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REVIEW

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p16/Ki-67 dual staining as a predictive value for cervical cancer compared to other conventional triage tools: a descriptive literature review

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Abstract

Cervical cancer (CC) poses a significant global health concern, ranking as the fourth most frequently diagnosed cancer and the fourth leading cause of death among women worldwide. Ecuador bears a substantial burden of CC, with a considerable number of new cases and deaths reported annually. The primary cause of CC is the human papillomavirus (HPV), a sexually transmitted virus that is usually eliminated by cell immunity. However, around 5% of infections persist and can lead to invasive cancer. This literature review assessed the predictive value of p16 and Ki-67 dual staining (DS) as a standalone method or combined with conventional triage methods to improve CC screening programs. A total of 42 relevant articles were analyzed, evaluating the performance of DS in predicting cervical intraepithelial neoplasia (CIN) of varying severities. DS exhibited a median sensitivity and specificity of 87.7% and 76.7% for detecting CIN2+ and 89.7% and 79.6% for CIN3+. When combined with liquid-based (LB) cytology, DS demonstrated superior sensitivity and specificity compared to other screening strategies. This review suggests that p16 and Ki-67 DS alone or in combination with liquid base (LB) could enhance the accuracy and effectiveness of CC screening.

Keywords

Dual staining; p16; Ki-67; Cytology; Cervical cancer

1. Introduction

Cervical cancer (CC) is the fourth most commonly diagnosed cancer and the fourth leading cause of death in women worldwide, with over 600,000 new cases and more than 300,000 deaths reported in 2020 [1, 2]. In Ecuador, CC ranks as the second most common type of cancer, with an estimated 1500 new cases and over 800 deaths in the same year [3, 4].

Human papillomavirus (HPV) is a sexually transmitted virus that is responsible for causing CC [5]. While most HPV infections typically clear within two years, persistent infections can lead to disease progression [6]. The literature categorizes HPV types based on their oncogenic potential. High-risk HPV types (HR-HPV) associated with precancerous lesions include 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68 [7]. Conversely, low-risk HPV types (LR-HPV) such as 6, 11, 42, 43 and 44 primarily cause genital warts [8]. Lastly, there are unclassified-risk HPV types, namely 26, 34, 40, 54, 55, 57, 61, 67, 69, 70, 71, 72, 73, 82, 83 and 84 [8, 9].

The screening tools commonly used for CC detection include cytology-based methods, liquid-based (LB) cytology, and HPV DNA testing. The Pap smear, also known as cytology-based screening, involves collecting superficial epithelial cells from the transformation zone and immediately fixing them on a glass slide. This technique has a sensitivity of approximately 60% and depends on the perspective of the observer [5, 8–10]. In LB cytology, epithelial cells are suspended in a liquid medium and then transferred to a slide for examination. LB cytology exhibits similar sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) to a Pap smear [5, 7, 11]. Another screening tool is HPV DNA testing, which determines the presence of the genetic material of the HPV virus in the sample and also indicates the genotype of the HPV infection in women. It can be done by self-sampling, a urine test, or taken by a physician [11, 12]. According to the literature, HPV testing has been used as a standard test for CC screening, with reported sensitivity and specificity ranging from 65% to 95%, and 50% to 85%, respectively [13–16].

Most screening programs use cytology and/or HPV testing as triage tools. However, these techniques do not provide a persistence or progression prognostic for CIN2+. To improve CC screening programs, different approaches have emerged, such as dual staining (DS) of the CC-related proteins p16 and Ki-67. This technique identifies the co-expression of p16 (a tumor suppressor marker) and Ki-67 (a proliferative

marker) in the same cervical epithelial cell [5, 17]. p16 is a cyclin-dependent kinase inhibitor that facilitates the re-binding of retinoblastoma protein (Rb) and E2F transcription factor. However, its function is disturbed by E7 oncoprotein from HR-HPV when it interrupts the Rb-E2F pathway, leading to an overexpression of p16. It indicates an HR-HPV-induced transformation in cervical epithelial cells [17–19]. The Ki-67 antigen, a nuclear protein expressed throughout the cell cycle except in G0, is typically limited to the basal layer of squamous epithelium in the uterine cervix under normal physiological conditions [17]. Dual expression is necessary to determine the risk of cervical cancer, since their presence is mutually exclusive in a normal cell [20]. Several studies have analyzed the sensitivity and specificity of DS of both proteins compared to or in combination with other screening tools to predict the risk of CC. However, little is known about the correct management of women in CC screening programs when one protein (either p16 or Ki-67) shows positivity in women with positive HR-HPV test or CIN2+ cytology.

To improve CC screening programs, we aimed to review the scientific evidence of p16 and Ki-67 DS as predictive values for CC and precancer alone or when combined with other conventional triage tools. This review also includes what is known about the independent expression of either protein and its relation to CC.

