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GENERAL DISCUSSION

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General Discussion

The role of hrHPV testing in cervical cancer screening

In many developed countries, cytology-based cervical cancer screening programmes have decreased the incidence and mortality of cervical cancer.[1-5] However, in recent years, this decreasing effect on the incidence of cervical cancer has levelled off.[4;6] This can be explained by the varying screening uptake among women, a relatively high number of false-negative cytology tests (lack of sensitivity)[7;8] and suboptimal follow-up of screen-positive women. In addition, although the incidence of squamous cell carcinoma has decreased, the incidence of cervical adenocarcinoma does not display a decreasing trend.[9] These findings confirm that adenocarcinoma and its precursor lesions are difficult to detect by cervical cytology. In addition, the incidence of adenocarcinoma seems to be increasing in young women.[9]

Since hrHPV testing has a higher sensitivity and offers better protection against high-grade cervical lesions and cervical cancer than cytology, implementation of hrHPV testing as primary cervical cancer screening test is presently considered in many countries.

Here, we will discuss whether cervical screening can be improved by 1. hrHPV testing to triage women with borderline or mild dyskaryosis and 2. implementing hrHPV testing as a primary screening test. Furthermore, we addressed the following questions: at what age should hrHPV testing be started? What is the appropriate triage algorithm for hrHPV-positive women to minimize the loss of specificity with hrHPV screening compared to cytology screening? In addition, we will discuss how cervical screening can be improved by hrHPV testing on self-collected cervicovaginal specimens. Finally, effects of HPV vaccination on screening and future aspects of hrHPV-based screening are discussed.

HrHPV testing to triage women with borderline or mild dyskaryosis

Currently, cervical cancer screening programmes are based on cytology. About 2.5% of the women participating in the Dutch population-base screening programme have a bordeline or mild dyskaryosis (BMD) test result.[10] Of these women, only 10%-20% harbor CIN2+ lesions.[11;12] These women are therefore not directly referred for colposcopy but are advised to repeat cytology at 6 and 18 months and are only referred for colposcopy if any of the repeat cytology tests is abnormal (threshold ≥ BMD). The majority of referred women, however, will have meaningless lesions that will regress spontaneously.[12] To reduce the number of referrals and colposcopies, the New Dutch guidelines[13] allowed laboratories to choose to include hrHPV testing at the repeat visit at 6 months (Figure 1). This guideline change was a first step in the transition towards hrHPV testing. The complexity of this follow-up testing strategy is considerable since women with a repeat smear of BMD and who test positive for hrHPV are referred to a gynecologist, while those with a negative hrHPV test still get a repeat smear at 18 months after the first test (Figure 1). Moreover, only women with a normal repeat smear and negative hrHPV test are referred back to the screening programme, whereas their hrHPV-positive counterparts are offered repeat cytology at 18 months. Women with a repeat smear of >BMD are, independent of the hrHPV test result, directly referred to the gynecologist.

