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Triage of HPV positive women in cervical cancer screening

Nicolas Wentzensen[1], **Mark Schiffman**[1], **Timothy Palmer**[2], and **Marc Arbyn**[3]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, MD, USA

[2]The University of Edinburgh, Edinburgh, UK

[3]Belgian Cancer Centre/Unit of Cancer Epidemiology, Scientific Institute of Public Health, Brussels, Belgium

Abstract

Despite HPV vaccines, screening will remain central for decades to control cervical cancer. Recently, HPV testing alone or with cytology was introduced as an alternative to cytology screening. However, most HPV infections are harmless and additional tests are required to identify women with progressing infections or precancer. With three options for primary screening, and without clear strategies for triage of screen-positive women, there is great confusion about the best approach. Also, increasing HPV vaccination coverage will lead to lower disease prevalence, and force new screening approaches. Currently recommended triage strategies for primary HPV screening include HPV genotyping for HPV16 and HPV18 and cytology. Other alternatives that are currently evaluated include p16/Ki-67 dual stain cytology, host methylation, and viral methylation testing. Clinical management of women with cervical cancer screening results is moving to use risk thresholds rather than individual test results. Specific risk thresholds have been defined for return to primary screening, repeat testing, referral to colposcopy, and immediate treatment. Choice of test algorithms is based on comparison of absolute risk estimates from triage tests with established clinical thresholds. Importantly, triage tests need to be evaluated together with the primary screening test and the downstream clinical management. An optimal integrated screening and triage strategy should reassure the vast majority of women that they are at very low risk of cervical cancer, send the women at highest risk to colposcopy at the right time, when disease can be colposcopically detected, and minimize the intermediate risk group that requires continued surveillance.

To whom correspondence should be addressed: Nicolas Wentzensen, M.D., Ph.D., M.S., Division of Cancer Epidemiology and Genetics, National Cancer Institute, 9609 Medical Center Drive, Room 7-E114, Bethesda, MD 20892-9774, Phone: (240) 276-7303, Fax: (240) 276-7838, wentzenn@mail.nih.gov.

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Introduction

This review covers a critical issue in the transition from cytology-based cervical screening to a reliance on human papillomavirus (HPV) testing, namely, the triage of the HPV-positive woman. We begin with a general introduction to the topic of cervical screening, and then address specifically the issues and possible options for managing HPV-positive results.

Cytology-based cervical cancer screening was introduced decades ago and subsequently implemented in many industrialized countries. It has led to substantial reductions in cervical cancer incidence and mortality, particularly in countries with organized screening programs.[1, 2] Long after the first introduction of cervical cancer screening, etiology and natural history studies of cervical cancer established that persistent infections with one of a group of carcinogenic HPV types are a necessary cause of almost all cervical cancers.[3] HPV infections are very common in the population, but most infections become undetectable after 1–2 years. Long-term persistent infections are highly associated with progression to cervical precancer. When left untreated, about 30% of CIN3s will progress to invasive cancer over the subsequent years to decades.[4, 5]

The understanding that HPV is a necessary cause of cervical cancer has led to major advances in primary and secondary prevention of cervical cancer. Vaccines targeting the two most important carcinogenic types, HPV16 and HPV18, were introduced a decade ago. These vaccines are highly effective at preventing HPV infections with these types, particularly when administered before onset of sexual activity.[6] Recently, a nonavalent vaccine was licensed that includes 7 carcinogenic types accounting for roughly 90% of cervical cancers and 2 non-carcinogenic types that cause genital warts.[7]

For secondary prevention, detection of HPV DNA or RNA has been introduced. [4] Initially, HPV DNA tests were approved for triage of women with ASC-US cytology results; later the regulatory approval was extended to HPV-cytology co-testing and recently, an HPV DNA test was the first to be approved for primary screening. A test detecting RNA instead of DNA has been approved by FDA for the first two indications.[8]

Risk-based approach to cervical cancer screening and triage

The main goal of cervical cancer screening is to prevent cancers, which is achieved by identifying cervical precancers that can be treated to prevent progression to invasive cancer. On the population level, cervical cancer screening needs to identify the small group of women at increased risk of cervical cancer, who need further workup and possibly treatment, while reassuring the majority of women that their cancer risk is very low.[9]

A cervical cancer screening program includes several steps: Population-based screening of a specific target age group, referral to colposcopy and biopsy of women at increased risk of precancer, and treatment of women with a confirmed precancer.

