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.

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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.
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 oncoproteins 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) have been found to identify risk groups amongst hrHPV DNA-positive women with normal cytology [28] and minimal 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 GPS+/6+–PCR-enzyme immunoassay (GPS+/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 Micro- Lab Star robotic system (Hamilton, Germany) according to manufacturers’ instructions, and subjected to GPS+/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 HPV16/18/31/33/35/45/52/58 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, Bextel, 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
assays [28], E7 mRNA testing was performed following a positive HPV16/18/31/33/45/52/58 genotype test only, and samples containing non-HPV16/18/31/33/45/52/58 types were considered ‘mRNA negative’. Comparisons of sensitivity, specificity and referral rates were performed using McNemar chi-squared tests. Comparison to cytology was restricted to women referred to the gynecologist because of non-cervix-related gynecological complaints, i.e., those referred on the basis of abnormal cervical cytology were excluded, to avoid bias towards a positive outcome (subgroup analysis).

4. Results

A flowchart of the study population is shown in Fig. 1. Of 1870 eligible women, 497 (27%) tested hrHPV DNA-positive on the self-sample and were invited for further study participation. During the study, 58 women (12%) were excluded for various reasons (Fig. 1), leaving 439 women from whom a cervical scrape was obtained. Women who either tested hrHPV DNA-negative on the cervical scrape (47/439; 11% – indicating that these women had cleared the virus in the weeks between self-collection and physician-taken cervical scrape), or who had an invalid hrHPV DNA result (2/439; 0.5%) did not undergo further triage tests. From the remaining 390, those with an invalid NASBA result (36/390; 9%), or invalid cytology (6/390; 2%) were excluded from further comparative analyses, leaving 348 hrHPV-positive participants (median age 31.6, range 18–63 years).

Cytology was abnormal (≥BMD) in 67% (234/348) of participants, HPV16/18 was detected in 53% (186/348), and HPV16/18/31/33/45/52/58 in 74% (259/348), with presence of E7 mRNA in 190 women of the latter group (i.e., 54% (190/348) of the total population).

A summary of histological endpoints is depicted in Fig. 1. Of 348 participants, 151 women (43%) had a ≥CIN2 and 80 women (23%) had a ≥CIN3, including two carcinomas. The remaining women had no histomorphological abnormalities or CIN 1 (CIN0/1).

As shown in Table 2, cytology was abnormal in 53% of CIN0/1 (104/197), 85% in CIN2 (60/71) and 87% in CIN3 (68/78). HPV16/18 DNA positivity was 48% both in women with CIN0/1 (94/197) and those with CIN2 (34/71) and increased to 73% (57/78) in participants with CIN3. The algorithm of E7 mRNA testing following HPV16/18/31/33/45/52/58 genotyping was positive in 45% (89/197) of CIN0/1, 55% (39/71) of CIN2 and increased to 78% (61/78) in CIN3. Of the two women with cervical cancer, one tested positive by all three algorithms (i.e., HPV16 DNA- and mRNA-positive, and >BMD cytology), while the other was only positive by cytology (i.e., HPV39 and >BMD cytology). In total, 22 women (6% of the total population) tested positive for HPV52 and/or HPV58 E7 mRNA, exclusively. Of these women, 7 had CIN2 and 4 had CIN3; the remaining 11 women had a CIN0/1.

Screening features of the triage algorithms are summarized in Tables 3A and 3B. The sensitivity for detection of ≥CIN2 of the triage algorithm including E7 mRNA testing was slightly higher than that of HPV16/18 DNA genotyping (66.9% versus 60.9%; ratio 1.10, 95% CI: 1.0002–1.21), while specificities were similar (54.8% versus 52.3%; ratio 1.05, 95% CI: 0.93–1.18). When using ≥CIN3 as an endpoint, the sensitivity of the algorithm including E7 mRNA testing did not differ significantly from that of HPV16/18 DNA genotyping (77.5% versus 72.5%; ratio 1.07, 95% CI: 0.99–1.16), and neither did the specificity (both 52.2%; ratio 1; 95% CI: 0.90–1.11; Table 3A).

A comparison of the E7 mRNA HPV16/18/31/33/45/52/58 triage algorithm to sole DNA detection of the corresponding 7 hrHPV types showed that E7 mRNA detection had a lower ≥CIN2 sensitivity (66.9% versus 80.9%; ratio 0.83, 95% CI: 0.76–0.90), but a higher ≥CIN2 specificity (54.8% versus 30.5%; ratio 1.80, 95% CI: 1.52–2.13; Table 3A). This was similar for ≥CIN3 detection (sensitivity: 77.5% versus 88.8%; ratio 0.87, 95% CI: 0.80–0.95; specificity: 52.2 versus 29.9%, ratio 1.75, 95% CI: 1.52–2.02; Table 3A).

