

Screening Tests for Cervical Cancer Up-To-Date

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1. Abstract

1.1. Objective: To evaluate the different strategies for cervical cancer screening, its risk stratification with the use of biomarkers to improve diagnosis and avoid overtreatment.

1.2. Methodology: a review was made in PubMed, Web of Science, Scopus, to see the sensitivity and specificity of the screening tests

1.3. Results: with cytology or Pap test, primary high-risk human papillomavirus (HPV-ar) test or Co-testing (Pap plus HPV-ar test) have been widely used in the identification of lesions High-grade squamous intraepithelial (HSIL) and cervical cancer, is of great importance in prevention and treatment; Although, Pap reports squamous intraepithelial lesions of undetermined significance (ASC-US) or borderline or low-grade squamous intraepithelial lesions (LSIL) generally need follow-up, some progress to HSIL, and women with positive HPV tests vary; p16 / Ki-67 dual staining cytology is effective for classification of previous screening results.

1.4. Conclusions: p16 / Ki-67 dual staining cytology is a biomarker, with high sensitivity and specificity in the identification of HSIL and cervical cancer.

2. Background

Cervical cancer has an incidence of 604,127 new cases, with 341,831 deaths, with an incidence of 13.3 cases per 100,000 and mortality of 7.3 per 100,000; it is the fourth most common malignant tumor in women worldwide [1]; it mainly affects emerging

countries. Persistent high-risk Human Papillomavirus (HPV) infection is the essential cause, and HPV-ar genotypes, particularly HPV-16 and 18, induce cervical High-Grade Squamous Intraepithelial Lesion (HSIL) Some of these return and others lead to cervical cancer; due to the effects of ar-HPV oncoproteins or genes, in particular E6 and E7; the integration of HPV-ar DNA into the host genome induces the overexpression of these [2]; the association of both, E7 binds and inactivates the Retinoblastoma protein (pRB), and E6 binds and inactivates the p53 protein, altering the regulation of the cell cycle and the expression of E7 and E6 effectively immortalizes human primary keratinocytes and they are necessary to induce and maintain the transformed phenotype of cervical cancer cells [3,4].

Historically, cytology or Papanicolaou (Pap) screening was useful in developed countries with organized screening programs; Pap smear is not considered adequate for screening in emerging countries, it requires specialized personnel, infrastructure, and frequent intervals due to its low specificity, and high rates of equivocal results; but, if with high specificity [5,7]. Currently, three main screening tests, Pap, HPV-ar test, primary, and Co-testing (Pap with HPV-ar test), are used for the detection of cervical cancer [6,7].

Pap is the method used for the detection of cervical cancer for decades, which reduced its morbidity and mortality rate, with high specificity from 86 to 100%, but lower sensitivity than an average of 51%, with subjectivity and intervariability between observers, a Unlike the HPV-ar test, the sensitivity is high 95%, it is an al-

ternative, with a longer interval time for its detection, although the specificity is lower than the Pap test; Co-testing is expected to overcome its deficiencies [8,9].

Patients with Pap Low-Grade Squamous Intraepithelial Lesion (LSIL) and cervical Atypical Squamous Cells of Undetermined Significance (ASC-US) diagnosed as mild or borderline lesions may progress to grade 2 or more severe Cervical Intraepithelial Neoplasia (CIN-2 +); the low specificity of HPV-ar tests, many women are referred for colposcopy, especially those under 30 years of age due to the higher prevalence of HPV infection; the prevalence of HPV-ar varies from 80 to 85% in LSIL, where they are sent for colposcopy or followed with Pap; women with HPV-ar, primary, positive for HPV-16 or HPV-18 are sent for colposcopy and for the other 12 HPV-ar, positive, they are followed up with Pap tests; if it is negative, the 12-month follow-up is recommended [10,11]; many ar-HPV-positive women need repeat Pap follow-up due to their low sensitivity; makes effective biomarkers required to classify ar-HPV-positive or normal Pap-16/18-negative women and identify women with HSIL on ASC-US / LSIL Pap. Evidence suggests that p16 / Ki-67 dual staining cytology is an alternative biomarker, with high sensitivity and general specificity for identifying HSIL [12-16].

