Cervical cancer screening: on the way to a shift from cytology to full molecular screening

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Cytology-based nation-wide cervical screening has led to a substantial reduction of the incidence of cervical cancer in western countries. However, the sensitivity of cytology for the detection of high-grade precursor lesions or cervical cancer is limited; therefore, repeated testing is necessary to achieve program effectiveness. Additionally, adenocarcinomas and its precursors are often missed by cytology. Consequently, there is a need for a better screening test. The insight that infection with high-risk human papillomavirus (hrHPV) is the causal agent of cervical cancer and its precursors has led to the development of molecular tests for the detection of hrHPV. Strong evidence now supports the use of hrHPV testing in the prevention of cervical cancer. In this review, we will discuss the arguments in favor of, and concerns on aspects of implementation of hrHPV testing in primary cervical cancer screening, such as the age to start hrHPV-based screening, ways to increase screening attendance, requirements for candidate hrHPV tests to be used, and triage algorithms for screen-positive women.

Key words: hrHPV DNA, cervical cancer screening, triage, self-sampling

introduction

Currently, cervical cancer is the fourth leading cause of cancer death in women worldwide, causing more than 275 000 deaths annually. The disease has a very uneven global distribution; over 85% of cases are found in low-resource countries, with incidence and death rates being the highest in sub-Saharan Africa, Central America, South-Central Asia, and Melanesia [1, 2]. This imbalance in disease burden can be explained by differences in background risk [exposure to human papillomavirus (hrHPV) infection], and the fact that cervical cancer is preventable by an effective screening and intervention system. Therefore, the lowest incidence and mortality rates are recorded in countries where screening is available to women. The impact of population-based screening is reflected in a substantial reduction in the incidence of cervical cancer over the past 50 years in countries with established cytology-based screening programs [3–6]. Especially, quality-assured population-based programs have shown to be very effective [6]. However, cytology-based cervical screening also has some limitations. The major problem is the low sensitivity of a single smear to detect high-grade precursor lesions (50%–70%), which require frequent testing [7]. In addition, cytology has low reproducibility, leading to variable accuracy [8, 9]. Moreover, by repeating cytology, the number of false positives increases substantially over time [10]. Finally, the decrease in the incidence of cervical cancer induced by cytology-based screening is mainly restricted to squamous cell carcinoma, whereas no change is observed in the incidence of cervical adenocarcinoma [11, 12], suggesting that cytology fails to detect adenocarcinomas and its precursors. Consequently, there is a need for a better primary screening test, and thus a new screening algorithm.

human papillomavirus testing in the prevention of cervical cancer

cross-sectional sensitivity

Infection with hrHPV is a necessary event in the multistep process of cervical carcinogenesis [13]. Thirteen hrHPV types...
have been identified [14], of which HPV16 and HPV18 are the most important types, causing ~70% of squamous cell carcinomas, and >90% of adenocarcinomas [15, 16]. Recently, two prophylactic vaccines against HPV16 and HPV18 (Cervarix GSK® and Gardasil Merck®) have shown good protection against vaccine type-related cervical intraepithelial neoplasia grade 2 or worse (CIN2+) precursor lesions. However, cervical screening is still required, because the current vaccines do not protect against all carcinogenic HPV types. In addition, despite the high vaccine uptake among women in countries with a school-based program [17–19], the uptake remains suboptimal in, e.g. the Netherlands, France, Germany, and the USA [20–23].