2. Materials and methods

2.1 Data source and search strategy

The PubMed database served as our sole data source. Four keywords were employed to locate articles related to our topic of interest: p16, Ki-67, cervical cancer, and women. In addition, three filters were applied in the search, targeting sex (female), age (adult: 19–44 years, middle-aged: 45–64 years), and a timeline (since 2013). The literature search identified 101 articles up to 15 February 2023. Forty-one articles lacking the specified keywords in their titles were removed from the PubMed list before proceeding to the screening stage.

2.2 Screening

This review included original research articles discussing the proteins of interest and CC. The remaining 60 studies were uploaded to the web tool Rayyan for systematic review by a researcher [21]. A blind first screening was done by two other researchers, who only read abstracts. After reaching a consensus, 11 articles were excluded. Finally, a second blind screening by the same two researchers was conducted to assess methods and materials, excluding seven articles. Studies were excluded for the following reasons: (1) being reviews or non-original articles; (2) being non-English articles; (3) not being related to the keywords; and (4) not being accessible. Fig. 1.

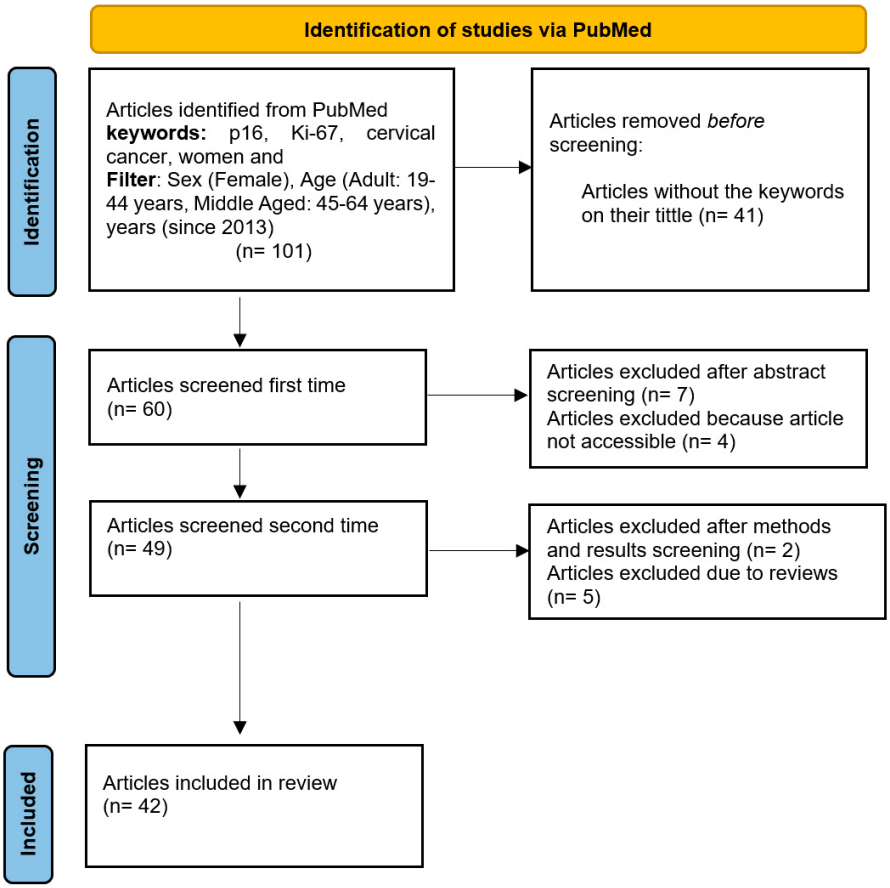

FIGURE 1. Flowchart of the screening process. Most articles were divided into Table 1 (n = 9) and Table 2 (n = 29), based on the classification of each table. However, four articles were included in both, since they met both requirements. The eight articles that were not included in the tables were analyzed in the results section.

2.3 Data collection and extraction

An online Excel document, containing the extraction matrix, was designed and used for this stage [22]. The data collected from the 42 selected articles by the two previous researchers encompassed various parameters, including the title, language, study year, publication year, country/city, study design, age group, median age, type of sample, recruitment method, sample size, final sample size, statistical analysis method, dependent variable, independent variable, objectives, results, conclusions, limitations and future studies, abstract and keywords. Additionally, the impact factor (IF) of the journal at the time each article was published was included as an additional parameter for evaluation.

