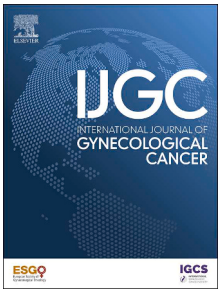
ORIGINAL RESEARCH

Diagnostic performance and clinical utility of p16 immunostaining in a population-based HPV DNA screening program



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ABSTRACT

Objective: This study aimed to evaluate p16 expression and its clinical utility in cervical biopsies from women who tested positive for high-risk human papillomavirus (HPV) in a real-world screening program, assessing its correlation with HPV genotype, lesion grade, clinical management, and diagnostic upgrading.

Methods: We conducted a diagnostic test study nested within the first round (2017-2022) of a population-based HPV DNA screening program in a Brazilian municipality. Women who tested positive for HPV were referred for colposcopy, and those who underwent cervical biopsy with p16(INK4a) immunohistochemistry were included. We analyzed age, HPV results, biopsy diagnoses, p16 status, procedures, and clinical outcomes using the χ^2 test or Fisher exact test and logistic regression ($p < .05$).

Results: Among 696 biopsies, 48.4% were p16-positive. Positivity increased with lesion severity, from 14.3% in negative biopsies to 80.0% in cervical intra-epithelial neoplasia grade 2 and 95.1% in cervical intra-epithelial neoplasia grade 3/adenocarcinoma in situ ($p <$ .001). No significant association was found between p16 and specific HPV genotypes (43·4%-60%), although 73% of p16-positive carcinomas were linked to HPV16/18. Excisional treatment was performed in 61.7% of p16-positive cases versus 7.5% of p16-negative cases ($p < .001$). Cervical intra-epithelial neoplasia grade 2 or worse was diagnosed in 69.4% of p16-positive cases ($p < .001$), with diagnostic upgrading 7 times more frequent than in p16-negative cases (19.9% vs 2.8%; $p < .001$). p16 showed a positive predictive value of 69% and a negative predictive value of 90% for cervical intra-epithelial neoplasia grade 2 or worse detection, reaching 91.6% in the non-HPV16/18 sub-group. The regression analysis confirmed p16 expression with 20-fold higher odds for significative cervical lesions (odds ratio 20.2, 95% confidence interval 13.3 to 30.6, $p < .001$).

Conclusions: Systematic p16 assessment in population-based screening improves risk stratification, diagnostic accuracy, and treatment allocation. These findings support integrating p16 into cervical cancer prevention strategies, particularly, in resource-limited settings where optimizing diagnostic precision and treatment balance is crucial.

Keywords:
Uterine Cervical Neoplasms; Human Papillomavirus DNA Tests; p16(INK4a) Protein Immunohistochemistry; Screening

WHAT IS ALREADY KNOWN ON THIS TOPIC

p16 is a well-established surrogate marker of oncogenic human papillomavirus (HPV) activity, widely used to improve the histopathologic diagnosis of cervical lesions, particularly, for differentiating cervical intra-epithelial neoplasia grade 2 from mimics. Most evidence comes from referral or research settings, with limited data on its systematic use in population-based screening. In addition, the association between p16 expression and specific HPV genotypes remains uncertain.

WHAT THIS STUDY ADDS

In a real-world, population-based HPV DNA screening program, p16 expression in cervical biopsies was strongly associated with lesion severity and diagnostic upgrading but not with specific HPV genotypes. p16-positive cases had significantly higher rates of excisional treatment and cervical intra-epithelial neoplasia grade 2 or worse confirmation compared with p16-negative cases. These findings confirm the independent value of p16 as a marker of clinically significant disease and underscore its potential for routine clinical practice.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE, OR POLICY

Incorporating p16 immunohistochemistry into diagnostic protocols may improve accuracy, support risk-based management, and help reduce over-treatment in cervical screening programs. Its routine use may be particularly valuable in settings with limited diagnostic expertise, supporting more consistent and safer clinical decision-making. These results support consideration of p16 as a complementary biomarker in population-based cervical cancer prevention strategies.

INTRODUCTION

Cervical cancer remains a major cause of cancer-related mortality among women worldwide, despite being largely preventable through the detection of precursor lesions via periodic

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screening.[1,2] Persistent infection with high-risk human papillomavirus (HPV) is the primary driver of cervical carcinogenesis, and HPV DNA testing has been incorporated into screening programs to identify women at risk of developing high-grade lesions.[3-5]

Compared with conventional cytology, HPV DNA testing offers superior sensitivity and reproducibility, enabling the detection of high-grade lesions and early-stage carcinomas up to a decade earlier.[5] Reflecting this improved performance, the World Health Organization endorsed in 2013 a revised screening strategy based on 5-yearly HPV DNA testing as the primary screening method.[6]

