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Article

# Impact of knowledge of human papillomavirus positivity on cervical cytology performance in Latin America

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## Abstract

**Background:** Cervical cytology is recommended by the World Health Organization as a triage option in human papillomavirus (HPV)-based cervical cancer screening programs. We assessed the performance of cytology to detect CIN3+ without and with knowledge of HPV positivity.

**Methods:** Women were screened with cytology and HPV across ESTAMPA study centers in Latin America. Screen-positives were referred to colposcopy with biopsy and treatment as needed. Cytology was initially interpreted without knowing HPV results. A subset of cytologies from HPV-positive women were reinterpreted at the same laboratories, with knowledge of HPV status, blinded to previous cytology and histological diagnosis. Performance indicators for cytology to detect CIN3+ without and with knowledge of HPV positivity were estimated.

**Findings:** A total of 4087 women were included, of which 490 had histologically confirmed CIN3+ (455 CIN3 and 35 cancers). Cytology sensitivity without knowledge of HPV positivity for CIN3+ was 47.2% (95% CI = 42.5 to 51.9), whereas with knowledge of HPV positivity, the sensitivity was higher (58.9%, 95% CI = 54.2 to 63.5; $P < .0001$). The specificity without knowledge of HPV was 89.4% (95% CI = 88.2 to 90.5), whereas with knowledge of HPV positivity was 78.9% (95% CI = 77.4 to 80.4; $P < .0001$). Performance estimates varied by study center for cytology without knowing the HPV positivity (range = 32.8%-61.5% for sensitivity; range = 80.7%-98.6% for specificity). Similarly, performance varied with knowledge of HPV positivity (36.1%-93.4% for sensitivity; 39.6%-98.6% for specificity).

**Conclusion:** The increase in sensitivity of cytology with HPV knowledge was limited and highly variable, reinforcing the need for alternative triage methods to support cervical cancer elimination goals.

## Introduction

Cervical cancer screening programs are adopting human papillomavirus (HPV) testing as the primary method to enhance current cervical cancer screening protocols worldwide. In Latin America (LA), there is a growing shift from cytology to HPV-based screening. Argentina, El Salvador, and Mexico have introduced HPV testing, and other countries such as Colombia, Costa Rica, Bolivia, Honduras, Peru, Paraguay, and Uruguay are implementing it regionally or conducting pilot studies.[1]

HPV testing is more sensitive than cytology for detection of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) and cervical cancer.[2,3] However, it is not highly specific, requiring secondary triage to select HPV-positive women needing colposcopy or treatment. In the screen-triage-and-treat algorithms

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recommended by the World Health Organization (WHO), cytology alone or in combination with partial genotyping (HPV16/18) is one triage option.[4] Additionally, cytology is already established in many countries in LA, which can facilitate transition to HPV screening followed by cytology triage.

It has been proposed that the performance of cytology triage for HPV-positive women would improve with prior knowledge of the HPV result, because cytology interpreters may perform a more careful assessment of smears.[5,6] In practice, such a program performs HPV testing with HPV-positive women undergoing "reflex" cytology. Workload reductions doing cytology in fewer women could also allow more time for interpreting smears, with a better inspection of slides and reduction of false negative results, increasing cytology sensitivity as triage.

To evaluate the impact of HPV positivity on cytology performance, we selected slides from HPV-positive women in ESTAMPA, which were initially evaluated without knowledge of HPV status, for a second reading after informing the interpreters that smears were from HPV-positive women and assessed the performance of cytology for CIN3+ and CIN2+ detection with and without knowledge of HPV positivity.

# Methods

## Study design

This is a cross-sectional study within ESTAMPA to evaluate whether knowledge of HPV positivity affects cytology performance as triage to detect CIN3+ and CIN2+.