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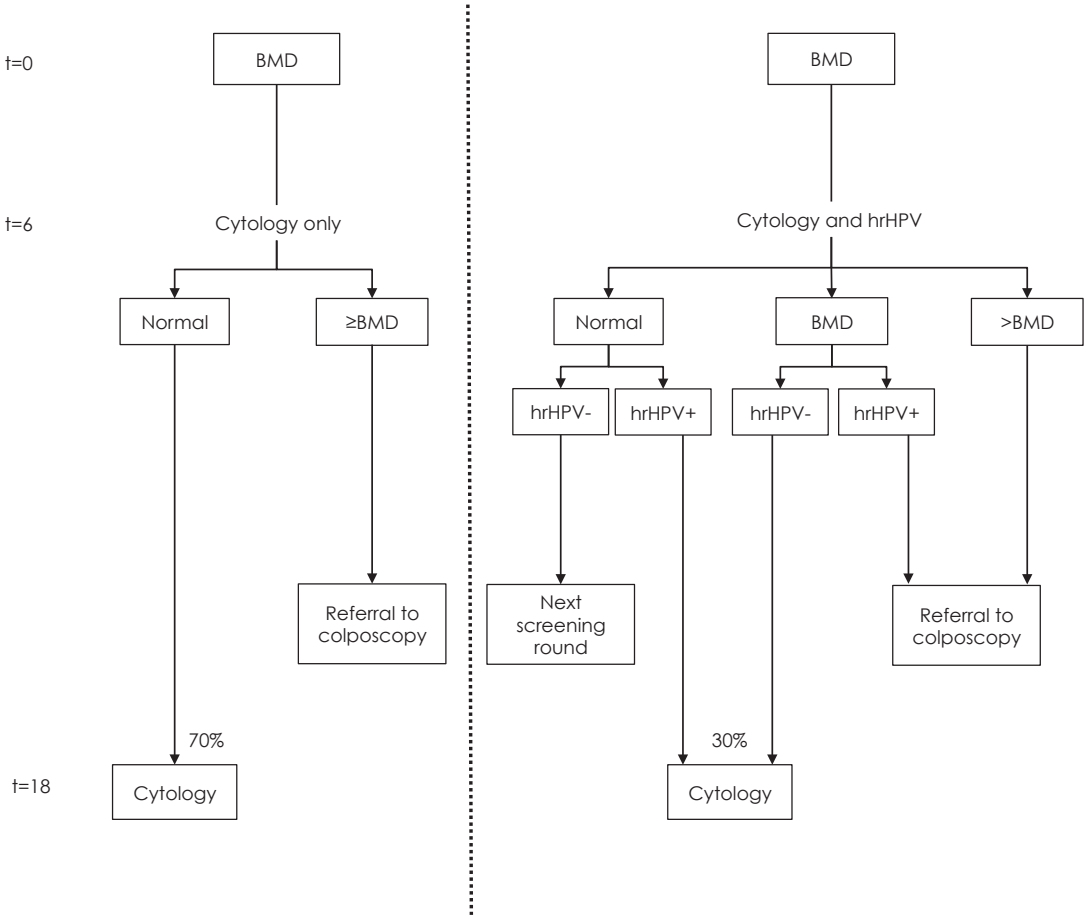

Figure 1 Flowchart for the follow-up of women with BMD cytology in the Netherlands [adapted from [13]].

We studied whether a less complex referral strategy is possible where women with BMD are triaged at baseline with hrHPV testing (Chapter 2).[14] In this algorithm women with BMD and a positive hrHPV test are directly referred for colposcopy while the women with BMD and a negative hrHPV test are advised to repeat cytology at 6 and 18 months.

Our study showed that hrHPV triaging of women with BMD resulted in a high CIN3 and CIN2+ detection rate (10.7% and 22.3%, respectively). This finding is consistent with meta-analyses that have shown that hrHPV triaging has a higher sensitivity than repeat cytology for detection of CIN2+.[12;15] Furthermore, in our study women with BMD and a negative hrHPV test had a low three-year risk to develop high-grade lesions (CIN3 of 1.2% and CIN2+ of 2.9%)[14] and therefore could be directly referred to the routine screening. The next regular screening round (interval 5 year) could serve as a safety net, at least for women aged ≤ 55 years who will still be invited for screening.

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Disadvantage of triage by hrHPV testing at baseline is that approximately 20% of the women will have cleared their hrHPV infection in the first 6 months of follow-up. As a consequence, hrHPV triaging at baseline might result in 51.5% higher colposcopy referral rates than repeat cytology (colposcopy referral rates of 57.6% versus 38.0%, respectively). However, our study showed that hrHPV triaging did not lead to an increase in the referral rate per detected CIN3. The medical costs per detected CIN3 were even slightly lower for hrHPV triaging than for repeat cytology testing.

Advantages of baseline triaging instead of repeat cytology are ease of implementation, low loss of follow-up, fast diagnosis and low distress for participating women.