In Brazil, where cervical cancer remains a significant public health problem and approximately 70% of cases are detected at advanced stages,[7] a demonstration program was launched in 2017 through a partnership between the University of Campinas (UNICAMP) and the city of Indaiatuba (São Paulo). The program introduced an organized, population-based screening model using HPV DNA testing with partial genotyping (Cobas Roche Molecular Systems), targeting women aged 25 to 64 years at 5-year intervals. Its design and outcomes have been described.[3-5,8] Key findings in the first round included high coverage, a 4-fold increase in detection of precursor lesions, and a marked shift toward earlier-stage diagnoses, with most invasive cases detected at stage I.[5]

As part of the program's histopathologic quality assurance protocol,[8] cervical biopsies were subjected to immunohistochemical analysis for the $p16^{INK4a}$ (p16) oncoprotein. During HPV-driven oncogenesis, the viral E7 oncoprotein inactivates the retinoblastoma (pRb) tumor suppressor, leading to E2F release and uncontrolled cell cycle progression. In response, host cells upregulate p16, a cyclin-dependent kinase inhibitor, making its overexpression a reliable surrogate marker for high-risk HPV-induced transformation.[9-11]

Accordingly, p16 has been proposed as an ancillary marker to improve diagnostic accuracy, particularly, in equivocal cases, such as cervical intra-epithelial neoplasia grade 2, by identifying lesions with higher risk of progression and supporting clinical decision-making.[12-14] Given the high sensitivity (up to 98.5%) and negative predictive value (up to 99.7%) of HPV DNA testing, p16 immunohistochemistry may serve as a valuable adjunct for distinguish between clinically significant and insignificant lesions.[15,16]

This study aimed to evaluate p16 expression and its clinical utility in cervical biopsies from women who tested positive for high-risk HPV in a real-world screening setting, assessing its correlation with HPV genotype, lesion grade, clinical management, and diagnostic upgrading.

METHODS

A diagnostic test study was conducted evaluating p16 protein expression in cervical biopsies performed on women who tested positive for HPV DNA during the first round of an organized, population-based screening program in Indaiatuba, São Paulo, Brazil. Between October 2017 to September 2022, 20,551 women were screened. Those with a positive high-risk HPV result who were referred for colposcopy and underwent a colposcopy-directed cervical biopsy were considered eligible for this study (Fig.). p16 immunohistochemistry was successfully performed in 696 cases, which comprised the final study population.

Referral to colposcopy was based on a positive result for HPV16 and/or HPV18 without cytology or a positive result for a pooled group of 12 other high-risk HPV types combined with an abnormal reflex liquid-based cytology result from the same sample.[3,8] Colposcopic findings were classified according to the 2011 guidelines of the International Federation for Cervical Pathology and Colposcopy.[17] Biopsies were obtained in all cases with positive colposcopic findings, defined as the presence of at least 1 colposcopic image suggestive of minor or doubtful abnormalities.

After routine histopathologic evaluation, preliminary diagnostic reports were issued. Paraffin-embedded tissue blocks were subsequently sent to an external laboratory for immunohistochemistry using CINtec p16 histology antibody (Roche Diagnostics, Switzerland), processed on the Ventana BenchMark ULTRA platform with the Ultraview DAB detection system. Preliminarily planned as a quality-control assessment limited to cervical intra-epithelial neoplasia grade 2 cases, p16 testing was extended in the final study protocol to all biopsies collected during the first 5 years of the program.

Preliminary histopathologic evaluations were performed by a pathologist experienced in gynecologic oncology and were subsequently then reviewed in light of the histopathologic diagnosis provided by an external laboratory that incorporated p16 expression. Based on the p16 results from cervical biopsies, pathologists reviewed and, when necessary, updated the preliminary diagnoses. Discordant cases from cervical biopsies or excisional specimens were reviewed by a senior pathologist at the University of Campinas and discussed until consensus was reached. These revised diagnoses guided clinical management according to the program's predefined flowchart, which included options ranging from routine follow-up to excision of the transformation zone.[3,8] The final diagnosis was defined as the most severe histopathologic finding across all procedures and determined subsequent clinical interventions.

Laboratory and follow-up data were retrieved from the municipal digital health system and dedicated program spreadsheets. The data were compiled into Microsoft Excel for analysis. Statistical analyses considered the following variables: age (years) as continuous or grouped, screening DNA HPV testing (HPV16- and/or HPV18-positive or a group of 12 other high-risk HPV), reflex cytology (negative, low-grade abnormalities, or high-grade abnormalities including abnormal glandular cells), p16-negative or -positive expression, and histopathology diagnosis (negative; cervical intra-epithelial neoplasia grades 1, 2, or 3; adenocarcinoma in situ; micro-invasive or invasive cancer) from biopsy and including other excisional procedures. The statistical tests applied were linear trend tests (linear regression), χ^2 tests, and Fischer exact tests and p16 performance evaluation considered a logistic regression, sensitivity, specificity, and predictive values calculated for cervical intra-epithelial neoplasia grade 2 or worse diagnosis. The flow of patients (HPV testing — cytology — biopsy — p16 — final diagnosis) was visualized using Sankey diagrams created in Python (version 3.13) with the Plotly library (Plotly Technologies Inc., Montreal, QC, Canada). Analysis was performed using the StatsDirect software version 3.0 (England, www.statsdirect.com) and a $p < .05$ was considered statistically significant. The study followed the Standards for Reporting of Diagnostic Accuracy guidelines (www.Equator-network.org).