ESTAMPA is a multicenter study investigating performance of emerging screening and triage techniques among women aged 30 years and older in 12 study centers across 9 countries in LA. At the screening visit, women received cytology and HPV. Those who tested negative for both exited the study, whereas those with abnormal cytology and/or HPV-positive results were referred to standardized colposcopy with biopsy and histological assessment, as needed. The protocol included conservative management, where women with negative colposcopy or with biopsy <CIN2 were recalled to follow-up at 18 months for a second HPV test; those HPV-positive had a second colposcopy with biopsy collection, as needed. Those with high-grade lesions at enrollment or 18 months were treated with large loop excision of the transformation zone (LLETZ) and exited the study.[7]

Six ESTAMPA centers participated in this study to reinterpret cytology results with knowledge of HPV positivity. Cytology smears from HPV-positive women were reinterpreted as described below. In 1 center (center 7), by design, cytology was mainly interpreted only for HPV-positive women; thus, cytology was not reinterpreted. However, because this center recruited women from several health institutions, there were scenarios where cytology was interpreted without knowledge of HPV positivity and routinely processed within the health-care system. On the other hand, at the local ESTAMPA study center cytology smears were prepared and interpreted only for HPV-positive women and with knowledge of HPV positivity. This allowed an additional comparison of the 2 approaches despite the samples not being paired.

## Participants and specimens

Participants were women aged 30-64 years without history of cervical cancer or precancer treatment. During screening visits, exfoliative cervical samples were taken with a Cervex Brush (Rovers Medical Devices, Oss, The Netherlands) and prepared as conventional cytology. The remaining cells on the brush were rinsed into vials containing 20 mL of ThinPrep PreservCyt medium (Hologic, Inc, Marlborough, MA, USA) for HPV testing with Digene HC2 or COBAS 4800.

## Cytology and reinterpretation procedures

Cytology smears were initially processed, stained, interpreted, and reported independently of HPV testing results by local laboratories. Cytology was reported according to the Bethesda System as negative for intraepithelial lesion or malignancy (NILM) or epithelial cells abnormalities (in either squamous and/or glandular cells).[8]

Cytology smears from HPV-positive women collected until March 2020 at the 7 participating centers were selected. In the 6 centers that conducted reinterpretation, slides were retrieved, relabeled with new codes, and marks removed. Cytology was reinterpreted blinded to previous cytological and histological diagnosis at the same laboratory where they were initially evaluated.[8] In centers 2, 3, and 7, cytology was interpreted by cytotechnologists, and cytopathologists read the positive ASC-US+ cases; in other centers, interpretation was exclusively by cytopathologists. Cytotechnologists/cytopathologists were informed that all slides were from HPV-positive women before review.

## Histology

Histological results were reported locally under the CIN nomenclature.[8] Additionally, 77% of local diagnosis were reviewed by an international panel of experts on cervical pathology using the Lower Anogenital Squamous Terminology (LAST) nomenclature, which uses p16 immunohistochemistry to improve diagnostic accuracy for histological high-grade squamous intraepithelial lesions (HSIL).[9]

## Study outcomes

Primary and secondary outcomes were histologically confirmed CIN3+ and CIN2+, respectively, based on biopsy, LLETZ, or histological endocervical sample specimens obtained at either the enrollment or 18-month visit. Women without high-grade cervical disease (<CIN2) included CIN1, negative histology, or negative colposcopy where cervical tissues were not collected. For women missing the 18-month visit, enrollment diagnosis was used.

Secondary analyses were conducted for histologically confirmed HSIL reported under the LAST nomenclature, including 77% of local diagnoses already reviewed by the expert panel at the time of analysis.

## Statistical analysis

Cytology results without and with HPV positivity knowledge were compared in a cross-classified table using standard Bethesda classifications. The threshold for cytology positivity was ASC-US or higher (ASC-US+).

Sensitivity, specificity, positive predictive value (PPV), complement of the negative predictive value (cNPV), and referral rate for CIN3+ (or CIN2+) detection were estimated with 95% CIs overall, by age (<50 or 50+ years), and by HPV positivity knowledge. CIN2 cases were excluded from estimates of predictive values for CIN3+ and specificity. Differences in performance estimations between cytology without and with knowledge of HPV positivity were assessed using a McNemar test for paired proportions (for sensitivities and specificities) or a $\chi^2$ test for unpaired proportions, as appropriate.