The causal relationship between infection with hrHPV and cervical cancer has stimulated the application of hrHPV DNA testing, which has been proposed, either alone or in combination with cytology, as a means to improve existing cervical screening programs. In the past 15 years, large randomized trials designed to evaluate the performance of hrHPV testing have provided important arguments for the implementation of this assay as a primary screening tool (Table 1). First, five of these trials showed, in cross-sectional studies, that hrHPV testing is ~30% more sensitive in detecting CIN2+, and four of these studies showed that it is also ~20% more sensitive in detecting CIN3+ (Figure 1). Most of these lesions are HPV16-associated. The higher cross-sectional sensitivity was confirmed by two more studies analyzing the baseline data of screening populations [29, 30]. The study published by Kitchener et al. [25] showed somewhat different results; first, it demonstrated similar cross-sectional sensitivity for hrHPV testing and liquid-based cytology (LBC) in primary cervical screening, and secondly, the data suggest that LBC, as used in the first round, has a lower specificity for CIN3+ than usually expected [31]. Possible reasons for these discrepancies could, first, be attributed to overdiagnosis of lesions, as LBC was just introduced at that time and secondly, to incomplete follow-up of hrHPV-positive women with normal cytology, resulting in lower detection rates of high-grade CIN in the hrHPV arm at baseline [31, 32].

**long-term protection**

At present, four randomized trials conducted in Europe have published longitudinal data on CIN3+ diagnosed at

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**Table 1. Trials comparing cytology and hrHPV testing in cervical cancer screening**

<table>
<thead>
<tr>
<th>Study</th>
<th>Description</th>
<th>Interval</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>POBASCAM</td>
<td>HPV (GP5+/6+-PCR) and cytology versus cytology alone</td>
<td>5 years</td>
<td>Bulkmans et al. [24, 109]</td>
</tr>
<tr>
<td>ARTISTIC</td>
<td>HPV (HC2) combined with cytology (LBC) versus cytology (LBC) alone</td>
<td>3 years</td>
<td>Kitchener et al. [25, 110]</td>
</tr>
<tr>
<td>SwedeScreen</td>
<td>HPV (GP5+/6+-PCR) and cytology versus cytology alone</td>
<td>3–5 years (by age)</td>
<td>Naucler et al. [26, 27]</td>
</tr>
<tr>
<td>NTCC</td>
<td>HPV (HC2) alone versus HPV (HC2) and cytology (LBC) versus cytology (LBC)</td>
<td>3 years</td>
<td>Ronco et al. [28, 111, 112]</td>
</tr>
<tr>
<td>CCCaST</td>
<td>HPV (HC2) and cytology versus cytology and HPV (HC2) (randomized order of collection)</td>
<td>1 year</td>
<td>Mayrand et al. [29]</td>
</tr>
<tr>
<td>Finnish screening trial</td>
<td>HPV (HC2) and cytology triage versus cytology alone</td>
<td>5 years</td>
<td>Leinonen et al. [113]</td>
</tr>
<tr>
<td>India screening trial</td>
<td>HPV (HC2) versus cytology versus visual inspection with acetic acid (VIA) versus no screening</td>
<td>–</td>
<td>Shankaranarayanan et al. [41]</td>
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</table>

**Figure 1.** Detection rate ratio in screening with hrHPV versus cytology in the first screening round of randomized trials for outcome CIN3+.
subsequent screening rounds, which took place in 3–5 years (Figure 2) [24–26, 28, 33]. All trials reported an ~50% lower CIN3+ detection rate in the second screening round among women who were hrHPV negative at baseline than those who had normal cytology. The pooled detection rate ratio of CIN3+, of hrHPV testing versus cytology, was 0.42 [95% confidence interval (CI): 0.32–0.55]. The consistency of results was reflected by the absence of interstudy heterogeneity (P = 0.68). Thus, a negative hrHPV test provides a better protection against CIN3+, than a negative Pap smear. Several cohort studies have also shown a consistently long-term cumulative incidence rate of CIN2+ among women negative for hrHPV than those with normal cytology [30, 34, 35]. In addition, in two trials, the detection rate of ‘cervical cancer’ at the second screening round was significantly lower among women, who were hrHPV negative at baseline [odds ratio (OR): 0.19 (95% CI 0.07–0.53)] [28, 33]. Most importantly, in a pooled analysis of four European trials following women for at least two screening rounds, it was recently confirmed that hrHPV-based screening provides better protection against cervical cancer than cytology [36]. Consequently, screening intervals might be extended when primary hrHPV testing has been introduced. Berkhof et al. [37] have shown that an extension to 6 or 8 years is possible without increasing the lifetime cancer risk, using a simulation model. However, it makes sense to extend the screening interval for hrHPV negatives only, as hrHPV-positive women, even those with normal cytology, have a non-negligible CIN3+ risk (5.2%) [33, 35]. The risk is too high to delay follow-up to the next screening round (3–5 years) [38, 39], but too low to refer these women for immediate colposcopy [40]. Therefore, hrHPV-positive women with normal cytology at baseline require further triage testing and/or follow-up, which will be discussed below.