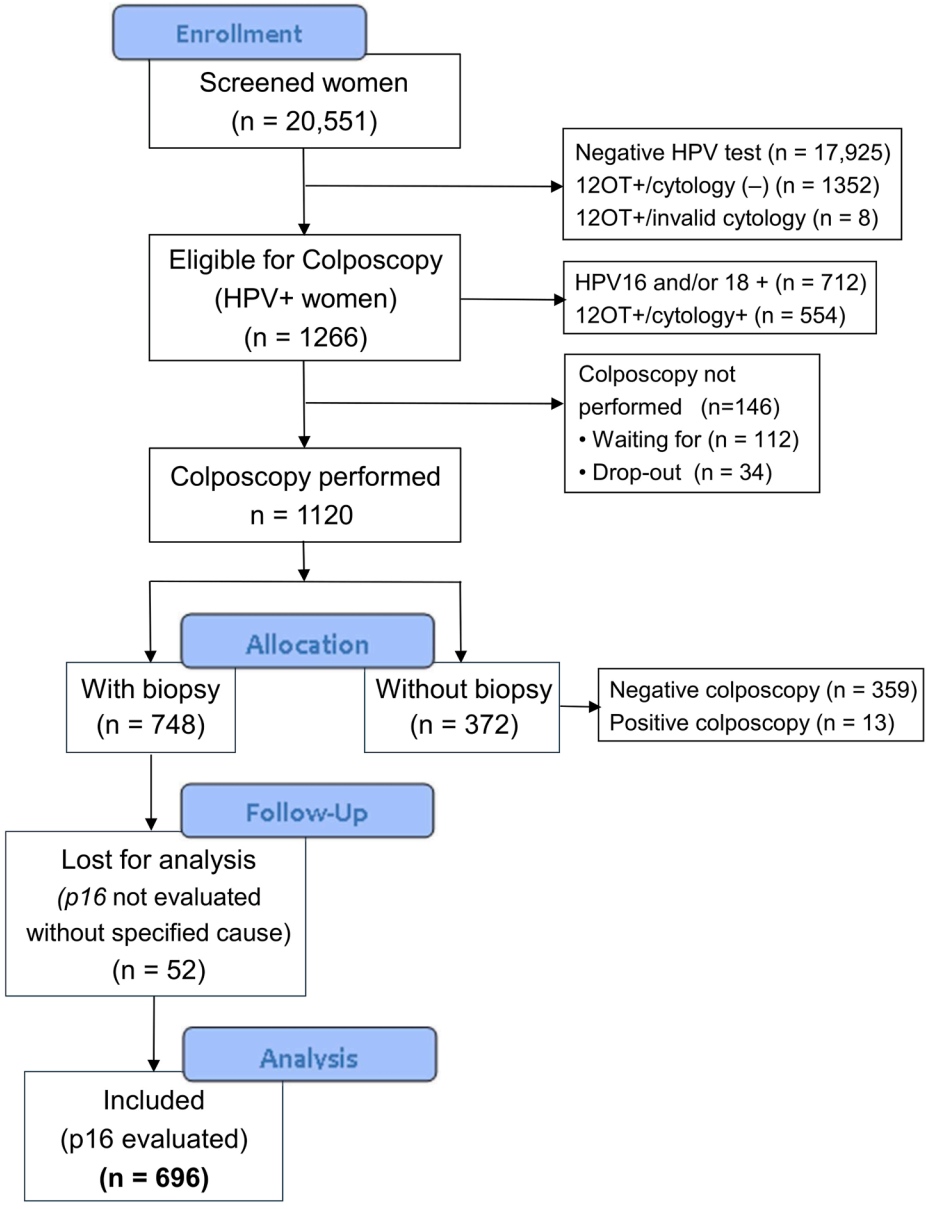

Figure Flowchart showing the process of participant inclusion in the study.

In accordance with the journal's guidelines, we will provide our data for independent analysis by a selected team by the Editorial Team for the purposes of additional data analysis or for the reproducibility of this study in other centers if such is requested.

RESULTS

Among the 696 participants, 98.7% (687 of 696) were within the program's target age range of 25 to 64 years. A total of 56.3% (392 of 696) tested positive for HPV-16 and/or HPV-18, whereas 43.7% (304 of 696) were positive for 12 other high-risk HPV types combined with abnormal reflex cytology. Overall, 48.4% (337 of 696) of cervical biopsies showed p16 positivity (Table 1).

