a high detection rate of CIN3+; however, a major disadvantage is the high transferral rate of more than 6% of the total screening population. Repeat Pap smear and repeat HPV testing reduce the referral rate to approximately 3% but will delay the diagnosis of cancer in a reasonable number of cases [19, 20]. HPV genotyping of all HC2 positives is suitable to determine the long-term risk of developing CIN3+, but this does not necessarily mean that genotyping is the optimal method to detect already underlying CIN3+
lesions. All large epidemiologic studies show that 60–70% of all invasive cancers and CIN3 are linked to either HPV16 or HPV18 but at least 30% are associated with other HR HPV types and will be missed by HPV16/18 genotyping. Even before HPV vaccines were introduced, epidemiologic studies indicated a decline in CIN3+ associated with HPV16 [21]. The best explanation for this observation is that HPV16-associated lesions are easier to detect by cytology and colposcopy. Studies already showed that the typical acetowhite reaction of CIN on colposcopy is more pronounced in HPV16-positive lesions in comparison with other HPV types. It is very likely that vaccination will accelerate this trend over the next decades even in individuals who are not vaccinated. Furthermore, even if the likelihood of a progression to CIN3+ is increased 5- to 6-fold for HPV16 and 18 in comparison with other HR HPV types, HPV16/18 infections typically represent just 25–30% of all HR HPV infections. Hence HPV16- or HPV18-positive individuals carry an increased risk but a focus only on this subgroup of HR HPV infections would finally miss again at least 30% of all prevalent CIN3+ lesions.

Expression of E6/E7 mRNA shows a correlation with the severity of associated lesions [22]. However, no valid statements on the performance of mRNA testing in the triage of HPV DNA+ can be made so far, because such an approach has not yet been investigated systematically by any trial. Within the Wolfsburg pilot project, triage of all HC2 positives with a dual-stained cytology using p16 and Ki67 showed a very high sensitivity of 96.4% for underlying CIN3+ and a specificity of 76.9% [23]. This approach seems to be the optimal algorithm for HPV+. Within the Wolfsburg project, just 2.2–2.5% of the screening population will be transferred for colposcopy and almost all CIN3+ lesions will be diagnosed without delay (fig. 1).

**Outlook**

Screening for cervical cancer already changed from a sole conventional Pap smear-based program to conventional or liquid-based Pap smear screening followed by HPV testing for the triage of borderline findings in most industrialized countries. Furthermore, HPV testing is already established as a proof of cure in women who were treated for CIN. The high evidence from large randomized trials that HPV testing is superior to cytology as a primary screening test in organized programs for women being 30 years and older in combination with new optimal triage tests for HPV-positive women offer so many benefits that it is very likely that it will eventually replace Pap smear screening.
HPV Testing in Primary Screening for Cervical Cancer

Fig. 1. Primary HPV screening followed by p16-Ki67 triage. This model allows for an optimal risk stratification in women aged 30 years or older. The HPV-negative majority (dark blue) has virtually no risk of developing cervical cancer for the coming 5 years. Extension of screening intervals avoids overdiagnosis and unnecessary treatment in this group. The small HPV-positive group with positive p16 and Ki67 (red) staining carries a high risk of underlying CIN3+ (approx. 30–40%) and benefits from immediate colposcopy. HPV-positive women with negative p16/Ki67 staining (orange and green) can be followed safely by repeat testing every 12 months. The majority will undergo spontaneous regression during follow-up, which will be confirmed by negative HPV testing (bright blue). A small subgroup will eventually show positive staining for p16/Ki67 including most women with newly developed CIN2+ (green).

References

1 Jagu S, Kwak K, Garcea RL, Roden RB: Vaccination with multimeric L2 fusion protein and L1 VLP or capsomers to broaden protection against HPV infection. Vaccine 2010;28:4478–4486.