When focusing on HPV16/18, a similar specificity advantage of E7 mRNA detection over DNA detection was found for both ≥CIN3 (sensitivity 68.8% versus 72.5%; ratio 0.95, 95% CI: 0.89–1.007; specificity 64.2% versus 52.2%; ratio 1.23, 95% CI: 1.14–1.32; Table 3A) and ≥CIN2 (sensitivity: 55.6% versus 60.9%; ratio 0.91, 95% CI: 0.86–0.97; specificity: 66.0% versus 52.3%; ratio 1.26, 95% CI: 1.16–1.38; Table 3A).

For comparison of E7 mRNA testing with cytology, 215 women were excluded who were referred to the gynecologist because of cervix-related gynecological complaints, i.e., those referred on the basis of abnormal cervical cytology, leaving a subgroup of 133 women referred because of non-cervix-related gynecological com-
Fig. 1. Composition of study population and summary of histological endpoints. Study participants were recruited amongst women visiting gynecologic outpatient departments, regardless of their reason for referral to the gynecologist (abnormal cervical cytology or other, non-cervix-related, complaints).


In this subgroup, 44% (59/133) of women had abnormal cytology. The ≥CIN2 sensitivities of the E7 mRNA test algorithm and cytology were not significantly different (68.8% versus 75.0%; ratio 0.92, 95% CI: 0.72–1.17), and neither were the specificities (59.4% versus 65.3%; ratio 0.91, 95% CI: 0.75–1.10). Although the ≥CIN3 sensitivity of E7 mRNA testing was lower than that of cytology, this difference was not significant (75.0% versus 91.7%; ratio 0.82, 95% CI: 0.62–1.08). Also the specificities for ≥CIN3 did not differ significantly (55.4% versus 60.3%; ratio 0.92, 95% CI: 0.76–1.11; Table 3B). For both ≥CIN2 and ≥CIN3, sensitivities and specificities of HPV16/18 DNA genotyping tended to be lower than those of cytology, but none of these differences was statistically significant (≥CIN2: sensitivity 62.5% versus 75.0%; ratio 0.83, 95% CI: 0.65–1.07; specificity 56.4% versus 65.3%; ratio 0.86, 95% CI: 0.70–1.07. ≥CIN3: sensitivity 66.7% versus 91.7%; ratio 0.73, 95% CI: 0.51–1.04; specificity 53.7% versus 60.3%; ratio 0.89, 95% CI: 0.72–1.09; Table 3B).

We also evaluated the performance of several combinations (Table 3B). The combinations of cytology with the algorithm including E7 mRNA testing, and the combination of cytology with HPV16/18 DNA genotyping both resulted in high sensitivities (≥CIN2: 84.4% and 81.3%, respectively; ≥CIN3: 91.7% for both) at the cost of a considerable decrease in specificities (≥CIN2: 43.6% and 38.0%, respectively; ≥CIN3: 39.7% and 36.4%, respectively; Table 3B).

5. Discussion

In search of a triage algorithm for hrHPV DNA-positive women, we compared the additional value of E7 mRNA testing of seven hrHPV types, to that of HPV16/18 DNA genotyping and single baseline cytology. In the studied gynecologic outpatient population, the sensitivity and specificity of the algorithm including E7 mRNA testing following HPV16/18/31/33/45/52/58 DNA genotyping were similar to those of HPV16/18 DNA genotyping. In a subpopulation (without cytology selection bias), sensitivities and specificities of both E7 mRNA testing following HPV16/18/31/33/45/52/58 DNA genotyping and HPV16/18 DNA genotyping tended to be lower
### Table 3A
Clinical performance to detect ≥CIN2 or ≥CIN3 in hrHPV DNA-positive women. Total study population (n = 348).

