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Short communication

High risk HPV testing for cervical cancer screening in a Puerto Rican population

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ABSTRACT

The Human Papillomavirus (HPV) causes cervical cancer, the fourth most common cause of death in women in the United States (US). Several major screening clinical trials have demonstrated that high risk HPV (HR-HPV) DNA primary screen is more sensitive at determining the risk of cervical intraepithelial neoplasia level 3 or higher (CIN $\geq$ 3) than cytology alone and is similar to co-testing. In this cross-sectional study, we characterized a Hispanic population of 18,052 women ages 21–70 years with HR-HPV DNA testing and cytology to determine the prevalence of HR-HPV in the population and determine the likelihood of high grade squamous intraepithelial lesion (HSIL). We also compared cytology, HR-HPV DNA testing, and co-testing strategies to determine sensitivity, specificity, positive predictive value, and negative predictive value for HSIL in cervical biopsies. Results show that HR-HPV had a slightly higher sensitivity (94.2% vs 92.3%) compared to cytology for all high-grade disease (CIN2/3).

1. Introduction

The human papillomavirus (HPV) is the principal cause of cervical cancer and can cause up to six different types of cancer (Saraiya et al., 2015). Cervical cancer is the fourth most common cancer among women globally, with an estimated 604,000 new cases and 342,000 deaths in 2020. About 90% of the new cases and deaths worldwide in 2020 occurred in low- and middle-income countries (Sung et al., 2021). Early detection of cervical cancer through routine screening is critical for early diagnosis and better survival outcomes. As evidence of this, cytological screening techniques are responsible for the large decrease in cervical cancer incidence worldwide (Adegoke et al., 2012).

Human papillomavirus is present in 99.7% of cervical squamous cell carcinomas (Walboomers et al., 1999). The most oncogenic strains identified by the International Agency for Research on Cancer (IARC) are genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. Among these strains, HPV 16 and HPV 18 cause approximately 70% of all cervical cancer cases (Saraiya et al., 2015). An alternative strategy to screening by cytology is to perform molecular based screening for HR-HPV to determine the risk of developing cervical cancer. Several large clinical trials and observational studies have demonstrated that HR-HPV testing as a primary screen or as co-testing with cytology improves the sensitivity of detecting CIN $\geq$ 3 over cytology alone (Mayrand et al., 2007, Katki et al 2011, Wright et al 2015, Ogilvie et al., 2018). One of these studies called the ATHENA study, was the first prospective US screening study to evaluate the performance of HR-HPV primary screening for women $\geq$ 25 years by comparing it to cytology alone or a hybrid strategy. The HR-HPV primary strategy (76.1%) was significantly more sensitive for CIN $\geq$ 3 compared to the cytology alone (47.8%) or hybrid strategies (61.7%) (Wright et al., 2015).

With these advancements in screening technology available today, it is surprising that in Puerto Rico (with a population of approximately 3.2 million), the incidence of cervical cancer in females between 2001 and 2017 increased from 9.2 to 13.0 cases per 100,000 (Ortiz et al., 2021). There is an indication that in Puerto Rico, compliance with testing guidelines may be lacking and that better screening strategies may help (Méndez et al., 2015). The aims of this study are to estimate the prevalence of HR-HPV genotypes in a Puerto Rican population and the likelihood of HSIL (Fig. 1) when compared to screening by cytology and co-testing. In addition, we provide estimates for the sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) for these three strategies.

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2. Materials and methods

2.1. Study design

This is a cross-sectional study (IRB# 1,810,001,213 Ponce Medical School Foundation, Inc) to evaluate as a primary aim the performance of the cobas HPV test for HR-HPV in women for the likelihood of HSIL compared to cytology and co-testing. A secondary aim will be to calculate the clinical performance of specificity, sensitivity, PPV and NPV for each test. The inclusion criteria for the study consisted of samples received for routine clinical screening at CorePlus Servicios Clínicos y Patológicos LLC, Carolina, Puerto Rico and processed for HPV and cytology from 2015 until 2018. Exclusion criteria were samples from patients < 21 years old and > 70 years old. In total, data from 18,052 anonymized Puerto Rican women received the HPV and cytology screening as part of their standard of care. The age distribution ranged from 24 to 70 years with a mean age of 43.6 years (SD $\pm$ 12.4).

