



## Research article

## Detection of high-risk human papillomavirus RNA in urine for cervical cancer screening with HPV 16 &amp; 18/45 genotyping

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## ABSTRACT

**Objective:** To detect high-risk human papillomavirus (hrHPV) messenger-RNA (mRNA) in urine samples, compare their concordance with cervical samples including HPV 16 & 18/45 genotyping, and to determine the utility in detecting  $\geq$  CIN 2 lesions.

**Methods:** A cohort of 189 non-pregnant patients (age  $\geq$  25) was recruited in three groups: Group 1 with abnormal pap-smears and hrHPV positivity, Group 2 with normal pap-smears and hrHPV positivity, and Group 3 with normal pap-smears and hrHPV negativity. Urine samples were tested for hrHPV-mRNA and subsequent hrHPV-mRNA genotype if positive. High-risk HPV detection and genotyping were performed using Aptima assays which are validated for cervical HPV testing. Colposcopy results from groups 1 & 2 were analyzed.

**Results:** The sensitivity of urine hrHPV-mRNA detection was 31.5% while the specificity and PPV were above 95% (96.9% & 95.1% respectively) ( $p < 0.001$ ). The kappa agreement with cervical samples was fair (0.22,  $p = 0.04$ ). The sensitivity and specificity of urine hrHPV-mRNA genotyping were 20.0% & 100% respectively ( $p < 0.001$ ) with 100% genotype-specific concordance. The kappa agreement with cervical samples was fair (0.25,  $p = 0.16$ ). For urine hrHPV-mRNA detection of ASC-H/HSIL when grouped by age  $\geq$  30, the sensitivity and specificity were 45.4% & 63.9% respectively ( $p = 0.009$ ). For urine hrHPV-mRNA detection of  $\geq$  CIN 2 for all ages, the sensitivity and specificity were 45.5% & 75.0% respectively ( $p = 0.03$ ).

**Conclusion:** Using the Aptima Assay, urine hrHPV-mRNA detection is suboptimal for cervical cancer screening but given the high specificity, it has the potential to identify high-grade lesions ( $\geq$  CIN 2). Urine hrHPV-mRNA genotyping via this modality is not beneficial in triage settings of normal or abnormal cytology to determine the need for colposcopy.

## 1. Introduction

A large proportion of the population is lost to cervical cancer screening due to poor resources, cultural barriers or avoidance of a pelvic exam. Based on 2015 CDC statistics, the rate for cervical cancer screening in USA was 81.8% for women ages 21 to 44, much higher than developing countries where rates are as low as 19–45% [1, 2]. In order to increase cervical cancer screening through self-sampling, alternate sources of detecting high-risk human papillomavirus (hrHPV) have been studied, one of which includes urine testing [3].

Most studies involving urine hrHPV detection are based on the L1 gene that is highly prevalent in HPV infections [4, 5, 6]. It encodes for the L1 capsid protein that facilitates entry of the virus into host epithelial cells [7]. Even though added testing for hrHPV-DNA is better in detecting high-grade

lesions than cytology alone in cervical samples, it cannot distinguish between transient or persistent infections, the latter which have a higher rate of progression to cervical cancer [8]. The overall conclusion has been that HPV DNA-based urine testing is concordant with cervical samples (>80%) with high sensitivity (81–100%) but low specificity (23–51%) in detecting  $\geq$  cervical intraepithelial neoplasia 2 ( $\geq$  CIN 2) lesions [8, 9, 10, 11, 12, 13]. Recently, HPV detection in cervical samples has been performed by targeting the E6/E7 messenger RNA (mRNA) which makes up for the specificity lacking in HPV-DNA assays while maintaining a high sensitivity, thereby proving its utility in population-based primary screening [4, 6]. The rationale is that cervical carcinogenesis involves the overexpression of viral oncogenes E6/E7 in concurrence with decreased host immunity [7, 8]. Furthermore, HPV E6/E7 mRNA has been shown to correlate with severity of cervical lesions [5].

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The objective and primary endpoint of this study was to detect hrHPV-mRNA in urine samples and compare their concordance with hrHPV-mRNA in cervical samples including HPV 16 and 18/45 genotyping. The secondary endpoint was to determine the utility of urine hrHPV-mRNA detection for high-grade histologic lesions ( $\geq$  CIN 2).

