High-Risk Human Papillomavirus Detection in Urine Samples From a Referral Population With Cervical Biopsy-Proven High-Grade Lesions

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Objective: The aim of the study was to evaluate the performance of the HPV-HR test to detect high-risk human papillomavirus (HPV) in urine samples in comparison with a commercial molecular HPV test.

Materials and Methods: This is a prospective study, in which 350 patients diagnosed previously with cervical intraepithelial neoplasia (CIN) grade 2 or higher were enrolled. Urine and cervical specimens were collected. Urine was tested with the HPV-HR test and cervical specimens were tested with the Cobas.

Results: Of the 336 evaluable patients, there were 271 cases of CIN 2+, of which 202 were CIN 3+ and the remaining 65 patients were less than CIN 2. Positivity was 77.1% (95% confidence interval [CI] = 72.5–81.5) for the urine samples and 83.6% (95% CI = 79.6–87.6) for the cervical samples. Agreement between cervical and urine samples for HPV detection was 79.8% (κ = 0.363; 95% CI = 0.243–0.484). Sensitivity for CIN 2+ was 83.4% (95% CI = 78.4–87.6) for urine and 90.8% (95% CI = 86.7–92.9) for cervical samples. The sensitivity for CIN 3+ was 85.6% (95% CI = 80.0–90.2) for urine and 92.6% (95% CI = 88.0–95.8) for cervical samples. Specificity for worse than CIN 2 was 50.8% (95% CI = 33.7–59.0) and 46.2% (95% CI = 33.7–59.0) for urine and cervical samples, respectively.

Conclusions: Although these results demonstrated slightly higher detection rates for HR-HPV and clinical sensitivity in cervical samples than in urine, when compared with histological diagnoses, urine sampling is a viable alternative to access women who do not participate in routine screening programs.

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Trovagene Inc provided the materials to molecular analysis. Some items as collection flasks, preservative medium, plastic consumables, and collection brushes were provided from Barretos Cancer Hospital.

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The study protocol (#107775/2012) was approved by the ethics committee of Barretos Cancer Hospital, Barretos, Brazil.

All women signed an informed consent before entering the study. The study protocol (#107775/2012) was approved by the ethics committee of Barretos Cancer Hospital, Barretos, Brazil.

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The use of human papillomavirus (HPV) testing for cervical cancer screening has several advantages in comparison with cytology-based screening, which includes greater sensitivity and better diagnostic reproducibility in many settings.[1] Moreover, HPV testing has the advantage of being an objective measure with easy implementation in areas lacking well-trained professionals for cytology evaluation.[2] However, HPV testing can be more costly, requiring more complex laboratory infrastructure. Thus, efforts have been made to reduce the cost of HPV testing and offer less invasive sampling options, thereby enabling HPV testing in regions with limited resources and low screening rates.[3]

Screening programs, organized or opportunistic, demand adherence of the target population (i.e., the ability to follow up) to be efficient and effectives. Unfortunately, many women are left out of these programs and/or remain many years without screening because of various reasons, which, in developing countries, include difficulty to access medical assistance.[4] The consequence of these inadequate intervals for screening is a high incidence of high-grade lesions in these populations. Self-sampling for HPV testing is a promising option for screening programs in developed and developing countries, with significant data suggesting its use in large population.[5] A successful self-sampling strategy requires, among other variables, recruiting of nonscreened women, the ability to efficiently trace women for follow-up, and selection of accurate and precise low-cost HPV testing and self-sampling methodologies. Thus, it is strongly recommended that implementation of a self-sampling strategy be preceded by pilot studies in the intended setting to assess these variables.[6]

Human papillomavirus testing in urine has been postulated as an alternative methodology to be considered for identification of high-risk HPV (hr-HPV)-positive women who may be at risk for cervical cancer. The main advantage to urine sampling versus cervical sampling is that it does not require the intervention of medical personnel and the sample can be collected outside of a clinic. Urine is more acceptable and can be used in low-income settings to enhance women's participation in screening programs. Moreover, the results of HPV testing using urine sampling have been very promising, and the limited sensitivity of the previous tests seems has been overcome.[7]

The HPV-HR test (Trovagene, Inc, San Diego, CA), a polymerase chain reaction–based test that targets the E1 region of the HPV genome, has shown high sensitivity for urine-based detection of cervical intraepithelial lesions in multiple studies.[8–10] The aim of this study was to evaluate the HPV-HR test in a referral population diagnosed with cervical intraepithelial neoplasia grade 2 or higher (CIN 2+) confirmed by biopsy and compare its performance to paired cervical specimens tested with the Cobas HPV test (Roche Molecular Systems, Inc, Pleasanton, CA).