Fixed-effects logistic regression models were used to obtain pooled sensitivity and specificity estimates for CIN3+ and, as a

supplementary result, for CIN2+. The results were displayed through forest plots and stratified by HPV positivity knowledge.

Additionally, as supplementary analysis, a random-effects meta-analysis[10] was conducted to pool proportions of differences in sensitivity with and without HPV positivity knowledge for CIN3+ detection. All analyses were performed using Stata, version 15.0.

## Ethical considerations

The ESTAMPA protocol was approved by the Ethics Committee of the International Agency for Research on Cancer of the World Health Organization (IARC/WHO) (IEC Project 12–27-A7), the Pan American Health Organization Ethical Committee, and Ethical Committees at each study center. The study is classified as minimal risk because procedures are standard clinical practice. All women signed informed consent.

# Results

Between December 2012 and March 2020, a total of 36 458 women aged 30-64 years were recruited in the 7 ESTAMPA participating centers. Among them, 5062 (13.9%) tested positive for HPV and were referred to colposcopy. For this specific analysis, 795 women for whom cytology smears were not available or had no results were excluded. Additionally, 121 with unsatisfactory cytology and 59 for whom disease ascertainment was not available were also excluded. However, all women excluded from this analysis received the necessary diagnostic and treatment procedures as required. The final analysis included 4087 HPV-positive women (Figure 1).

A total of 2746 cytologies had paired cytology interpretations, without and with knowledge of HPV positivity. Additionally, there were 1341 cytologies from the study center where cytology was performed only for HPV-positive women, including 645 interpreted without knowledge of HPV result and 696 interpreted knowing that they were from HPV-positive women. Thus, a total of 3391 cytology smears were interpreted without knowledge of HPV positivity, and 3442 with knowledge of HPV positivity.

In total, 534/3391 (15.7%) participants had ASC-US+ when cytology was interpreted without knowledge of HPV positivity, whereas 909/3442 (26.4%) had ASC-US+ when slides were interpreted with knowledge. Among women included in the analysis, 743 CIN2+ cases were detected, including 253 CIN2, 455 CIN3, and 35 cancers (Figure 1).

Table S1 presents the characteristics of the study population. A total of 3044 women (74.5%) were younger than 50 years; 2288 (56.0%) reported having been screened with cytology every year, 1225 (30.0%) within 2-5 years, and 564 (13.8%) more than 5 years.

In the paired results analysis, knowledge of HPV positivity reduced cytologies classified as NILM from 2327 (85%) to 1997 (72%). When the HPV positivity was known, 82, 98, and 210 cytologies classified as NILM without HPV knowledge were reclassified as ASC-US, LSIL, and HSIL+, respectively (Table 1). Knowing of HPV positivity increased cytologic abnormalities for LSIL from 6% to 10% and for HSIL+ from 5% to 12%.

Overall sensitivity, specificity, PPV, cNPV, and referral rate of cytology without knowledge of HPV positivity for CIN3+ detection were 47.2% (95% CI = 42.5 to 51.9), 89.4% (95% CI = 88.2 to 90.5), 40.5% (95% CI = 36.2 to 44.9), 8.3% (95% CI = 7.3 to 9.4), and

