ORIGINAL ARTICLE

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Diagnostic performance and concordance of triage tests for high-grade cervical premalignant lesions

Rendimiento diagnóstico y concordancia de pruebas de triaje para lesiones cervicales premalignas de alto grado

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ABSTRACT

Introduction: Early detection of cervical cancer is performed using triage tests such as liquid-based cytology (LBC) and HPV genotyping. Objectives: To evaluate the diagnostic performance and agreement of LBC and HPV genotyping for the triage of high-grade cervical premalignant lesions. Methods: A retrospective cohort study of women aged 30–65 years seen between 2020–2024 at a clinic in Lima, Peru. Diagnostic performance measures and agreement were calculated between LBC and genotyping results (16/18 and pool) and those of high-grade cervical premalignant lesions confirmed by cervical biopsy. Results: A total of 102 women were included, 53.9% of whom presented with high-grade lesions. Positivity rates were 13.7% for LBC and 71.6% for genotyping (16/18=26.5% and pool=45.1%). HPV 16/18 genotyping demonstrated higher sensitivity (32.7%) and agreement (kappa=0.130; p=0.061), whereas LBC showed higher specificity (93.6%). Conclusions: HPV 16/18 genotyping offered better sensitivity and slight agreement as a triage test for high-grade cervical premalignant lesions, while LBC was notable for its specificity.

Keywords: Biopsy, Uterine cervical neoplasms, Precancerous lesions, Genotyping techniques, Sensitivity and specificity, Cytology, Triage, Diagnosis, Genotype.

RESUMEN

Introducción: La detección temprana del cáncer cervicouterino se realiza mediante pruebas de triaje como la citología en base líquida (CBL) y la genotipificación de VPH (Gtp). Objetivos: Evaluar el rendimiento diagnóstico y concordancia de la CBL y Gtp para el triaje de lesiones cervicales premalignas de alto grado (LCPAG). Métodos: Estudio de cohorte retrospectivo, de mujeres entre 30-65 años atendidas entre 2020-2024 en una Clínica de Lima, Perú. Se calcularon medidas de rendimiento diagnóstico y concordancia entres los resultados de CBL y genotipificación (16/18 y pool) con los de LCPAG por biopsia cervical. Resultados: Se incluyeron 102 mujeres, 53,9% con LCPAG, 13,7% y 71,6% positivos por CBL y Gtp (16/18=26,5% y pool=45,1%). Gtp-16/18 tuvo mejor sensibilidad (32,7%) y concordancia (kappa=0,130; p=0,061), CBL mayor especificidad (93,6%). Conclusión: La Gtp-16/18 ofreció mejor sensibilidad y concordancia leve como prueba de triaje para LCPAG, mientras que la CBL destacó por su especificidad.

Palabras clave: Biopsia, Neoplasias del cuello uterino, Lesiones precancerosas, Técnicas de genotipaje, Sensibilidad y especificidad, Citología, Triaje, Diagnóstico, Genotipo.

Introduction

Cervical cancer (CC) is the third leading cause of cancer-related mortality among women and ranks fourth in incidence worldwide, according to data from the Global Cancer Observatory for 2022[1]. Approximately 90% of cases occur in low- and middle-income countries[2]. Its primary etiological factor —though not the sole cause— is infection with the human papillomavirus (HPV), comprising more than 200 genotypes, classified as low-risk (non-oncogenic) and high-risk (oncogenic). The latter includes 14 genotypes, among which types 16 and 18 are the most prevalent[3] and are strongly associated with the development of cervical cancer[4,5].

The early detection of precancerous lesions, including cervical intraepithelial neoplasia (CIN), is critical in preventing progression to cervical cancer;

accordingly, screening tests constitute a fundamental strategy for secondary prevention. According to the World Health Organization (WHO), there are different screening methods, such as the Pap smear (PAP) and the molecular HPV test (mtHPV) [6]. The former is used in developing countries due to its low cost and wide availability, with sensitivity ranging from 51% to 74%, which is considerably lower than that of mtHPV (98.1%)[7]. Given the characteristics of the test, it has been implemented worldwide with varying protocols[8].

