Research Article

Cancer Prevention Research

High-Risk HPV Testing in Primary Screening for Cervical Cancer in the Public Health System, São Paulo, Brazil

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Abstract

Every year there are approximately 16,000 new cases of cervical cancer in Brazil. Novel screening technologies may reduce this number by expanding the population coverage but also by improving the detection rate of precursor lesions. We aimed to evaluate human papillomaviruses (HPV)-DNA testing in the context of routine cervical cancer screening in the public health system of the city of São Paulo, Brazil. Women participating in the primary screening program were invited to enroll. Liquid-based cytology samples were collected and cytology and Hr-HPV DNA testing were performed in parallel. Cytologists were blind to HPV results. Women older than 24 years with a positive high-risk HPV test and/or cytology class $\geq$ ASC-US were referred to colposcopy. From December 2014 to December 2016, 16,102 women joined the study. High-risk human papillomavirus (HR HPV) DNA prevalence was 14.9%, whereas cytologic abnormalities were found in 7.2% of the women. Per protocol, 1,592 Hr-HPV$^+$ women, in addition to 72 patients with cytologic classification > low-grade squamous intraepithelial lesion (LSIL) were referred. A total of 80 cervical intraepithelial neoplasia (CIN2$^+$) cases were diagnosed, 79 were Hr-HPV DNA$^+$ and 18 had normal cytology. Hr-HPV DNA detected a significant number of patients with premalignant lesions missed by cytology and all 16 CIN3$^+$ cases were Hr-HPV DNA$^+$. HPV genotyping may be useful in the management of Hr-HPV$^+$ women, reducing the burden of colposcopic referral for those harboring genotypes with a weaker association to CIN3$^+$. Use of HPV-DNA testing was shown to be feasible and advantageous over current cytologic screening in the public health system.

Introduction

Cervical cancer incidence rates are lowest in developed countries with well-established organized screening programs, whereas the rates are highest in some developing countries with inefficient opportunistic screening programs. This scenario is further aggravated in certain African countries, and elsewhere, by the frequent coinfection with human immunodeficiency virus (HIV; ref. 1; Table 1). On the basis of reported cause of death and local cancer registries (where available), the Brazilian National Cancer Institute (INCA) estimated an incidence of 16,370 cervical cancer cases in 2018, corresponding to an adjusted rate of 17.1 cases/100,000 women (2). Being a country with huge geographic and socioeconomic disparities, this number actually reflects the mean from high and low incidence areas. The north region, comprising the Amazon states, shows the highest rate in the country (24.9/100,000) highlighted by the appalling incidence in the Amazonas state (47.3/100,000) and its capital Manaus (61/100,000). In contrast, more developed areas display a much lower rate, like the state of São Paulo with 6.7 of 100,000 (2). This discrepancy can be partially attributed to the inconsistent coverage by the cervical cancer program, being higher in São Paulo, and the inherent geographic barriers that make it difficult to enroll women in Amazonas. Interestingly, there are no major differences in human papillomaviruses (HPV) prevalence among women from these two states (this article; ref. 3).

The unequivocal causal association between HPV and cervical cancer provided the basis for the introduction of screening tests that detect viral DNA or mRNA (4). First

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Table 1. Cervical cancer incidence per 100,000 women in selected countries

Country	Crude rate	Age-standardized rate
Bolivia	39.5	47.7
Uganda	22	44.4
Angola	20.4	35.5
Peru	31.3	32.7
Romania	39.4	28.6
Mexico	23.7	23.3
India	20.2	22
Argentina	23.6	20.8
Colombia	19.3	18.7
Thailand	23	17.8
Brazil	18.4	16.3
Germany	12	8.2
China	9.4	7.5
Italy	9.4	6.7
USA	8.1	6.6
Turkey	4.5	4.3

NOTE: From http://www.hpvcentre.net, data updated on November 15, 2015.