2.1. The Clinical Importance of Dual-Stained Cytology p16 and Ki-67

The characteristics and function of p16INK4A (p16) is a tumor suppressor oncoprotein, known as a Cyclin-Dependent Kinase 2A (CDKN2A) inhibitor [17-18], encoded by the CDKN2A gene located on the short arm of chromosome 9 (9p21.3), which receives its name from its molecular weight and function in the inhibition of Cyclin-Dependent Kinases 4 (CDK4) [17-20]; it binds to CDK4 and CDK6, which is important in cell cycle regulation. CDK4 / 6 forms a protein complex with cyclin D to phosphorylate pRB. After phosphorylation, pRB dissociates from the transcription factor E2F1, which leads to the translocation of E2F1 to the nucleus, where E2F1 induces the transcription of target genes promoting the cellular transition from G1 to S phase; acting as an inhibitor of CDK by preventing its interaction with cyclin D, and inhibiting cell cycle progression [17,18].

La regulación a la baja de p16 conduce a cáncer a través de la aceleración en la regulación de la progresión del ciclo celular; esta p16 está mutado con frecuencia y se asocia con mayor riesgo de cánceres [20]. Las células infectadas con VPH-ar, la E7 compite al unirse a las proteínas reguladoras del ciclo celular pRb, liberando E2F1 de la pRb y activación del ciclo celular [3] (poner imagen que ya tengo) La alteración de la vía pRb-E2F1 por E7 induce la sobreexpresión y acumulación de p16 en las células a través de un circuito de retroalimentación negativa [2,21-23]. La expresión citoplasmática, nuclear fuerte y difusa de p16 en CaCu escamoso se asocia con la infección por VPH-ar, y la p16 es marcador susti-

tuto de infección por VPH-ar, persistente; con sobreexpresión de p16 en la mayoría de HSIL y CaCu [2,5,19,20]. Las características y función del biomarcador Ki-67; es un marcador de proliferación celular, definido por su ciudad de origen (Kiel) y número de clones originales [21]; proteína nuclear no histona, que está codificada por el gene MKI-67 y se expresa en todas las fases del ciclo celular, excepto durante la fase G0; ejerce múltiples funciones en la regulación de la progresión del ciclo celular [22], con la progresión del ciclo celular, se relaciona con su distribución en células, necesario para la distribución celular normal y asociación nuclear de la heterocromatina durante la interfase [22]; en la mitosis, participa en la formación de la capa pericromosómica, que funciona como una vaina protectora alrededor de cromosomas y proporciona una plataforma de unión nucleolar, donde Ki-67 es surfactante biológico para prevenir la agregación de cromosomas mitóticos después de la desintegración de la envoltura nuclear [23]; como marcador de proliferación celular, predice el potencial maligno de los tumores; por lo que se ha utilizado en el pronóstico y predicción en muchos tumores y existe correlación positiva entre la expresión de la tinción dual p16/Ki-67 (2+ y 3+) en HSIL, tabla 1.

[Downregulation of p16 leads to cancer through the regulation of cell cycle progression; this p16 is frequently mutated and is associated with increased risk of cancers [20]. Cells infected with HPV-ar, E7 competes by binding to the regulatory proteins of the pRb cell cycle, releasing E2F1 from pRb and activating the cell cycle [3] (put the image I already have) Alteration of the pRb-E2F1 pathway by E7 induces the overexpression and accumulation of p16 in cells through a negative feedback loop [3]. Strong and diffuse cytoplasmic, nuclear expression of p16 in squamous cervical cancer is associated with ar-HPV infection, and p16 is a persistent surrogate marker for ar-HPV infection; with overexpression of p16 in the majority of HSIL and CaCu [2,5,19,20]. The characteristics and function of the biomarker Ki-67; it is a marker of cell proliferation, defined by its city of origin (Kiel) and number of original clones [21]; non-histone nuclear protein, which is encoded by the MKI-67 gene and is expressed in all phases of the cell cycle, except during the G0 phase; it exerts multiple functions in the regulation of cell cycle progression [22], with cell cycle progression, it is related to its distribution in cells, necessary for normal cellular distribution and nuclear association of heterochromatin during interphase [22]; in mitosis, it participates in the formation of the perichromosomal layer, which functions as a protective sheath around chromosomes and provides a nucleolar attachment platform, where Ki-67 is a biological surfactant to prevent the aggregation of mitotic chromosomes after the disintegration of the nuclear envelope [23]; as a marker of cell proliferation, it predicts the malignant potential of tumors; therefore it has been used in the prognosis and prediction in many tumors and there is a positive correlation between the expression of dual staining p16 / Ki-67 (2+ and 3+) in HSIL, Table 1]