2.2. Cytology preparation and screening

Cervical cytology specimens were collected in either ThinPrep (Hologic, Marlborough, MA) or SurePath (Becton, Dickinson and Company, Franklin Lakes, NJ) media and processed according to the manufacturer's instructions. The screening procedure starts with an evaluation by a certified cytotechnologist. The criteria for rescreening of the sample includes negative cases with the presence of actinomyces, herpes simplex virus, and trichomonas vaginalis, reactive changes, high-risk cases, positive HR-HPV and those randomly chosen for the 10% QC review. Cases interpreted as abnormal or malignant are reviewed by the senior cytotechnologist and pathologist.

2.3. HR-HPV testing

Cervical cytology specimens collected in ThinPrep or SurePath liquid based cytology media were processed via DNA extraction and PCR analysis for the presence of HR-HPV including genotypes HPV-16 and HPV-18. Samples were processed using the cobas HPV PCR test on the Roche cobas 4800 instrument according to the manufacturer's instructions (Roche Diagnostics, Indianapolis, IN).

2.4. Statistical analysis

Prior to any statistical analysis, we assessed the data for outliers, out of range values, and the need for data cleaning and editing by performing a series of frequencies, proportions, descriptive statistics and figures. Bivariate analyses were performed to determine any associations between the study explanatory variables (i.e., HR-HPV strains, etc.) and each HSIL category. Such analyses included Fisher exact tests. Multivariate logistic regression analyses were also conducted to determine the odds of HSIL categories for different HR-HPV strains. Sensitivity, specificity, positive predictive value and negative predictive value estimates and their 95% confidence intervals were calculated for HR-HPV testing, cytology screening, and co-testing using the biopsy results as the gold standard. All analyses were done using Stata statistical software package version 15 (Stata Corporation, College Station, TX). The usual two-sided 0.05 Type I error threshold for statistical significance was used for all analyses.

3. Results

3.1. Prevalence of HR-HPV

The prevalence of HR-HPV for different age groups was estimated. The HR-HPV DNA results positive for genotypes 16, 18, or the 12 other HR-HPV genotypes, included women that were positive for those genotypes and negative for all other genotypes (Table 1A). HPV positive results for genotypes 16 and 18 were analyzed individually as well as combined (genotypes 16 and/or 18). Samples positive for HR-HPV were positive for any of the 14 HPV genotypes. The overall prevalence of HR-HPV (14 genotypes) detected was 16.2% and the overall prevalence rates for HPV-16, HPV-18, the 12 other HR-HPV genotypes and HPV-16/HPV-18 were 2.4%, 1.3%, 12.6% and 3.6%, respectively (Table 1A). The overall prevalence of HR-HPV (14 types), as well as HPV-16 and HPV-18 individually, decreased with increasing age. The prevalence of abnormal cytology independent of HPV status was 9.5% in our study sample (data not shown).