introduced and well accepted as a tool for the triage of smears with mild abnormalities, HPV testing went on to be compared with cervical cytology for primary screening in several large randomized studies (5, 6). These studies demonstrated HPV nucleic acid tests (NAT) to be more sensitive than cytology; the specificity was slightly lower as most HPV infections are asymptomatic and regress spontaneously. Moreover, HPV NATs are more objective, straightforward, and amenable to automation, whereas cytology presents inherent variability in its performance, influenced by both stringent preanalytical demands and observer skill. Not surprisingly, the detection rate of cervical intraepithelial neoplasia grade 3 and cervical cancer lesions by HPV-DNA testing was shown to be 60%–70% higher than using cytology alone (6).

Cytology has been the sole cervical cancer screening tool in the Brazilian program since its introduction in the 1970s. While some countries in Latin America (7) and Europe (8) have introduced HPV testing in combination with or in replacement of cytology, Brazilian government guidelines, last issued in 2016 (9), continue to exclusively recommend the Pap test for cervical cancer screening. However, a critical analysis of the historical results achieved by the program identified several technical deficiencies that may produce false-negative results, leading experts to advocate HPV DNA as an attractive alternative for cervical cancer screening (10). It must be recognized that a reduction in cervical cancer mortality in the state capitals has been demonstrated. This may be attributed both to a higher coverage of the cervical cancer program in addition to improvements in cervical cancer treatment and medical assistance in these cities (11).

One of the most frequent arguments against the adoption of HPV screening in Brazil is that despite the superiority of molecular testing observed in selected settings, like those provided by research protocols, similar results would not be possible under "real life" conditions (12).

In this study, we aimed to evaluate the performance of an HPV NAT in primary cervical cancer screening in the public health system in São Paulo. To comply with the national guidelines, all participating women were tested simultaneously with HPV-DNA and liquid-based cytology (LBC).

Materials and Methods

Study participants

Women attending Basic Health Units (UBS) in São Paulo city, belonging to the Brazilian universal access public health system (SUS), were invited to enroll in the study. All women were already participating or newly engaged in the cervical cancer screening program at the participating UBSs, in the majority under the direction of the Fundação Faculdade de Medicina (FFMUSP), a nongovernmental organization associated with São Paulo Medical School, part of the Universidade de São Paulo (São Paulo, Brazil). UBSs from the following neighborhoods participated in the study: Jardim Boa Vista, Jardim D'Abril, Jardim São Jorge, Paulo VI, Vila Dalva Guiherme, Vila Sônia, and Hospital e Maternidade Interlagos (not affiliated with FFMUSP).

Ethics statement

Institutional review board approval was provided by the Ethics Committee of the Faculty of Medicine, University of São Paulo (No. 075/13; São Paulo, Brazil) and all patients enrolled provided written informed consent. The study was carried in accordance to the Declaration of Helsinki ethical guidelines.

Samples

Cervical samples were collected from the endo- and ectocervix with a soft brush provided in the SurePath collection kit. The whole brush head was transferred to a vial containing BD SurePath Liquid (BD Diagnostics) and transported at room temperature to the Fundação Oncocentro de São Paulo (FOSP) laboratory for preparation of slides for cytology. A residual aliquot was taken for the HPV Assay (BD Onclarity). BD Totalys equipment was used to process the samples.

Hr-HPV-DNA testing

HPV was detected and genotyped using the BD Onclarity HPV assay, a real-time PCR running on the fully integrated Viper LT platform, which targets the E6/E7 genes of 14 high-risk HPV types. This assay provides individual genotyping information for six HPV types, 16, 18, 31, 45, 51, and 52, whereas another eight Hr-HPV types are reported in three distinct groups: P1 (HPVs 33/58); P2 (56/59/66), and P3 (35/39/68). The BD Onclarity HPV assay also amplifies the human *β-globin* gene as a control for both sample and process adequacy (13).