Table 1: Positivity P16 and K I -67 According to Positivity Percentage

Marker	Negative	Low positive	Moderately positive	High positive
		1	2	3
p16	< 5%	5–25%	26–50%	> 50%
Ki -67	< 5%	5–25%	26–50%	> 50%

P16 / Ki-67 dual staining cytology and its clinical implication; p16 is a tumor suppressor and Ki-67 is a proliferation cell marker; their overexpression of p16 and Ki-67 in physiological situations are mutually exclusive and do not manifest themselves in the same cervical epithelial cell, the co-expression of p16 / Ki-67 implies the alteration of the cell cycle induced by HPV-ar, the detection of the co-expression of p16 / Ki-67 predictive marker of cellular transformation by HPV-ar, and presence of HSIL [18,19,36]. The co-expression of p16 / Ki-67 is detected with antibodies against

p16 and Ki-67. Cytoplasm / nucleus staining brown with p16 alone, and red staining with Ki-67 alone. Cells with positive p16 / Ki-67 dual staining stain the cytoplasm brown for p16 expression, and dark red nucleus staining reflects both p16 and Ki-67 locations in the same cell (Figure 1); one or more cervical epithelial cells that simultaneously stain for p16 and Ki-67 are classified as positive regardless of cell morphology [24]. Positive dual p16 / Ki-67 staining is associated with ar-HPV infection, particularly HPV-16 and 18 [24].

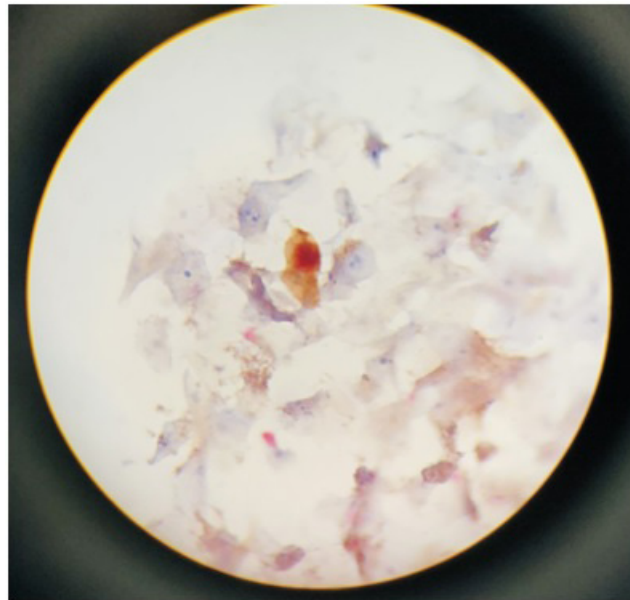


Figure 1: p16 / Ki-67 dual-staining positive cells with HSIL morphological characteristics. A, Liquid-based cytology B. A was double stained with p16 / Ki-67. The cell stained with p16 alone (blue arrow) is characterized by a brown nuclear / cytoplasmic signal and the cell with Ki-67 staining alone (red arrow) is presented in a red nuclear signal. Dual-stained p16 / Ki-67 positive cells (dark arrow) are characterized by a brown cytoplasmic signal for overexpression of p16 and a dark red nuclear signal for co-expression of p16 / Ki-67 in the same cell.

The p16 / Ki-67 positivity rate in HPV-positive women was 78.9%, higher than 9.4% in HPV-negative patients [25,26]. The association of p16 / Ki-67 positivity with HPV16 and / or 18 infections was 2 to 4 times stronger compared to cases infected with other ar-HPV genotypes [27].