Another important and consistent finding in the five trials with combined hrHPV and cytology co-testing was that co-testing has virtually no additional value compared with single hrHPV screening [40]. Although slightly more sensitive, no significant differences for CIN2+ or CIN3+ detection were found [sensitivity ratio cytology and hrHPV versus hrHPV alone: 1.06 (95% CI 0.96–1.18) for CIN2+, and 1.03 (95% CI 0.89–1.20) for CIN3+] [31]. A recent study from the USA also showed that hrHPV and cytology co-testing have no advantage over sole cytology screening [35]. Collectively, these data show that sole hrHPV testing is sufficient for cervical screening, and argue against the use of combined hrHPV and cytology co-testing in women aged 30–60 years, as recently recommended by the American Cancer Society (ASC) [41]. Moreover, the data from the POBASCAM trial, in which hrHPV and cytology co-testing were used in both study arms in the second screening round, show that the total number of CIN3+ detected over two rounds is equal in both arms. These results indicate that at least a part of the surplus of CIN2/3+ lesions detected in the first round by hrHPV testing represent non-regressing, clinically relevant lesions [33].

Finally, an Indian cluster-randomized trial [42] found that, in a low-resource setting, a single round of hrHPV screening was associated with a significant decline in the rate of advanced cervical cancers (FIGO stage II+), when compared with an unscreened control group [hazard ratio 0.47 (95% CI 0.32–0.69)]. No significant reduction was observed in the study arms with cytology or visual inspection with acetic acid screening.

Collectively, the available evidence indicates that sole hrHPV testing should replace cytology as a primary screening tool in cervical screening.

hrHPV detection methods

hrHPV detection methods include both HPV DNA assays and E6/E7 mRNA assays. The drawback of hrHPV testing is that it has an (apparently) unavoidable tradeoff between sensitivity and specificity. Overall, hrHPV testing has a 3–4% lower specificity than cytology [at cutoff atypical squamous cells of undetermined significance or worse (ASC-US+)] [40] due to its inability to distinguish between persistent hrHPV infections associated with (precursor lesions of) cervical cancer and transient hrHPV infections. The specificity could even be lowered >25% when an ultra-sensitive hrHPV test was applied [43]. From a clinical point of view, testing for hrHPV is only useful when a positive hrHPV test result is informative about the presence or absence of CIN2+ (clinical sensitivity and specificity). Thus, in order to prevent excessive follow-up procedures for women with transient hrHPV infections or hrHPV-positive women without cervical lesions, candidate hrHPV tests to be used for cervical screening should be clinically validated. Two hrHPV DNA tests, i.e. HC2 and GP5+/6+, have shown, in large clinical trials, to perform better in reducing the incidence of CIN3+ and are thus considered as clinically validated prototype assays. Guidelines for hrHPV test requirements and clinical