The decisions about further management or reassurance are typically based on risk thresholds. When the absolute risk for cervical precancer approaches a certain level, women are referred to colposcopy-biopsy. For a subset of these women, absolute risk of precancer is so high that immediate treatment is an option. Historically, these management thresholds have been based on results from the long-standing primary screening test, Pap cytology. For example, women with high grade intraepithelial lesions (HSIL), for whom the risk of precancer is high, are immediately referred for colposcopy with an option for treatment at the first visit in some settings. Women with low grade squamous intraepithelial lesions (LSIL) or ASCUS, whose risk of significant disease is lower, may be referred immediately for colposocopy, or followed cytologically with only persistent disease warranting referral. [10]

Of note, a large fraction of non-normal cytology results are equivocal rather than definitely abnormal; atypical squamous cells of undetermined significance (ASC-US) account for the majority of abnormal cytology results.[11] While up to half of ASC-US represents cellular changes not associated with HPV or cervical precancer, many precancers come from the HPV-positive half of the group just because it is so large. Referring all women with ASC-US to colposcopy is not efficient, because of the large number of women without precancer or anything related to cervical carcinogenesis in that group and the limited colposcopy capacity in most health systems. The first application of HPV testing in cervical cancer screening was for triage of ASC-US cytology. The risk of HPV-positive ASC-US is equal to that of LSIL, justifying referral to colposcopy.[12–14] Triage of ASC-US with HPV testing has been part of cervical cancer screening guidelines for more than a decade and represents the first example of risk-based management recommendations in cervical cancer screening.[15–17]

Figure 1 shows the relevant groups of risk of cervical precancer with respect to clinical management. Women testing negative in primary screening are in the lowest risk category and can return after regular screening intervals (minimal risk group). Women with ASC-US are at increased risk and cannot be returned to regular screening intervals, but since up to half are HPV-negative, their risk is not high enough for immediate referral to colposcopy (low risk group). Women with LSIL or HSIL are at high enough risk for referral to colposcopy (medium risk group), and women with HSIL are at high enough risk that allows to immediately treat women as an option (high risk group). Additional testing can move women from one group to another, e.g. HPV triage of ASC-US moves HPV-positive women from the low risk to the medium risk group.[12, 18]

The same thresholds can be used for other screening and triage assays. For example, women testing positive for HPV in primary screening are at increased risk, but there are too many women (and the likelihood of finding immediately the many associated small, early precancers is too low) to refer everybody to colposcopy (low risk group).

Importantly, there are no universal thresholds for any of these management strategies. Risk thresholds depend on many factors, including a societal perception of risk, established clinical practice, different weight on benefits and harms, health economic considerations, and health infrastructure. Consequently, the risk thresholds considered for different management options differ across healthcare settings. Also, the approach to setting risk thresholds differs. For example, in European guidelines, often a 10% risk of CIN3 is often proposed as the colposcopy referral threshold.[19] In the US, the principle of risk benchmarking is used: Risk of cervical precancer similar to a LSIL cytology result is used as a risk benchmark for colposcopy referral.[12, 18] The risk benchmarking approach is less susceptible to absolute risk differences between screening populations, since the relative order between the risk groups is very consistent. Despite these different approaches, the conclusions drawn from clinical trials and observational studies and the management approaches implemented have been very similar in the US and in Europe.[16,20, 21]

Options for primary screening for cervical precancers

There are now three major alternatives for cervical cancer screening: cytology, HPV testing, and cytology-HPV co-testing.[22] Cytology-based screening is still the most widely used modality. Cytology screening has lower sensitivity compared to HPV and co-testing, which results in lower reassurance against prevalent and incident precancers. Therefore, cytology-based screening needs to be repeated at shorter intervals to achieve good program sensitivity. Conversely, while HPV testing has high sensitivity and allows extending screening intervals, it can double the number of screen-positive women compared to cytology and it is not feasible to send all HPV-positive women to colposcopy. Figure 2 shows two algorithms for primary HPV based screening from the US and the Netherlands. Co-testing is currently recommended as the preferred screening option in the US, while it is not in Europe or Australia.[23] While it is the safest of all primary screening option, the additional reassurance provided by cytology over HPV testing alone is actually quite limited,[24, 25] suggesting that many unnecessary tests are performed.