Colposcopy referral

The national guidelines stipulate that all women with a Pap smear categorized by the updated Bethesda system (14) as ASC-H, AGC, HSIL, or carcinoma should be referred for colposcopy. In some cases, depending on the patient's age and Pap history, LSIL and ASC-US may also require referral. In this study the above guidelines were observed. In addition, all women over 24 years of age with a positive HPV-DNA result were also referred to colposcopy.

Colposcopy

The SUS colposcopy centers that routinely receive patients from the participating UBSs were warned of the potential increase in the number of referrals. Colposcopies were performed by medical doctors aware of both cytology and HPV status. Biopsies were taken at suspicious cervical sites or acetowhite areas.

Histology

Biopsied lesions were sent in 4% buffered formalin to FOSP where they were processed routinely, they were stained with hematoxylin and eosin, and classified using the most recent WHO classification (15).

Data analysis

Histology proven CIN3$^+$ was used as the gold-standard criterion for the evaluation of the sensitivity, specificity, and predictive value for cytology and HPV-DNA testing.

Results

Participants

Between December 2014 and December 2016, 16,102 women joined the study. The mean age was 42 years $\pm$ 14.7 (12–90 year), with a median age of 41 years. A total of 2,121 participants were younger than 25 (13.2%).

Cytology

Overall, 82.8% of the participants reported ever having performed a Pap test, more than 90% of which were in the last 3 years (the interval recommended by the national guidelines). In 72.0% of the cases, cytologists confirmed the presence of cells from the squamocolumnar junction; however, among women older than 60 years this rate was below 50%. A total of 47 slides were classified as unsatisfactory for cytologic evaluation for different reasons (slide lost, broken, too much blood etc.), giving a total of 16,055 valid smears. The rate of cytologic abnormalities was 7.2%. ASC-US was the most frequently encountered class of altered cells, followed by LSIL, ASC-H, HSIL, and Carcinoma (Table 2).

Hr-HPV DNA

All samples were considered adequate by the onclarity test as the internal control gene (*β-globin*) was successfully amplified in all cases. Less than 1% of the samples failed on the first run but were successfully processed on a second attempt. Thus, all 16,102 samples had an evaluable Hr-HPV DNA test. Hr-HPV DNA prevalence was 14.9% (N = 2,400). Hr-HPV DNA frequency among women $\geq$30 years was 9.6% and 32.3% in those younger than 30 years (Fig. 1).

Colposcopy

According to the national guideline, which relies exclusively on cytology, 404 women would have been offered colposcopy; however, per study protocol, 1,664 women were finally referred. A total of 72 were Hr-HPV$^-$ but had

Table 2. Distribution of cytology results by age

	NILM	ASC-US	AG-US	ASC-H	LSIL	HSIL	Carcinoma	Total	AS-CUS$^+$
<25	1,834	93	0	24	151	19	0	2,121	
	86.5%	4.4%	0.0%	1.1%	7.1%	0.9%	0.0%		13.5%
25–29	1,470	65	1	14	55	15	0	1,620	
	90.7%	4.0%	0.1%	0.9%	3.4%	0.9%	0.0%		9.3%
30–34	1,721	81	0	12	56	13	0	1,883	
	91,4%	4.3%	0.0%	0.6%	3.0%	0.7%	0.0%		8.6%
35–39	1,706	50	1	15	36	9	0	1,817	
	93.9%	2.8%	0.1%	0.8%	2.0%	0.5%	0,0%		6.1%
40–44	1,645	59	1	14	32	13	1	1,765	
	93.2%	3.3%	0.1%	0.8%	1.8%	0.7%	0,1%		6.8%
45–49	1,637	62	0	9	23	4	1	1,736	
	94.3%	3.6%	0.0%	0.5%	1.3%	0.2%	0,1%		5.7%
50–54	1,508	47	1	13	20	8	0	1,597	
	94.4%	2.9%	0.1%	0.8%	1.3%	0.5%	0,0%		5.6%
55–59	1,232	30	1	9	12	2	0	1,286	
	95.8%	2.3%	0.1%	0.7%	0.9%	0.2%	0,0%		4.2%
60–64	974	25	1	5	7	3	0	1,015	
	96.0%	2.5%	0.1%	0.5%	0.7%	0.3%	0,0%		4.0%
$\geq$ 65	1,172	33	0	3	5	2	0	1,215	
	96.5%	2.7%	0.0%	0.2%	0.4%	0.2%	0,0%		3.5%
TOTAL	14,899	545	6	118	397	88	2	16,055	
	92.8%	3.4%	0.0%	0.7%	2.,5%	0.5%	0,0%		7.2%