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Risk Ratio</th>
<th>Year</th>
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<tr>
<td>Naucler 2007</td>
<td>0.53 [0.29, 0.98]</td>
<td>2007</td>
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<tr>
<td>Kitchener 2009</td>
<td>0.52 [0.28, 0.97]</td>
<td>2009</td>
</tr>
<tr>
<td>Ronco 2010</td>
<td>0.34 [0.15, 0.75]</td>
<td>2010</td>
</tr>
<tr>
<td>Rijkaat 2012</td>
<td>0.39 [0.27, 0.56]</td>
<td>2012</td>
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<tr>
<th>Total (95% CI)</th>
<th>Risk Ratio</th>
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<tr>
<td></td>
<td>0.42 [0.32, 0.55]</td>
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Figure 2. Detection rate ratio of screening with hrHPV versus cytology in the second screening round, for outcome CIN3+, among women who were hrHPV negative versus cytology negative in the first screening round.
validation were developed, based on the available data from large prospective screening studies [44]. These guidelines can be used to assess the clinical performance of a candidate HPV test, relative to one of two prototype hrHPV tests with proven good clinical performance (i.e., HC2 or GP5+/6+–PCR) by a cross-sectional clinical equivalence analysis in a screening setting [45]. In short, the candidate test should have a clinical sensitivity for CIN2+ not less than 90%, and a clinical specificity not less than 98% of that of the reference assays.

Thus far, three additional hrHPV DNA tests, i.e., Cobas 4800 Roche®, Real-Time (RT) PCR Abbott Molecular®, and Papillocheck Bio-Greiner® (when only 14 hrHPV types are considered), have fulfilled the criteria provided in these guidelines, with sensitivities ranging between 100% and 95.8%, and specificities from 96.7% to 92.3% [46–52]. Thus, these assays can be considered as clinically validated for primary hrHPV-based cervical cancer screening.

In contrast to the HPV DNA tests, the APTIMA HPV Assay (GenProbe) relies on aggregate detection of mRNA of 14 hrHPV types [53]. Two studies [54, 55] have evaluated the performance of this assay for primary screening compared with the HC2 test, and the results were summarized in a recent review [40]: the relative sensitivity ratio (outcome CIN2+) of APTIMA versus HC2 was 1.02 (95% CI 0.86–1.20), and the relative specificity ratio was 1.07 (95% CI 1.05–1.08). Thus, the APTIMA assay seems to perform with sensitivity closely to that of the DNA test and with a possible slightly higher specificity. A recent study comparing the APTIMA HPV Assay with GP5+/6+–PCR showed similar results, and indicated that also the APTIMA HPV Assay is clinically non-inferior to GP5+/6+–PCR with respect to sensitivity and specificity for CIN2+ [56]. Another approach in trying to validate new hrHPV tests was used by Cuzick et al. [57] in the predictors study: the sensitivity was primarily analyzed in a cytology-based referral population [58], and specificity in a cytology-based screening population [57]. This study evaluated six HPV tests and established that four tests (Roche Cobas, GenProbe APTIMA, Abbott Real-Time PCR, and Becton, Dickinson and Company HPV assay) achieved the required sensitivity and specificity compared with HC2. This would also qualify the BD test as clinically validated. However, the predictors screening study only included cytology-driven CIN2+ lesion, which made the sensitivity criterion too soft to consider it in line with the guideline described earlier [44]. In addition, no details of any previous screening history of participants were available. The use of validated HPV test assays alone, however, is not sufficient to reduce the number of false-positive tests among healthy women; triage testing of hrHPV-positive women is necessary to keep the number of invasive follow-up examinations, and thus the costs within acceptable limits [59].

**Management of hrHPV-positive women**

**Cytology and hrHPV genotyping**

Epidemiological studies have estimated that HPV16 and HPV18 cause ∼70% of cervical cancers worldwide, and that the cumulative 10-year risk of CIN3+ in HPV16/18-positive women ranges from 10% to 20% [60, 61]. These facts have led to several studies evaluating the value of hrHPV genotyping, with or without cytology, to triage hrHPV-positive women.