For primary HPV screening, all HPV positive results need additional triage, but the management of HPV positive women with negative cytology is particularly challenging, since the risk in this group is too high to return women to regular screening, but too low for colposcopy referral. [15, 24, 26, 27] Importantly, triage cannot be evaluated in isolation, but it needs to be considered in the context of primary screening and of downstream management options.

Triage tests

Cytology

Cervical cytology is an option for triage of HPV-positive women. Restricting cytology to HPV-positive women eliminates the group of HPV-negative ASC-US results that has virtually no increased risk of precancer or cancer while constituting a major proportion of abnormal cytology results in primary screening. Thus, with increased baseline risk in HPV-positive women, and without a large group of unspecific results (HPV-negative ASC-US excluded), cytology is expected to have better performance for triage of HPV-positive

women compared to primary screening. A recent study suggested that cytology is more sensitive for detection of precancer when it is evaluated with knowledge of HPV status. [28] However, another study revealed substantial loss in specificity.[29] These contrasting findings are not surprising given the subjective character of cytology.

HPV-positive women triaged with cytology who are found to have ASC-US or worse cytology are referred to colposcopy, while HPV-positive women with normal cytology require re-testing at a shorter interval than primary screening.[20] In an alternative screening algorithm recently approved by the FDA, cytology is recommended to triage HPV-positive women who are negative for HPV16 or HPV18 (see next section, Figure 2). In this algorithm, for HPV-positive women, those who are HPV16/18-negative and cytology-negative are followed up after 12 months.[30] Other algorithms for primary HPV screening are currently under development in several countries.

The altered composition of cytology results among HPV-positive women also opens up new possibilities for automated evaluation of cytology slides. A recent feasibility study demonstrated that automated cytology evaluation based on the FocalPoint system may achieve a performance close to that of unaided microscopically interpreted cytology. This could lead to development of integrated approaches using HPV testing and automated cytology, minimizing the subjectivity of cervical cancer screening.

HPV genotyping

Natural history studies have demonstrated that the risk of precancer and cancer varies substantially for different carcinogenic HPV types, with HPV16 being associated with by far the highest risk of cancer and precancer.[31] HPV18 is the second most important type for cancers, but is less prevalent in precancers. Several types compete for the next most important roles, with some geographic variation.[32] HPV18 and HPV45 are considered particularly important because of their association with cervical adenocarcinoma. In contrast, several other types that are included in the 13 or 14 carcinogenic types detected by commercial HPV assays, such as HPV56 and HPV51, have a very low risk of cervical cancer and precancer. Because of this remarkable risk stratification, HPV genotyping has been evaluated for triage of HPV-positive women. The risk of precancer in HPV16/18 positive women (without cytology testing) surpasses that of women with pooled HPV-positive ASC-US.[33, 34] In current US cervical cancer screening guidelines for co-testing, HPV16/18 triage is recommended for HPV-positive women with normal cytology (NILM).[20] If positive for HPV16 or HPV18, immediate referral to colposcopy is recommended. The cobas assay that was recently approved for primary screening, includes genotyping for HPV16 and HPV18 in two separate channels, thereby providing an immediate triage option for HPV-positive women. In the FDA-approved algorithm for primary HPV screening, women testing positive for HPV16 or HPV18 are referred to immediate colposcopy, while the remaining HPV-positive women are recommended to have cervical cytology triage. [30] Other assays, partially or completely fulfilling requirement for cervical cancer screening, also provide immediate genotyping to various extents.[35–40]