2.4 Data analysis

Microsoft Excel was used to calculate averages, maximums and minimums. In addition, to determine the strategy that optimally combines high sensitivity and specificity, an analysis was conducted to generate a likelihood ratio plot illustrating the performance characteristics of different triage tests. RStudio (version 2023.03.0) and the ggplot2 package (version 3.4.2) (Posit Software, Boston, MA, USA) were utilized for this purpose [23, 24]. In constructing the likelihood ratio graph, 1 minus specificity was plotted on the x-axis, and sensitivity on the y-axis. Additionally, LR+ and LR− slopes were computed using the mean values of sensitivity and specificity reported for all diagnostic methods. LR+ was calculated as (sensitivity/(1 − specificity), and LR− as (1 − sensitivity)/specificity. For the Youden Index line, a slope of 1 and the calculated Youden Index value were used as the cut-off point. For the LR+ line, the LR+ value served as the slope, and the cut-off point was set at (1,1). Lastly, for the LR− line, the cut-off point was (0,0), and the slope was determined by the LR− value. This approach facilitated the identification of distinct regions on the graph that determined the most effective overall strategies, the least effective overall strategies, those best suited for detecting the presence, and those best for detecting the absence [25].

3. Results

Detailed information about articles reporting the specificity and sensitivity of p16/Ki-67 DS is presented in two main tables.

Table 1 compares p16/Ki-67 DS alone as a triage strategy to predict CIN2+ and CIN3+ in nine articles. The final sample ranges from 93 to 25,577 women, with an average of 3193 participants. The age range is from 15 to 88 years old; however, some studies also classified their results from women older and younger than 30 years old [26–30]. Most studies used histopathology as a gold standard, and only one used cytology [31]. To identify CIN2+/3+ lesions, the highest sensitivity for p16/Ki-67 DS alone was 90.9% and 93.1% [32], and the lowest was 80.7% and 86.8% [28], with an average of 87.8% and 89.7%, respectively. On the other hand, the highest specificity for predicting CIN2+/3+ lesions were 95.2% and 94.8% [29], and the lowest was 63% [27] and 69.4% [28], with an average of 76.7% and 79.6%, respectively. For women older than 30 years old, the highest sensitivity found was 91% [27] and 91.3% [26], and the lowest was 81.2% and 85.4% [28], for CIN2+/3+ respectively. For specificity, the highest was 96.2% and 95.9% [29], and the lowest was 67% [27] and 75.2% [28], correspondingly. In addition, the average IF of the articles from Table 1 is 5.34.

Table 2 reports the results of 29 articles that used p16/Ki-67 DS in combination with other triage strategies to predict CIN2+ and CIN3+. To assess the sensitivity and specificity of strategies involving various cytology types (including LB), there exist variations among authors in terms of the cytology categories included in the analysis. Table 2 elucidates these differences, with specific notations to convey the inclusions for each cytology category. An asterisk (*) appended to a particular cytology category indicates that the authors considered data solely from that specific cytology category. On the other hand, if a cytology category is denoted with a plus sign (+), it indicates that the analysis encompassed that category and more severe cases. Lastly, in cases where no mark is added, it is implied that all the cytology categories under the Bethesda System were encompassed in the sensitivity and specificity analysis.

Using the likelihood ratio graph, the strategies found to have the highest sensitivity and specificity were those that combined LB cytology plus p16/Ki-67 DS, as shown in Fig. 2A. Strategies placed in the upper left corner correspond to the best fit for sensitivity and specificity, as explained in Fig. 2B. Furthermore, the mean IF from articles listed in Table 2 is 4.20.

Articles analyzing p16/Ki-67 DS alone and in combination are included in both tables. The remaining eight articles were not included in either table, as they lacked data on sensitivity or specificity. However, they were still included in the review because they provide additional information about the performance of p16/Ki-67 DS as a predictor of HSIL and CC. Studies show similar results when comparing p16/Ki-67 DS positivity with increasing cytology or histology severity [60–66], including a study that also links HR-HPV with high expression of p16/Ki-67 [65]. Moreover, one study found a correlation between positive p16/Ki-67 DS results and significantly higher cumulative five-year risks of ≥CIN2 [60]. In contrast, another study concluded that p16/Ki-67 DS did not provide any information about the progression or persistence of HSIL/CIN2± in HR-HPV-positive women. However, the authors also mentioned that the difference among the study populations could be the reason for such discordance with the literature [67]. Finally, only two studies included the analysis of the expression of p16 or Ki-67 individually. One study found differences in the positivity of one of the two proteins in CIN2/3 patients. Three cases of p16-negative CIN2/3 showed Ki-67 positivity, while six cases of Ki-67-negative CIN2/3 exhibited p16 positivity [63]. Another study also found a few cases of CIN2/3 where only one protein was positive. In addition, the authors analyzed the expression of Ki-67 in the lower, medium, and higher third of the epithelium from cervical biopsies in comparison with CC lesion severity. The authors concluded that a combination of p16 negativity and the absence of Ki-67 staining beyond the lower third of the epithelium almost ruled out high-grade lesions [61].

TABLE 1. Sensitivity and specificity of p16/Ki-67 DS alone as a triage strategy to predict CIN2+ and CIN3+.