In the VUSA-screen study, evaluating 14 triage strategies [59], cytology triage at baseline and repeat cytology testing at 12 months emerged as the optimal strategy; it showed the highest positive predictive value [PPV 37.5% (95% CI 32.6–42.6)] in combination with a high negative predictive value (NPV) for CIN3+ [99.3% (95% CI 89.1–99.8)]. A CIN3+ risk of <2% (corresponding with a NPV for CIN3+ of >98%) was considered to be acceptable for dismissal from further follow-up. This threshold was based on the 5-year CIN3+ risk of women with borderline or mild dyskaryosis (BMD) cytology at baseline, and normal cytology at 6- and 18-month follow-up (1.2%), which is presently accepted in the Netherlands [38].

In addition, we have recently carried out a post hoc analysis of data from the POBASCAM trial [39] to evaluate useful triage strategies, including HPV16/18 genotyping and cytology. Three triage strategies met the criteria for both the NPV (≥98%) and PPV (≥20%) that were set to minimize the risk of overinvestigation and excessively aggressive management; i.e. the NPVs ranged between 98.1% and 99.6%, while the PPVs ranged between 25.6% and 34.0%. The eligible triage strategies were: (i) cytology and HPV16/18 genotyping at baseline without repeat testing, (ii) cytology at baseline with repeat cytology testing after 6 months, and (iii) cytology and HPV16/18 genotyping at baseline followed by repeat cytology examination at 6 months [39]. Furthermore, a nested evaluation of the Swedish SWEDSCREEN study suggested to follow-up hrHPV-positive women with normal cytology at baseline, by one repeat hrHPV test [27]. This strategy showed comparable results in terms of sensitivity for CIN3+ (96.0%), however, with somewhat lower PPV (22.0%) than obtained with the preferred strategies in the POBASCAM sub-study. Moreover, implementation of this strategy leads to a substantial increase in the colposcopy referral rate, and thus possible overtreatment [39, 59].

Finally, Castle et al. [62] evaluated the performance of Cobas HPV testing (Roche Molecular Systems, USA) as a primary screening test among women aged 25 years and older. The preferred strategy in this sub-analysis of the ATHENA study was cytology triage (threshold low-grade squamous intraepithelial lesion or worse) in combination with the detection of HPV16, HPV18, or both types, resulting in a sensitivity for CIN3+ of 72.2% (95% CI 66.4–77.4) and a PPV of 13.9% (95% CI 12.8–15.0). Although the authors recommended re-testing of screen negatives after 1 year, because the sensitivity for CIN3+ was <80% and the NPV <98%.

Thus, several triage strategies seem to be feasible, because the results of the different studies also indicate that it does not appear that the exact triage protocol has any effect on the outcome. However, as preferences and the quality of cytology will vary between countries, policymakers will have to weigh the pros and cons of the different triage strategies when making a choice. Especially, the balance between the safety of a triage strategy (NPV) and the burden of screening on patients and clinicians (PPV and referral rate) is important [63].

**HPV mRNA, p16INK4a IHC, and methylation markers**

In future cervical screening, it might be expected that the role of cytology, as a triage test, will become more limited, as the hrHPV test result may influence the subjective reading of
cytology. Promising biomarkers involved in cervical carcinogenesis have already emerged. Especially, biomarkers that indicate a shift from the productive phase of hrHPV infection to the transforming phase are valuable. hrHPV E6/E7 oncogenes are highly expressed in (para)basal cells of high-grade CIN and interact with p53 and pRB, respectively; in this way, they interfere with cell cycle control [64]. As a consequence, uncontrolled proliferation and chromosomal instability occur, resulting in additional (epi) genetic changes [65, 66]. Therefore, detection of elevated E6/E7 mRNA levels in cervical smears has been suggested to be an attractive biomarker [67].

Two commercial hrHPV mRNA assays can be used for triage testing, i.e. the PreTect HPV-Proofer (NorChip) and the NucliSENS Easy Q HPV (bioMérieux). Both are based on the same technology and are marketed under different brand names in different countries. These assays detect HPV E6/E7 mRNA from the five most prevalent hrHPV types in cervical cancer (HPV16, 18, 31, 33, and 45) [68]. A recent study [69] showed that the HPV-Proofer assay is particularly of value to triage hrHPV DNA-positive women with normal cytology, given their markedly increased risk of CIN2+ in case of a positive mRNA result [55% (95% CI 34%–76%)]. However, this study also revealed that hrHPV-positive cytological normal women who are mRNA test negative would still need follow-up, as their CIN2+ risk was 20% (95% CI 7%–33%). Thus, as a primary screening test, the clinical applicability of these HPV mRNA tests is insufficient.