MATERIALS AND METHODS

Study Design

This study recruited 350 women who were diagnosed with CIN 2+ and referred for treatment (i.e., conization) at the Molecular Oncology Center of Barretos Cancer Hospital, Barretos, São Paulo, Brazil, from October 2013 to December 2014.

Sample Collection

Paired urine and cervical samples were obtained from each study participant before the conization procedure. The urine was sampled as soon as the woman arrived at the hospital and before she underwent loop electrosurgical excision procedure (LEEP), and at least 20 mL of urine was collected in a sterile flask, to which 10 mL of a urine preservative solution (Trovagene, Inc) was immediately added after the collection. Samples were preserved between 4°C and 8°C ("cold chamber") until the DNA extraction (up to 4 weeks). Cervical samples were obtained by a trained professional using a Rovers Cervex-Brush equipped with a removable brush head (Rovers Medical Devices BV, Oss, the Netherlands) and placed into a vial containing SurePath preservative fluid (Becton & Dickinson, Burlington, NC). Histopathological analysis was performed on the tissue obtained from the conization procedure, stained with hematoxylin, and evaluated by an experienced pathologist according to national routine protocols, which is similar to the World Health Organization classification.[11]

HPV-HR Test of Urine Samples

Testing of the blinded urine samples was performed at Trovagene, Inc. Results of those tests were sent to Barretos' investigators, who independently performed all statistical analyses, with no interference of Trovagene, Inc. Briefly, DNA was extracted from 500 μL of the preserved urine sample using the QIAamp MinElute Virus Vacuum Kit (QIAGEN, Germantown, MD) according to the manufacturer's instructions. Isolated DNA (5 μL) was tested with the HPV-HR test, a PCR-based assay that uses degenerate primers to amplify a conserved region in the E1 gene of hr-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Amplification of the RNaseP gene was performed as a control for the presence of amplifiable DNA. The HPV and RNaseP PCR products were subjected to capillary electrophoresis for fragment size analysis on the ABI 3130 Genetic Analyzer instrument (ThermoFisher, Carlsbad, CA). Results were reported as HR-HPV positive or negative based on the presence or absence of appropriately sized fragments of the HPV and RNaseP amplicon relative to a predefined cutoff.

Cobas HPV Test of Cervical Samples

A 2 mL aliquot of the cervical samples, collected into SurePath Preservative Fluid, was transferred to a specific kit tube and tested with the HPV test on the COBAS 4800 system (Roche Molecular Systems, Inc, Pleasanton, CA). This test identifies 14 types of hr-HPV, 16 and 18 individually, and 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 collectively. Testing was performed according the manufacturer's instructions at Molecular Oncology Center, Barretos Cancer Hospital.

Statistical Analyses

The HPV test results were evaluated against the histology result obtained from the conization sample. The results were analyzed using the IBM SPSS Statistics software, Version 20.0, for MAC (IBM Corporation, Somers, NY). The χ^2 test was used to compare the frequencies among groups, accompanied by the McNemar test when indicated. In all tests, p values less than .05 were considered to be statistically significant.

Role of the Funding Source

The materials to do the molecular analysis were provided by Trovagene Inc. Some items as collection flasks, preservative medium, plastic consumables, and collection brushes were provided by Barretos Cancer Hospital.

RESULTS

Population

In total, 350 women previously diagnosed with CIN 2+ were enrolled in this study. There were 14 women excluded from the analysis because of invalid HPV-HR test or inadequate sample for HPV testing (not viable or shortage of material) on Cobas HPV test results, yielding 336 evaluable women. The mean age was 37 years (range = 18–83 years; median = 35 years). Among the participants, 65.1% (228) were younger than 40 years. The median time between colposcopy and treatment (LEEP) was 124 days (range = 20–439).