First Author; Year	Final Sample	Age Group	Gold Standard	Sensitivity % 95% Confidence interval (CI)	Specificity % 95% Confidence interval (CI)
S.K. Zhang *et al.* [26] 2019	537	20–79	Histopathology	CIN2+ 88.1 (83.0–91.8) CIN3+ 91.3 (85.9–94.7)	CIN2+ 85.0 (80.7–88.4) CIN3+ 76.8 (72.3–80.8)
A. Celewicz *et al.* [27] 2018	93	16–64	Histopathology	CIN2+ 90	CIN2+ 63
M. El-Zein *et al.* [28] 2021	492	19–73	Histopathology	CIN2+ 80.7 (75.0–85.6) CIN3+ 86.8 (79.7–92.1)	CIN2+ 69.4 (60.9–77.1) CIN3+ 69.4 (60.9–77.1)
H. Ikenberg *et al.* [29] 2013	25,577	≥18	Histopathology	CIN2+ 86.7 (81.1–90.9) CIN3+ 87.4 (79.5–92.5)	CIN2+ 95.2 (94.9–95.4) CIN3+ 94.8 (94.5–95.1)
K. Prigenzi *et al.* [30] 2018	151	15–62	Histology	HSIL 61.5 (31.6–86.1)	HSIL 91.1 (80.4–97.0)
P.J. Toliman *et al.* [31] 2020	243	30–59	LB Cytology	HSIL+ Cervical Specimens 100.0 (84.6–100.0) Vaginal Specimens 68.2 (45.1–86.1)	HSIL+ Cervical Specimens 79.6 (70.0–87.2) Vaginal Specimens 84.9 (76.0–91.5)
L. Yu *et al.* [32] 2016	1290	30–69	Histopathology	CIN2+ 90.9 (86.5–94.0) CIN3+ 93.1 (88.6–96.0)	CIN2+ 79.5 (77.0–81.8) CIN3+ 77.2 (74.6–79.6)
R. Zhang *et al.* [33] 2018	223	20–73	Histopathology	CIN2+ 90.2 (84.5–94.3)	CIN2+ 68.3 (55.0–79.7)
S. Amaro-Filho *et al.* [34] 2013	130	24–88	Histopathology	FIGO III+ 66.7 FIGO II+ 53.3	FIGO III+ 70.0 FIGO II+ 81.8

Notes: CIN2+: Cervical Intraepithelial Neoplasia Grade 2+; CIN3+: Cervical Intraepithelial Neoplasia Grade 3+; HSIL: High-grade squamous intraepithelial lesion; FIGO III+: International Federation of Gynecology and Obstetrics System Grade 3; FIGO II+: International Federation of Gynecology and Obstetrics System Grade 2; LB: Liquid-based; DS: Dual staining.

TABLE 2. Sensitivity and specificity of p16/Ki-67 DS in combination with other triage strategies to predict CIN2+ and CIN3+.

First Author; Year	Final Sample	Histology	LB cytology + p16/Ki-67 DS	HR-HPV + p16/Ki-67 DS	HR-HPV (other) + p16/Ki-67 DS	HPV16/18 + p16/Ki-67 DS	HR-HPV + LB cytology + p16/Ki-67 DS
					Sensitivity % (95% CI) Specificity % (95% CI)		
A. Celewicz *et al.* [27] 2018	93	CIN2+	86 74				
M. El-Zein *et al.* [28] 2021	492	CIN2+	ASC-US+ 96.1 (92.6–98.2) 40.3 (31.9–49.1)	85.0 (77.7–90.6) 48.4 (30.2–66.9)			
		CIN3+	96.9 (92.3–99.2) 40.3 (31.9–49.1)	86.4 (77.0–93.0) 48.4 (30.2–66.9)			
L. Yu *et al.* [32] 2016	1290	CIN2+	LSIL+ 91.7 (87.3–94.9) 53.0 (44.2–61.7)				
		CIN3+	96.1 (91.2–98.7) 53.0 (44.2–61.7)				
		CIN2+	ASC-US and LSIL 87.5 (75.3–94.1) 66.4 (59.7–72.4)	92.7 (88.4–95.4) 52.7 (46.4–58.8)			
		CIN3+	89.7 (73.6–96.4) 62.1 (55.7–68.2)	95.0 (90.7–97.3) 47.7 (42.0–53.5)			
R. Zhang *et al.* [33] 2018	223	CIN2+	ASC-US 89.0 (81.7–96.4) 71.4 (60.7–82.0) LSIL 89.1 (80.5–97.8) 61.5 (48.0–75.0)	90.8 (85.1–94.9) 70.2 (55.1–82.7)			
P. Giorgi Rossi *et al.* [35] 2021	3147	CIN2+		75.2 (68.1–81.6) 74.8 (72.4–77.1)		90.1 (76.9–96.5) 53.7 (49.9–57.5)	
		CIN3+		80.6 (70.9–88.3)		100.0 (85.8–100.0)	
M. Magkana *et al.* [36] 2021	196	CIN2+	ASC-US 90.4 (68.0–98.0) 97.2 (89.0–99.0) LSIL 95.0 (85.0–99.0) 95.2 (83.0–99.0)				

TABLE 2. Continued.