Given the presence of low-risk HPV genotypes, a positive mtHPV result does not provide sufficient diagnostic specificity. Accordingly, the World Health Organization (WHO) recommends the use of a triage test—such as liquid-based cytology (LBC) or HPV genotyping with emphasis on types 16 and 18[9,10] —following a positive result. This approach facilitates the early identification of high-grade precancerous cervical lesions (HGCPL), which should subsequently be confirmed by biopsy[6,10]. Moreover, this strategy optimizes resource utilization and enhances the efficiency of care by reducing unnecessary referrals and minimizing the risk of overtreatment[11,12].

Given the wide range of evidence regarding the choice of triage test to be used, it is necessary to establish consensus tailored to each specific context [13]; for this reason, the objective of this study was to evaluate the diagnostic performance and concordance of LBC and genotyping as triage tests for the detection of HGCPL, based on biopsy results from patients in routine care.

Methods

A retrospective cohort study was conducted at a private healthcare facility in Lima, Peru, providing services through self-pay and private insurance schemes. The study included patients managed by a gynecologic oncologist between January 1, 2020, and July 31, 2024, whose samples were processed at the private laboratory Patología Oncológica.

Women aged 30 to 65 years with positive mtHPV results, irrespective of risk classification, were eligible for inclusion if they met the following criteria: (i) availability of screening and triage test results, including LBC and HPV genotyping for types 16/18 (Gtp-16/18) as well as other high-risk genotypes (Gtp-pool); and (ii) histopathological results obtained from cervical biopsy. This age range was selected because it corresponds to the population routinely undergoing the screening and triage procedures evaluated in this study at the participating institution.

To assess diagnostic performance in a routine clinical setting, we excluded women with a previously established diagnosis of cervical cancer or precancerous lesions, regardless of whether they were undergoing treatment, as well as those who had undergone a total hysterectomy. We did not perform sampling, as we included all women who met the eligibility criteria.

The triage tests evaluated were genotyping (Gtp-16/18 and Gtp-pool) and CBL, while the reference test was cervical biopsy. All results were obtained from medical records. At the study health facility, genotyping was performed using real-time PCR (qPCR) with the Cobas® 4800 HPV Test kit (Roche Molecular Diagnostics), which detects both genotypes 16/18 and 12 other high-risk genotypes (pool: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68)[14]; sample processing was performed following standardized protocols, ensuring diagnostic validity. Results were classified as positive based on the presence of genotype 16/18 (Gtp-16/18) or another high-risk genotype (Gtp-pool).

For LBC, the ThinPrep® system (Hologic Inc.) was used, employing vials with PreservCyt® preservative solution and automated processing using the ThinPrep 5000® system. Cytological interpretation was performed according to the Bethesda guidelines[15], with a five-level classification: i) negative or unidentifiable, ii) atypical squamous cells of undetermined significance (ASC-US), iii) atypical squamous cells that do not exclude a high-grade lesion (ASC-H), iv) low-grade squamous intraepithelial lesion (LSIL, equivalent to: CIN1, mild dysplasia), and v) high-grade squamous intraepithelial lesion (HSIL, equivalent to: CIN2, CIN3, moderate dysplasia, severe dysplasia, carcinoma in situ). The first four categories were classified as negative LBC results for HGCPL, and the fifth category was considered a positive result because it is specific to high-grade lesions equivalent to CIN2 and CIN3.

Cervical biopsies were processed using hematoxylin-eosin staining and classified as negative, CIN1, CIN2, CIN3, adenocarcinoma in situ (AIS), or invasive cancer according to the Bethesda cytological classification[16]. The presence of CIN2 or CIN3 was considered a positive result for high-grade precan-

cerous lesions, while negative and CIN1 findings were considered negative, in accordance with the guidelines of the American Society for Colposcopy and Cervical Pathology (ASCCP)[17].

In addition to the biopsy result, the colposcopy result was considered, with categories of negative, positive (grade 1: thin, punctate, and fine mosaic acetowhite epithelium; grade 2 or higher: dense, punctate, and coarse mosaic acetowhite epithelium), suspected invasion, and nonspecific[18].

The patient's age was categorized into five-year age groups; parity was defined as an ordinal variable (nulliparous, primiparous, multiparous). The age at first sexual intercourse was categorized into two groups: early (<16 years) and late (>16 years) [19]. Finally, the number of sexual partners was categorized into two groups: fewer than five partners, and five or more sexual partners[20].