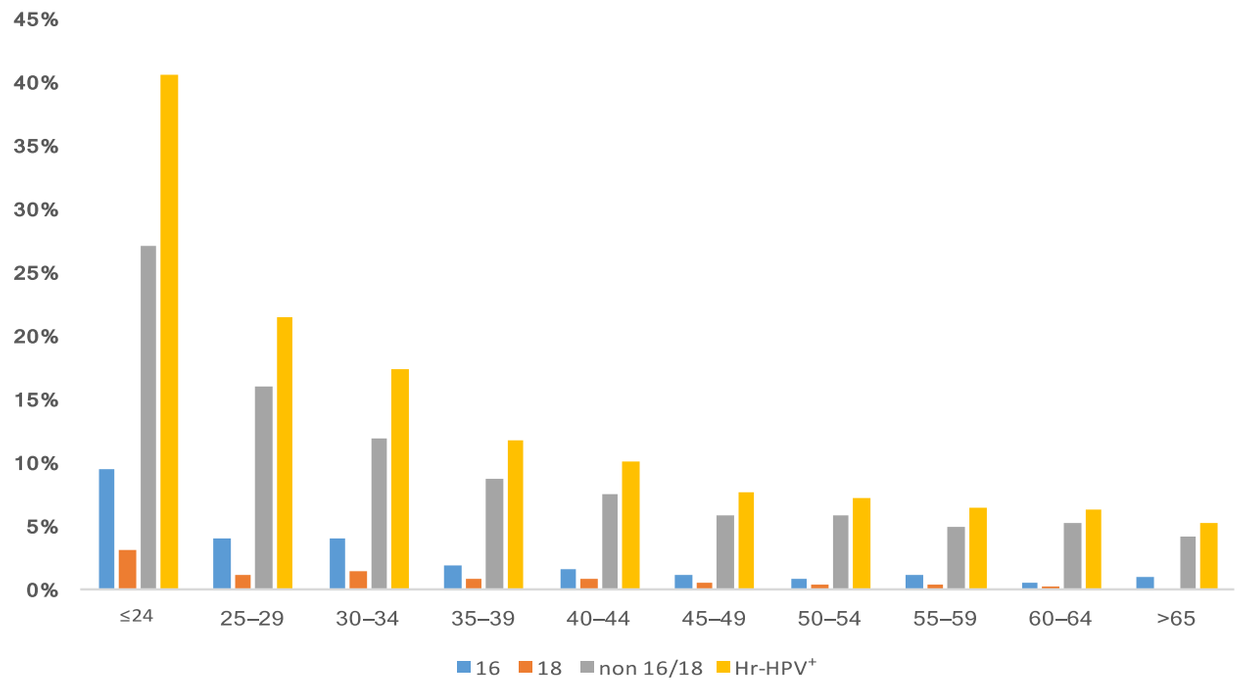

Figure 1.
Hr-HPV DNA prevalence by age and HPV genotypes.