<table>
<thead>
<tr>
<th>Triage algorithm</th>
<th>n1/N1</th>
<th>Sensitivity (95% CI)</th>
<th>n2/N2</th>
<th>Specificity (95% CI)</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
<th>Referral rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>≥CIN2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E7 mRNA testing following HPV16/18/31/33/45/52/58 DNA genotyping</td>
<td>101/151</td>
<td>66.9% (59.4–74.4%)</td>
<td>108/197</td>
<td>54.8% (47.9–61.8%)</td>
<td>53.2% (46.1–60.3%)</td>
<td>68.4% (61.1–75.6%)</td>
<td>54.6%</td>
</tr>
<tr>
<td>HPV16/18/31/33/45/52/58 DNA genotyping</td>
<td>122/151</td>
<td>80.8% (74.5–87.1%)</td>
<td>60/197</td>
<td>30.5% (24.0–36.9%)</td>
<td>47.1% (41.0–53.2%)</td>
<td>67.4% (57.7–77.2%)</td>
<td>74.4%</td>
</tr>
<tr>
<td>HPV16/18 DNA genotyping</td>
<td>84/151</td>
<td>55.6% (47.7–63.6%)</td>
<td>130/197</td>
<td>66.0% (59.4–72.6%)</td>
<td>55.6% (47.7–63.6%)</td>
<td>66.0% (59.4–72.6%)</td>
<td>43.3%</td>
</tr>
<tr>
<td>HPV16/18 DNA genotyping</td>
<td>92/151</td>
<td>60.9% (53.1–68.7%)</td>
<td>103/197</td>
<td>52.3% (45.3–59.3%)</td>
<td>49.5% (42.3–56.6%)</td>
<td>63.6% (56.2–71.0%)</td>
<td>33.4%</td>
</tr>
<tr>
<td>Cytology</td>
<td>130/151</td>
<td>86.1% (80.6–91.6%)</td>
<td>93/197</td>
<td>47.2% (40.2–54.2%)</td>
<td>55.6% (49.2–61.9%)</td>
<td>81.6% (74.5–88.7%)</td>
<td>67.2%</td>
</tr>
<tr>
<td>E7 mRNA testing following HPV16/18/31/33/45/52/58 DNA genotyping</td>
<td>62/80</td>
<td>77.5% (68.3–86.7%)</td>
<td>140/268</td>
<td>52.2% (46.3–58.2%)</td>
<td>32.6% (26.0–39.3%)</td>
<td>88.6% (83.7–93.6%)</td>
<td>54.6%</td>
</tr>
<tr>
<td>HPV16/18/31/33/45/52/58 DNA genotyping</td>
<td>64/80</td>
<td>68.8% (63.0–74.7%)</td>
<td>80/268</td>
<td>54.8% (48.4–61.3%)</td>
<td>34.9% (27.4–42.4%)</td>
<td>82.6% (75.8–89.4%)</td>
<td>48.1%</td>
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<tr>
<td>HPV16/18 DNA genotyping</td>
<td>58/80</td>
<td>62.5% (56.1–69.0%)</td>
<td>80/268</td>
<td>52.2% (46.3–58.2%)</td>
<td>36.4% (28.7–44.1%)</td>
<td>89.9% (83.6–96.2%)</td>
<td>43.3%</td>
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<td>67.2%</td>
</tr>
</tbody>
</table>

CIN – Cervical intraepithelial neoplasia (grade 2 or 3 or higher); CI – confidence interval; PPV – positive predictive value; NPV – negative predictive value; n1 – number of test positive disease cases; N1 – total number of disease cases; n2 – number of test negative non-disease cases; N2 – total number of non-disease cases.

a Out of 122 women who tested positive for HPV16/18/31/33/45/52/58 DNA.
b Including 60 women who tested negative for HPV16/18/31/33/45/52/58 DNA.
c Out of 71 women who tested positive for HPV16/18/31/33/45/52/58 DNA.
d Including 80 women who tested negative for HPV16/18/31/33/45/52/58 DNA.

### Table 3B
Clinical performance to detect ≥CIN2 or ≥CIN3 in hrHPV DNA-positive women. Subpopulation of women who were referred to the gynecologist because of non-cervical general complaints (n = 133).