First Author; Year	Final Sample	Histology	LB cytology + p16/Ki-67 DS	HR-HPV + p16/Ki-67 DS	HR-HPV (other) + p16/Ki-67 DS	HPV16/18 + p16/Ki-67 DS	HR-HPV + LB cytology + p16/Ki-67 DS
					Sensitivity % (95% CI) Specificity % (95% CI) (12)†		
Y. Hu *et al.* [37] 2020	846	CIN2+		63.4 (54.4–71.9) 85.2 (82.5–87.8)	86.5 (79.3–91.9) 62.5 (58.8–66.0)		
		CIN3+		64.6 (55.2–73.3) 84.7 (82.0–87.3)	87.0 (79.6–92.6) 61.9 (58.3–65.5)		
					(12)†		
M.Y. Jiang *et al.* [38] 2020	1757	CIN2+		89.0 (86.3–91.4) 49.1 (42.1–56.0)	97.1 (95.5–98.3) 41.5 (34.8–48.5)		
		CIN3+		89.8 (87.1–92.2) 44.8 (38.6–51.2)	98.3 (96.9–99.2) 38.1 (32.1–44.4)		
					(8)‡		
		CIN2+			96.1 (94.3–97.5) 48.6 (41.7–55.5)		
		CIN3+			97.4 (95.8–98.5) 44.4 (38.2–50.8)		
R. Luttmer *et al.* [39] 2016	446	CIN2+		85.5 (80.2–90.9) 60.0 (54.3–65.7)			
		CIN3+		93.8 (88.6–99.1) 51.2 (46.1–56.4)			
		CIN2+		83.4 (77.1–88.6) 58.9 (56.2–61.6)			
N. Wentzensen *et al.* [40] 2015	1509	CIN3+		86.9 (78.6–92.8) 56.9 (54.2–59.5)			
							ASC-US
		CIN2/3		88.0 (79.0–94.0) 31.0 (23.0–40.0)			71.0 (60.0–80.0) 49.0 (40.0–59.0)
I.T. Ovestad *et al.* [41] 2017	266	CIN3		94.0 (82.0–98.0) 28.0 (22.0–36.0)			86.0 (73.0–94.0) 50.0 (41.0–58.0)

TABLE 2. Continued.

First Author; Year	Final Sample	Histology	LB cytology + p16/Ki-67 DS	HR-HPV + p16/Ki-67 DS	HR-HPV (other) + p16/Ki-67 DS	HPV16/18 + p16/Ki-67 DS	HR-HPV + LB cytology + p16/Ki-67 DS
					Sensitivity % (95% CI) Specificity % (95% CI)		
D. Gustinucci *et al.* [42] 2016	6272	CIN2+		87.6 (75.7–93.6) 74.9 (69.0–79.0)			ASC-US+ 93.8 (85.0–98.3) 59.2 (53.4–64.6)
		CIN3+		92.3 (74.9–99.1)			100.0 (89.1–100.0)
		CIN2+					HSIL 89.2 (79.1–95.6) 74.2 (68.4–78.5)
		CIN3+					96.2 (80.4–99.9)
R.M. Ebisch *et al.* [43] 2017	462	CIN2+		86.0 (79.0–92.0) 73.0 (65.0–81.0)			ASC-US and LSIL 89.0 (82.0–94.0) 79.0 (70.0–85.0)
		CIN3+		92.0 (84.0–97.0) 61.0 (54.0–69.0)			92.0 (84.0–97.0) 64.0 (56.0–71.0)
		CIN2+					NILM 97.0 (92.0–99.0) 55.0 (46.0–63.0)
		CIN3+					97.0 (91.0–100.0) 43.0 (35.0–51.0)
P. Ziemke *et al.* [44] 2014	260	CIN2+	74.5 (67.8–80.3) 90.0 (78.8–95.9)				NILM, ASC-US and LSIL 92.0 (85.0–96.0) 71.0 (62.0–78.0)
		CIN3+					96.0 (89.0–99.0) 58.0 (50.0–65.0)
L. Pirtea *et al.* [45] 2018	310	CIN2/3	ASC-US 66.0 93.0 LSIL 59.0 79.0				

TABLE 2. Continued.

