Molecular-based triage on hrHPV-positive women to detect cervical cancer

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CHALLENGES WITH CURRENT CERVICAL CANCER SCREENING METHODS

Cervical cancer remains the fourth most common cancer type in women, with over 500,000 cases, and 300,000 deaths in 2018 worldwide[1]. Regular cytology-based screening programs have shown to increase detection of precancer lesions, resulting in a 50% reduction of premature cervical cancer deaths. However, despite the benefits, many women are often reluctant to undergo a Pap smear and uptake varies greatly from region to region[1].

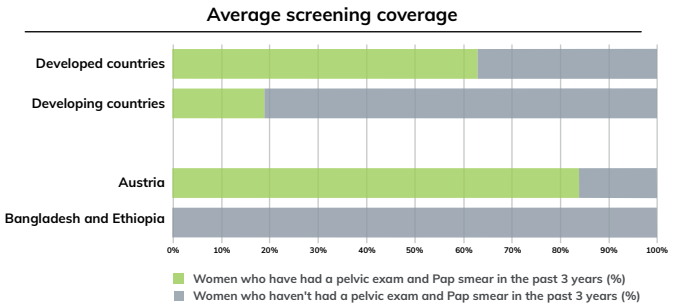


Figure 1: Average screening coverage

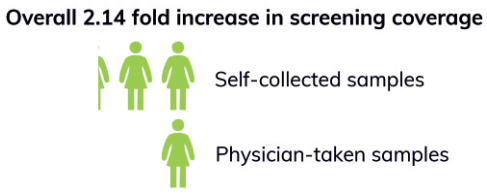
Some reasons women are reluctant to undergo a Pap smear is because the process is invasive and can be associated with physical discomfort. Women have also listed lack of time, inconvenient clinic hours or lack of transportation as reasons to avoid regular testing. Additionally, religion and culture can also influence participation for gynecological examinations[1].

A NEW ERA IN CERVICAL CANCER SCREENING USING URINE AS A SAMPLE TYPE

Most cases of cervical cancer have been linked to types of high-risk Human Papillomavirus (hrHPV), a common sexually transmitted infection. Using primary hrHPV-based testing as a screening tool for cervical cancer is promising[2]. Additionally, compared to cytology, hrHPV DNA detection can be more effective and efficient for preventing cervical cancer and the associated mortality[3].

However, as most cervical cancers cases occur in women who do not participate in any sort of screening, switching to HPV-based screening may not necessarily lead to a substantial reduction in cases without improving participation rates[4].

The benefit of HPV-based testing, compared to cytology is that it allows the potential of self-sampling to initial non-participants, thereby reaching populations that would otherwise not be screened[5]. Self-sampling techniques, including brush-based cervical-vaginal self-sampling methods and urine-based sampling have shown potential in HPV detection and have proven effective in increasing participation and screening coverage of target populations[2]. A meta-analysis reported an overall 2.14-fold increase in screening coverage due to the use of self-collected samples, highlighting the impact self-sampling methods could have in cervical cancer screening uptake[6].



Women have shown a significantly higher preference to urine as a sample type for hrHPV testing as it is completely non-invasive and allows for repetitive sampling if needed[3].

LIMITATIONS OF PRIMARY HPV SCREENING

An important limitation of primary hrHPV-testing is that it has a 2.5 - 4% lower specificity for cervical (pre)cancer compared to primary Pap smear cytology testing[7].

Most hrHPV-positive women have transient (so-called productive) infections, which will not result in any clinical symptoms and the virus will clear spontaneously. Only a minority of hrHPV-positive women (20%) harbor a transforming infection, in which the normal viral life cycle is aborted and the viral early genes *E6* and *E7* are overexpressed in proliferating cells, leading to the development of clinically relevant cervical intraepithelial neoplasia (CIN) for cervical cancer[7].