Regarding the p16 expression by age group and screening results, p16 positivity remained approximately 50% across age groups, with no significant linear trend ($p = .40$). Women aged $\geq$50 years showed a lower positivity rate (38.0%, 35 of 92, $p = .033$; Table 1). p16 positivity ranged from 43.4% to 60.0% according to the screening test results ($p = .16$). Within the positive 12 other high-risk HPV types group, positivity was higher in women with atypical squamous cells of undetermined significance, which cannot exclude high grade, or high-grade cytology (59.4%, 2 of 19) compared with those with atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion (43.4%, 118 of 272), although this difference was not statistically significant (Table 1).

Considering clinical management, conservative approach was adopted in 92.5% (332 of 359) of p16-negative cases, including 168 negative biopsies, 155 cervical intra-epithelial neoplasia grade 1, and 9 cervical intra-epithelial neoplasia grade 2. In contrast, an excision of the transformation zone was performed in 61.7% (208 of 337) of p16-positive cases, and 19 subsequently underwent hysterectomy ($p < .0001$) (Table 2). Final diagnosis revealed cervical intra-epithelial neoplasia grade 2 or worse in 69.4% (234 of 337) of p16-positive cases, including 21 carcinomas (17 superficially invasive, stage IA1). Among p16-negative cases, only 1

Table 1 p16 Protein Expression in Cervical Biopsies According to Age Group and HPV DNA Screening Results Among Women in Routine Screening ($n = 696$).

Variable	p16-Negative ($n = 359$)		p16-Positive ($n = 337$)		*p*-Value
	n	(%)	*n*	(%)	
Age group					.40[a]
21-24 y	4	(44.4)	5	(55.6)	≥.05
25-29 y	98	(50.3)	97	(49.7)	≥.05
30-34 y	75	(53.8)	67	(46.2)	≥.05
35-39 y	58	(51.8)	54	(48.2)	≥.05
40-49 y	67	(45.0)	79	(55.0)	≥.05
50 y or older	57	(62.0)	35	(38.0)	.033
HPV DNA testing					.16[a]
HPV16+	142	(49.0)	148	(51.0)	≥.05
HPV18+	42	(51.2)	40	(48.8)	≥.05
HPV16+ and 18+	8	(40.0)	12	(60.0)	≥.05
HPV 12 other+ (non 16/18) plus cytology+	167	(54.9)	137	(45.1)	≥.05
Low-grade cytology	154	(56.6)	118	(43.4)	≥.05
High-grade cytology[b]	13	(40.6)	19	(59.4)	≥.05

Abbreviation: HPV, human papillomavirus.
High-grade: atypical squamous cells of undetermined significance, which cannot exclude high-grade and high-grade squamous intra-epithelial lesion.
Low-grade: squamous cells of undetermined significance and low-grade squamous intra-epithelial lesion.
[a] Linear trend test; χ^2 test for categories.
[b] Included 1 "atypical glandular cytology" case.

Table 2 p16 Protein Expression in Cervical Biopsies According to Histopathologic Diagnosis Severity and Post-Biopsy Management Procedures ($n = 696$).

Variable	p16-Negative ($n = 359$)		p16-Positive ($n = 337$)	
	n	(%)	*n*	(%)
Cervical biopsy				
Negative	168	(85.7)	28	(14.3)
Grade[a] 1	156	(64.5)	86	(35.5)
Grade[a] 2 or worse	35	(13.6)	223	(86.4)
Grade[a] 2	30	(20.0)	120	(80.0)
Grade[a] 3	4	(4.0)	96	(96.0)
Adenocarcinoma in situ	1	(33.3)	2	(66.7)
Suspected invasion	0	(0)	5	(100)
Post-biopsy procedure[b]				
None	332	(72.0)	129	(28.0)
Excisional procedure	26	(12.1)	189	(87.9)
Hysterectomy	1	(5.0)	19	(95.0)
Final diagnosis				
Negative	172	(86.0)	28	(14.0)
Grade[a] 1	151	(66.8)	75	(33.2)
Grade[a] 2 or worse	36	(13.3)	234	(86.7)
Grade[a] 2	21	(20.0)	84	(80.0)
Grade[a] 3	13	(9.4)	126	(90.6)
Adenocarcinoma in situ	1	(25.0)	3	(75.0)
Squamous cell carcinoma	1	(5.9)	16[c]	(94.1)
Adenocarcinoma	0	(0)	5[d]	(100)

The linear trend test for all 3 variables was $p < .001$.
[a] Grade refers to cervical intra-epithelial neoplasia graduation.
[b] Last procedure performed.
[c] A total of 15 of 16 squamous cell carcinoma cases were superficially invasive with p16 positivity.
[d] Adenocarcinoma included 2 micro-invasive, 2 invasive, and 1 adenosquamous.

superficially invasive squamous cell carcinoma was identified (Table 2). Carcinoma final diagnoses showed a 95% (21 of 22) p16 expression, whereas 73% (16 of 22) were associated with HPV16 and/or HPV18 ($p = .047$).