Recommendations

- HrHPV triaging of women with BMD has shown to be at least as effective for detection of CIN3 as repeat cytology. Women with BMD should be directly triaged by hrHPV testing instead of by repeat cytology testing to achieve a fast diagnosis and therefore reduce unnecessary distress for women.
- Women with BMD and hrHPV-positive test should be referred for colposcopy, since these women have a high risk to develop CIN3 and CIN2+ (20.4% and 41.9%, respectively).
- Women with BMD and hrHPV-negative test result have an acceptable low 3 year CIN3+ risk of 1.3% and CIN2+ risk of 2.9% and therefore could be referred to routine screening.

Primary hrHPV based screening

We evaluated hrHPV testing in two prospective population-based screening studies: the POBASCAM trial that followed 40,105 women aged 29-56 for two screening rounds (9 year interval) and the VUSA-screen study that followed 48,088 women aged 29-61 for 3 years.

Both studies showed that hrHPV testing has a higher cross-sectional sensitivity, but lower specificity than cytology for detecting CIN2+ (Chapter 3 and 4).[16;17] These results are in line with other screening studies.[12;18-22] These trials showed that the cross-sectional sensitivity of hrHPV testing for CIN2+ was 49% and for CIN3+ was 30% higher than that of cytology, while the specificity of hrHPV testing was 2.5-4% lower.[23]

The POBASCAM trial furthermore showed that hrHPV testing in combination with cytology resulted in higher detection of CIN2+ lesions compared to cytology testing alone at the first round. This resulted in an improved protection against CIN3+ at the second screening round compared with cytology alone (relative risk 0.73, 95% CI0.55-0.96) (Chapter 4).[16] These findings are in agreement with other randomised controlled trails, [22;24;25] and our interim analysis.[26] Additionally, as was the case in the NTCC trial,[27] our study showed that hrHPV testing provides better protection against cervical cancer in the second screening round than cervical cytology (relative risk 0.29, 95% CI 0.10-0.87). Collectively, all trials showed that hrHPV testing significantly reduces detection of CIN3+ lesions in the second screening round relative to cytology.

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These results suggest that an extension of the screening interval may be considered when hrHPV testing is implemented.[22;26] This is also consistent with the results of the VUSA-Screen study and other studies,[28-31] showing a very low detection of CIN3+ and CIN2+ lesions after a negative hrHPV test (Chapter 3).[17] A model study based on data from the POBASCAM study [32] indicate that an extension of screening interval to 7.5 years is possible without increasing the cancer risk as presently observed with 5-yearly cytology.

Questions have risen whether the increased sensitivity of hrHPV testing results in over-diagnosis of lesions that otherwise would have regressed spontaneously. The long screening interval used in the POBASCAM trail (5 years) may provide some information on whether a cervical lesion is persistent or regressive. The POBASCAM trial showed that over-diagnosis in the women studied (30-60 year) does not seem a clinically relevant problem since the cumulative number of women with CIN3+ over both screening rounds did not differ between intervention (hrHPV & cytology) and the control (cytology only) group (259 of 19,999 vs 272 of 20,106; 0.96, 95%CI 0.81-1.14). This result supports the idea that hrHPV testing leads to earlier detection of clinically relevant lesions.

Another important question is whether hrHPV testing should be offered in combination with cytology or as a single, primary screening instrument. Because hrHPV testing has a very high sensitivity, others and we showed that combined testing with cytology was not better than hrHPV screening alone in detecting CIN3+ and CIN2+ lesions (pooled detection ratios of 1.04 (95%CI 0.92-1.17 for CIN3+ and 1.06 (95%CI 0.97-1.16 for CIN2+, respectively).[16;17;23;27] For this reason, cervical screening with a primary stand-alone hrHPV test seems preferable. This recommendation is confirmed by cost-effectiveness studies.[32-34]