Positive p16 / Ki-67 dual staining indicates NIC-2 + or HSIL. Positive rates of dual p16 / Ki-67 staining in ar-HPV positive women with negative diagnosis of intraepithelial lesion or malignancy (NILM), ASCUS, LSIL, atypical squamous cells cannot exclude HSIL (ASC-H) and HSIL were 3, 23.6, 25.8, 78.6 and 100%, respectively [28]. The detection of Ki-67 is used by diagnostic support of HSIL and cervical cancer. The positive rate increased 31% in women with negative cytology to 92% in women with HSIL, clinicosonology.com

similar to the positive rate of p16 / Ki-67 in women with CIN-3 was 86%, which is greater than 24 % in women without biopsy (Table 1). All patients with CaCu showed double positive staining for P16 / Ki-67; furthermore, the positive rate of p16 / Ki-67 increased with the severity of cytological and histopathological abnormalities [27,28]. The sensitivity and specificity of dual p16 / Ki-67 staining for CIN-2 + were 74.9–90.9% and 72.1–95.2%, respectively [29,30]. The positive rate of CIN-2 + detected by dual p16 / Ki-67 staining was 92.7%, with a higher sensitivity of 71.1% for HPV-16/18 genotypes alone [29]; detection of HPV, dual p16 / Ki-67 staining has greater specificity in the detection of CIN-2 + and reduce the number of patients referred to colposcopy, especially in young women with a high rate of HPV infection [20,29]

The application of dual p16 / Ki-67 staining in the triage of HPV-positive women eliminates unnecessary follow-up, with timely classification [31]. Pap is generally used to classify HPV-positive women who are negative for HPV-16 or 18, but pos-

itive for the other 12 HPV-ar; dual p16 / Ki-67 staining is useful in classification (Figure 2). The sensitivity of cytology with dual p16 / Ki-67 staining was 74.9%, greater than 51.9% for Pap and comparable to its specificity [30].

Table 2: Cytology with dual p16 / Ki-67 staining in the detection of HSIL and CaCu.

Indicators	Sensitivity (%)	Specificity (%)
p16	85.4	94.6
Ki-67	95.2	86.7
p16/Ki-67	94.8	93.2

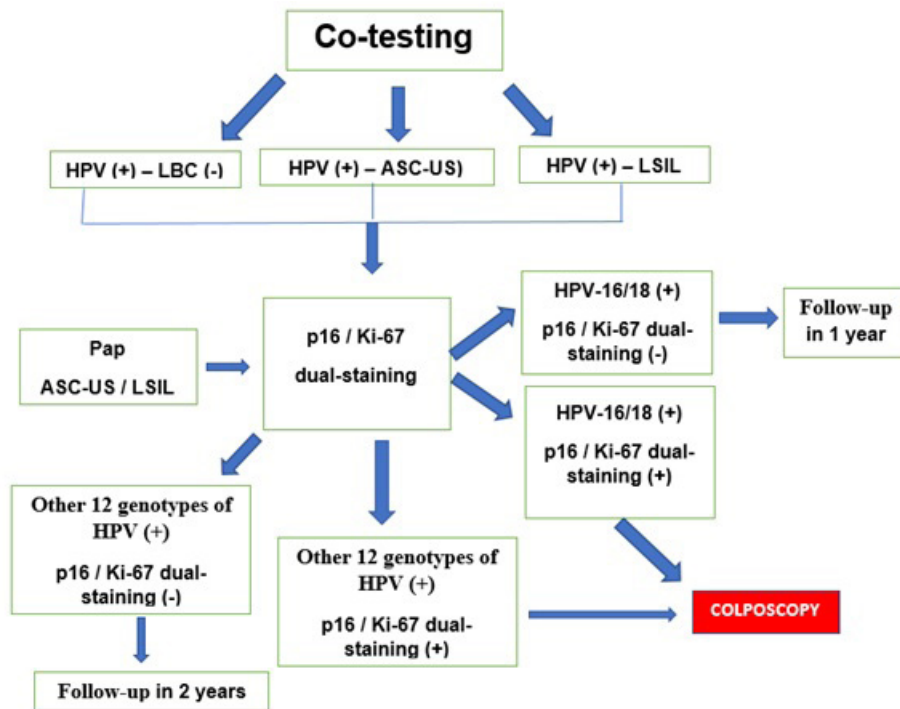


Figure 2: Detection and classification of cervical cancer and use of cytology with dual p16 / Ki-67 staining. Women diagnosed as ASC-US / LSIL, or positive for HPV-ar and free of cytological abnormalities, or positive for the other 12 genotypes of HPV-ar and negative for HPV-16 and 18 are recommended for triage with stained cytology. dual p16 / Ki-67

