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Comparing triage algorithms using HPV DNA genotyping, HPV E7 mRNA detection and cytology in high-risk HPV DNA-positive women

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ABSTRACT

Background: High-risk human papillomavirus (hrHPV) DNA positive women require triage testing to identify those with high-grade cervical intraepithelial neoplasia or cancer (≥CIN2).
Objective: Comparing three triage algorithms (1) E7 mRNA testing following HPV16/18/31/33/45/52/58 genotyping (E7 mRNA test), (2) HPV16/18 DNA genotyping and (3) cytology, for ≥CIN2 detection in hrHPV DNA-positive women.
Study design: hrHPV DNA-positive women aged 18–63 years visiting gynecology outpatient clinics were included in a prospective observational cohort study. From these women a cervical scrape and colposcopy-directed biopsies were obtained. Cervical scrapes were evaluated by cytology, HPV DNA genotyping by bead-based multiplex genotyping of GP5+6+-PCR-products, and presence of HPV16/18/31/33/45/52/58 E7 mRNA using nucleic acid sequence-based amplification (NASBA) in DNA positive women for respective HPV types. Sensitivities and specificities for ≥CIN2 were compared between E7 mRNA test and HPV16/18 DNA genotyping in the total group ($n = 348$), and E7 mRNA test and cytology in a subgroup of women referred for non-cervix-related gynecological complaints ($n = 133$).
Results: Sensitivity for ≥CIN2 of the E7 mRNA test was slightly higher than that of HPV16/18 DNA genotyping (66.9% versus 60.9%; ratio 1.10, 95% CI: 1.0002–1.21), at similar specificity (54.8% versus 52.3%; ratio 1.05, 95% CI: 0.93–1.18). Neither sensitivity nor specificity of the E7 mRNA test differed significantly from that of cytology (sensitivity: 68.8% versus 75.0%; ratio 0.92, 95% CI: 0.72–1.17; specificity: 59.4% versus 65.3%; ratio 0.91, 95% CI: 0.75–1.10).
Conclusion: For detection of ≥CIN2 in hrHPV DNA-positive women, an algorithm including E7 mRNA testing following HPV16/18/31/33/45/52/58 DNA genotyping performs similar to HPV16/18 DNA genotyping or cytology.

1. Background

The upcoming implementation of hrHPV DNA testing in cervical screening in western countries follows strong evidence that a persistent hrHPV infection is necessary for the pathogenesis of cervical cancer [1]. The main drawback of primary hrHPV DNA testing is its 2–5% lower specificity for cervical intraepithelial neoplasia grade 2 or worse (≥CIN2) compared to current cytology-based screening [2]. Therefore, triage algorithms that allow identification of women with clinically relevant lesions in need of treatment are required. Repeat cytology testing has been described as a cost-effective triage strategy [3,4]. However, cytology triage requires repeat testing which is associated with considerable loss-to-follow-up, is subjective, and requires high overhead resources to ensure high quality

Abbreviations: AIS, adenocarcinoma in situ; BMD, borderline or mild dyskariosis; CI, confidence interval; CIN, cervical intraepithelial neoplasia; DNA, deoxyribonucleic acid; EIA, enzyme immunoassay; HPV, human papillomavirus; hrHPV, high risk human papillomavirus; LLETZ, large loop excision of the (cervical) transformation zone; mRNA, messenger ribonucleic acid; NASBA, nucleid acid sequence based amplification; NPV, negative predictive value; ntd, not to determine; PCR, polymerase chain reaction; PPV, positive predictive value; snRNP, small nuclear ribonucleoprotein; UE, uterus extirpation.

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[5,6]. Therefore, more objective (molecular) triage markers, suitable for automated and standardized processing, are warranted.

HPV16/18 DNA genotyping is suggested for triage of hrHPV-positive women testing cytology negative [7]. HPV16 and HPV18 are associated with approximately 70% of cervical cancers [8]. Therefore, HPV16/18 DNA genotyping identifies a subgroup of hrHPV-positive women with an increased risk of ≥CIN2 [9,10]. However, HPV16/18 DNA genotyping fails to detect ≥CIN2 lesions associated with non-16/-18 types and therefore still needs to be combined with cytology.

An alternative molecular tool is the detection of hrHPV E6/E7 mRNA [11–34]. The activity of hrHPV oncogenes E6 and E7 is essential for initiation and maintenance of the malignant phenotype [35]. Previous studies have shown that the level of hrHPV E6/E7 transcripts correlates with severity of histological abnormality [11–34]. Analysis for E6/E7 transcripts of five hrHPV types (HPV16/18/31/33/45) by commercially available nucleic acid sequence-based amplification (NASBA) tests (PreTect HPV-Proofer, Norchip and NucliSENS EasyQ test, Biomerieux) has been found to identify risk groups amongst hrHPV DNA-positive women with normal cytology [28] and minor cytological abnormalities [34] in need of immediate colposcopy. However, despite its relatively high specificity, the sensitivity of this E6/E7 NASBA test is limited [13,15,16,18,21,24–26,28,34], implying that follow-up of test-negatives remains necessary. Under the assumption that the sensitivity of currently available E6/E7 NASBA tests is restricted by the limited number of targeted hrHPV types, we developed a NASBA assay that allows detection of E7 transcripts of a broader spectrum of hrHPV types (i.e., HPV16/18/31/33/45/52/58). With the addition of HPV52 and HPV58, this assay targets the seven HPV types responsible for development of over 90% of cervical cancers [8] and 74% of ≥CIN2 lesions [36].