Diagnostic upgrading occurred 19.9% (66 of 332; 1 in 5) of p16-positive cases compared with 2.8% (10 of 359; 1 in 36) of p16-negative cases ($p < .0001$). Among cervical intra-epithelial neoplasia grade 2 biopsies, upgrading occurred in 26.7% (8 of 30) of p16-negative cases, including 1 superficially invasive carcinoma, and in 39.2% (47 of 120) of p16-positive cases, including 8 cancers; this difference was not statistically significant ($p = .20$) (Table 3).

Considering all 696 cases, p16 expression showed good diagnostic performance for detecting cervical intra-epithelial neoplasia grade 2 or worse, with an area under the curve of 0.81, sensitivity of 87% (232 of 268), specificity of 75% (323 of 428), positive predictive value of 69% (232 of 337), and negative predictive value of 90% (323 of 359) (Table 4). The Sankey diagram is shown in the supplementary material (Fig. S), illustrating patient flow from positive DNA HPV screening, stratified by testing considering the HPV16 and/or 18 genotyping, through reflex cytology results (low-grade or high-grade squamous intra-epithelial lesions), which was performed only when high-risk non-HPV16/18 types were detected. The diagram then depicts progression from the initial cervical biopsy results to p16 expression status and the final histological diagnosis, allowing visual comparison of how HPV16/18 versus 12 other high-risk HPV infections are distributed across each diagnostic step and, ultimately, across outcomes of cervical intra-epithelial neoplasia grade 2 or worse versus less severe diagnoses.

The multi-variable logistic regression analysis, including age, HPV testing results (HPV16/18 versus 12 other high-risk HPV types), and p16 expression, with cervical intra-epithelial neoplasia grade 2 or worse as the outcome, showed that only p16 expression remained strongly associated with cervical intra-epithelial neoplasia grade 2 or worse (odds ratio 20.2, 95% confidence interval 13.3 to 30.6, $p < .001$). Neither age (odds ratio ~1.00, $p = .71$) nor HPV16/18 positivity (odds ratio 1.21; 95% confidence interval 0.82 to 1.79, $p = .33$) showed a significant independent association.

Considering the HPV genotyping included in the screening test, the positive and negative predictive values of p16 expression for

Table 3 Diagnostic Upgrading at Final Diagnosis According to p16 Expression in the Initial Cervical Biopsy (n = 691).

Biopsy category (n)	Maintained diagnosis		Upgraded diagnosis					
			Total		Final diagnosis (n)			
	n	(%)	n	(%)	Grade[a] 2	Grade[a] 3	Superficially invasive	Invasive
p16-Negative (359)	**349**	**(97.2)**	**10**	**(2.8)**	**0**	**9**	**1**	**0**
Negative or Grade[a] 1 (324)	322	(99.4)	2	(0.6)	0	2	0	0
Grade[a] 2 (30)	22	(73.3)	8	(26.7)	0	7	1	0
Grade[a] 3 (5)	5	(100)	0	-	0	0	0	0
p16-Positive (332)	**266**	**(80.1)**	**66**	**(19.9)**	**9**	**41**	**13**	**3**
Negative or Grade[a] 1 (114)	103	(90.4)	11	(9.6)	9	2	0	0
Grade[a] 2 (120)	73	(60.8)	47	(39.2)	0	39	5	3[b]
Grade[a] 3 (98)	90	(91.8)	8	(8.2)	0	0	8	0

Note: Five cases with suspected invasion on biopsy were excluded. Upgrading from negative/grade[a] 1 with p16 positivity (9.6%, 11 of 114) versus p16-negative (0.6%, 2 of 324): $p < .001$ (Fisher exact test); grade[a] 2 upgrading with p16 positivity (39.2%, 47 of 120) versus p16-negative (26.7%, 8 of 30): $p = .20$ (χ^2 test).

[a] Grade refers to cervical intra-epithelial neoplasia graduation.

[b] One case of each histology: squamous cell carcinoma, adenocarcinoma, and adenosquamous.

Table 4 Final Diagnosis According to p16 Expression at Initial Biopsy, Stratified by Screening HPV Test Results (n = 696).