## 2. Methods

To gain a power of 80%, a sample size of 186 patients was calculated to estimate a 95% confidence interval (CI) for the sensitivity of the urine hrHPV-mRNA test for detecting hrHPV (assuming the prevalence of HPV in the study population is 50%).

### 2.1. Study population

Inclusion criteria entailed patients  $\geq$  age 25 who had a pap-smear with concurrent hrHPV testing performed (with or without hrHPV 16 and hrHPV 18/45 genotyping) within the past 360 days. Exclusion criteria entailed patients who were pregnant or had a history of HPV vaccine administration.

Between November 2016 and February 2018, 192 patients who presented for their scheduled visit at Staten Island University Hospital (SIUH), NY, were recruited. Of these patients, 189 met the inclusion criteria.

The study design incorporated three groups of women: Group 1 with abnormal pap-smears and hrHPV positivity, Group 2 with normal pap-smears and hrHPV positivity, and Group 3 with normal pap-smears and hrHPV negativity (Figure 1).

Pap-smear results were reported using the Bethesda system. A normal pap-smear included results reported as “negative for intraepithelial lesion or malignancy [NILM].” An abnormal pap-smear included results reported as atypical squamous cells of undetermined significance [ASCUS], atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion [ASC-H], low-grade squamous intraepithelial lesion [LSIL], high-grade squamous intraepithelial lesion [HSIL], and atypical glandular cells [AGC].