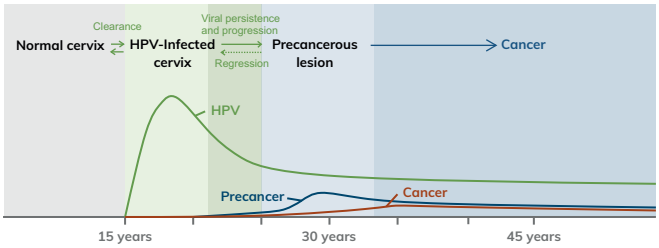


Figure 2: peak prevalence of transient infections
Figure adapted from PMID: 16291978

Therefore, referral of all hrHPV-positive women to a gynecologist for colposcopy would be unnecessary, leading to overdiagnosis and overtreatment, adding to unnecessary costs and higher risks of obstetric complications[7].

TRIAGE TESTS FOR HPV-POSITIVE WOMEN

To reduce overdiagnosis and overtreatment of clinically non-relevant hrHPV infections, hrHPV testing programs must incorporate triage before colposcopy referral[2]. State-of-the-art triage markers for hrHPV infections in cervical samples can be divided in two major groups: microscopy-based biomarkers and molecular biomarkers.

	Microscopy-based biomarkers	Molecular biomarkers
Type of assay	• Conventional cytology • p16INK4a staining • Ki-67/p16 dual stained cytology	• HPV16/18 genotyping • HPV E6 protein • E6/E7 mRNA based biomarkers • miRNA based biomarkers • Viral load • Integration of HPV DNA into host DNA • Methylation of host and viral genes
Sample type	• Cell rich sample material	• Cervicovaginal material
Pros and Cons	• Subjective • Need for trained cytotechnologist	• Objective • Highly reproducible • Suitable for high throughput screening
Self-collection	NA	Self-collected cervicovaginal material

Table 1: Overview of triage tests for HPV positive women
Based on PMID: 28689156

A general limitation of microscopy-based strategies is they can only be adequately performed on cervical scrapes collected by health-care professionals. As highlighted, fear or embarrassment related to the cervical scraping procedure and other practicalities reasons related to visiting a physician prevent eligible women from participation in cytology-based screening in many countries[7,8].

Alternatively, molecular biomarkers offer a high-throughput capacity and reproducible results compatible with self-sampled specimens. Consequently, they address non-adherence to screening and distinguish between clinically relevant, transformative hrHPV infections from productive infections[3].

MOLECULAR BIOMARKERS

HPV GENOTYPING

The detection of specific hrHPV types, referred to as HPV genotyping, can be used for triage given the carcinogenic potential differs between hrHPV types. HPV16 is the genotype associated with the highest (pre)cancer risk, followed by HPV18[7].

Together, HPV16 and HPV18 are responsible for the development of 52–64% of CIN3 lesions and approximately 70% of cervical carcinomas. Women with a single HPV16 and/or HPV18 positive test have a five-fold increased risk of developing ≥CIN3 during the following 10 years compared to women infected with other hrHPV types. Therefore, the detection of HPV16 and/or HPV18 might serve to identify women with an elevated risk of cervical (pre)cancer[7].

RNA-BASED BIOMARKERS

In contrast to DNA based methods, in which the genomic absence or presence of certain hrHPV types is detected, RNA-based assays detect changes or differences in gene expression profiles related to cancer development. Messenger RNAs (mRNAs) play an important role in protein synthesis, whereas their function is regulated by small non-coding micro RNAs (miRNA). Overexpression of *E6/E7* mRNA has been associated with cellular transformation and increased severity[3].

PROTEIN BIOMARKERS

Elevated levels of *E6/E7* proteins represent important cervical-specific biomarkers and are the main mediators of hrHPV induced malignant transformation[3].

METHYLATION BIOMARKERS

An important asset of host cell DNA methylation biomarkers is their ability to distinguish productive from transforming hrHPV infections, especially those with a high short-term risk of cancer progression. Methylation levels have also been found to rise in parallel with increased severity and duration of disease[3].