a cytology diagnosis >LSIL ($n = 63$; 47 ASC-H, 11 HSIL, and 5 AGC) or an ASC-US/LSIL result on repeat testing ($n = 9$); 151 were both Hr-HPV$^+$ and had a smear classified as HSIL ($n = 77$), ASC-H ($n = 71$), carcinoma ($n = 2$), or AGUS ($n = 1$). In addition, another 1,441 referred subjects were Hr-HPV$^+$/cytology $\leq$ LSIL. Of those referred, 1,395 were actually submitted to colposcopy (84%). Patients that failed to perform colposcopy ($n = 369$) were both from the Hr-HPV$^+$/cytology $\leq$ LSIL ($n = 331$, all older than 24 years) and cytology $\geq$ LSIL ($n = 38$) groups. In 11 cases, the procedure was considered unsatisfactory. A total of 1,020 colposcopies did not reveal any abnormality, whereas in 364 cases, biopsies were taken from abnormal cervical areas (Table 3A). In total, 17.4 colposcopies were performed per CIN2$^+$ case detected, and 87.2 per CIN3$^+$ case.

Histopathology

Of the 364 biopsies, 197 were classified as normal (54%), with 87, 64, and 12 classified as CIN1, CIN2, and CIN3, respectively. In addition, two squamous cell carcinomas and two adenocarcinomas were diagnosed (Table 3B). All ($n = 80$) but one CIN2$^+$ lesions were Hr-HPV$^+$.

HPV genotypes

Among the 2,400 Hr-HPV$^+$ women, 1,762 (10.9% of the overall study population) were infected by a single genotype, with 638 (4.1%) infected with two or more genotypes (Table 4A). Probes P3 and P2 represented the most frequent Hr-HPV$^+$ results, followed by HPV 16 (Table 4B). Among the 16 CIN3$^+$ cases, HPV 16 was found in nine; HPV33/58 in three (one coinfection with HPV 16 and another with HPV45); HPV 31 and HPV 52 were each found in two cases; whereas HPV 45 and HPV 18 were identified in one CIN3$^+$ case each. Table 5 shows the frequency of each genotype in the study population and the rates of Hr-HPV infection in CIN3$^+$ over negative for intraepithelial lesion or malignancy (NILM) cytology as an index of oncogenicity.

Clinical performance

Cytology and HPV-DNA testing were independently evaluated taking histology proven CIN3$^+$ as the gold-standard. Sixteen cases of CIN3$^+$ were diagnosed. All 16 cases were identified by the HPV test, while two were classified as NILM by cytology, one of which was an adenocarcinoma. Consequently, the sensitivity of the HPV test was 100% while cytology, at the ASC-US cut-off, showed a slightly higher specificity (85.2% vs. 92.9%) and a significantly lower sensitivity (87.5%; Table 6).

Discussion

This study aimed to evaluate HPV-DNA testing for routine cervical cancer screening at public health units in the city of São Paulo, Brazil. The large number of subjects enrolled provides robustness to the findings; these include a high incidence of precursor lesions and

Table 3A. Colposcopic findings by Hr-HPV DNA and cytology results

	Hr-HPV$^+$/ CYTO$^-$ (%)	Hr-HPV$^+$/ CYTO > LSIL (%)	Hr-HPV$^-$/ CYTO^{+a} (%)	Total (%)
COLPO				
Invalid	11 (0.9)	0 (0)	0 (0)	11 (0.8)
Normal	937 (78.5)	34 (26.2)	49 (68.1)	1,020 (73.2)
Abnormal	245 (20.5)	96 (73.8)	23 (31.9)	364 (26)
Total	1,193	130	72	1,395

Abbreviation: CYTO, cytology.
[a]CYTO$^+$, >LSIL or repeated ASCUS/LSIL in the indicated time span (6 months–1 year after the initial altered smear).