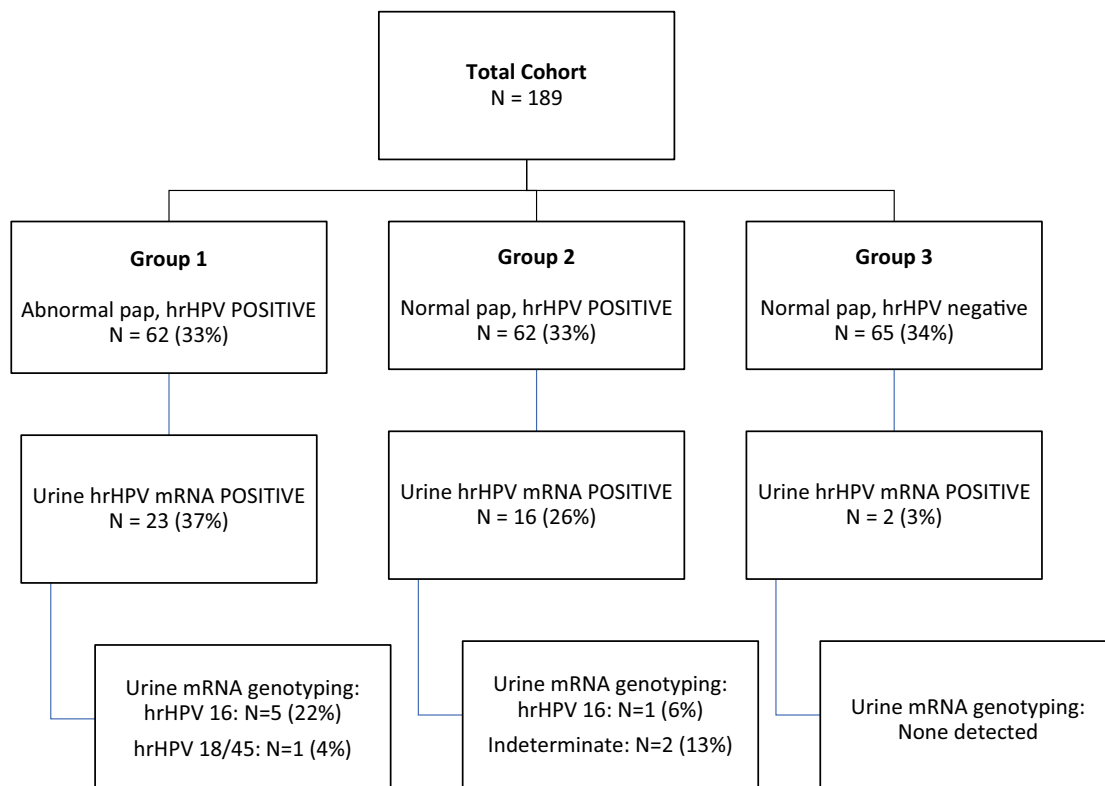
### 2.2. Sample collection

All patients enrolled in the study had their pap-smear and hrHPV testing performed via a liquid based collection media (ThinPrep, Hologic Inc, San Diego, CA, USA). High-risk HPV testing was performed using an HPV-mRNA assay (Aptima HPV assay, Hologic, Inc., San Diego, CA, USA) validated and FDA approved for cervical samples, which detects qualitative E6/E7 mRNA for the following hrHPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

After informed consent was obtained, initial stream urine was collected in sterile containers. Within one hour of collection, 2–2.5 ml of urine was transferred to the urine specimen transport tube (Aptima urine specimen collection kit, Hologic, Inc., San Diego, CA, USA), stored at 2°–4 °C and processed within 30 days of collection. Concurrent cervical samples were not collected.

### 2.3. HPV testing

Processing of urine specimens was performed using a transcription-mediated amplification system (Panther Hologic System, Hologic, Inc.,



hrHPV: high-risk human papillomavirus  
mRNA: messenger RNA  
RNA: ribonucleic acid

**Figure 1.** Study design and results. hrHPV: high-risk human papillomavirus, mRNA: messenger RNA, RNA: ribonucleic acid.

San Diego, CA, USA). Urine samples were tested using the same Aptima HPV assay as was used for cervical samples. The Aptima HPV assay involves three main steps involving HPV-mRNA target capture, transcription-based nucleic acid amplification of the target molecules and their subsequent detection via hybridization with chemiluminescent labels.

Urine samples which were positive for hrHPV-mRNA further underwent hrHPV genotyping using an HPV-mRNA 16 and 18/45 genotype assay (Aptima HPV 16, 18/45 Genotype Assay, Hologic, Inc., San Diego, CA, USA) validated and FDA approved for cervical samples, which detects E6/E7 HPV-mRNA.

Final assay results were interpreted based on analyte signal-to-cut off (S/CO) ratio incorporating pre-determined relative light unit (RLU) values used for cervical samples. Colposcopy results of patients from Groups 1 and 2 were collected.

#### 2.4. Statistical analysis

Comparison of categorical values was performed using Pearson and chi-square tests. Comparison of urine and cervical hrHPV test results was performed using McNemar's test. All comparisons were two-sided and p-values for  $\leq 0.05$  were considered statistically significant. Percent agreement adjusted for chance agreement was calculated using the Cohen's kappa coefficient. Statistical analyses were performed using SPSS software (IBM Corp, 2012, Version 21.0, Armonk, NY, USA).

### 3. Results

The total cohort involved 189 patients (Figure 1). Group 1 included 62 (62/189: 33%) patients of which 23 (23/62: 37%) tested positive for urine hrHPV. Of those 23 patients, 5 (5/23: 22%) tested positive for hrHPV 16 and 1 (1/23: 4%) tested positive for hrHPV 18/45. Group 2 included 62 (62/189: 33%) patients of which 16 (16/62: 26%) tested positive for urine hrHPV. Of those 16 patients, 1 (1/16: 6%) tested positive for hrHPV 16 and 2 (2/16: 13%) tested as indeterminate. Group 3 included 65 (65/189: 34%) patients of which 2 (2/65: 3%) tested

positive for urine hrHPV. None of the patients in this group tested positive on urine genotyping. The median time from pap-smear collection to urine collection was 97 days.

The cohort characteristics are listed in Table 1. The statistically significant differences among the characteristics were age (grouped as  $<30$ ,  $\geq 30-49$ ,  $\geq 50$ ,  $p = 0.006$ ) and race (grouped as Hispanic and non-Hispanic,  $p = 0.004$ ).