Table 1 presents the comparison of the histology diagnoses for the biopsy and conization samples, for which 43% agreement (145/336) was observed. Of the 118 women with a biopsy diagnosis of CIN 2, 33% (39/118) progressed (had a more severe diagnosis at the time of conization), 28.8% regressed (had a less severe diagnosis at the time of conization), and 38% (45/118) had the same CIN 2 diagnosis at the time of conization. For those women with a CIN 3 diagnosis at biopsy; 20.9% (37/177) progressed, 27% (48/177) regressed, and 51.9% (92/177) had the same CIN 3 diagnosis. Of the 40 women with carcinoma in situ (CIS) at biopsy, 2.5% (1/40) progressed, 77.5% (31/40) regressed, and

TABLE 1. Comparison of Biopsy Histology and Conization Histology, Barretos, Brazil

	Conization						
Biopsy	**Negative**	**CIN 1**	**CIN 2**	**CIN 3**	**CIS**	**SSC**	**Total**
CIN 2	15	19	**45**	29	10	0	118 (35.1%)
CIN 3	16	13	19	**92**	31	6	177 (52.7%)
CIS	1	1	5	24	**8**	1	40 (11.9%)
SCC	0	0	0	1	0	**0**	1 (0.3%)
Total	**32 (9.5%)**	**33 (9.8%)**	**69 (20.5%)**	**146 (43.5%)**	**49 (14.6%)**	**7 (2.1%)**	**336**

Bold values indicates evidence to the results obtained and to give visually, the differences between the categories.

CIN indicates cervical intraepithelial neoplasia; CIS, carcinoma in situ; SCC, squamous cell carcinoma.

TABLE 2. Agreement Between HPV Positivity in Cervical (Cobas HPV Test) and Urine (HPV-HR Test) Samples

Urine samples of HPV-HR test	Cervical samples of Cobas HPV Test		
	Positive	Negative	Total
Positive	236 (70.2%)	23 (6.8%)	259 (77.1%)
Negative	45 (13.4%)	32 (9.5%)	77 (22.9%)
Total	281 (83.6%)	55 (16.4%)	336 (100%)
Overall agreement	79.8 (268/336)		
Positive agreement	84.0 (236/281)		
Negative agreement	58.2 (32/55)		

McNemar test, $p = .01$, and $\kappa = 0.363$ (95% CI = 0.243–0.484).

HPV indicates human papillomavirus; HPV-HR, molecular test to detect high-grade human papillomavirus.

20% (8/40) has the same CIS diagnosis. The one woman with invasive cancer at biopsy had a CIN 3 diagnosis on the conization sample.

High-Risk HPV Detection in Urine and Cervical Samples

Human papillomavirus positivity was 77.1% (259/336) in urine and 83.6% (281/336) in cervical samples (see Table 2). Negative results were observed with both sample types for 9.5% (32/336) of women. The overall agreement between urine and cervical samples for HPV detection was 79.8% ($\kappa = 0.363$; 95% confidence interval [CI] = 0.243–0.484). Overall, 6.8% (23/336) of women had a positive urine sample and a negative cervical sample, and 13.4% (45/336) of women had a negative urine sample and a positive cervical sample (discrepant results, Table 2). From the 23 urines positive/cervical negative women, 47.7% (11/23) were disease negative (<CIN 2: inflammation, CIN 1, and CIN 2) and 52.2% (12/23) were disease positive (CIN 2+: CIN 2, CIN 3, adenocarcinoma in situ, and invasive carcinoma). Of the 45 urine negative/cervical positive, 28.9% (13/45) were disease negative and 71.1% (32/45) were disease positive. Of the 32 women who were HPV negative with both urine and cervical samples, 13 (40.6%) had a diagnosis of CIN 2+ and 19 (59.4%) had a diagnosis of worse than CIN 2. Of the 236 women HPV positive with urine and cervical samples, 214 (90.7%) had a diagnosis of CIN 2+ and 22 (9.3%) had worse than CIN 2.

Clinical Performance for Detection in High-Grade Lesions

Table 3 depicts that sensitivity for CIN 2+ detection was 83.4% (95% CI = 78.4–87.6) for urine samples and 90.8% (95% CI = 86.7–92.9) for cervical samples. The sensitivity for CIN 3+ detection was 85.6% (95% CI = 80.0–90.2) for urine samples and 92.6% (95% CI = 88.0–95.8) for cervical samples.

TABLE 3. Performance of the HPV-HR and Cobas Tests to Detect hr-HPV in High-Grade Intraepithelial Neoplasia

		Sensitivity (95% CI)	Specificity (95% CI)
CIN 2+	Urine	83.4% (78.4–87.6)	50.8% (38.1–63.4)
	Cervical	90.8% (86.7–92.9)	46.2% (33.7–59.0)
CIN 3+	Urine	85.6% (80.0–90.2)	35.8% (27.7–44.6)
	Cervical	92.6% (88.0–95.8)	29.9% (22.3–38.4)

CI indicates confidence interval; CIN, cervical intraepithelial neoplasia.