<table>
<thead>
<tr>
<th>Triage algorithm</th>
<th>n1/N1</th>
<th>Sensitivity (95% CI)</th>
<th>n2/N2</th>
<th>Specificity (95% CI)</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
<th>Referral rate</th>
</tr>
</thead>
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<td></td>
</tr>
<tr>
<td>E7 mRNA testing following HPV16/18/31/33/45/52/58 DNA genotyping</td>
<td>22/32</td>
<td>68.8% (52.7–84.8%)</td>
<td>60/101</td>
<td>59.4% (49.8–69.0%)</td>
<td>34.9% (23.1–46.7%)</td>
<td>85.7% (77.5–93.9%)</td>
<td>47.4%</td>
</tr>
<tr>
<td>HPV16/18 DNA genotyping</td>
<td>20/32</td>
<td>62.5% (45.7–79.3%)</td>
<td>57/101</td>
<td>56.4% (46.8–66.1%)</td>
<td>31.3% (19.9–42.6%)</td>
<td>82.6% (73.7–91.6%)</td>
<td>48.1%</td>
</tr>
<tr>
<td>Cytology</td>
<td>24/32</td>
<td>75.0% (60.0–90.0%)</td>
<td>66/101</td>
<td>65.3% (56.1–74.6%)</td>
<td>40.7% (28.1–53.2%)</td>
<td>89.2% (82.1–96.3%)</td>
<td>44.4%</td>
</tr>
<tr>
<td>Cytology and/or E7 mRNA testing following HPV16/18/31/33/45/52/58 DNA genotyping</td>
<td>27/32</td>
<td>84.4% (71.8–97.0%)</td>
<td>44/101</td>
<td>43.6% (33.0–53.2%)</td>
<td>32.1% (22.2–42.1%)</td>
<td>89.8% (81.3–98.3%)</td>
<td>63.2%</td>
</tr>
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<td>E7 mRNA testing following HPV16/18/31/33/45/52/58 DNA genotyping</td>
<td>26/32</td>
<td>81.3% (67.7–94.8%)</td>
<td>39/101</td>
<td>38.0% (29.1–48.1%)</td>
<td>29.5% (20.0–39.1%)</td>
<td>86.7% (76.7–96.6%)</td>
<td>66.2%</td>
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<tr>
<td>HPV16/18 DNA genotyping</td>
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<td>55.4% (46.5–64.2%)</td>
<td>14.3% (5.6–22.9%)</td>
<td>95.7% (91.0–100%)</td>
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<td>66.7% (40.0–93.3%)</td>
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<td>51.7% (44.8–62.6%)</td>
<td>12.5% (4.4–20.6%)</td>
<td>94.2% (88.7–99.7%)</td>
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<td>Cytology</td>
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<td>91.7% (76.0–100%)</td>
<td>73/121</td>
<td>60.3% (51.6–69.0%)</td>
<td>18.6% (8.7–28.6%)</td>
<td>98.6% (96.0–100%)</td>
<td>44.4%</td>
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<tr>
<td>Cytology and/or E7 mRNA testing following HPV16/18/31/33/45/52/58 DNA genotyping</td>
<td>11/12</td>
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<td>44/121</td>
<td>36.4% (27.8–44.9%)</td>
<td>12.5% (5.6–19.4%)</td>
<td>97.8% (93.5–100%)</td>
<td>66.2%</td>
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CIN – Cervical intraepithelial neoplasia (grade 2 or 3 or higher); CI – confidence interval; PPV – positive predictive value; NPV – negative predictive value; n1 – number of test positive disease cases; N1 – total number of disease cases; n2 – number of test negative non-disease cases; N2 – total number of non-disease cases.

a Out of 27 women who tested positive for HPV16/18/31/33/45/52/58 DNA.
b Including 60 women who tested negative for HPV16/18/31/33/45/52/58 DNA.
c Out of 10 women who tested positive for HPV16/18/31/33/45/52/58 DNA.
d Including 36 women who tested negative for HPV16/18/31/33/45/52/58 DNA.

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than those of cytology, but these differences were not statistically significant.