Within the total cohort of 189 patients, when compared to cervical samples, the sensitivity of urine hrHPV-mRNA detection was 31.5% and specificity was 96.9%. The positive predictive value (PPV) was 95.1% and negative predictive value (NPV) was 42.6%. McNemar's comparison was statistically significant at  $p < 0.001$ . The overall percent agreement (OPA) was 54.0% and positive percent agreement (PPA) was 31.5%. Refer to Table 2. The Cohen's kappa agreement was fair at 0.22 (0.10–0.34 95% CI,  $p = 0.04$ ).

Within Groups 1 and 2, 39 patients had positive urine hrHPV samples of which 7 (7/39: 18%) tested positive for either hrHPV 16 or hrHPV 18/45. Concurrent cervical hrHPV genotype information was available for only 4 out of the 7 patients. The genotype-specific concordance for HPV 16 and 18/45 was 100% between the urine and cervical samples.

In Groups 1 and 2, 57 patients had genotyping done on both cervical and urine samples. The sensitivity of urine hrHPV genotype detection was 20.0% and specificity was 100%. The PPV was 100% and NPV was 69.8%. McNemar's comparison was statistically significant at  $p < 0.001$ . The OPA was 71.9% and PPA was 20.0%. Refer to Table 2. The Cohen's kappa agreement was fair at 0.25 (0–0.56 95% CI,  $p = 0.16$ ).

A sub-analysis was performed for age  $\geq 30$ . Comparison was made for urine hrHPV-mRNA detection of high-grade cytology (ASC-H/HSIL) versus low-grade cytology (ASCUS/LSIL) in Group 1. When grouped by age  $\geq 30$ , statistical significance was reached with sensitivity of 45.4% and specificity of 63.9%. This translated to a NPV of 79.3% ( $p = 0.009$ ). Refer to Table 3. A higher proportion of patients  $\geq$  age 30 with ASC-H/HSIL had positive urine hrHPV samples (5/11: 46%) than patients with ASCUS/LSIL (13/36: 36%) (Figure 2).

Of the 124 patients in Groups 1 and 2, 11 patients did not have colposcopy biopsies performed based on physician judgment. The results of

Table 1. Cohort characteristics.

	Total		Group 1 = Abnormal pap, hrHPV POSITIVE		Group 2 = Normal pap, hrHPV POSITIVE		Group 3 = Normal pap, hrHPV negative	
	N	%	N	%	N	%	N	%
N	189		62	32.8%	62	32.8%	65	34.4%
Age (years)								
Median	41		36		42		45	
Age Groups (years) **								
$<30$	24	12.7%	15	24.2%	7	12.3%	2	3.1%
$\geq 30-49$	108	57.1%	34	63.0%	35	61.4%	39	60.0%
$\geq 50$	57	30.1%	13	24.0%	20	35.1%	24	36.9%
Race**								
Hispanic	92	48.7%	29	53.7%	40	70.2%	23	35.4%
Non-Hispanic	97	51.3%	33	61.1%	22	38.6%	42	64.6%
Parity <sup>(NS)</sup>								
0-1	47	24.9%	22	40.7%	8	14.0%	17	26.2%
2-4	117	61.9%	34	63.0%	42	73.7%	41	63.1%
$\geq 5$	15	7.9%	3	5.6%	6	10.5%	6	9.2%
Smoking <sup>(NS)</sup>								
Yes	30	15.9%	12	22.2%	10	17.5%	8	12.3%
No	159	84.1%	50	92.6%	52	91.2%	57	87.7%
History of past or present sexually transmitted diseases (STD) (NS)								
Yes	28	14.8%	14	26.0%	9	15.8%	5	7.7%
No	161	85.2%	48	89.0%	53	93.0%	60	92.3%

hrHPV: high-risk human papillomavirus; (NS): Not Significant.

\*\* $p < 0.01$ .