While HPV genotyping can predict an increased risk of precancer concurrently and over the next years, it cannot definitively differentiate between a transient infection, an early

persistent infection that may develop to precancer, and a prevalent precancer. While these conditions represent very different steps in the natural history of cervical precancer and cancer, the consequence of HPV16/18 positivity in current clinical algorithms is immediate referral to colposcopy. As a result, some women are referred to colposcopy who are at increased long-term risk of precancer, but who have no visible or only very small lesions at the time of colposcopy. This is a challenge for colposcopists and may motivate excessive biopsy protocols to counter the limited colposcopy performance in this population, possibly yielding overdiagnosis and increased morbitity.[41] As HPV-vaccinated women form an increasing proportion of women in the screened population, the utility of genotyping for 16/18 and immediate referral will be reduced as their prevalence decreases.

p16/Ki-67

The cellular protein p16 is upregulated in transforming HPV infections and serves as an HPV-type independent biomarker of cervical precancer. p16 immunostaining protocols have been developed for cervical cytology and histology.[42] A study nested in a large Italian trial of HPV-based primary screening evaluated the performance of p16 cytology for triage of HPV-positive women. p16-positive women were at high enough risk for immediate colposcopy referral, while p16-negative women had low enough risk that they were judged in the Italian context to not need repeat testing for at least two years.[43, 44] The original p16 assay evaluated in the Italian trial required morphological evaluation of p16-stained cells, which is subjective and time-consuming. To overcome this limitation, the assay was expanded by adding Ki-67, a cell proliferation marker. In this assay, the detection of at least one cell stained for both p16 and Ki-67 is considered a positive result.[45] It has been demonstrated that the dual stain assay can be implemented with little training and that it is highly reproducible.[46, 47] As part of a HPV-cytology co-testing demonstration project, the dual stain was evaluated for triage of HPV-positive cytology-negative women.[48] In a large study within the Kaiser Permanente Northern California (KPNC) Health System, the dual stain was evaluated for triage of HPV-positive women. At KPNC, cytology is evaluated with knowledge of HPV status, numerous quality checks, and FocalPoint adjunct testing: as a result, it has a very high sensitivity. Compared to the cytology at KPNC, the dual stain had both higher sensitivity and higher specificity with lower colposcopy referral rates. HPV-positive women testing positive for the dual stain are at high enough risk for immediate referral to colposcopy, while dual-stain negative women could be retested at extended intervals, similar to the observations in the Italian study of p16 alone.[49] Similar results were found for triage of HPV-positive, cytology-negative women. Automated evaluation of dual stain slides may lead to further improvement of dual stain performance. Initial studies have demonstrated the feasibility of whole-slide scanning and automated detection of dual stain positive slides.[50, 51]

Host methylation

Increased methylation of host genes has been observed in women with precancer and cancer compared with those with acute HPV infection. Several of these genes have been evaluated as candidates for triage of HPV-positive women. Three methylation markers, CADM1, MAL, and miR-124-2 have been evaluated extensively in cervical cancer screening studies in the Netherlands. Across these studies, methylation testing has demonstrated similar

performance as cytology for triage of HPV-positive women.[52, 53] Molecular testing for methylation markers offers several advantages over cytology triage: Molecular tests are not subjective, may offer higher throughput, and can be conducted from a variety of specimen types, including self-sampling specimens. A randomized controlled trial in women undergoing self-sampling demonstrated that triage of HPV-positive women using MAL and mir-124-2 methylation testing was non-inferior to cytology based on a physician-collected specimen.[54] This offers the possibility of conducting primary screening and triage from a self-collected specimen, averting the need for an office visit to collect a cytology specimen for triage of HPV-positive women. The list of potential host methylation markers for triage of HPV-positive women keeps growing, with several candidates reported recently,[55, 56] and more methylation profiling studies are underway. Finding an optimal combination of methylation markers needs consideration of the sample type (self vs. physician-collected), the assay platform, and specific applications (e.g., triage of all HPV-positive women or triage of HPV-positive, HPV16/18 negative women). The increased post-test risk for precancer in methylation+/hrHPV+ women is a promising characteristic underlying its positive triage capacity. However, more longitudinal research is needed to demonstrate the longitudinal safety of a methylation-negative result.