Table 3B. Histopathology classification by Hr-HPV DNA and cytology results

	Hr-HPV$^+$/ CYTO$^-$ (%)	Hr-HPV$^+$/ CYTO >LSIL (%)	Hr-HPV$^-$/ CYTO^{+a} (%)	Total (%)
Biopsy negative	155 (63.3)	23 (24.0)	19 (82.6)	197 (54.4)
CIN 1	57 (23.3)	27 (28.1)	3 (13.0)	87 (24.0)
CIN 2	30 (12.2)	33 (34.4)	1 (4.3)	64 (17.7)
CIN 3	2 (0.8)	10 (10.4)	0 (0.0)	12 (3.3)
Carcinoma	0 (0.0)	2 (2.1)	0 (0.0)	2 (0.6)
Adenocarcinoma	1 (0.4)	1 (1.0)	0 (0.0)	2 (0.6)
Total	245	96	23	364

Abbreviation: CYTO, cytology.
[a]CYTO$^+$, >LSIL or repeated ASCUS/LSIL in the indicated time span (6 months–1 year after the initial altered smear).

Table 4A. Number of HPV genotypes in the study population

Number of HPV genotypes	Frequency	%
0	13,702	85.1
1	1,762	10.9
2	450	2.8
3	122	0.8
4	46	0.3
5	15	0.1
6	4	<0.1
9	1	<0.1
Total	16,102	100

Table 4B. Hr-HPV genotypes prevalence in the HPV$^+$ study population (N = 2,400)

Hr-HPV Genotypes	Frequency	%
35/39/68 (P3)	597	24.9
56/59/66 (P2)	582	24.3
16	506	21.1
52	389	16.2
33/58 (P1)	368	15.3
31	311	13.0
51	231	9.6
18	199	8.3
45	137	5.7

invasive cancers. The majority of the enrolled women (83%) were participating regularly in the cervical cancer screening program and had a Pap smear performed in the preceding 3 years. Despite this, four carcinomas were diagnosed among the over 16,000 women enrolled in the 2-year study, corresponding to an unadjusted incidence of 12.4/100,000 women/year. This rate is higher than the estimate of 10.05/100,000 (2) provided by the Brazilian National Cancer Institute for São Paulo city, suggesting that the incidence of cervical cancer in Brazil may be underestimated.

The overall rate of 7.2% abnormal cytologic preparations observed (i.e., ASC-US$^+$) was similar to our previous experience when LBC was introduced at FOSP (16). However, this value is significantly different from the numbers reported in the Brazilian National Cervical Cancer Screening program, mostly conventional cytology, that remained below 3% from 2006 to 2013 (10). LBC was enthusiastically adopted by the participating UBSs mostly due to the ease of sample collection, as well as the possibility of longer room temperature storage times when compared with conventional smears.

All participants received both cytology and HPV results. Before beginning the study, we anticipated that Hr-HPV$^+$/cytology normal results could lead to anxiety and stress for such patients. According to the UBS staff this did not prove to be a significant issue. Furthermore, we considered it to be unethical not to provide HPV results to participants. After the conclusion of the study, results were presented to the health professionals at the participating UBSs; they provided positive feed-back concerning a hypothetical change in the primary screening strategy.

This study clearly shows a higher detection rate of high-grade cervical abnormalities using molecular screening compared with cytology alone. It must be emphasized that had the comparator been conventional cytology, an even superior performance of the HPV-DNA test would be expected, because we previously observed lower rates of abnormal smears at the same institution when conventional and LBC were compared (16). However, there are several obstacles to the substitution of cytology by HPV testing. First, the cultural resistance of generations of health professionals and the public used to the periodic Pap smear as the single tool for cervical cancer screening. Bringing the established and accepted scientific evidence in support of HPV testing to the various stakeholders in cervical cancer screening will be key to the introduction of this innovative approach, which has become routine in some countries in recent years (17). Indeed, the recent publication of a recommendation from the Brazilian Federation of Gynecology and Obstetrics Associations supporting screening based on HPV testing indicates a growing acceptance by the national medical community (18). A second aspect always to be considered is the associated cost. Although HPV testing has been proven cost-effective in countries with striking differences, for example Mexico (19) and