**Table 2.** Urine hrHPV and hrHPV genotype detection compared to cervical samples.

Groups compared	Urine samples	Cervical samples		Prevalence % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	McNemar p-value	Overall percent agreement	Positive percent agreement
		hrHPV positive	hrHPV negative								
1, 2, 3 Urine hrHPV detection (compared to cervical hrHPV detection)	hrHPV positive	39	2	65.6 (58.3–72.2)	31.5 (23.6–40.5)	96.9 (88.3–99.5)	95.1 (82.2–99.2)	42.6 (34.6–51.0)	<0.001	54.0%	31.5%
	hrHPV negative	85	63								
		hrHPV 16 or 18/45 positive		hrHPV 16 or 18/45 negative							
1,2 # Urine hrHPV genotyping (compared to cervical hrHPV genotyping)	hrHPV 16 or 18/45 positive	4	0	35.1 (23.2–48.9)	20.0 (6.6–44.3)	100 (88.3–100.0)	100 (39.6–100.0)	69.8 (55.5–81.3)	<0.001	71.9%	20.0%
	hrHPV 16 or 18/45 negative	16	37								

# (57 patients had concurrent genotyping at time of pap smear collection); hrHPV: high-risk human papillomavirus; PPV: Positive predictive value; NPV: Negative predictive value; CI: Confidence interval.

**Table 3.** Urine hrHPV detection by cervical cytology and histology.

Groups compared	Age	Urine samples	Cervical samples		Prevalence % (95% CI)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)		
			ASC-H/HSIL	ASCUS/LSIL							
1 Urine hrHPV detection (for high grade cytology (ASC-H/HSIL) versus low grade cytology (ASCUS/LSIL))	All <sup>(NS)</sup>	hrHPV positive	5	18	22.6 (12.3–35.3)	35.7 (14.0–34.4)	62.5 (47.3–75.7)	21.7 (8.3–44.2)	76.9 (60.3–88.3)		
		hrHPV negative	9	30							
	≥ 30 <sup>**</sup>	hrHPV positive	5	13	23.4 (12.8–38.4)	45.4 (18.1–75.4)	63.9 (46.2–78.7)	27.8 (10.7–53.6)	79.3 (59.7–91.3)		
		hrHPV negative	6	23							
		≥ CIN 2		≤ CIN 1							
1,2 # Urine hrHPV detection (for ≥ CIN 2 versus ≤ CIN 1)	All <sup>*</sup>	hrHPV positive	15	20	29.2 (21.2–38.6)	45.5 (28.5–63.4)	75.0 (63.8–83.7)	42.8 (26.7–60.5)	76.9 (65.8–85.4)		
		hrHPV negative	18	60							
	≥ 30 <sup>(NS)</sup>	hrHPV positive	12	16	30.3 (21.2–41.1)	44.4 (26.0–64.3)	74.1 (61.2–84.1)	42.8 (25.0–62.6)	75.4 (62.4–85.1)		
		hrHPV negative	15	46							

# (11 patients did not have colposcopies); hrHPV: high-risk human papillomavirus; PPV: Positive predictive value; NPV: Negative predictive value; CI: Confidence interval; (NS): Not Significant; CIN: Cervical intra intraepithelial neoplasia; ASC-H: Atypical squamous cells, cannot rule out HSIL; HSIL: High-grade squamous intraepithelial lesion; ASCUS: Atypical squamous cells of undetermined significance; LSIL: Low-grade squamous intraepithelial lesion.

\*\*p < 0.01.

\*p < 0.05.

113 patients who underwent colposcopies were grouped into two categories: ≤ CIN 1 (including benign results) and ≥ CIN 2 (CIN 2, CIN 3). None of the patients were diagnosed with carcinoma.

When the two categories were compared, the sensitivity for detecting urine hrHPV-mRNA for patients of all ages with ≥ CIN 2 was 45.5% and specificity was 75.0%. The PPV was 42.8% and NPV was 76.9%. All results were statistically significant (p = 0.03). Refer to Table 3. A higher proportion of patients of all ages with ≥ CIN2 had positive urine hrHPV-mRNA samples (15/18: 83%) than patients with ≤ CIN 1 (20/60: 33%) (Figure 3).