Viral methylation

HPV genome-wide methylation studies of carcinogenic HPV types have demonstrated an increase of viral methylation associated with precancer and cancer compared with HPV infection.[57–59] Across a number of carcinogenic types, a characteristic pattern was observed with increased methylation particularly in the E2, L2, and L1 regions. Individual CpG sites from these regions showed a good discrimination between HPV infection and precancer, suggesting that HPV methylation testing could serve as triage marker for HPV-positive women. A combined methylation assay for HPV16, HPV18, HPV31 and three host genes has shown promise for triage of HPV-positive women, but further studies are needed, particularly in comparison to established markers, to assess the value of HPV methylation for triage. If successful, a HPV methylation assay that covers the majority of carcinogenic types could provide combined HPV test results, HPV genotyping, and HPV methylation results in a single assay that would provide risk stratification for placing women in all but the highest risk groups. A methylation-based triage assay would not require cytology infrastructure and could be adapted to high- and low-resource settings, with the possibility of using self-collected samples.[60]

HPV E6 protein

Expression of the viral oncogenes E6 and E7 is much higher in cervical precancers compared to transient HPV infections. A lateral flow assay was developed to detect the E6 oncoprotein from the most important carcinogenic types, HPV16, 18 and 45 (OncoE6 test). A pilot study evaluating the assay showed higher specificity, but lower sensitivity for the detection of CIN3+ compared to HPV-DNA tests. Clinical evaluation of the assay in rural China showed a high positive predictive value for detection of CIN3+, but the sensitivity was limited at only 54%.[61, 62] Given the high PPV for precancer and the limited laboratory equipment needed for the assay, it may have triage applications in low-resource settings, following up on HPV assays targeted to low-resource settings like careHPV.

Other assays

Several other markers have been proposed for specific detection of cervical precancers, including assays that detect chromosomal abnormalities,[63] HPV oncogene mRNA,[64, 65] or other cellular proliferation-associated proteins.[66] However, clinical data are very limited for these assays and they have not been formally evaluated in a HPV triage setting so far.

Evaluation and implementation of triage tests

An optimal integrated screening and triage strategy should reassure the vast majority of women that they are at very low risk of cervical cancer, send the women at highest risk to colposcopy at the right time, when disease can be colposcopically detected, [67] and minimize the intermediate risk group that requires frequent re-testing and repeat colposcopy. Studies will need to evaluate the effect of increasingly vaccinated populations on screening and triage strategies, by studying screening and triage performance in truly vaccinated women and by simulating different vaccination rates using HPV genotyping. Low-resource settings have different needs for triage strategies, because the capacity for treatment is limited and getting women back for repeat testing is challenging or impossible. Therefore, in low-resource settings, there is a focus on strategies with higher positive predictive values to identify women in need of immediate interventions. Self-sampling can increase screening coverage both in high- and low-resource settings, but it eliminates any cell-based screening and triage options. Finally, apart from these considerations, integrated screening and triage strategies may be constrained by corporate strategies that can limit the optimal combination of different screening and triage strategies, due to incompatibilities of the sampling and assay platforms.

With so many options available, large head-to-head comparisons of promising markers are needed to identify the best screening and triage strategies. Several of these efforts are currently underway in different parts of the world, including the FRIDA study in Mexico, ESTAMPA in several Latin American countries, and a new large effort conducted by the NCI in the United States. Another approach for validation of triage markers is the testing of well conserved specimens stored in a biobank using case-control designs sampling HPV+ women with subsequent CIN3+ and HPV-positive controls which did not develop significant disease.[68] Together, these studies will be able to provide important guidance on optimal screening and triage strategies for different settings, from low-resource countries without current screening to industrialized countries with long-term established screening programs.

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Highlights

- Evidence suggests that primary HPV testing can be more effective than cytology
- Additional triage tests are needed to decide who among the HPV-positive women requires further management or treatment
- Evaluation of triage test candidates is based on absolute risk estimates compared to established clinical action thresholds
- An optimal integrated screening and triage strategy should reassure the vast majority of women that they are at very low risk of cervical cancer while sending the women at highest risk to colposcopy and treatment