About 20% of all cervical cancers are adenocarcinomas.[35] A problem of the present cytology-based programmes is that in contrast to squamous cell carcinomas the incidence of adenocarcinomas is not decreasing.[36-38] Reasons for this finding are 1) the poor cytological definition of the precursor lesions of adenocarcinomas except for adenocarcinoma in situ resulting in poor recognition by the cyto-pathologists and 2) the localization of these lesions higher in the endocervical canal. Exfoliation of representative abnormal cells is therefore limited and abnormal cylinder cells are more difficult to detect by the cytopathologist. Studies demonstrated that hrHPV testing has a higher sensitivity for precursor lesions of adenocarcinoma of the cervix than cytology testing.[39;40] The NTCC trial [27] reported a higher occurrence of adenocarcinomas after cytology screening compared to hrHPV screening, confirming the notion that cytology is particularly less effective in preventing adenocarcinomas. However, in the POBASCAM trail no significant differences in detection of adenocarcinomas between intervention (hrHPV & cytology) and control arm (cytology) was detected, probably because the number of cervical adenocarcinomas in this study was small. Therefore, the results from prospective trials need to be pooled to determine whether hrHPV testing prevents adenocarcinomas better than cytology. Presently, these pooled trial data are analysed in the PreHdict consortium (www.ecca.info/campaigns/prehdict.html).

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Another issue is how much each of the HPV genotypes contributes to the detection of CIN3+ and CIN2+. The POBASCAM study showed that hrHPV testing mainly resulted in early detection of HPV16 associated CIN2+ lesions in the first screening round followed by a reduction of HPV16 CIN3+ lesions in the second round. This finding is in agreement with the finding that HPV16 is the most common genotype in cervical cancer.[41] The contribution of other non-HPV16 genotypes could not be assessed because of the limited numbers of women in the study.

Recommendations

- HrHPV testing in cervical screening provides better protection against CIN3+ and cervical cancer in the second screening round than cytology by earlier detection of clinically relevant CIN2+ lesions in the first round.
- HrHPV testing alone should become the primary screening method.
- Women with a negative hrHPV test have an extremely low risk to develop high-grade cervical lesions. Longer screening intervals (up to 7.5 years) may therefore be used.

Age to begin screening

An important issue is the age at which hrHPV testing should be offered in primary screening. Women under the age of 30 years have a high prevalence of hrHPV infections and the majority of these infections are generally cleared in a relatively short time.[42;43] Screening young women under 30 years by hrHPV testing results in detection of mostly transient hrHPV infections. As women become olders the prevalence of hrHPV decreases and stabilizes at the age of 35 years. Older women are more likely to have a persistent infection and they are therefore more likely to benefit from intervention. In addition, incidence and mortality rates for cervical cancer among women younger than 30 are low.[44;45] In the Netherlands, 4.5% of all carcinomas are found under the age of 30 year.[9] The benefit of screening all women between 25-30 years does not outweigh the negative screening effects (high referral rates for colposcopy, which in turn might result in unnecessary treatment and the risk of preterm births and substantial anxiety). Therefore, it has been decided in the Netherlands that hrHPV testing should not be used to screen women younger than 30 years.[46]

Ronco and colleagues [27] reported that hrHPV testing in women aged 25-34 years could lead to substantial over-diagnosis of regressive CIN2+ lesions. As a result, they suggested that hrHPV screening should not start in women younger than 35 years. In the POBASCAM trial, our separate analyses of women attending screening for the first time (age 29-33 years) and older women (age 34-56 years) revealed that the CIN3+ detection rates over two rounds were similar for the intervention and control group within both age subgroups. Also for CIN2+, the detection rates were similar for the intervention and control group (see Figure 3. Chapter 4). These data therefore indicate that hrHPV testing in women aged 29-33 does not result in excessive diagnosis of cervical lesions and argues for implementation of hrHPV testing in programmed cervical screening at a starting age of 30 years.

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In addition, we observed in the VUSA-Screen study that the CIN3+ and CIN2+ detection rate in hrHPV-positive women was similar for women invited for cervical screening for the fist time (age 29–33 years) and for older women (>34 years).[47] Thus also the data from the VUSA-Screen study support the notion that screening should start at the age of 30.

Recommendations

- Screening by primary hrHPV testing should start at the age of 30 years.

Management of hrHPV-positive women

An adverse effect of using an hrHPV test for cervical screening is that transient infections are detected. In population-based screening, specificity of the screening test is of utmost importance, as it basically determines the costs of the programme and the occurrence of adverse effects (repeat screening test(s), colposcopy referrals, treatment of regressive lesions, anxiety) in the generally healthy population. At first, it is therefore important to use a clinically validated hrHPV test in primary cervical screening.[48] Secondly, management of hrHPV-positive women deserves attention.