#### 4. Discussion

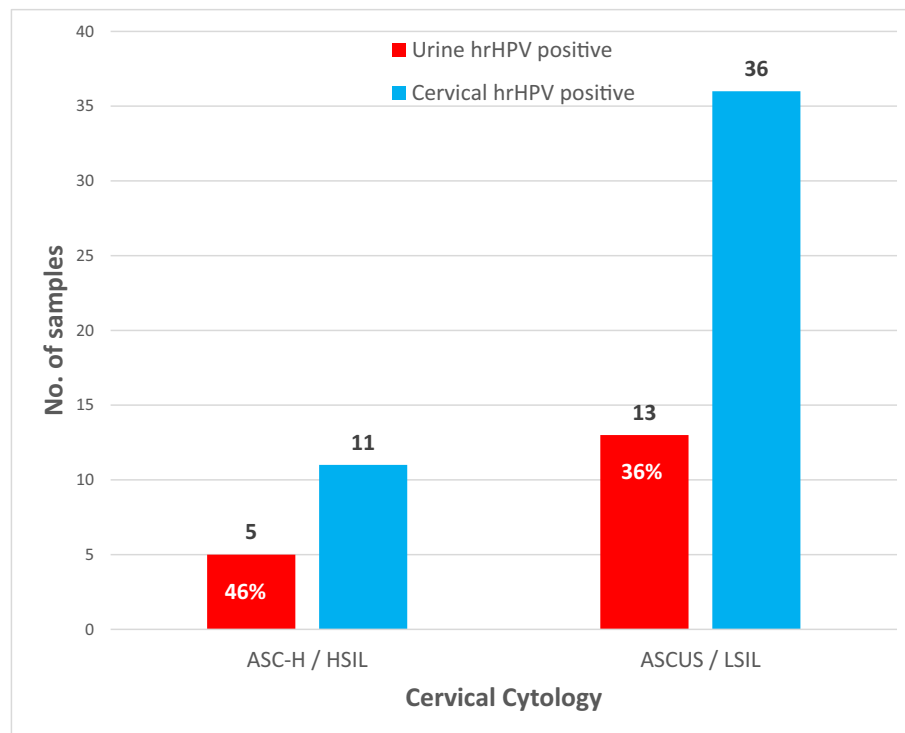
In this study, the overall sensitivity of urine hrHPV-mRNA detection is low (31.5%), however the specificity and PPV are above 95% (96.9% and 95.1% respectively). Given the fair agreement between cervical and urine samples (k = 0.22) and low OPA of 54%, this modality of urine hrHPV-mRNA testing is not appropriate for primary cervical cancer screening. Similar results were noted by Ascutto et al who demonstrated a 48.1% sensitivity and 82.8% specificity of urine hrHPV-mRNA detection using the Aptima HPV assay in 209 patients [3]. The decreased OPA of urine hrHPV-mRNA detection can be attributed to the use of assay cut-offs for positive results based on cervical samples, which in general yield higher concentrations of infected cells. The addition of Proteinase K to increase HPV-RNA extraction with the Aptima HPV assay has been shown to increase agreement rates between urine and cervical hrHPV-mRNA detection but was not used in this study [14].

A sub-analysis was performed on patients ≥ age 30 because it is known that HPV detection in this age group represents a more persistent

infection with higher risk of progression, thereby used to improve sensitivity of CIN 3 detection versus cytology alone [15, 16]. In this study, a high specificity of 63.9% of urine hrHPV-mRNA detection in patients ≥ age 30 who had high-grade cytology (ASC-H/HSIL) was noted in Group 1 with a sample size of 47 patients. This is comparable to the specificity determined by Ascutto et al of 61.9% of urine hrHPV-mRNA detection of high-grade lesions (HSIL/AIS/cancer) [3]. The specificity in this study is higher than the 41% specificity that was observed in biopsy-confirmed ≥ HSIL in cervical samples using the Aptima HPV Assay [17]. Although the ultimate end-point is to detect high-grade histology (CIN 2/CIN 3/carcinoma), there is a benefit in detecting high-grade cytology which generally correlates to high-grade cervical dysplasia. A larger sample size would be necessary to enhance precision of the statistically significant results obtained.