Screening category	Final diagnosis				Total (n)	Predictive value (%)
	Negative or grade[a] 1		Grade[a] 2 or worse			
	n	(%)	n	(%)		
HPV16- and/or HPV18-positive						
p16-Positive	59	(29.5)	141	(70.5)	200	70.5
p16-Negative	170	(88.5)	22	(11.5)	192	88.5
HPV 12 other positive plus low-grade cytology						
p16-Positive	46	(39.0)	72	(61.0)	118	61.0
p16-Negative	143	(92.9)	11	(7.1)	154	92.9
HPV 12 other positive plus high-grade cytology[b]						
p16-Positive	0	(0)	19	(100)	19	100
p16-Negative	10	(77)	3	(23)	13	76.9

HPV 12 other: pooled non-16/18 high-risk HPV types.
Low-grade: squamous cells of undetermined significance and low-grade squamous intra-epithelial lesion.
High-grade: atypical squamous cells of undetermined significance, which cannot exclude high-grade and high-grade squamous intra-epithelial lesion.
For all 696 cases: Negative predictive value = 90%; positive predictive value = 69%.
For all 304 HPV 12 other positive cases (any cytology): Negative predictive value = 91.6%; positive predictive value = 66.4%.

[a] Grade refers to cervical intra-epithelial neoplasia graduation.

[b] Included 1 atypical glandular cytology case.

the detection of cervical intra-epithelial neoplasia grade 2 or worse showed balanced performance for HPV16- and/or HPV18-positive tests, with positive and negative predictive values of 70.5% and 88.5%, respectively. Among the positive cases for the 12 other high-risk HPV types with high-grade squamous intra-epithelial lesion reflex cytology, performance improved, with a positive predictive value of 100% and a negative prediction of 76.9%. In contrast, among cases with low-grade squamous intra-epithelial lesion cytology, the positive predictive value decreased to 61%, whereas the highest negative predictive value was observed (92.9%) (Table 4).

DISCUSSION

Summary of Main Results

This diagnostic test study, conducted within a population-based HPV test screening program, demonstrated a strong association between p16 protein expression and cervical lesion severity. p16 positivity increased progressively from 14.3% in negative biopsies to 35.5% in cervical intra-epithelial neoplasia grade 1 and exceeded 86.4% in cervical intra-epithelial neoplasia grade 2 or worse. Women who were p16-positive underwent significantly more excisional procedures or hysterectomies than women who were p16-negative, of whom 72% required no further intervention. Diagnostic upgrading was 7 times more frequent among p16-positive cases, particularly, within the cervical intra-epithelial neoplasia grade 2 sub-group, in which 39.2% of p16-positive lesions were subsequently re-classified to more severe diagnoses, including 8 carcinomas. p16 immunohistochemistry achieved a positive predictive value of 69% and negative predictive value of 90.0% for cervical intra-epithelial neoplasia grade 2 or worse, confirming its value as a biomarker for risk stratification and diagnostic refinement.

Results in the Context of Published Literature

Although p16 overexpression is a well-established surrogate marker of HPV-induced oncogenic activity, our analysis did not find a significant association between p16 expression and specific HPV genotypes.[18] This finding aligns with previous studies showing that p16 reflects cellular transformation rather than viral genotype alone.[19,20] In our series, 95% of carcinomas expressed p16 and 73% were associated with HPV16/18, highlighting the added value of p16 for identifying malignant transformation beyond genotype-based risk assessment.

Previous studies have demonstrated the utility of p16, particularly, when combined with Ki-67 in cytology, to improve

diagnostic accuracy after positive HPV testing.[20-22] Ebisch and colleagues[22] evaluated p16 in 326 cervical biopsies from women who tested positive for HPV aged over 30 years, supporting its role within management algorithms. However, to the best of our knowledge, no previous population-based screening program has systematically implemented p16 evaluation as part of routine histopathologic protocols, underscoring the originality of our study.

Strengths and Weaknesses

The strengths of this study include its population-based design, integration within an organized screening program, standardized testing procedures, and a real-world applicability within the Brazilian public health system. The use of systematic follow-up and excisional procedures as reference standards provided robust diagnostic confirmation.

The limitations include the absence of formal use of p16 for clinical decision-making, which may have introduced clinician-driven management biases. Although rare, a small number of cervical intra-epithelial neoplasia grade 2 or worse lesions showed negative p16 staining, potentially due to technical issues, biological variability in the p16/Rb pathway, or non—HPV-related carcinogenesis.[16,23] In this study, we did not consider follow-up after diagnosis, limiting the confirmation of results to a posteriori, such as in the p16-negative or p16-positive sub-groups managed conservatively or without lesions. In addition, sampling errors or interpretative variability in borderline immunohistochemistry cases cannot be fully excluded.