First Author; Year	Final Sample	Histology	LB cytology + p16/Ki-67 DS	HR-HPV + p16/Ki-67 DS	HR-HPV (other) + p16/Ki-67 DS	HPV16/18 + p16/Ki-67 DS	HR-HPV + LB cytology + p16/Ki-67 DS
					Sensitivity % (95% CI) Specificity % (95% CI)		
C. Bergeron *et al.* [46] 2015	25,577		ASC-US				
		CIN2+	87.5 (47.3–99.7) 81.1 (75.8–85.7)				
		CIN3+	100.0 (54.1–100.0) 80.8 (75.5–85.4)				
			LSIL				
		CIN2+	86.5 (71.2–95.5) 56.0 (48.3–63.5)				
		CIN3+	88.2 (63.6–98.5) 51.8 (44.5–59.0)				
T. Wright *et al.* [47] 2022	5250	CIN2+		86.5 (83.3–89.1) 57.5 (55.8–59.1)		90.2 (87.4–92.5) 40.9 (39.3–42.6)	
		CIN3+		89.5 (84.9–92.9) 54.0 (52.4–55.6)		94.3 (90.5–96.7) 38.6 (37.0–40.2)	
C. White *et al.* [48] 2016	471		ASC-US+				
		CIN2+	75.4 (72.3–78.8) 88.3 (87.2–89.4)				
		CIN3+	79.2 (74.5–83.8) 75.2 (73.4–77.0)				
			LSIL				
		CIN2+	77.8 (74.0–81.5) 88.6 (87.1–90.1)				
		CIN3+	85.7 (81.7–89.8) 72.7 (70.2–75.2)				
			ASC-US				
		CIN2+	71.9 (66.7–77.2) 87.9 (86.2–89.6)				
		CIN3+	71.4 (60.7–82.1) 78.7 (76.2–81.2)				

TABLE 2. Continued.

First Author; Year	Final Sample	Histology	LB cytology + p16/Ki-67 DS	HR-HPV + p16/Ki-67 DS	HR-HPV (other) + p16/Ki-67 DS	HPV16/18 + p16/Ki-67 DS	HR-HPV + LB cytology + p16/Ki-67 DS
					Sensitivity % (95% CI) Specificity % (95% CI)		
M. Uijterwaal *et al.* [49] 2015	762	CIN2+					68.8 (53.7–81.3) 72.8 (67.9–77.3)
		CIN3+					73.3 (44.9–92.2) 70.0 (65.2–74.6)
Q.P. Qian *et al.* [50] 2018	108	HSIL+	96.0 (82.0–100.0) 60.0 (48.0–71.0)		(21)[§] 96.0 (82.0–100.0) 35.0 (25.0–46.0)		
M. Stoler *et al.* [51] 2019	8067	CIN3+		85.9 60.1			
Y.J. Koo *et al.* [52] 2013	70	CIN2+	ASC-H 94.6 (84.1–99.0) 75.8 (64.0–80.7)				
		CIN3+	100.0 (79.7–100.0) 50.9 (44.4–50.9)				
C. Solares *et al.* [53] 2015	160	CIN2+					82.4 (61.28–100.0) 78.3 (71.2–85.4)
G. Trutnovsky *et al.* [54] 2014	27	CIN2+	ASC-US+ 100 66.7				
J. Ordi *et al.* [55] 2014	1123	HSIL/CC					ASC-US 90.9 (87.9–93.9) 72.1 (68.7–75.4)

TABLE 2. Continued.

First Author; Year	Final Sample	Histology	LB cytology + p16/Ki-67 DS	HR-HPV + p16/Ki-67 DS	HR-HPV (other) + p16/Ki-67 DS	HPV16/18 + p16/Ki-67 DS	HR-HPV + LB cytology + p16/Ki-67 DS
					Sensitivity % (95% CI) Specificity % (95% CI)		
T. Fujii *et al.* [56] 2014	479	CIN2+	ASC-US and LSIL 87.3 (78.0–93.8) 76.4 (71.6–80.8)				
J. Killeen *et al.* [57] 2013	515	CIN2/3	ASC-US+ 94.2 61.9				
C. Areán-Cuns *et al.* [58] 2018	3810	CIN2+		98.0 (93.1–99.8) 39.1 (32.3–46.2)			NILM 100.0 (50.0–100.0) 71.4 (41.9–91.6) ASC-US 91.6 (61.5–99.8) 51.6 (33.1–69.8) LSIL 97.9 (88.9–100.0) 34.4 (26.8–42.7)
N. Lorenzi *et al.* [59] 2022	232	CIN2+	HP 84.4 (74.3–91.6) 59.2 (38.8–77.6) SP 70.6 (59.0–80.6) 85.7 (67.3–95.9)				