In the Netherlands, about 5% of the women between 30 and 60 years of age are hrHPV-positive,[10;49] and approximately 13% of them have an underlying CIN3+ and about 22% a CIN2+ lesion.[47] Therefore, not all hrHPV-positive women should be directly referred because this would result in a substantial increase in colposcopies and may result in overtreatment. The latter is particularly problematic because unnecessary excisional treatment of cervical lesions may result in preterm delivery in subsequent pregnancies.[50;51]

An option to improve the specificity of an hrHPV-based screening algorithm is to narrow the definition for a positive hrHPV screening test, for example by increasing the threshold of the hybrid capture 2 HPV DNA test (> 1 RLU).[52-54] Kotaniemi-Talonen et al.[52] and Ronco et al.[53] concluded that the cutoff can be increased to, respectively 10 RLU/CO and 2 RLU/CO. However, in our VUSA-Screen study (Chapter 6),[54] we found that there was no HC2 threshold for which single hrHPV screening resulted in both superior sensitivity and specificity compared to cytology screening. Therefore, we conclude that changing the threshold of the HC2 test is not sufficient and that some form of triage and/or follow-up testing is required for hrHPV-positive women.

Several triage suggestions for hrHPV-positive women have been made in the literature. Because cytology has a relatively high specificity,[12] it seems suitable to triage hrHPV-positive women. Also genotyping for HPV16 and HPV18 has been considered as triage option as those types are associated with strongly increased CIN2+ risk.[55;56]

We compared 14 triage/follow-up testing strategies using cytology, hrHPV testing and/or HPV genotyping (Chapter 5).[49] A triage strategy was considered feasible if the 2-year CIN3+ risk was equal to or lower than 2% (corresponding with a NPV of at least 98%), which is presently considered acceptable according to the Dutch screening guidelines. The most attractive strategy was cytology testing at baseline and at 12 months because it has a low 2-year CIN3+ risk (below 1%), a high PPV of 37.5% and

results in only a modest colposcopy referral rate in the total population (1.70%, 95% CI 1.54-1.85). In addition, this strategy is easy to communicate to physicians and women. Another attractive but more costly strategy is combined cytology and HPV16/18 genotyping at baseline followed by repeat cytology at 12 months. This strategy has a low CIN3+ risk (0.3%) and a lower PPV of 25.6% but results in a relatively high colposcopy referral rate in the total population (2.53%, 95CI 2.34-2.73). Both strategies entail short-term recalls of hrHPV-positive (for the second strategy hrHPV-positive but HPV16/18 negative), cytology negative women and demand a high level of compliance to follow-up. One baseline strategy without repeat testing, i.e. combined cytology with HPV16/18/31/33/45 genotyping may be considered as an alternative because it resulted in a CIN3+ risk of test negative women of <1% and does not depend on follow-up compliance. However, such a strategy has a low PPV of 20.7% and will lead to a high overall colposcopy referral rate in the total population (2.95% (95%CI: 2.75-3.17).

It should be kept in mind that in the Netherlands, cytology has a high quality. The cytology test shows a relatively high sensitivity and specificity and the cytological abnormality rates are low. As a result, the most attractive strategy to triage hrHPV-positive women is cytology testing at baseline and at 12 months. However, in countries where the quality of cytology is worse, cytology and HPV16 and HPV18 genotyping might be an alternative triage tool.

Loss to follow-up is a major risk factor of a screening strategy with a repeat test. Several studies have shown that attendance at repeat testing may be poor, particularly after a cytologically normal test result.[24;26;57] Appropriate communication strategies are therefore necessary to establish high attendance at repeat testing. In addition, the logistics of a triage and follow-up strategy should preferably be simple.