3.2. Odds ratio for HSIL by HR-HPV test result

Table 1B shows the estimated odds ratios and 95% confidence

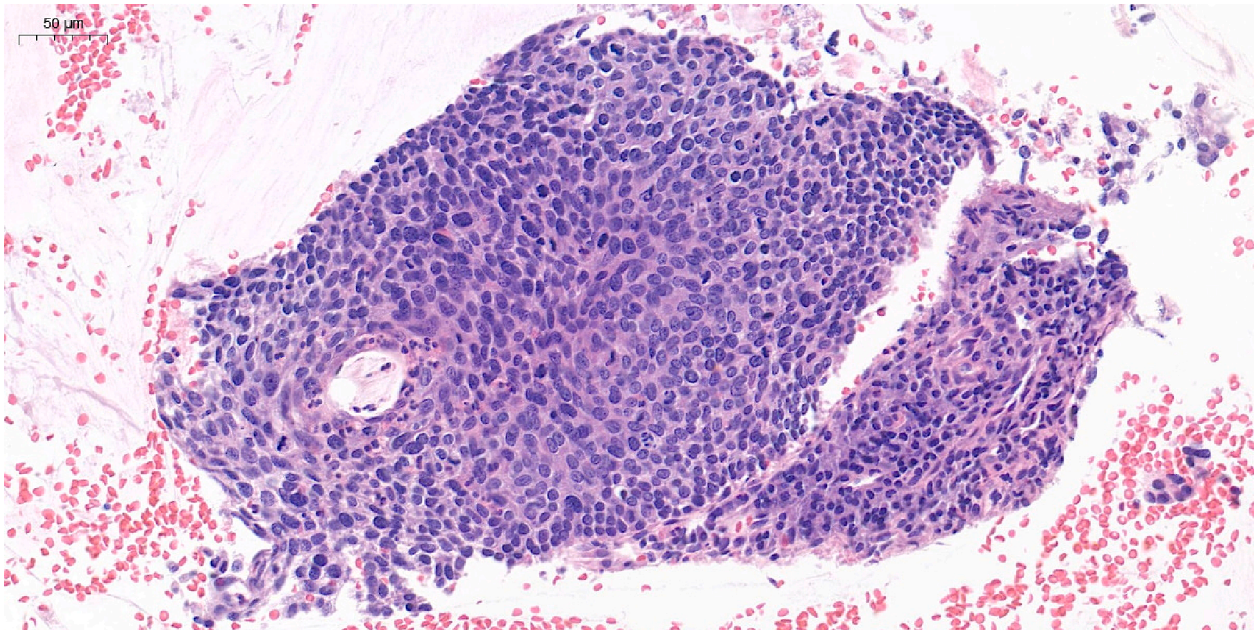

Fig. 1. High grade squamous intraepithelial lesion upon biopsy (H&E 20x WSI).

Table 1
A. Prevalence of HPV as percentages by genotype and B. Odds ratios for high grade disease in Puerto Rican women 21 years or older.

A.

Age Group,	Total	HPV Test Result, % HR-HPV+	HPV-16+	HPV-18+	12 Other HR-HPV+	HPV-16+/HPV-18+
21–29	2,644	31.3	4.7	1.7	24.9	6.5
30–39	5,003	18.6	2.9	1.7	14.0	4.6
40–49	4,449	13.4	1.8	0.9	10.7	2.7
50–59	3,524	10.1	1.3	0.9	7.9	2.2
60–69	2,287	8.8	1.1	1.0	6.6	2.2
>=70	145	11.0	2.1	1.4	7.6	3.5
Overall	18,052	16.2	2.4	1.3	12.6	3.6

B.

HPV Test Result	Estimated Odds Ratios (95% Confidence Intervals) CIN 2	CIN 3	CIN 2/3
HR-HPV+ vs HPV–	-*	5.4 (1.6, 17.8)	7.0 (2.1, 22.7)
HPV-16+ vs HPV–	9.3 (2.7, 31.3)	6.7 (3.4, 13.1)	7.1 (3.9, 13.2)
HPV-18+ vs HPV–	-*	1.6 (0.6, 4.9)	1.3 (0.4, 3.7)
12 other HPV+ vs HPV–	0.8 (0.2, 2.5)	0.5 (0.3, 1.0)	0.6 (0.3, 1.0)
HPV-16+/HPV-18+ vs HPV–	5.6 (1.7, 12.7)	6.0 (3.1, 11.5)	5.9 (3.2, 10.6)