Notes: + Includes the cases of that cytology category and more severe. 12† HR-HPV genotypes, except for 16/18. 8‡ HR-HPV genotypes (HPV31/33/58/52/45/59/56/66). 21§ HPV genotypes (HR: 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68; LR: 6, 11, 42, 43, 44 and CP8304(81)). CIN2+: Cervical Intraepithelial Neoplasia Grade 2+; CIN3+: Cervical Intraepithelial Neoplasia Grade 3+; NILM: Negative for intraepithelial lesions or malignancy; ASC-H: Atypical squamous cells; ASC-US: Atypical squamous cells of undetermined significance; LSIL: Low-grade squamous intraepithelial lesion; HSIL: High-grade squamous intraepithelial lesion; HSIL/CC: High-grade intraepithelial lesion or carcinoma; LB: Liquid-based; HP: Health professional; SP: Self-sampling; DS: Dual staining; HR-HPV: High-risk human papillomavirus; CI: Confidence interval.

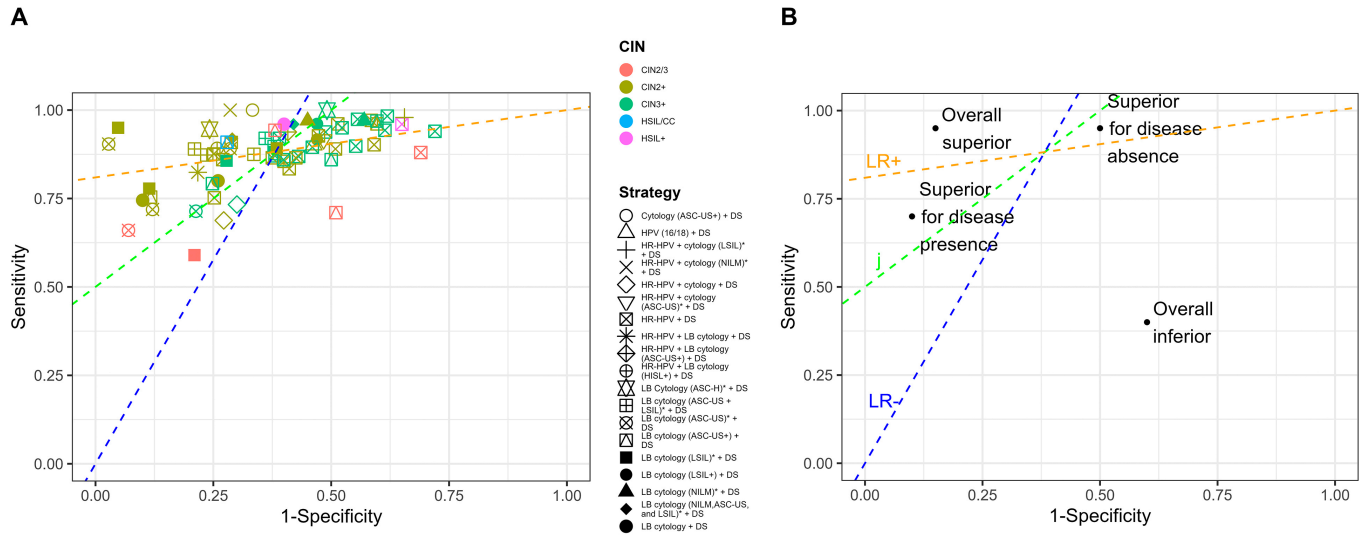

FIGURE 2. Sensitivity and specificity of different standalone or combined screening strategies plus p16/Ki-67 DS. (A) Likelihood ratio graph of the combined strategies from Table 2, by region. (B) Regions of comparison of the likelihood ratio graph. CIN2/3: Cervical Intraepithelial Neoplasia grades 2 and 3; CIN2+: Cervical Intraepithelial Neoplasia grade 2 or higher; CIN3+: Cervical Intraepithelial Neoplasia grade 3 or higher; HSIL/CC: High-Grade Squamous Intraepithelial Lesion/Cervical Cancer; HSIL+: High-Grade Squamous Intraepithelial Lesion; ASC-US: Atypical Squamous Cells of Undetermined Significance; DS: Dual Staining; HPV: Human Papillomavirus; HR-HPV: High-Risk HPV; LSIL: Low-Grade Squamous Intraepithelial Lesion; NILM: Negative for Intraepithelial Lesion; LB: Liquid-base cytology; ASC-H: Atypical Squamous Cells.

4. Discussion

Analyzing the latest available data on the predictive capabilities of p16/Ki-67 DS in the identification and prevention of CC, this review explores its applicability in screening programs and draws comparisons with established techniques. According to the literature, cytology and HPV testing, when used independently, have shown low sensitivity and specificity for the diagnosis of CIN2+ [68, 69].