With prior authorization from the healthcare facility, authors D.A., X.H., and X.P. equally reviewed 2,615 medical records from the gynecologic oncology clinic. After verifying compliance with the previously detailed eligibility criteria, 102 medical records were included for data extraction using an ad hoc form in REDCap (Figure 1).

For the statistical analysis, we used the STATA v.17.0 software, setting a significance level of 5% and a 95% confidence interval. We estimated Cohen's Kappa agreement coefficient for each of the triage tests in relation to biopsy, focusing on the identification of high-grade premalignant lesions. The Kappa coefficient was categorized as weak agreement (k <0.4), moderate agreement (k 0.4–0.6), and strong agreement (k >0.6), according to Landis and Koch[21]. Additionally, we estimated the sensitivity and specificity of LBC and 16/18 genotyping relative to biopsy. Finally, we describe the frequency of positive cases identified by the triage tests and confirmed by biopsy.

The research protocol was approved by the Research Ethics Committee of the University of Piura (File No. T0624-09). The analysis was performed on an anonymized database; this process was achieved during the export from REDCap, where the platform removed the patients' identifying information.

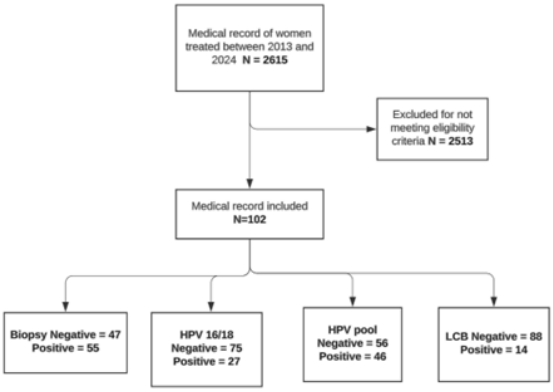

Figure 1. Screening flowchart for women aged 30–65 years with positive HPV test results and histology findings indicative of high-grade precancerous lesions (CIN2–CIN3). HC: medical history; HPV: human papillomavirus; LBC: liquid-based cytology.

Results

Among the 102 medical records included in the study, the highest proportion of patients undergoing triage was observed in the 30–34-year age group (46.1%). With respect to gynecological characteristics, 40.2% of participants reported initiating sexual activity at age 16 years or older. In terms of sexual history, 38.2% reported having fewer than five sexual partners. Regarding parity, nulliparous women constituted the largest proportion of the cohort (35.3%) (Table 1).

Through histopathological evaluation by biopsy, 55 confirmed cases (53.9%) of HGCPL were identified, of which 28 were CIN2 (50.9%) and 27 were CIN3 (49.1%). Of the latter, LBC identified 14 patients (13.7%) as positive cases; of these, 11 were HGCPL according to biopsy (78.57%), comprising 3 CIN2 and 8 CIN3 cases. On the other hand, 27 positive cases (26.5%) were identified by Gtp-16/18; of these, 18 were HGCPL according to biopsy (66.7%), most of which were NIC2 (n=10, 55.6%); 46 positive cases were identified by Gtp-pool (45.1%), 17 of which were HGCPL (37.0%) with 9 cases (52.9%) of NIC2 (Table 2).

In terms of diagnostic performance among the tests evaluated, Gtp-16/18 had the highest sensitivity (32.7; 95% CI: 20.7–46.7), followed by Gtp-pool (30.9; 95% CI: 19.1–44.8); while for specificity, the highest value corresponded to LBC (93.6; 95% CI: 82.5; 98.7), followed by Gtp-16/18 (80.9; 95% CI: 66.7; 90.9) (Table 3).

Table 1. Characteristics of the women included in the analysis (n = 102).