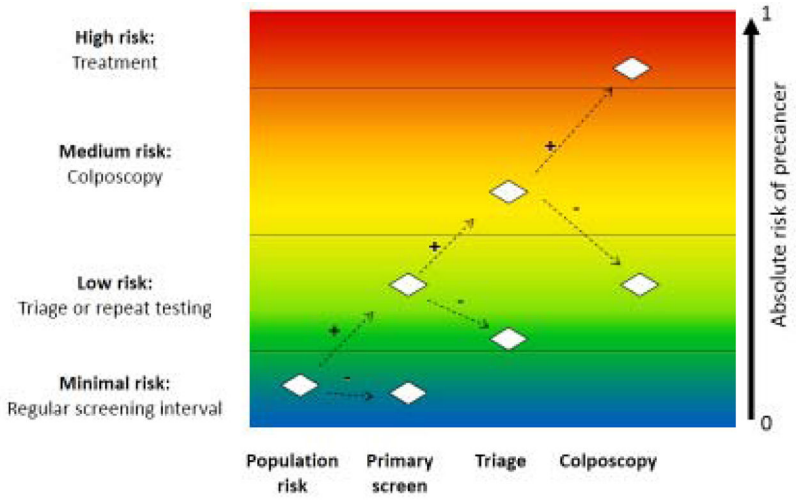

Figure 1.

Risk-based management in cervical cancer screening.

The absolute risk of precancer from 0 to 1 is shown on the y-axis. Clinically relevant risk strata are shown, ranging from minimal to high risk of precancer. On the population level, the risk of precancer is very low. A positive primary screening test (e.g. HPV testing) increases the risk of precancer, but not high enough to refer women to colposcopy. A triage test is applied in women with a positive screening result and should distinguish women who need colposcopy referral from women who need continued surveillance or who can be released to primary screening. At colposcopy, women with precancer are identified who need treatment. An idealized example is shown. The risk thresholds may vary between health systems and populations. Different primary screening and triage tests may group women in different risk strata.

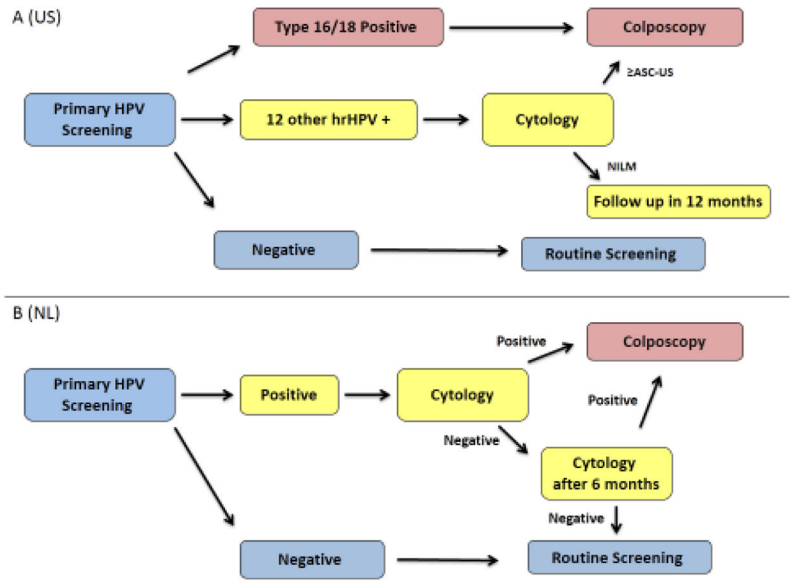

Figure 2.

Two examples of algorithms for primary HPV screening

A: In the United States, primary HPV screening using the cobas assay (Roche) was approved by the FDA. Women negative for HPV will go back to routine screening with 3-year intervals. Women with HPV16/18-positive results are referred to colposcopy immediately. Among women positive for other carcinogenic types, cytology is performed and women with ASC-US or higher are referred to colposcopy, while women with normal cytology are re-tested after 12 months B: In the Netherlands, a primary HPV screening approach has been proposed and approved for implementation. All HPV-positive women are triaged with cytology. If cytology is positive, women are referred to colposcopy. Women with negative cytology will have repeat cytology after 6 months and are referred to colposcopy when cytology is positive, otherwise they are released back to routine screening. For women with a previous negative HPV screening test, the screening interval is 5 years if <45 years and 10 years for women aged >= 45. For women 45 years or older with a positive HPV test but with double negative cytology triage, the next invitation will be sent after 5 years instead of after 10 years.