Table 5. Frequency of individual HPV genotypes in the NILM HPV$^+$ population, CIN2, and CIN3$^+$ cases and rate of CIN3$^+$/NILM among HPV$^+$ women

Hr-HPV Genotypes	Frequency % Among Hr-HPV$^+$ (NILM)	CIN2	CIN3$^+$	Rate CIN3$^+$/NILM
16	13.6	52	56	4.1
33/58 (P1)	10.4	25	19	1.8
45	4.4	2	6	1.4
31	9.7	14	13	1.3
52	12.1	11	13	1.1
18	6.1	5	6	0.98
51	6.8	6	0	0.0
35/39/68 (P3)	18.7	16	0	0.0
56/59/66 (P2)	18.2	11	0	0.0

Table 6. Sensitivity, specificity, and positive and negative predictive values for cytology and HPV-DNA testing having CIN3$^+$ as the gold-standard

	HISTO CIN3$^+$			Sensitivity	Specificity	PPV	NPV
Cytology	Positive	Negative		87.5%	92.9%	1.2%	99.9%
≥ASC-US	14	1,142	1,156				
Negative	2	14,897	14,899				
	16	16,039	16,055				
	HISTO CIN3$^+$			**Sensitivity**	**Specificity**	**PPV**	**NPV**
HPV-DNA	Positive	Negative		100.0%	85.2%	0.7%	100.0%
Positive	16	2,384	2,400				
Negative	0	13,702	13,702				
	16	16,086	16,102				

Abbreviations: HISTO, histology; NPV, negative predictive value; PPV, positive predictive value.

Australia (20), an evaluation under the Brazilian health economics structure is pending. However, it is quite likely that the costs associated with the treatment of such a high number of cervical cancer cases in addition to the loss of working years from affected women and associated social costs would tip the balance in favor of screening with HPV testing.

Implementation of massive screening by molecular testing should not represent a challenge to the Brazilian public health system. Years ago, a network of laboratories performing HIV viral load determination was established in all Brazilian states, currently performing more than 1 million tests per year (21). Moreover, since 2015 it is mandatory to supplement serologic screening of donated blood units with NAT for Hepatitis C Virus (HVC) and HIV, representing another 3–4 million tests per year performed in 14 blood centers located in different Brazilian regions (22). This previous and ongoing experience demonstrates that implementing HPV testing is feasible. Initially, this could take place in a few centralized laboratories, also equipped for cytology triage and related IHC and histochemistry methods. This would greatly improve screening, as well as accurate diagnosis of precursor lesions and cervical cancers in Brazil. HPV testing may be carried out by high-throughput, fully automated method platforms, certainly more suitable for a program that foresees the evaluation of millions of samples yearly. However, it would be naïve to suppose that simply changing the screening method will solve the cervical cancer problem in Brazil. Identifying women harboring Hr-HPV DNA is only the first step in a necessary cascade of secondary and tertiary care. It is equally important to triage Hr-HPV$^+$ women properly and to provide timely intervention in confirmed CIN3$^+$ cases. Thus, specialized regional centers must be prepared to attend to the referred population, offering colposcopy, surgical pathology, as well as surgical and clinical services. As an example of the limitations to the current system, in this study it took, on average, 172 days from the altered cytology and/or Hr-HPV$^+$ result to colposcopy execution. Clearly, there is a bottleneck in scheduling colposcopy, as well as an important delay in getting histopathology results and ultimately in offering the recommended treatment.