In Pap-based screening, colposcopy is recommended at risk greater than the threshold for HSIL [20,32] and in positive HPV tests (HPV +), the risk of HSIL in women with positive dual p16 / Ki-67 staining is greater than the threshold risk of Pap, while the risk of women with negative dual staining is lower, indicating the classification of HPV + women from 3 to 7% of women with normal Pap and HPV-ar tests, +; they are still at risk for CIN-3 [24], dual p16 / Ki-67 staining classifies these patients, and 25.4% are positive. The sensitivity for the detection of CIN-2 + and CIN-3 + was 91.9 and 96.4%, respectively, while the specificity was 82.1 and 76.9% respectively [24,35], the similar results, of the sensitivity for CIN-2 + was higher than HPV-16/18 genotyping (Table 1) [33,34]. In the follow-up of HPV + women with normal Pap smears, the 5-year cumulative incidence risks (CIR) for CIN-2 + and CIN-3 + were 12.2 and 6.9%, respectively. Even if HPV-16/18 genotyping was negative, the 5-year RIC incidence risk for CIN-3 + was 3.6%. If these women are negative for p16 / Ki-67, the 5-year CIR incidence risk for CIN-3 + decreases 3.3%, dual p16 / Ki-67 staining

classifies HPV + women with normal Pap than colposcopy identifies with a higher probability of CIN-2 + [20,33].

Pap triage with LSIL / ASC-US, in women diagnosed in Pap with ASC-US and LSIL have a 5-year risk of 2.6 and 5.2% of CIN-3 +, respectively [32] to identify HSIL in ASC-US / LSIL; the efficacy of double staining with p16 / Ki-67 [25,27,34], its specificity for the detection of CIN-3 was 75.2%, higher than 40.4% than the HPV-ar tests, although the sensitivity in the first it was slightly lower [40]. dual p16 / Ki-67 staining has higher specificity and comparable sensitivity than the HPV-ar test [25,34]. Dual p16 / Ki-67 staining presented high positive predictive value (PPV) for HSIL, especially in women under 30 years of age, reducing unnecessary colposcopy [25,31]

The risk of CIN-3 in women with HPV + test was 15.6% [20,34] and with dual p16 / Ki-67 staining and HPV + tests, the risk increased 27%, while if both were negative, it decreased 1.2 % [34], the combination of the HPV-ar test, and dual staining of p16 / Ki-67 identify HSIL that borderline Pap

2.2. Follow-Up, Recurrence, and Supportive Diagnosis

The combination of p16 / Ki-67 double staining and HPV-ar detection is used to monitor the recurrence of treated CIN-2 + (rNIC-2 +); with close monitoring; Co-testing is recommended, to follow-up and avoid loss of CIN-2 + in negative HPV tests [20,35,36], the specificity of Pap detection or Co-testing detection is limited; the sensitivity and specificity of Pap smears, HPV-ar detection and dual p16 / Ki-67 staining for CIN-2 + in women treated for CIN-2/3; the sensitivities were 82.1, 84.6 and 69.2%, respectively, but the specificity of p16 / Ki-67 was 90-4%, significantly higher than 70.8% in Pap and 76.2% in HPV-ar tests. The sensitivity of the combined detection of p16 / Ki-67 and HPV-ar is similar to the Co-testing detection (87.2 vs 89.7%), but the specificity improved (74.2 vs 58.1%), resulting in higher PPV and minor sent for colposcopy [20].