In Groups 1 and 2, there was a high specificity of 75.0% for patients with ≥ CIN 2 histology. This is equivalent or higher than the specificities of detection of ≥ CIN 2 in several studies (40–75%) using the same hrHPV-mRNA assay on cervical samples in a referral population [18, 19, 20, 21, 22]. Iftner et al, however, was able to demonstrate a significantly high specificity of 96.1% for ≥ CIN 3 using the Aptima HPV assay on cervical samples in a screening population [23]. The increased specificity in urine samples can potentially be used to identify high-grade lesions (≥ CIN 2). A high percentage of patients (83%) with ≥ CIN 2 histology had positive urine hrHPV results compared to patients with ≤ CIN 1 (33%), which further substantiates the potential for this mode of urine hrHPV detection for triage of abnormal cytology to pursue colposcopy in positive samples.

For HPV 16 or HPV 18/45 genotyping, the OPA was 71.9% with a low PPA of 20.0%. Nevertheless, the genotype-specific concordance with



hrHPV: high-risk human papillomavirus  
 HSIL: High-grade squamous intraepithelial lesion  
 ASC-H: Atypical squamous cells, cannot rule out HSIL  
 LSIL: Low-grade squamous intraepithelial lesion  
 ASCUS: Atypical squamous cells of undetermined significance

**Figure 2.** Proportion of urine hrHPV mRNA positive samples in Group 1 separated by cytology for age  $\geq 30$ . hrHPV: high-risk human papillomavirus, HSIL: High-grade squamous intraepithelial lesion, ASC-H: Atypical squamous cells, cannot rule out HSIL, LSIL: Low-grade squamous intraepithelial lesion, ASCUS: Atypical squamous cells of undetermined significance.

cervical samples was 100% and the specificity for urine hrHPV-mRNA genotyping was 100%. As demonstrated by the CLEAR trial using the Aptima HPV genotype assay on cervical samples, women who tested positive for HPV 16 or HPV 18/45 had a 29.1% absolute risk of  $\geq$  CIN 2 and 16.2% absolute risk of CIN 3/AIS [24]. The low PPA between urine and cervical samples in this study deters the use of urine HPV-mRNA genotype testing in a triage setting for normal or abnormal cytology to determine the need for colposcopy.

This was not a longitudinal study and disease progression was not studied, which is a limitation. A study involving low-risk population with 11220 women showed an overall cumulative rate of 19.7% over 72 months of  $\geq$  CIN 3 in patients who were mRNA-HPV positive versus 0.62% in patients who were mRNA-HPV negative in cervical samples [25]. Furthermore, Bruno et al demonstrated that 97.1% of patients who were HPV-DNA positive but E6/E7 mRNA negative and with ASCUS/LSIL cytology at baseline did not progress during a follow-up period of 3 years [26]. In women who were E6/E7 mRNA positive, 45.2% in the ASCUS group and 12.9% in the LSIL group developed  $\geq$  CIN 2 during the 3 years of follow-up [26]. Long-term follow-up will be required to ascertain the significance of positive urine hrHPV-mRNA detection in the setting of disease course.

As to hrHPV-DNA studied in urine samples, there is no consensus on urine collection modes or HPV-DNA extraction methods from urine samples [13, 27, 28]. Some studies have found higher sensitivity and specificity for HPV-DNA detected in first-void samples based on the theory that initial-flow urine collects most of the debris [28, 29]. In 30 patients with  $\geq$  CIN 2 or worse, Senkomago et al found no difference on hrHPV-DNA detection for first-void, initial-stream and mid-stream urine samples for unfractionated and pellet fractions with an overall high

sensitivity of 89% [13]. Future studies are required to assess the different methods of urine collection and voiding times to determine the highest yield of infected cells for detection and genotyping.