The fact that public oncogynecologic services are overwhelmed emphasizes the importance of adopting appropriate triage of Hr-HPV-DNA$^+$ women. Several strategies have been proposed and implemented worldwide (17). It is well-known that HPV prevalence is directly correlated with age as an indirect marker of sexual activity. As demonstrated in this study, there is a substantial difference in the prevalence of Hr-HPV between women ages 30 years and over and those younger than 30 years (9.6% vs. 32.3%, respectively). Consequently, HPV-based cervical cancer screening in the younger population will inevitably display a very low positive predictive value and likely lead to unnecessary interventions that may harm the reproductive capacity of some young women. It is well documented that cervical cancer in women younger than 30 years is an uncommon event. These facts led to the almost universal recommendation that, where adopted, HPV screening be restricted to women ≥30 years old, which is the current guideline in the Netherlands and Italy. Other countries like Mexico and Sweden employ a mixed strategy, relying on cytology screening for those younger than 30 years (23). In our casuistic, the prevalence of Hr-HPV among cytology NILM women in between 25 and 29 years was 21.5% (Fig. 1), which certainly demands additional tests on HPV$^+$ women, to avoid a colposcopy referral rate impossible to be managed.

As demonstrated in several longitudinal studies (24–26), Hr-HPV genotypes harbor different oncogenic potential. Triage algorithms that take this into consideration have been proposed. In the simplest example, deployed in the United States and Australia, HPV16/18$^+$ women ≥30 years are directly referred to colposcopy, whereas those with non-16/18 Hr-HPV are submitted to reflex cytology triage. This approach is corroborated by data from the ATHENA trial (27). The Hr-HPV prevalence in this study (14.9%) was strikingly similar to that (14.7%) reported in the U.S. Onclarity HPV trial (28) including genotype-specific frequencies of HPV16 (3.1% vs. 2.7%), HPV18 (1.2% vs. 0.8%), and the 12 other HPV genotypes (11.7% vs. 11.2%). As shown in Table 5, HPV16, 33 or 58 (P1), 45, and 31 have a high rate (>1) of CIN3$^+$/NILM among HPV$^+$ patients, indicating higher risk of incident CIN3$^+$, hence demanding immediate referral.

Our study was cross-sectional and therefore unable to evaluate the longitudinal risk of CIN3$^+$ in cytology$^-$/Hr-HPV$^+$ women according to the HPV genotypes. Longitudinal observation of the women in this study will assist in the elaboration of an algorithm based on clinical–epidemiologic evidence linked to specific HPV genotypes, as allowed by the extended genotyping capability offered by the onclarity assay. Our evaluation of 12 referral algorithms showed that the use of 16/18 HPV genotyping and cytologic triage for the other Hr-HPVs provided the best balance between sensitivity and specificity, number of tests, and colposcopies required for detection of CIN2$^+$. A positive side-effect of adopting HPV as the primary screening tool is the improvement in cytologic diagnosis achieved when the observer is aware of the HPV status of the patient (29). The substantial improvement in cytology performance when HPV status is known suggests that further evaluation of cytologic triage must use this approach, in contrast to this study where blind reading was adopted.

This study has some limitations. Because neither colposcopy nor blinded biopsies were performed on cytology$^-$/HPV$^-$ patients, adjustment for false negatives could not be performed. Another drawback is the absence of follow-up that restricts the findings to the performance of HPV testing on the diagnosis of cervical abnormalities and prevents conclusions on the prognosis or long-term safety for the large HPV$^-$ group. Finally, abiding to the national guidelines, we did not refer HPV$^+$/cytology$^-$ women younger than 25 years to colposcopy and may have missed high-grade disease in this subset. With all these acknowledged limitations, the data are convincing for the superior sensitivity of Hr-HPV-DNA testing in comparison with cytology. However, the strategy for the HPV$^+$ women would be improved by adding a biomarker with high specificity for cervical dysplasia, to be performed as a reflex test on the original LBC. Proliferative markers such as p16/Ki-67 and methylation of host genes represent promising advances under evaluation in clinical trials (17).

This study provides practical evidence that may assist policy makers in offering the Brazilian female population the standard of care in secondary prevention and management of cervical cancer, embodied by HPV primary screening.

Disclosure of Potential Conflicts of Interest

J.E. Levi is a consultant/advisory board member for Abbott. L.L. Villa is consultant/advisory board member for Merck and Co for HPV Vaccines, BD, Occasional Consultant. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

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