* Some cells have zero cases and estimates cannot be calculated.

interval (CI) for the relative risk of HSIL (CIN2, CIN3 and CIN2/3) according to the HPV test result in women 21 years or older. The odds for HSIL were the highest in women with HPV-16; the odds for CIN2 were 9.3 (95% CI, 2.7–31.3) for CIN3 were 6.7 (95% CI, 3.4–13.1) and for CIN2/3 were 7.1 (95% CI,3.9–13.2) compared with HR-HPV negative women. This was similar to the odds of HSIL across all 14 high-risk types. The chances were lowest in the 12 HR genotypes other than HPV-16 and HPV-18 and was 0.8 (95% CI, 0.2–2.5) for CIN2, 0.5 (95% CI, 0.3–1) for CIN3 and 0.6 (95% CI, 0.3–1) for CIN2/3.

3.3. Clinical performance comparison

Table 2 shows the results of clinical sensitivity and specificity as well as positive predictive value and negative predictive value for the ability of HR-HPV testing, cytology or co-testing to detect HSIL. HR-HPV had a slightly higher sensitivity (94.2% vs 92.3%) compared to cytology for all high-grade disease (CIN2/3). Co-testing was 100% sensitive for high grade disease with 100% negative predictive value. Results between

Table 2
Sensitivity, Specificity, PPV and NPV for HSIL by HR-HPV testing, cytology testing, and co-testing in Women 21 Years or older.

HPV Testing Type		HSIL CIN 3 n = 539 (95% CI)	CIN 2/3 n = 550 (95% CI)
HR-HPV+	Sensitivity	92.7 (80.1–98.5) 29.9	94.2 (84.1–98.8) 29.9
	Specificity	(25.9–34.2) 9.8	(25.9–34.2) 12.3
	PPV	(7.0–13.2) 98.0	(9.3–15.9) 98.0
	NPV	(94.3–99.6)	(94.3–99.6)
Cytology	Sensitivity	90.2 (76.9–97.3) 35.5	92.3 (81.5–97.9) 35.5
	Specificity	(31.3–39.9) 10.3	(31.3–39.9) 13.0
	PPV	(7.4–14.0) 97.8	(9.8–16.9) 97.8
	NPV	(94.4–99.4)	(94.4–99.4)
Co-testing	Sensitivity	100.0 (91.4–100.0)	100.0 (93.2–100.0)
	Specificity	14.1	14.1
	PPV	(11.1–17.4) 8.7	(11.1–17.4) 10.8
	NPV	(6.4–11.7) 100.0 (94.9–100.0)	(8.2–14.0) 100.0 (94.9–100.0)

PPV = Positive Predictive Value
NPV = Negative Predictive Value

testing strategies were not significantly different.

4. Discussion

This study investigated a sample of Puerto Rican women 21 years or older for the prevalence of HPV, the likelihood of HSIL and the screening characteristics of three testing scenarios (HR-HPV, cytology and co-testing). Our data showed that women between 21 and 29 years old have a high prevalence of HPV (31.3%) that decreases with increasing age with an overall prevalence of 16.2% across all ages. Additionally, the study showed the odds of HSIL is higher with the presence of all 14 types of HR-HPV but especially with HR-HPV16 and HR-HPV18 compared to HR-HPV negative women. This agrees well with the known relative oncogenicity of the HR-HPV genotypes in which HPV-16 and HPV-18 cause ~ 70% of cervical cancer cases (Saraiya et al.,2015). Lastly, the data show that there is no significant difference between HR-HPV, cytology or co-testing to indicate HSIL in this cross-sectional study of Puerto Rican women.