Although cytology is an appropriate triage tool for hrHPV-positive women, triage can be improved since the test is subjective and has a rather low reproducibility. Several new molecular biomarkers identified by molecular carcinogenesis research are developed to use as triage test. One of them is p16/ki67 dual-stain cytology. This test may be used as surrogate marker of cell cycle deregulation mediated by transforming hrHPV infections. Promising results have been shown and the test (CINTEC®) seems to perform better than cytology.[58;59] However, application of the CINTEC® test still requires cyto-pathological experience and prospective studies are needed to validate the published data. Another marker may be the HPV-Proofer E6/E7 mRNA test (Norchip®), detecting an active infection with cell- transforming potential for five hrHPV types.[60;61] Indeed a positive E6/E7 mRNA test is associated with CIN3+/CIN2+ but the risk of women with a negative E6/E7 mRNA test is too high to dismiss these women from further follow-up. In addition, promoter methylation analyses of tumor suppressor genes have been proposed as triage tool for hrHPV-positive women. CADM1 and MAL methylation markers showed to be promising candidates [62-64] but prospective studies are needed. These studies are presently ongoing. These molecular biomarkers may pave the way to a complete molecular-based cervical screening program, potentially also suitable to medium/low resource countries.

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Recommendations

- HrHPV-positive women need to be triaged to prevent over-diagnosis, over-treatment and high costs.
- In countries with a high quality of cytology, cytology testing at baseline followed by repeat cytology testing at 12 months is an attractive triage tool.
- In countries where the quality of cytology is less good, triage by combined cytology and HPV16/18 genotyping at baseline followed by repeat cytology at 12 months might be considered for implementation.

Improve screening participation by hrHPV testing on self-collected material

In the Netherlands about 35% of the invited women do not respond to a screening invitation.[65] More than half of the cervical cancer cases detected in the Netherland did not have a previous cervical smears.[8;66] Therefore, attempts should be made to increase the number of women participating in the programme. Offering women a user-friendly self-sampling device for collecting cervico-vaginal material for hrHPV testing has shown to increase participation rate.[67-69]

Self-sampling devices enable women to take their own (cervico-)vaginal sample at a suitable time and place. About 30% of the non-participating women responded actively by returning a self-sampling device,[68;70;71] which is considerably higher than the rates of 10-17% obtained when women were sent one or two reminders for regular screening. Furthermore, the yield of high-grade lesions was higher in self-sampling responders (non-responders to an invitation of the regular screening program who positively reacted to offering hrHPV testing on self-collected material) compared to screening participants.[68;70;71] The hrHPV test performed comparable in terms of sensitivity for CIN2+ on self-sample material and on smears taken by the general practitioner.[72-75]

In addition, self-sampling may improve participation in countries with cultural and religious programme barriers by increasing acceptance and access to cervical cancer screening. Furthermore, self-sampling can improve screening in countries with lack of medical staff and screening facilities. Indeed, self-sampling has shown to facilitate access to cervical screening in developing regions [76;77] and may well lead to increased screening participation.

However, cytology cannot be performed on self-collected material, most likely due to the fact that self-sampled material mostly contain admixed vaginal cells and relatively few intact cervical cells (so called cervical indicator cells) indicative for a high-grade precursor lesion of the cervix. For triage testing the women who test hrHPV-positive on self-collected (cervico-)vaginal material have therefore to be referred to the general practitioner for a cervical cytology smear. This extra visit to the physician results in discomfort for the women and loss to follow-up , and is unfeasible in under-resourced settings lacking medical services. Application of triage testing directly on the self-sampled specimens by a non-morphological biomarker test would

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be an ideal alternative to select hrHPV-positive women in need of colposcopy. Studies nowadays evaluate new molecular triage markers that are directly applicable on self-sampling tests, such as methylation markers.[64;78] In this way a complete molecular objective non-morphological cervical screening can be offered. This will make cervical screening also available to medium and low resource countries.

Recommendations

- Offering self-sampling for hrHPV testing is a promising, effective alternative to sampling by clinicians and improves screening compliance rates.