The specificity for worse than CIN 2 was 50.8% (95% CI = 38.1–63.4) and 46.2% (95% CI = 33.7–59.0) for urine and cervical samples, respectively. The positivity rates of Cobas and Trovagene tests were significantly different (respectively, 83.6% and 77.1%, McNemar test: $p = .010$).

Table 4 demonstrates the type of HPV according to the test. The HR-HPV Trovagene test missed some cases that were positive according to Cobas assay (45 samples were detected only by Cobas). In addition, 23 samples were missed by Cobas test, but those were positive by Trovagene test.

DISCUSSION

This study reports the results of a comparison of HPV detection in paired urine and cervical samples tested with the HPV-HR test and Cobas HPV test, respectively. Other studies have been conducted comparing HPV-HR test results from urine samples to other HPV tests from cervical samples,[8–10] but this is the first study to compare the HPV-HR test to the Cobas HPV test.

The use of urine to detect HPV infection is not new, but HPV detection in urine was found to vary greatly depending on the methodology used for collection, preservative, storage of samples, extraction of DNA, and the detection method as well as the sex, age, and risk profile of the population tested, as summarized in the review by Vorsters et al.,[12] with HPV detection rates ranging from less than 10% to more than 80%. The authors concluded that HPV testing from urine was feasible but required more standardized methods for further analysis of its utility. As described on Tables 3 and 4, it missed 23 samples that were not detected on Cobas but only on HR-HPV Trovagene test. On the other hand, 45 samples were detected only by Cobas test, including HPV 18 and other hr-HPV types. We concluded that urine HPV detection is a very interesting approach to increase the number of women included in a screening program; however, accuracy is still a manner of concern, because larger number of clinically relevant cases was missed by Trovagene test than Cobas. In a more recent review, Pathak et al.[7] reported on HPV detection from urine samples in a more limited population of sexually active women, with sensitivity for HPV detection of 80%. This was similar to the detection rate observed in our current study. Detection of hr-HPV was high in both the urine samples (77.1%) and the cervical samples (83.6%) from our study, which is expected in the population evaluated, women referred for treatment of cervical disease due to a CIN 2+ diagnosis of a biopsy sample collected at the time of a colposcopy examination. The agreement for HR-HPV detection between the two samples types/assays was fair, with a κ value of

TABLE 4. Relationship Between Cobas Test (Cervical) and the hr-HPV Test (Urine) According to HPV Types

		HR-HPV test (Trovagene test)		
		Not detected	Detected	Total
Cobas test	Negative	32 (58.2%)	23 (41.8%)	55 (100.0%)
	HPV 16	18 (11.4%)	140 (88.6%)	158 (100.0%)
	HPV 18	0 (0.0%)	7 (100.0%)	7 (100.0%)
	Other hr-HPVs	27 (23.3%)	89 (76.7%)	116 (100.0%)
	Total	77 (22.9%)	259 (77.1%)	336 (100%)

HPV indicates human papillomavirus; hr-HPV, high-risk human papillomavirus.

0.363. This was quite similar to previously reported results using the HPV-HR test from the Predictors 4 study, in which a fair to moderate agreement ($\kappa = 0.467$) was observed between urine and cervical samples, both tested with the HPV-HR test.[10] Higher agreement between urine and cervical samples was observed in a pilot study evaluating a prototype of the HPV-HR test (urine samples) as compared with the Linear Array HPV genotyping test (cervical samples), in which a substantial agreement ($\kappa = 0.65$) was observed.[8]

Most studies that included the assessment of urine only evaluated HPV positivity and did not necessarily assess clinical performance to a histologically confirmed disease end point. In our study, despite a significant difference in the sensitivity for the urine and cervical samples, the point estimate was only approximately 7% lower for urine (83.4% vs 90.8% for CIN 2 cases and 85.6% vs 92.6% for CIN 3 in urine and cervical samples, respectively). The high sensitivity observed with the urine samples tested with the HPV-HR test is consistent with other reported estimates for this test, 92.3% by Sahasrabuddhe et al.[8] and 89.0% by Cuzick et al.,[10] which were approximately 4% and 5% lower than cervical samples in both respective studies. There have been limited published data on the clinical sensitivity to a histologically confirmed disease end point by other tests with urine samples. However, sensitivity estimates that have been reported with other tests have been more variable. Sahasrabuddhe et al.[8] reported 80.8% sensitivity for urine samples tested with the Linear Array test, whereas Stanczuk et al.[13] reported 63.1% sensitivity for urine samples tested with the Cobas HPV test. The variation in sensitivity for detection of cervical precancer and cancer from HPV testing of urine samples may be related to sampling, storage, and test methods, as indicated by Vorsters et al.[12] It is important to consider that vaginal self-sampling and urine collection are two interesting strategies to improve the chance of women who do not attend the screening to do a molecular test or be included in a screening program. However, the urine is not used as a screening method yet because the mechanism to detect HPV in urine is not completely understood. A recent study[13] compared urine and vaginal self-sampling and observed that sensitivity rates were similar to detect CIN 2+. Although urine had lower sensitivity, the authors considered the urine as a potential biological source to detect HPV.