Many studies have found a strong association between the methylation of host and viral genome with the development of CIN2, CIN3, and cancer. Various combined quantitative DNA methylation assays are currently under evaluation[2].

Host gene DNA methylation

Increased methylation of host genes has been observed in women with (pre)cancer and cancer compared to those with acute HPV infection[9]. Methylation levels of certain host cell genes (such as *CADM1*, *MAL*, *mir124-2*, *JAM3*, *TERT*, *C13ORF*, *EPB41L3*, *ANKRD18CP*, *GFRA1*, *CDH6*, *LHX8*, *GATA4*, *PRDM14*, and *FAM19A4*) in cervical scrapes have been shown to increase with the severity of underlying cervical (pre)cancer[7]. In the Predictors 1 study, six human genes: *EPB41L3*, *EDNRB*, *LMX1*, *DPYS*, *MAL* and *CADM1* showed significantly elevated methylation in *CIN2* and *CIN3* (CIN2/3) versus ≤CIN1 ($p < 0.01$)[9].

Recently, the FAM19A4/miR124-2 methylation test has been introduced as a commercial CE-IVD triage test for screening and diagnostic purposes. In a large European study, this new standardized test for the methylation biomarker combination FAM19A4/miR124-2 did show results that were equal to or better than triage by cytology, in determining which hrHPV-positive women should be referred for colposcopy[10].

Viral gene DNA methylation

HPV genome-wide DNA methylation studies of carcinogenic HPV types have demonstrated an increase of viral DNA methylation associated with (pre)cancer and cancer compared with HPV infection[11].

However, in contrast to host gene DNA methylation, a unique assay needs to be developed and validated for each hrHPV type due to the genetic variation between HPV genotypes[3].

Although many DNA methylation targets have been identified, extensive validation for clinical usability and the development of commercial/research methylation assays has only been performed for few targets.[3]

URINARY METHYLATION-BASED TRIAGE

DNA methylation marker testing is feasible on urine and enables the detection of cervical cancer. Analysis of 6 DNA methylation markers (*FAM19A4*, *GHSR*, *PHACTR3*, *PRDM14*, *SST* and *ZIC1*) showed a strong correlation between DNA methylation levels detected in urine sediment versus native urine and cervical scrapes, indicating the potential of urinary hrHPV DNA and DNA methylation testing for the detection of cervical cancer[12].

Detection of both hrHPV DNA and molecular biomarkers in the same urine sample offers the potential to reduce efforts and costs associated with cervical cancer screening and to encourage women who are currently reluctant to participate in screening programs.

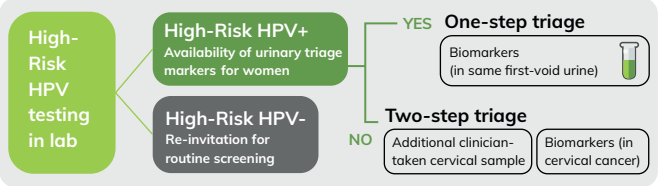


Figure 3: HPV triage
Figure adapted from Van Keer et al. PMID: 28689156

First-void urine has shown to contain higher concentrations of HPV DNA than subsequent fractions[13]. Novosanis' urine collection device, Colli-Pee® allows for volumetric and standardized first-void urine collection and stabilization.

CONCLUSION

As not all HPV infections lead to cervical cancer, it is important to identify and follow-up only those HPV-positive women with clinically relevant cervical cancer to avoid overreferral, and overtreatment. Currently, this requires further cytology and repeated testing, resulting in loss of follow-up.

Molecular-based triage methods on HPV positive women using self-collected samples, such as urine, offers potential to reach more women and distinguish clinically relevant infections. First-void urine sampling also offers the ability to test both primary hrHPV and biomarkers in the same sample, avoiding repeated sample collection.

Reach out to Novosanis to find out more!

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