Characteristics	Frequency	Percentage
Sociodemographic		
Age group (years)		
30–34	47	46.1
35–39	17	16.7
40–44	18	17.6
45–49	10	9.8
50–54	6	5.9
55–59	2	2.0
60–65	2	2.0
Gynecological		
Risk by age at first sexual intercourse		
Not recorded	41	40.2
Low risk (≥16 years)	57	55.9
High risk (<16 years)	4	3.9
Number of sexual partners		
Not recorded	40	39.2
Low risk (<5)	39	38.2
High risk (≥5)	23	22.5
Parity		
Nulliparous	36	35.3
Primiparous	19	18.6
Multiparous	26	25.5
Not recorded	21	20.6
From the tests		
LBC results		
Positive	14	13.7
Negative	88	86.3
LSIL	26	29.5
ASCUS	24	27.3
ASCH	4	4.5
Unclassified	34	38.6
Colposcopy results		
Negative	20	19.6
Positive	82	80.4
Thin, acetowhite epithelium with fine punctate staining and a fine mosaic pattern	30	36.6
Dense, acetowhite epithelium with coarse punctate staining and a coarse mosaic pattern	52	63.4
Cervical biopsy results		
Negative	12	11.8
Low-grade premalignant lesion (CIN1)	35	34.3
High-grade premalignant lesion	55	53.9
CIN2+	28	50.9
CIN3	27	49.1
Molecular test result: 16/18 and/or pool		
Negative	29	28.4
Positive	73	71.6
16/18	27	37.0
Pool	46	63.0

LBC: Liquid-based cytology of the cervix; ASCUS: atypical squamous cells of undetermined significance; ASCH: atypical squamous cells that cannot exclude a high-grade lesion; LSIL: low-grade squamous intraepithelial lesion/CIN 1/mild dysplasia.

The LBC test showed a weak but significant agreement with biopsy for the identification of high-grade premalignant lesions (k = 0.128, p = 0.023), whereas 16/18 genotyping did not show significant agreement (k = 0.130, p = 0.061). Pooled genotyping showed negative agreement (k = −0.304 (95% CI: −0.487; −0.121)), with 65.7% of cases yielding discordant results compared to biopsy. (Table 3).

Discussion

In this study, we found that, in terms of diagnostic performance relative to cervical biopsy results, Gtp-16/18 demonstrated the highest accuracy, correctly classifying 54.9% of cases (TP and TN), followed by LBC (53.9%); specifically, Gtp-16/18 had higher sensitivity, although LBC stood out in terms of specificity. We also found that agreement was higher between Gtp-16/18 and cervical biopsy, although this did not reach statistically significant levels; meanwhile, LBC came in second but with significant agreement potentially attributable to sample size.

Our findings demonstrate that although genotyping, as a triage test, focuses on identifying viral genetic material from HPV, it is useful in the field for detecting positive cases of HGCPL, since this virus causes this type of lesion; whereas LBC is useful for determining negative cases because it is based on morphological evaluation. Both triage tests should be used with caution as diagnostic tools without replacing cervical biopsy.

In our study, LBC and Gtp-16/18 showed similar diagnostic performance. Although Gtp-16/18 identified more true-positive cases and thus had higher sensitivity, and LBC had higher specificity, when analyzing the confidence intervals of these diagnostic parameters, we observed overlapping ranges, which prevents us from conclusively affirming the statistical superiority of one screening method over another, although it does suggest potentially better performance.

High-income countries report clearer advantages for the use of molecular techniques over LBC. An Israeli study reported that primary screening with Gtp-16/18 detected 70% more CIN2-CIN3 cases than CBL, with higher sensitivity (91% vs. 85.7%) and similar specificities (88% vs. 87.5%)[23]. Similarly, a study in China evaluated six genotyping tests with sensitivities ranging from 79% to 97%, which could reduce unnecessary referrals for colposcopy, thereby improving the efficiency of the screening program[24]

Table 2. Distribution of positive triage test results relative to cervical biopsy results.

Biopsy Results	Gtp-16/18		Value of p†	Gtp-pool		Value of p†	LBC		Value of p††
	Yes n (%)	No n (%)		Yes n (%)	No n (%)		Yes n (%)	No n (%)	
Negative	2 (16.7)	10 (83.3)	0.438	9 (75.0)	3 (25.0)	0.012	1 (8.3)	11 (91.7)	0.064
NIC1	7 (20.0)	28 (80.0)		20 (57.1)	15 (42.9)		2 (5.7)	33 (94.3)	
NIC2	10 (35.7)	18 (64.3)		9 (32.1)	19 (67.9)		3 (10.7)	25 (89.3)	
NIC3	8 (29.6)	19 (70.4)		8 (29.6)	19 (70.4)		8 (29.6)	19 (70.4)	
Total	27 (26.5)	75 (73.5)		46 (45.1)	56 (54.9)		14 (13.7)	88 (86.3)	

†Pearson's chi-square test, ††Fisher's exact test. LBC: liquid-based cytology.