An alternative is the detection of cellular host genes that are specifically up-regulated and overexpressed, or silenced in cells that have undergone the shift into the transforming phase of hrHPV infection [70]. Cyclin-dependent kinase inhibitor p16INK4a is up-regulated as a consequence of hrHPV E7 expression in proliferating cells [71, 72], and several studies have shown that p16INK4a-based cytology, either alone or in combination with hrHPV testing, can detect underlying CIN2+ with high sensitivity [73–78]. However, the results for p16INK4a were less favorable in the predictors studies [58, 79], comparing the sensitivity and specificity of several tests for the detection of high-grade CIN in a cytology-based referral population. In addition, even after p16INK4a immunostaining, still morphological interpretation necessary to differentiate hrHPV-transformed cells from endometrial cells with non-hrHPV induced p16INK4a expression [80]. To overcome this limitation, a double staining kit for p16INK4a and Ki-67 has been developed that allows simultaneous detection of p16INK4a and nuclear Ki-67 expression in dividing cervical cells (CINtec® Plus, MTM laboratories). The potential of this double staining kit to identify women at risk for underlying high-grade disease was shown in women with abnormal cytology [81, 82], as well as in hrHPV positives with normal cytology, when used as a reflex test [83]. At present, these good results will need to be confirmed in prospective studies. Furthermore, a recently published retrospective study evaluating different triage strategies in a cytology-based referral population suggested that further testing for p16INK4a and HPV16 genotyping may also be an important strategy to determine which women are in need of referral. They advocate this strategy, especially for hrHPV screen positives with normal cytology [84]. Currently, these strategies should be evaluated in prospective studies.

Although, a hrHPV infection can induce immortalization and trigger chromosomal instability, additional changes in oncogene expression and loss of function of tumor suppressor genes are necessary, to obtain a full blown invasive cancer cell. Methylation of CpG islands is an epigenetic modifier of gene expression. In many cancers, tumor suppressor genes were found to be inactivated by hypermethylation of their promoter region. Therefore, detection of hypermethylation of tumor suppressor genes involved in cervical cancer genesis may provide powerful biomarkers for cancer detection [85, 86], especially as methylation has been detected already at precancerous stages [87, 88]. In fact, a recent study of Bierkens et al. showed that methylation levels of two genes (i.e. CADM1 and MAL) increased with the grade of underlying CIN and were highest in carcinomas. Moreover, cervical scrapes of women with CIN 2/3 lesions with long-lasting hrHPV infections (≥5 years) had higher methylation levels than those with a shorter duration of preceding hrHPV infection (<5 years) [89]. These findings indicate that at least lesions with a longer duration of existence are detected by such methylation markers, further strengthening their value for triage testing of hrHPV-positive cervical scrapes. Recently, a study on scrapes from women participating in screening was carried out to evaluate the value of an objective real-time PCR assays that assess the methylation status of the promoter regions of CADM1 and MAL to triage hrHPV-positive women for CIN3+ [90]. Because of the design of the study, the results were compared with CIN3+ sensitivity and specificity of cytology only, and of cytology combined with HPV16/18 genotyping. An optimal threshold resulted in a sensitivity of 84.2%, and a corresponding specificity of 52.5% for the methylation assay; for cytology, these were 65.8% and 78.8%, and for cytology with HPV16/18 genotyping, these were 84.2% and 54.0%, respectively. Consequently, the authors concluded that, in hrHPV positive women, this methylation marker panel was at least equally discriminatory for high-grade CIN as cytology, or as cytology with the detection of HPV16/18. These results indicate that complete cervical screening by objective, non-morphological molecular methods seems to be feasible. However, further validation is needed before any of these tests can be considered for screening. Currently, a large randomized, controlled trial to validate methylation markers to triage hrHPV-positive women is being carried out in the Netherlands.