The Pap diagnosis of cervical glandular lesions is difficult to distinguish from inflammatory and hyperplastic changes in neoplasms [20]. In cervical adenocarcinoma, 92.5% of p16 / Ki-67 dual staining was positive, 1 of 16 cervical tissue samples without glandular lesions was positive for p16 / Ki-67 dual staining, makes it a diagnostic test for glandular lesions cervical [37-40]; double p16 / Ki-67 staining has a higher value in the classification according to how severe the lesion is and on average it is reported that for CIN-2 + the sensitivity and specificities were 87.35 and 64.1%, for CIN-3 + 85.7 and 71.95% and for cervical cancer 91.7 and 72.7% respectively; for Pap, the sensitivity was 76.92.8% and specificity 46.65%; cytology with dual p16 / Ki-67 staining identifies patients at high risk of cervical cancer and reduces the rate of misdiagnosis, which is of great value for the differential diagnosis of HSIL and cervical cancer, [41,42] Table 2.

3. Discussion

Pap and HPV-ar, primary, have been used in the detection of HSIL and cervical cancer, which is of great importance in their prevention and treatment; however, HPV + women need triage tests to determine their referral to colposcopy [41]. The search for biomarkers to detect HSIL due to inter and intraobserver variability in Pap samples and cervical biopsy, cytology with dual staining p16 / Ki-67 improves the sensitivity and specificity of these tests improve diagnosis. Positive rates of p16 / Ki-67 increase with higher risk genotypes from 65.0 to 88.0% in women HPV-16/18 + (P <0.001) was an effective method for the risk stratification of CIN-2 + in HPV + women, it is considered a strategy for the detection and classification of CaCu [42,43]. As CaCu screening progresses to primary ar-HPV testing, effective triage and treatment of HPV + women is critical to avoid unnecessary colposcopy referrals with associated damage while maintaining high sensitivity for HSILs. Triage with dual-stained p16 / Ki-67 cytology has high sensitivity and specificity for the detection of cervical HSIL [44]. Patients diagnosed as ASC-US / LSIL need follow-up, some will become CIN-2 +; and HPV + women, vary from person to person; some

progress to HSIL and eventually CaCu. dual staining cytology p16 / Ki-67 is an alternative, with high sensitivity and specificity to identify them [20].

Women with HPV + tests, need efficient triage tests to determine referral to colposcopy, cytology with dual staining p16 / Ki-67 is effective for the risk stratification of CIN-2 + in these women [43] The use of dual staining cytology p16 / Ki-67 to differentiate common HPV infections, which disappear spontaneously, from persistent and transforming infections, towards CIN-2 +; Furthermore, the association of HPV-ar, with the positivity of the dual staining p16 / ki-67 is in agreement with the higher risk of HSIL; this is an excellent classification test for HPV + women [35]. Most biomarkers correlate with the percentage of positivity, presence, classification and recurrence of HSIL. Glandular involvement is closely related to HSIL] and its glandular extension is frequently associated with HSIL and requires further follow-up [30]. As screening for cervical cancer moves to primary ar-HPV testing, finding an effective classification and treatment for HPV + women is critical [10]. Risk thresholds guide whether a woman should return to routine screening or be referred for repeat testing, colposcopy, or immediate treatment [12,37]; provides long-term risk stratification compared to 5-year Pap triage; the risk of HSIL in HPV + / negative women and dual-staining cytology p15 / ki-67 is identical to the risk at 3 years in HPV + women and Pap negative at 1 year and is safely extended to 3-year intervals in these women [42]. The immunohistochemical biomarkers p16 and Ki-67 correlate positively with the presence of HSIL and have described the relationship between its expression and the presence, classification and severity of the lesions. [45]

4. Conclusions

Dual staining cytology p16 / Ki-67 is of great importance in detection and classification, as a risk marker for stratification of HPV + women, including normal Pap, and identification of HSIL in ASC-US or LSIL; comparing the Pap with the HPV-ar test, it has greater sensitivity and specificity in the detection of HSIL and cervical cancer; useful for the diagnosis of cervical glandular lesions. Detection with the combination of dual staining p16 / Ki-67 and HPV-ar testing is recommended as a strategy for follow-up and surveillance of women treated for HSIL with broad perspectives in the diagnosis and treatment of cervical cancer.

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