Table 3. Diagnostic performance characteristics of screening tests for precancerous lesions of the cervix, compared with biopsy results.

Test	TP n	FP n	TN n	FN n	S (IC95%)	E (IC95%)	PV+ (IC95%)	PV- (IC95%)	Kappa (IC95%)	*P* value
Gtp-16/18	18	9	38	37	32.7 (20.7; 46.7)	80.9 (66.7; 90.9)	66.7 (46.0; 83.5)	50.7 (38.9; 62.4)	0.130 (-0.031; 0.292)	0.061
Gtp-pool	17	29	18	38	30.9 (19.1; 44.8)	38.3 (24.5; 53.6)	37.0 (23.2; 52.5)	32.1 (20.3; 46.0)	-0.304 (-0.487; -0.121)	0.999
LBC	11	3	44	44	20.0 (10.4; 33.0)	93.6 (82.5; 98.7)	78.6 (49.2; 95.3)	50.0 (39.1; 60.9)	0.128 (0.007; 0.250)	0.023

S: Sensitivity, E: Specificity, TP: True positive, FP: False positive, TN: True negative, FN: False negative, 95% CI: 95% confidence interval, PPV: Positive predictive value, NPV: Negative predictive value, GT 16/18: 16/18 genotyping, Gtp-pool: Pool genotyping, LBC: Liquid-based cytology using the Pap test.

The higher sensitivity values for genotyping reported in the international literature[23,24] can be explained by the nature of the technique, which allows for the detection of viral DNA in the early stages of infection, even before the appearance of histological changes. Since many HPV infections are transient, a considerable number of positive results do not correspond to high-grade precancerous lesions[25]. Although genotyping provides sensitivity at the population level, in clinical practice its concordance with confirmed disease may be reduced depending on the duration of infection and the extent of cellular involvement.

Regarding LBC, its sensitivity can be affected by multiple factors such as staff training for sample collection, lack of follow-up, geographical barriers, a deficient healthcare system, and careless transport and processing of samples[15]. Gtp-pool showed poorer diagnostic performance, despite covering a wide variety of genotypes; however, not all of them have a strong association with the development of cervical precancer or cancer [26].

This study has limitations, most notably the small sample size, due to the requirement that patients have results for both Gtp and LBC—a practice that is not always common in routine clinical settings in developing countries that are implementing Gtp as a triage tool. Another limitation worth noting is that only patients from a single private health center were included, which does not allow for generalizations to other populations; however, this spatial restriction does help reduce sample heterogeneity to some extent. Likewise, although our study included women with positive mtHPV, not all tested positive for Gtp-16/18 and Gtp-pool because the mtHPV test used not only detected high-risk genotypes but could also yield false positives, reinforcing the need for confirmatory tests such as cervical biopsy.

The triage tests evaluated (Gtp and LBC) as well as biopsy report polytomous results, which were dichotomized for the purposes of this study; this could introduce classification bias, particularly for LBC since it was considered positive when HSIL results were present; however, this classification was adopted to assess HGCPL, as the standard classification takes into account the presence of cervical abnormalities of any grade,[22] a fact that deviates from our objective.

In conclusion, within our sample of women with positive mtHPV results, genotyping for HPV 16/18 (Gtp-16/18) demonstrated favorable diagnostic performance for the identification of HGCPL when compared with biopsy, with slightly adequate agreement rates; however, because this triage test is not yet fully implemented nationwide in all countries, it is important to bear in mind the performance of LBC for these lesions, particularly its high specificity.

Although the findings should be interpreted with caution due to the aforementioned limitations, this study represents an initial effort in our context to directly compare both screening approaches. As such, it provides preliminary evidence that may inform future research and contribute to the strengthening of secondary prevention strategies for cervical cancer.

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