**primary hrHPV testing: at what age should we start?**

Another important issue is the age at which hrHPV testing should be offered for primary screening. Most experts agree that, in women younger than 25 years of age, hrHPV testing is not recommended, because the prevalence is very high, and at this age, hrHPV infections are commonly transient.

Ronco et al. [28] reported that hrHPV testing in women aged 25–34 years could lead to substantial overdiagnosis of regressive CIN2+ lesions, particularly when hrHPV positives in this age group are directly referred for colposcopy, without further triage testing. The results from the POBASCAM trial [33] showed that hrHPV screening does not have to be postponed until age 36 years or older, but can be started at age 30 years. The long-term follow-up results of this study showed that the cumulative detection of CIN2+ and CIN3+ lesions over two screening rounds
did not differ between women aged 29–33 years, and women of 34 years of age and older [33]. Thus, hrHPV screening does not result in excessive diagnosis of lesions destined to regress, even in women in the 30–34 years of age category. These findings indicate that primary hrHPV screening should be recommended for women aged 30–60 years. Additional follow-up, after the age of 60, is indicated for women with a positive hrHPV test at the last screening round. The residual risk of CIN3+ is too high to dispense them from follow-up, even when triage testing in hrHPV-positive women is negative [91]. These women can only be discharged from further hrHPV testing after they have cleared the virus. Future studies should investigate whether methylation markers might be used as a more specific triage test for hrHPV-positive women in the younger age range (below 30 years) to detect clinically relevant lesions, as high methylation levels seem to be related to the degree and duration of underlying CIN and highest levels are found in carcinomas [89].

**increasing the screening coverage**

One other problem concerning the effectiveness of current cervical screening programs remains nonattendance [92, 93]. Especially nonparticipating women are at increased risk of cancer [3, 94], as half of the cervical carcinomas are found in non-attending women. Therefore, targeting non-attendees is important in achieving optimal protection from screening programs.

Self-sampling is a less costly and less invasive collection method [95], and several studies have shown that non-attendees actually do take part in self-sampling studies [92, 96–99]. Thus, there is a basis for self-sampling in cervical cancer screening. In addition, self-collection makes cervical screening accessible to women in medium- and low-income countries [100–102]. That is why, in recent years, several studies have focused on the use of self-collected samples for hrHPV testing.

In most studies, a moderate-to-good agreement between hrHPV test positivity in self-collected and physician-taken samples was found. The data have been summarized in (systematic) reviews and meta-analyses [95, 103], in which study parameters included concordance in hrHPV detection rates between self- and physician-collected samples through kappa values, which varied between 0.45 and 1.00 (Table 2). However, the hrHPV test positivity rates varied across the studies. Most likely due to differences in study populations (age range and country of origin), the use of different collection devices for self- and physician-sampling, as well as differences in hrHPV tests and protocols. For example, in studies using the HC2 method, a higher hrHPV detection rate in self- compared with physician-collected samples was observed [97, 100, 104, 105]. The HC2 assay, however, is known to show some cross-reactivity to low-risk HPV types [106], and these types tend to more commonly affect vaginal than cervical mucosa. In other studies, a higher hrHPV detection rate was found in the physician-collected cervical samples [107, 108]. Yet, overall the data show that self-sampling is concordant with physician-sampling in detecting hrHPV DNA.