2. Objective

We compared the performance of three triage algorithms for ≥CIN2 detection on cervical scrapes of hrHPV DNA-positive women, being (1) E7 mRNA testing following HPV16/18/31/33/45/52/58 DNA genotyping, (2) HPV16/18 DNA genotyping and (3) cytology. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and referral rate were evaluated.

3. Study design

3.1. Study subjects

Participants were recruited amongst women visiting gynecology outpatient departments of six Dutch hospitals in December 2010–December 2013. The study was approved by the VU University Medical Center Ethical Committee. All women 18–70 years, regardless of their reason for visiting the gynecologist, were invited to participate in this prospective study. Exclusion criteria included history of treatment for ≥CIN2, current cancer, pregnancy or lactation. In total, 1870 women provided informed consent and performed a cervicovaginal lavage with a self-sampling device (Delphi); lavage material was tested for hrHPV DNA using GP5+/6+-PCR-enzyme immunoassay (GP5+/6+ PCR EIA; Diassay B.V., Rijswijk, Netherlands) at VU University medical center (Amsterdam, Netherlands). Women who tested positive for hrHPV DNA on the self-sample were subjected to physician-collection of a cervical scrape using a Cervex-Brush (Rovers, Oss Netherlands) or a cytobrush, which was placed in Thinprep Preservcyt (Hologic, Marlborough, USA). Next, colposcopy was performed, with collection of cervical biopsies from every visible lesion and, in case no lesions were visible, two mandatory biopsies (6 and 12 o'clock). In case of an invisible squamocolumnar junction, an endocervical curettage was performed.

3.2. Cytology

Cervical scrapes were classified according to the CISOE-A classification (reporting on composition, inflammation, squamous, other and endometrium, endocervical cylindrical epithelium, and adequacy) used in the Netherlands. The results can be translated into the Bethesda classification [37], in which borderline or mild dyskaryosis (BMD) equals ASC-US/ASC-H/LSIL, and >BMD equals high-grade squamous intraepithelial lesion (HSIL). Cytotechnicians were aware of the hrHPV-positive status of the scrapes in this study.

3.3. HPV DNA genotyping

DNA was isolated from 1/10th of cervical scrapes using the Nucleo-Spin 96 Tissue kit (Macherey-Nagel, Germany) and a Microlab Star robotic system (Hamilton, Germany) according to manufacturers' instructions, and subjected to GP5+/6+-PCR-EIA [38,39]. Genotyping for HPV16/18/31/33/35/39/45/51/52/56/58/59/66/68 DNA was performed using a microsphere bead-based assay (Luminex) as previously described [40].

3.4. HPV16/18/31/33/45/52/58 NASBA

Samples that tested positive for DNA of HPV16/18/31/33/45/52/58 were subjected to type-specific E7 mRNA detection using NASBA. Hereto, total RNA was extracted from 1/10th of the cervical scrape by the NucliSENS easyMAG procedure according to manufacturer's recommendations (bioMerieux, Boxtel, Netherlands) [28]. Subsequent type-specific NASBA reactions were performed using NucliSENS EasyQ reagents (bioMerieux) and a Microplate Fluorescence Reader FL600 (BioTek Instruments, Winooski, USA). Primers and beacons are depicted in Table 1. Primer pairs targeting mRNA from the human U1 small nuclear ribonucleoprotein (snRNP)-specific protein A (U1A) were included for all samples to ensure sample mRNA integrity. Fluorescence intensity data were recorded real-time during NASBA reaction. Samples showing no NASBA amplification for U1A and no positivity for HPV targets were regarded 'invalid' and excluded from analyses. In case of a positive U1A result and/or positivity for one or more HPV targets, samples were considered valid. To evaluate run validity, positive controls for U1A and HPV16/18/31/33/45/52/58 were included.

3.5. Histology

Biopsies taken at colposcopy were histologically assessed locally in participating hospitals and classified as normal (CIN0), CIN1, CIN2, CIN3 or invasive cancer, according to international criteria [41]. Most women with CIN2 (73%) and all women with CIN3 underwent large loop excision of the transformation zone (LLETZ) or cervical conisation. Women diagnosed with cervical cancer were treated according to standard procedures in the Netherlands.

3.6. Statistical analysis

The primary study endpoint was the histological outcome of the colposcopy-directed biopsy, or, if classified worse, the histology result of the specimen excised by LLETZ or conisation. The primary study outcome was ≥CIN2. Secondary study outcome was ≥CIN3. Sensitivity, specificity, PPV, NPV and referral rate for ≥CIN2 and ≥CIN3 detection were calculated for each triage algorithm. Given high type concordance between DNA and mRNA