## 5. Conclusion

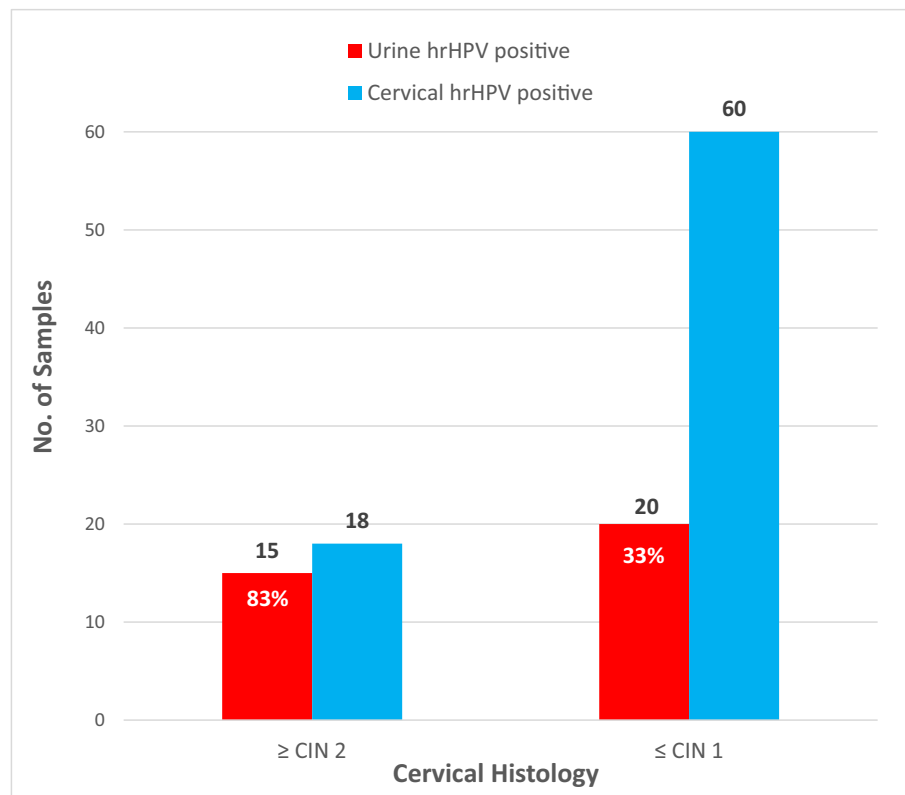
The detection of hrHPV-mRNA in urine samples using the Aptima HPV assay is suboptimal for cervical cancer screening. However, due to the comparably high specificities to cervical samples as determined by numerous studies, urine hrHPV-mRNA detection may have a utility in identifying high-grade lesions ( $\geq$  CIN 2) if substantiated by future studies [18, 19, 20, 21, 22]. This would potentially lower the number of colposcopies and accommodate increased testing frequency during surveillance. It may also be used in triage of abnormal cytology with respect to concurrent colposcopies, thereby being cost effective.

This is the first known study to test for urine hrHPV-mRNA genotype using the Aptima HPV 16 and 18/45 genotype assay. The low PPA with cervical samples discourages its use in triage settings, especially for low-grade lesions, irrespective of a high specificity.

The rationale for the study design was to include equal number of patients within each test group for a uniform statistical comparison. It accommodates a sample size to attain certain significant results which may be used as basis for future large population-based studies.

## 6. Limitations

First-void urine samples were not used which theoretically have a higher concentration of infected cells. Urine collection at time of visit was done to mimic the clinical setting as close to reality as possible where



hrHPV: high-risk human papillomavirus  
CIN: Cervical intraepithelial neoplasia

**Figure 3.** Proportion of urine hrHPV mRNA positive samples in Groups 1 and 2 separated by histology. hrHPV: high-risk human papillomavirus, CIN: Cervical intraepithelial neoplasia.

obtaining first-void samples would unlikely procure a high compliance rate.

Additionally, the lack of paired cervical and urine samples is a weakness. The median time between pap-smear and urine collection was 97 days and it is well-known that cytology and HPV status are prone to change over time. In these cases, regression of disease was not accounted for. Therefore, there is a deficiency in clinical accuracy due to non-simultaneous comparison of urine and cervical samples using the same HPV assay under standardized conditions. The VALHUDES protocol was recently undertaken in 2018 to target this deficiency on first-void urine samples [30]. Future studies will need to be undertaken with emphasis on paired cervical and urine samples to nullify regression of disease as a confounding factor.

### Declarations

#### Author contribution statement

R. Padhy: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

A. Davidov: Conceived and designed the experiments; Performed the experiments.

L. Madrigal, G. Alcide and A. Spahiu: Contributed reagents, materials, analysis tools or data.

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#### Competing interest statement

The authors declare no conflict of interest.

#### Additional information

No additional information is available for this paper.

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