However, before its use in cervical cancer screening, it is most important to know how self-sampling performs with regard to relevant disease outcomes. In other words, hrHPV self-sampling procedures should be clinically validated in terms of sensitivity and specificity to detect CIN2+ lesions. Various cross-sectional studies have been carried out to compare the value of hrHPV testing on self- with physician-collected samples to detect CIN2+, and the results have recently been summarized by Snijders et al. [99]. This review showed that hrHPV testing on self-sampled specimen is as sensitive for CIN2+ in several studies as hrHPV detection on physician-taken cervical samples, although sometimes less specific. However, the authors also reported that both the type of self-sampling device and the type of hrHPV test seem to influence the clinical performance of hrHPV testing on self-samples. Thus, for reaching clinical equivalence, in terms of detecting high-grade CIN, between self- and physician-sampling, a certified combination of self-sampling device and validated hrHPV test is important. Overall, the data show that self-sampling for primary hrHPV testing offers possibilities to increase screening coverage by reaching non-responders, and in future, might even be offered as a safe alternative screening method to regular attendees.

Additionally, hrHPV-oriented cervical screening programs will increase public awareness of the link between hrHPV and cervical cancer, which may, in turn, lead to a higher uptake of the prophylactic HPV vaccines and consequently a further reduction in cervical cancer incidence.

**Table 2.** Systematic reviews and meta-analyses of the literature comparing self-collected and physician-taken samples for the detection of hrHPV DNA

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of studies included</th>
<th>Kappa value for agreement (range)</th>
</tr>
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<tbody>
<tr>
<td>Ogilvie et al. [103]</td>
<td>12 studies</td>
<td>0.45–1.00</td>
</tr>
<tr>
<td>Petignat et al. [95]</td>
<td>18 studies</td>
<td>0.50–0.82</td>
</tr>
<tr>
<td>Schmeink et al. [114]</td>
<td>19 studies</td>
<td>0.45–0.81</td>
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</table>

**Conclusion**

All the evidence collected so far suggest that the time has come for the implementation of hrHPV testing as a primary screening test, as it provides a superior protection against cervical (pre)-cancerous lesions compared with cytology. hrHPV testing detects 30% more CIN2+, and 20% more CIN3+ lesions in women over 30 years of age. Although any hrHPV test used should be clinically validated, hrHPV-based primary screening should be implemented preferably within a population-based screening program with a call and recall system. The somewhat lower specificity, which may cause excess false-positive tests among healthy women, can be overcome by triage of hrHPV-positive women. At this time, triaging these women by cytology at baseline and repeat cytology testing after 6 months, possibly in combination with baseline HPV16/18 genotyping, seems to be very suitable for this purpose. Although, as preferences and the quality of cytology will vary between countries, policymakers will have to weigh the pros and cons of the different triage strategies when making a choice. In future screening, however, it is likely that the role of cytology becomes more limited and validated (molecular) biomarkers gain attention;
among these, p16\(^{INKA}\)/Ki-67 double staining and host genome or viral DNA methylation markers appear to be promising.

As for the age to commence screening, we advocate the introduction of primary hrHPV testing in women from the age of 30, to ensure that mostly clinically relevant, non-regressing high-grade lesions are detected. Furthermore, since a negative hrHPV test offers a better protection (50%) against CIN3+ than normal cytology, hrHPV testing can be implemented together with an extension of the screening interval for hrHPV screen negative women. In addition, to achieve optimal protection and screening coverage, self-sampling might be offered to non-attendees, and in future possibly as an alternative method to regular attendees, under the condition that a validated combination of self-sampling device and hrHPV assay is used.

Collectively, these data show that the time has come to implement primary hrHPV testing in population-based cervical screening, thereby offering women maximum protection against high-grade CIN with less uncertainty and fewer screening rounds.

disclosure

PJFS declares that he has received speakers’ fee from Roche, Abbott, GenProbe, and Qiagen. He is also shareholder of Self-screen B.V. JB declares that he has received speakers’ fee from Qiagen and a consultancy fee from GSK. CJLMM declares that he has received speakers’ fee from Qiagen, Roche, and Merck. He has received consultancy fees from Qiagen until 2010, and he is minority shareholder of Self-screen B.V. All remaining authors have declared no conflicts of interest.

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