Currently, various triage strategies are being evaluated, including p16/Ki-67 DS. In the Bethesda System for Reporting Cervical Cytology 2014 edition, DS was recommended as a complementary test for cytological diagnosis [70]. This review found that the median sensitivity and specificity of p16/Ki-67 DS for diagnosing CIN2+ were 87.7% and 76.7%, respectively. For CIN3+, the median sensitivity was 89.7% and specificity 79.6%. These findings are consistent with other studies that have demonstrated that DS exhibits higher sensitivity and specificity in detecting CIN3+ compared to CIN2+ [13, 17, 71]. Another study performed in China revealed that DS sensitivity and specificity were not higher than cytology in HPV-positive women [32]. Therefore, although DS reduces the repeat cytology and colposcopy referral rate, there is a need to enhance its sensitivity and specificity. There is increasing evidence that p16/Ki-67 DS is an alternative biomarker to improve screening programs. Some reviews also concluded that p16/Ki-67 DS increases the sensitivity of detecting precancerous lesions and is a marker for transforming HPV infections [14, 72, 73]. Some authors have also evaluated the interobserver reproducibility and accuracy of the technique. They mostly found satisfactory results above 80%, suggesting its implementation in screening programs [74, 75].

Our review found higher sensitivities and specificities for the combined strategy of DS plus LB cytology. These findings are consistent with those from other reviews or original research where the sensitivities and specificities were higher compared to other triage strategies such as HR-HPV [13, 76]. While HPV testing is expected to replace cytology as a triage tool worldwide due to its high sensitivity, it may also lead to overtreatment, since many HPV infections in young women are transient and likely to resolve within the next two years [77–79]. Considering the evidence previously presented and based on our results, DS plus LB cytology demonstrates effective predictive accuracy specifically for high-grade lesions [77, 80].

This review also found a positive relationship between p16/Ki-67 expression and CC lesion severity. Other studies further confirmed that a higher number of positive cells relate to worse histopathology results [20, 81, 82]. These results contribute to the understanding of p16/Ki-67 DS as an important predictor of HSIL/CC. On the other hand, the present review did not find extensive information when only one protein is expressed in a patient with cervical lesions. Studies that analyzed the expression of the proteins independently usually found very few cases where only one protein was expressed in this context. However, the overexpression of either p16 or Ki-67 still correlated with the degree of neoplasia [61]. A possible explanation could be that if left untreated, approximately 30% of CIN3 lesions and approximately 10% of CIN2 lesions will develop into invasive cancer [83, 84]. Thus, the regression of CIN2 and CIN3 lesions might change the overexpression of the proteins. Another study suggested that since Ki-67 had significantly higher positive cells for CIN3+ than CIN2+, p16

overexpression might be an early event, and Ki-67 expression increases throughout CIN progression [63]. However, a consensus on this matter has not been reached based on the available information.

Some limitations of this review are that only English articles and one database were used for the analysis.

5. Conclusions

In summary, this review presented sensitivity and specificity estimates of DS alone and in combination with other techniques for CC screening. When detecting precancerous lesions, DS alone exhibited higher sensitivity and specificity for CIN3+ compared to CIN2+. Among the combined strategies, DS along with LB cytology demonstrated higher sensitivities and specificities compared to other reviewed strategies. However, evidence suggests that HPV testing may be more suitable for triage screening. When analyzing the immunohistochemistry staining of p16 and Ki-67 independently, together, or in combination with other strategies, it has high predictive value for CC and its precursors. This review found that using p16/Ki-67 DS alone or in combination with LB cytology could improve the accuracy and efficacy of CC screening and therefore, the potential to enhance CC screening programs. Future literature and systematic reviews are needed to accurately analyze the effects, performance, and cost-effectiveness of DS and cytology/HPV co-testing.

ABBREVIATIONS

LB, Liquid-based; DS, Dual staining; NILM, Negative for intraepithelial lesions or malignancy; ASC-US, Atypical squamous cells of undetermined significance; ASC-H, Atypical squamous cells; LSIL, Low-grade squamous intraepithelial lesion; HSIL, High-grade squamous intraepithelial lesion; HSIL/CC, High-grade intraepithelial lesion or carcinoma; CIN2+, Cervical Intraepithelial Neoplasia Grade 2+; CIN3+, Cervical Intraepithelial Neoplasia Grade 3+; FIGO II+, International Federation of Gynecology and Obstetrics System Grade 2; FIGO III+, International Federation of Gynecology and Obstetrics System Grade 3; HP, Health professional; SP, Self-sampling.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are openly available at https://rpubs.com/rgrivasp71/1075694.

AUTHOR CONTRIBUTIONS

AAB, DDL, COA and VAN—conducted the research study. AAB, DDL and RRP—implemented the methodology, curated the data, handled the visualization. BVC—performed the validation of the study. AAB and DDL—conducted the investigation, prepared the original draft of the article. AAB, DDL and VV—contributed to the writing, review and editing. COA and VAN—supervised the investigation, managed project administration. VAN and VV—acquired funding. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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