Implications for Practice and Future Research

In resource-limited settings, access to colposcopy and specialized follow-up is often restricted, and variability in pathological expertise may compromise diagnostic accuracy. The use of p16 immunohistochemistry in cervical biopsies has the potential to increase the safety of clinical management decisions, whether conservative or interventional, by reducing uncertainty related to less experienced colposcopic assessments and improving the quality and reproducibility of histopathologic evaluation. Our findings reinforce the role of p16 immunohistochemistry as a valuable adjunct to histopathologic diagnosis in population-based cervical cancer screening. Although p16 testing entails additional costs and infrastructure, its use can enhance diagnostic precision, guide risk-based management, and minimize over-treatment, particularly relevant for younger women or those wishing to preserve fertility.[24,25]

Incorporating p16 into diagnostic algorithms may reduce misclassification of cervical intra-epithelial neoplasia grade 2, identify lesions at a higher risk of progression, and support clinical decisions when histology is equivocal. Future studies should evaluate cost-effectiveness, impact on long-term outcomes, and potential integration of p16 with molecular biomarkers in diverse screening settings.

CONCLUSIONS

Evaluation of p16 protein expression in cervical biopsies within a population-based HPV DNA screening program confirmed its strong correlation with lesion severity and contribution to clinical management. Although not associated with specific HPV genotypes, p16 significantly improved diagnostic accuracy for intermediate lesions, enabling more targeted interventions and reducing unnecessary interventions in women who were p16-negative. These findings support integrating p16 immunohistochemistry into cervical cancer screening protocols, particularly, in settings with limited pathology expertise, to improve diagnostic precision, guide risk-based care, and, ultimately, reduce disease burden through earlier and more accurate detection.

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Ethical Statement This study is part of a larger research project approved by the ethics committee of UNICAMP in May 2015 (approval No. 1.045.580; CAAE: 43815315.9.0000.5404), which covers the evaluation of the program's first 5 years. The study involved monitoring data entered into the municipal digital system without direct participant contact or intervention and focused solely on monitoring and technical advice. The municipal program was implemented as an official public health initiative and informed consent was not required. Throughout the study, strict data confidentiality, privacy, and security measures were observed. The data set used for statistical analysis was anonymized and securely stored, remaining under the responsibility of the coordinating researcher (JT).

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Author Contributions Conception and design: MGD, RMT, DBV, LCZ, and JCT. Acquisition of data: All authors. Analysis and interpretation: TGS, DBV, MGD, RMT, LCZ, and JCT. Drafting of the manuscript: TGS, DBV, and JCT. Critical revision and final approval: All authors.

Declaration of Competing Interests None declared.

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Data Availability Statement The data set from this study is available at https://data.mendeley.com/ (doi: 10.17632/2mzjbrvp9j.1).

Supplemental material Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijgc.2025.104451.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71(3):209—249. https://doi.org/10.3322/caac.21660.
2. Bouvard V, Wentzensen N, Mackie A, et al. The IARC perspective on cervical cancer screening. *N Engl J Med.* 2021;385(20):1908—1918. https://doi.org/10.1056/NEJMsr2030640.
3. Teixeira JC, Vale DB, Campos CS, Bragança JF, Discacciati MG, Zeferino LC. Organization of cervical cancer screening with DNA-HPV testing impact on early-stage cancer detection: a population-based demonstration study in a Brazilian city. *Lancet Reg Health Am.* 2021;5:100084. https://doi.org/10.1016/j.lana.2021.100084.