One limitation of the study is that we must have caution interpreting histology results, particularly given the disagreements between LEEP and biopsy samples, mainly because biopsy tends to pick up more severe cases than conization. The consistently high sensitivity of the HPV-HR test's performance with preserved urine samples makes it a useful option for use in remote areas lacking medical professionals to implement cervical cancer prevention programs. Performance observed with the HPV-HR test and urine samples is also comparable with reported sensitivity estimates using cytology alone, which was the criterion standard for cervical cancer screening for decades.[14]

In conclusion, despite the significant differences observed between urine and cervical samples in our study, performance of the HPV-HR test with urine samples is consistent with previously reported studies using this method. Urine sampling with this assay offers an alternative for accessing under- or never-screened women to better identify those at risk for cervical disease.

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REFERENCES

1. Goodman A. HPV testing as a screen for cervical cancer. *BMJ* 2015; 350:h2372.
2. Lorenzi AT, Syrjanen KJ, Longatto-Filho A. Human papillomavirus (HPV) screening and cervical cancer burden. A Brazilian perspective. *Virol J* 2015;12:112.
3. Lorenzi AT, Fregnani JH, Possati-Resende JC, et al. Self-collection for high-risk HPV detection in Brazilian women using the careHPV™ test. *Gynecol Oncol* 2013;131:131–4.
4. Mauad EC, Nicolau SM, Moreira LF, et al. Adherence to cervical and breast cancer programs is crucial to improving screening performance. *Rural Remote Health* 2009;9:1241.
5. Arbyn M, Verdoodt F, Snijders PJ, et al. Accuracy of human papillomavirus testing on self-collected versus clinician-collected samples: a meta-analysis. *Lancet Oncol* 2014;15:172–83.
6. Arbyn M, Castle PE. Offering self-sampling kits for HPV testing to reach women who do not attend in the regular cervical cancer screening program. *Cancer Epidemiol Biomarkers Prev* 2015;24:769–72.
7. Pathak N, Dodds J, Zamora J, et al. Accuracy of urinary human papillomavirus testing for presence of cervical HPV: systematic review and meta-analysis. *BMJ* 2014;349:g5264.
8. Sahasrabuddhe VV, Gravitt PE, Dunn ST, et al. Evaluation of clinical performance of a novel urine-based HPV detection assay among women attending a colposcopy clinic. *J Clin Virol* 2014;60:414–7.
9. Senkomago V, Des Marais AC, Rahangdale L, et al. Comparison of urine specimen collection times and testing fractions for the detection of high-risk human papillomavirus and high-grade cervical precancer. *J Clin Virol* 2016;74:26–31.
10. Cuzick J, Ahmad A, Ho L, et al. Performance of the trovagene urine HPV assay in a referral population. In: *EUROGIN*. Spain: Sevilla; 2015.
11. Kurman RJ, Carcangiu ML, Herrington CS, et al. *WHO Classification of Tumours of Female Reproductive Organs*. 4th ed. IARC/WHO Classification of Tumours; 2014.
12. Vorsters A, Micalessi I, Bilcke J, et al. Detection of human papillomavirus DNA in urine. A review of the literature. *Eur J Clin Microbiol Infect Dis* 2012;31:627–40.
13. Stanczuk G, Baxter G, Currie H, et al. Clinical validation of hrHPV testing on vaginal and urine self-samples in primary cervical screening (cross-sectional results from the Papillomavirus Dumfries and Galloway-PaVDaG study). *BMJ Open* 2016;6:e010660.
14. Richardson LA, El-Zein M, Ramanakumar AV, et al. HPV DNA testing with cytology triage in cervical cancer screening: Influence of revealing HPV infection status. *Cancer Cytopathol* 2015;123:745–54.