HPV vaccination

The HPV vaccination programme started in the Netherlands in 2009 for girls of 12 years old with a catch-up for 13-16-year old girls.[79] The prophylactic HPV vaccines have shown to be highly efficacious to prevent high-grade cervical lesions at a population level.[80-88] It is assumed that HPV16/18 VLP L1 vaccines will prevent about 70%-80% of the squamous cell carcinomas and 90% of the adenocarcinomas.[41;89] Since the HPV vaccines only have a prophylactic effect when women are hrHPV-negative, they are given to young, hrHPV naïve girls. However, HPV vaccination coverage for the first dose in the Dutch population targeted by the catch-up campaign was about 55%, lower than the aimed 70%.[90;91] There was a lower uptake among women in conservative religious and ethnic communities and among women with lower socioeconomic status.[91] Therefore, better communication strategies are needed to increase the vaccination attendance rate.

It is, however, of note that the potential benefit of HPV vaccination might be larger than the coverage would indicate because of indirect (herd immunity) protection to non-vaccinated women.

Vaccination does not eliminate the need for cervical cancer screening. The vaccinated women remain at risk of developing cervical cancer associated with non-HPV16/18 genotypes.[81-83;92;93] Even if hrHPV cross-protection is taken into account, about 20% of the cervical carcinomas will not be prevented by vaccination.

HPV vaccination is expected to have an impact on the effectiveness of cytology and hrHPV screening. Vaccination probably decreases hrHPV positivity, cytological abnormalities and reduces the number of high-grade cervical lesions. In countries with effective screening programmes and high coverage rates (i.e., Denmark Finland, Iceland, the Netherlands, and the UK), the impact of vaccination on the reduction of cervical cancer cases is expected to be relatively small. The impact of vaccination would be further attenuated if vaccination leads to a false sense of security resulting in lower adherence to the screening programme. Therefore, it is very important to educate and motivate women to attend cervical screening programme, even if they are vaccinated. The effect of vaccination is expected to be higher on the incidence of adenocarcinomas because HPV16 and 18 are associated with more than 90% of the adenocarcinomas. HPV vaccination may negatively influence current cytology

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based screening since the decrease of cytological abnormalities and high-grade lesions may deteriorate the quality of smear reading.[94] This is an additional reason to implement hrHPV testing as primary screening test since this test is objective. Cytology could be used as triage test for hrHPV-positive women, because in this group the occurrence of abnormal smear is increased.

Recommendations

- Besides HPV vaccination screening for cervical abnormalities will remain important to prevent cervical cancer because 1. the current vaccines protect against about 70%-80% of the cervical cancer cases and 2. the participation rate of vaccination will never reach 100% leaving a considerable number of women at risk of getting infected by hrHPV.

Future perspectives

We showed that hrHPV testing is superior to cytology as a primary screening test in cervical cancer screening programmes for women 30 years and older. Additionally, we presented feasible triage algorithms for hrHPV-positive women. Taken together, time has come for implementing hrHPV testing in nationwide cervical screening programmes.

Offering self-sampling to women who do not attend regular screening resulted in a response rate of about 30%.[67;68;70;71] Moreover, the number of CIN3+ and CIN2+ lesions detected by hrHPV testing in non-responders was significantly higher than in women responding to the regular screening programme. These results support implementation of self-sampling in the cervical screening programme, at least for non-attendees.[68;70]

In the future, self-sampling may be offered as primary screening test to all women eligible for cervical screening. Ideally, women would be given the choice for either self-sampling or physician-collection. These options need to be further analysed in implementation studies followed by cost-effectiveness analyses.

Topics of further research include ways to improve triage testing of hrHPV-positive women. Although cytology has shown to be an effective triage method in an hrHPV screening setting, this approach is labor intensive, subjective and unfeasible in under-resourced settings lacking medical services. Objective and reproducible biomarkers may improve triage of hrHPV-positive women. Studies have shown that methylation markers (methylation of the promoter regions of tumor suppressor genes involved in cervical carcinogenesis) are promising triage tools to distinguish women with clinically meaningful cervical disease amongst those who are hrHPV-positive without an underlying cervical high-grade lesion. Further research about the potential of different biomarkers in triaging hrHPV-positive women is needed.

Appropriate communication and education of both women and physicians about hrHPV and cervical cancer is very important to increase vaccination coverage and to motivate women to participate in the screening programme. In this way cervical cancer morbidity and mortality can be reduced.

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