4. Teixeira JC, Vale DB, Discacciati MG, Campos CS, Braganca JF, Zeferino LC. Cervical cancer screening with DNA-HPV testing and precancerous lesions detection: a Brazilian population-based demonstration study. *Rev Bras Ginecol Obstet*. 2023;45(1):21–30. https://doi.org/10.1055/s-0043-1763493.
5. Teixeira JC, Vale DB, Campos CS, et al. Transition from opportunistic cytological to organized screening program with DNA-HPV testing detected prevalent cervical cancers 10 years in advance. *Sci Rep*. 2024;14(1):20761. https://doi.org/10.1038/s41598-024-71735-2.
6. Global strategy to accelerate the elimination of cervical cancer as a public health problem. World Health Organization. Accessed July 7, 2025. https://www.who.int/publications/i/item/9789240014107.
7. Estimate 2023: cancer incidence in Brazil. National Cancer Institute, Ministry of Health Brazil. Accessed July 7, 2025. https://www.inca.gov.br/sites/ufu.sti.inca.local/files//media/document//estimativa-2023.pdf.
8. Teixeira JC, Vale DB, Bragança JF, Campos CS, Discacciati MG, Zeferino LC. Cervical cancer screening program based on primary DNA-HPV testing in a Brazilian city: a cost-effectiveness study protocol. *BMC Public Health*. 2020;20(1):576. https://doi.org/10.1186/s12889-020-08688-4.
9. Benevolo M, Mottolese M, Marandino F, et al. Immunohistochemical expression of p16(INK4a) is predictive of HR-HPV infection in cervical low-grade lesions. *Mod Pathol*. 2006;19(3):384–391. https://doi.org/10.1038/modpathol.3800551.
10. Miyamoto S, Hasegawa J, Morioka M, Hirota Y, Kushima M, Sekizawa A. The association between p16 and Ki-67 immunohistostaining and the progression of cervical intraepithelial neoplasia grade 2. *Int J Gynaecol Obstet*. 2016;134(1):45–48. https://doi.org/10.1016/j.ijgo.2015.12.005.
11. Damgaard R, Jenkins D, Stoler MH, et al. $p16^{INK4a}$ and HPV E4 immunohistochemistry for the prediction of regression of cervical intraepithelial neoplasia grade 2-a historical cohort study. *Int J Cancer*. 2025;157(7):1294–1303. https://doi.org/10.1002/ijc.35469.
12. Dijkstra MG, Heideman DAM, de Roy SC, et al. p16(INK4a) immunostaining as an alternative to histology review for reliable grading of cervical intraepithelial lesions. *J Clin Pathol*. 2010;63(11):972–977. https://doi.org/10.1136/jcp.2010.078634.
13. Miralpeix E, Genovés J, Maria Solé-Sedeño J, et al. Usefulness of $p16^{INK4a}$ staining for managing histological high-grade squamous intraepithelial cervical lesions. *Mod Pathol*. 2017;30(2):304–310. https://doi.org/10.1038/modpathol.2016.168.
14. Gustafson LW, Tranberg M, Christensen PN, et al. Clinical utility of p16/Ki67 dual-stain cytology for detection of cervical intraepithelial neoplasia grade two or worse in women with a transformation zone type 3: a cross-sectional study. *BJOG*. 2022;130(2):202–209. https://doi.org/10.1111/1471-0528.17248.
15. Rao A, Young S, Erlich H, et al. Development and characterization of the cobas human papillomavirus test. *J Clin Microbiol*. 2013;51(5):1478–1484. https://doi.org/10.1128/JCM.03386-12.
16. Stoler MH, Wright TC Jr, Ferenczy A, et al. Routine use of adjunctive p16 immunohistochemistry improves diagnostic agreement of cervical biopsy interpretation: results from the CERTAIN study. *Am J Surg Pathol*. 2018;42(8):1001–1009. https://doi.org/10.1097/PAS.0000000000001072.
17. Bornstein J, Bentley J, Bösze P, et al. 2011 colposcopic terminology of the International Federation for Cervical Pathology and Colposcopy. *Obstet Gynecol*. 2012;120(1):166–172. https://doi.org/10.1097/AOG.0b013e318254f90c.
18. Wei F, Georges D, Man I, Baussano I, Clifford GM. Causal attribution of human papillomavirus genotypes to invasive cervical cancer worldwide: a systematic analysis of the global literature. *Lancet*. 2024;404(10451):435–444. https://doi.org/10.1016/S0140-6736(24)01097-3.
19. Darragh TM, Colgan TJ, Cox JT, et al. The Lower anogenital squamous Terminology Standardization Project for HPV-Associated Lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. *Arch Pathol Lab Med*. 2012;136(10):1266–1297. https://doi.org/10.5858/arpa.LGT200570.
20. Zhang Y, Li H, Li X, et al. Associations of multi-human papillomavirus infections with expression of p16 in a cohort of women who underwent colposcopy: a retrospective study of 5165 patients. *Front Oncol*. 2023;13:1265726. https://doi.org/10.3389/fonc.2023.1265726.
21. Wright TC Jr, Stoler MH, Ranger-Moore J, et al. Clinical validation of p16/Ki-67 dual-stained cytology triage of HPV-positive women: results from the IMPACT trial. *Int J Cancer*. 2022;150(3):461–471. https://doi.org/10.1002/ijc.33812.
22. Ebisch RMF, Rijstenberg LL, Soltani GG, et al. Adjunctive use of p16 immunohistochemistry for optimizing management of CIN lesions in a high-risk human papillomavirus-positive population. *Acta Obstet Gynecol Scand*. 2022;101(11):1328–1336. https://doi.org/10.1111/aogs.14459.
23. Petry KU, Cox JT, Johnson K, et al. Evaluating HPV-negative CIN2+ in the ATHENA trial. *Int J Cancer*. 2016;138(12):2932–2939. https://doi.org/10.1002/ijc.30032.
24. de Sam Lazaro S, Newbill CP, Berlin M, Morgan TK. p16 Staining of Cervical Biopsies may decrease the frequency of unnecessary Loop electrosurgical excision procedures. *J Low Genit Tract Dis*. 2016;20(3):201–206. https://doi.org/10.1097/LGT.0000000000000189.
25. Kyrgiou M, Athanasiou A, Paraskevaidi M, et al. Adverse obstetric outcomes after local treatment for cervical preinvasive and early invasive disease according to cone depth: systematic review and meta-analysis. *BMJ*. 2016;354:i3633. https://doi.org/10.1136/bmj.i3633.