Previous studies demonstrate that the status of HR-HPV is an important predictor for the prevention, detection, and treatment of HSIL in women and is more sensitive than cytology screening alone. However, US guidelines still include recommendations for cytology and co-testing. Current cervical cancer screening guidelines in the US are driven by the US Preventive Services Task Force (USPSTF) recommendations. USPSTF recommends screening for cervical cancer every three years using cytology alone in women aged 21 to 29 years. For women aged 30 to 65 years, screening every 3 years with cervical cytology alone, every 5 years with HR-HPV testing alone, or every 5 years with HR-HPV testing in combination with cytology is recommended.

Continued reliance on co-testing is partly due to reports that HR-HPV tests may miss some invasive cancers (Blatt et al., 2015) and the increased rate of colposcopies required with an HR-HPV primary screen. However, an analysis of the Kaiser Permanente Northern California study, which screened more than a million women 30 years and older with three-year co-testing, concluded that the contribution of the cytology component of co-testing for identifying pre-cancers and cancers was extremely small and worth questioning (Schiffman et al., 2018). Likewise, the ATHENA study which evaluated over 42,000 women $\geq$ 25 years with HR-HPV testing as a primary screen and compared it to cytology, co-testing and the USPSTF recommended hybrid strategy noted that although HR-HPV primary screen resulted in more colposcopies, the number of colposcopies required to detect a single CIN $\geq$ 3 case was the same as for the hybrid strategy (Wright et al., 2015).

Contrary to USPSTF, the cervical cancer screening guidelines from the American Cancer Society (ACS) lean more heavily on HR-HPV primary screening. ACS recommends primary HR-HPV screening for women $\geq$ 25 years of age every five years, with testing every 3 years by cytology as an acceptable alternative only when the primary HPV test is not available. Additionally, after a transition period at which time HR-HPV testing is widely available, the cytological screening will not be part of further updated cervical cancer screening guidelines from ACS (Fontham et al., 2020).

Despite the effectiveness of cervical cancer screening and risk management, the rate of cervical cancer in Puerto Rico increased between 2001 and 2017. This is likely due to the suboptimal cancer screening rates among Puerto Rican women (Ortiz et al., 2021) and the relatively high HR-HPV prevalence, especially in younger women (this study). It follows that an effective screening strategy started at a later age (25 years) with more time between follow up, may be advantageous to this population. HR-HPV PCR testing in Puerto Rico is widely available, with many public and private laboratories readily providing the service in most population centers, making this a viable option. Several studies in different geographies have indicated that HR-HPV screening is more cost effective than cytology or co-testing, with costs being moderated by the longer screening interval and genotyping to inform the need for

colposcopy (Bains et al., 2019, Cromwell et al., 2021). As an additional consideration, HPV self-collected samples for screening have been investigated in Puerto Rico and compared to clinician collected samples and no statistically significant differences were observed (Ortiz et al., 2013). Self-sampling along with HR-HPV testing may be another way to increase testing compliance.

In conclusion, our study demonstrates a high prevalence of HPV in this Puerto Rican population. Results comparing screening with HR-HPV testing, cytology and co-testing agree with the already well demonstrated evidence of the effectiveness of HR-HPV screening for HSIL. Due to the historical precedent in Puerto Rico of relatively low cervical screening participation rates, we would propose that screening guidelines recommended by the ACS are followed in Puerto Rico. This would mean starting cervical cancer screening at age 25 with HR-HPV testing. Women testing negative would be re-screened in five years. Management of positive results would follow the 2019 ASCCP risk-based management consensus guidelines also recommended by ACS. Early evidence from vaccination studies (Fontham et al 2020) indicates that HR-HPV testing may become even more effective than cytology screening as the HPV vaccinated population enters the screening age.

CRediT authorship contribution statement

Erik A. Gustafson: Writing – original draft, Methodology. **Juan C. Santa Rosario:** Writing – review & editing, Project administration, Supervision, Methodology. **Carlos Rios-Bedoya:** Formal analysis, Data curation, Visualization. **Mariano de Socarraz:** Conceptualization, Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [CorePlus was funded in part for this work by a research agreement with Roche Diagnostics (RD003764)].

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