Previous studies have addressed the value of E6/E7 mRNA NASBA tests for ≥CIN2 detection. These studies, however, used different approaches: primary stratification by, for example, cytology results [13–15,17,21,25,28–32,42–45] or by HPV16/18 DNA genotyping results [26]. Secondly, these studies addressed NASBA assays targeting five hrHPV types. We evaluated whether additional testing for E7 mRNA of HPV52 and HPV58 increases the clinical utility of the NASBA test. E7 mRNA detection showed a specificity advantage over DNA genotyping. Still a suboptimal specificity of the algorithm including the E7 mRNA assay is seen, which might be explained by the fact that E7 expression is present in both transforming and productive HPV infections (albeit apparently at a lower level in the latter) [35]. Although the sensitivity of our newly proposed algorithm including E7 mRNA testing following HPV16/18/31/33/45/52/58 DNA genotyping was very reasonable, it did not surpass that of HPV16/18 DNA genotyping or cytology. The still limited number of seven targeted HPV types probably restricts its sensitivity. Indeed, one carcinoma, eight CIN3 lesions and twenty CIN2 lesions were considered negative by the algorithm as they harboured HPV types not targeted by the NASBA assay. Thus, further enhancement of the sensitivity might be envisioned by further increasing the number of hrHPV types targeted by the mRNA assay, though this would likely also have a drawback on its specificity. This is illustrated by features of the APTIMA test (Hologic), a mRNA assay targeting a larger number of hrHPV types than our NASBA assay, i.e., 14 hrHPV types. The estimated sensitivity of this test is high, but its specificity ranges between 42% and 54% [16]. The assay characteristics more resemble those of clinically validated hrHPV-DNA tests for primary cervical screening [46] than those of a triage test. While performing similar to HPV16/18 DNA genotyping or cytology for detection of ≥CIN2 in hrHPV DNA-positive women, it should be taken into account that the algorithm including E7 mRNA testing uses a type-specific NASBA assay – and therefore is complex and laborious, in contrast to the more straightforward HPV DNA genotyping assays. The described testing algorithm furthermore performed type-specific mRNA detection only if DNA of the corresponding HPV type was present. Although a previous study showed high type-specific concordance of HPV-DNA and mRNA detection [28], theoretically mRNA detection could have outperformed DNA testing if an independent approach had been used. Another limitation is that a considerable percentage of cervical scrapes (36/390; 9%) had an invalid E7 mRNA result and could therefore, not be included in comparative analyses. Of these 36 cases, five (11%) had a CIN2 and five (11%) a CIN3. Another opportunity lies in the use of E7 mRNA tests on self-collected specimens. So far, this has only been done with the APTIMA test for primary hrHPV detection [47–49].

Regarding HPV16/18 DNA genotyping, our study confirms the suggestion of previous studies [9,10] that the assay has insufficient sensitivity to be applied as a single triage marker and can therefore only be used in combination with an additional test, for example cytology [7]. Additionally, since in the future the vaccination coverage against HPV16 and HPV18 in developed countries will rise, HPV16/18 DNA genotyping will likely gradually lose value for the triage of hrHPV DNA-positive women for risk of ≥CIN2. An alternative strategy, targeting a broader spectrum of HPV types, might then be preferable.

Although the PPVs of the three evaluated tests are considered acceptable in US settings (>10%) [50], we found that the NPVs of both the algorithm including E7 mRNA testing and the one with HPV16/18 DNA genotyping (88.6% and 86.4%, respectively, in the total study population, and 95.7% and 94.2%, respectively, in the subpopulation corrected for cytology selection bias) are too low to safely dismiss hrHPV-DNA positive women, who are triage-negative, from further follow-up. Interestingly, cytology (as assessed in the subpopulation corrected for cytology selection bias) has a very high NPV (98.6%). The remarkably good performance of cytology in this population, even after elimination of a possible selection bias, could be partly explained by the known hrHPV-positive status of the study participants. This might have influenced cytological screening, as is illustrated by the relatively low specificity of cytology in our population (65.3% for ≥CIN2 and 60.3% for ≥CIN3) compared to those in other large Dutch cohorts (POBASCAM study: 81.4% for ≥CIN2 and 78.0% for ≥CIN3 [3]; VUSAScreen study: 89.1% for ≥CIN2 and 85.6% for ≥CIN3 [4]).

As both evaluated molecular triage algorithms were not capable of providing direct and safe triage, we studied the combined use of these markers with cytology. Both the combination of cytology with the E7 mRNA testing algorithm, and the combination of cytology with HPV16/18 DNA genotyping yielded high sensitivities (both 91.7%), acceptable NPVs (98.0% and 97.8%) and relative low specificities (39.7% and 36.4%, respectively), resulting in undesirably high referral rates (63.2% and 66.2%, respectively).

In conclusion, in hrHPV DNA-positive women, a triage algorithm of E7 mRNA testing following HPV16/18/31/33/45/52/58 DNA genotyping performs similar to HPV16/18 DNA genotyping and cytology. As the NPVs of both molecular triage algorithms do not provide enough safety to use them as single triage strategies, these can in clinical practice only be considered in combination with cytology.

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Competing interests

CJLMM, PJFS, and DAMH are shareholders of Self-screen BV, a spin-off company of VU University Medical Center. JB has performed consultancies for Qiagen and Roche, and has received travel reimbursements from DDL. All other authors declare that they have no conflict of interest.

Ethical approval

The study was approved by the Ethical Committee of the VU University Medical Center (reference number 2009/178 METc VUmc).

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References