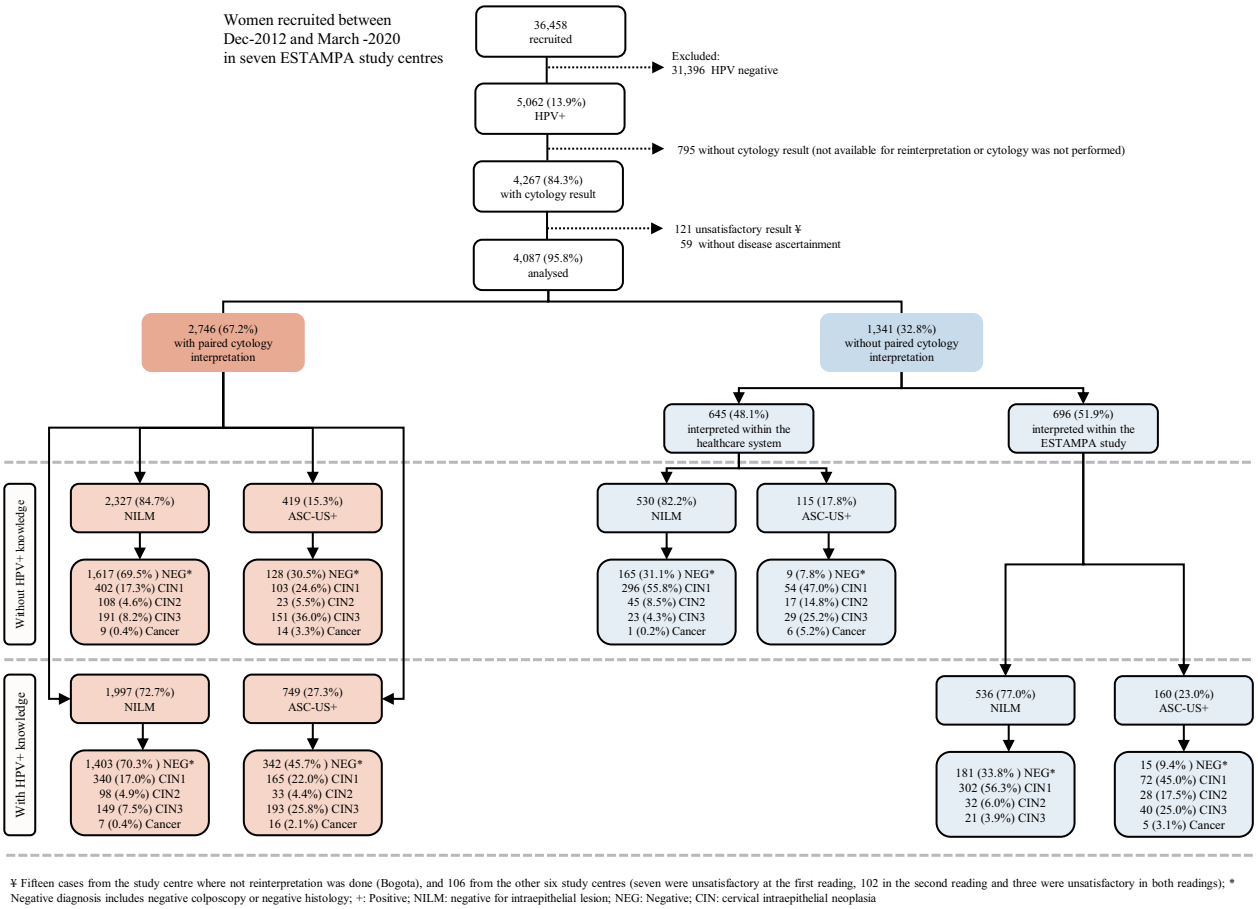


**Figure 1.** Study population. ESTAMPA participants recruited between December 2012 and March 2020.

**Table 1.** Cytology results for the paired comparison (without and with knowledge of HPV positivity).

| Without HPV positivity knowledge | Cytology results with HPV positivity knowledge | | | | |
|---|---|---|---|---|---|
| | NILM | ASC-US | LSIL | HSIL+ | Total (%[b]) |
| **NILM** | 1937 | 82 | 98 | 210 | 2327 (85%) |
| **ASC-US** | 31 | 39 | 23 | 9 | 102 (4%) |
| **LSIL** | 23 | 16 | 117 | 13 | 169 (6%) |
| **HSIL+** | 6 | 10 | 24 | 108 | 148 (5%) |
| Total (%[a]) | 1997 (72%) | 147 (5%) | 262 (10%) | 340 (12%) | 2746 |

[a] Percentage of cytology results with knowledge of HPV positivity.
[b] Percentage of cytology results without knowledge of HPV positivity.
Abbreviations: NILM = negative for intraepithelial lesion and malignancy; ASC-US = atypical squamous cells of undetermined significance; LSIL = low-grade squamous intraepithelial lesions; HSIL+ = high-grade squamous intraepithelial lesions or worse.

15.4% (95% CI = 14.2 to 16.7), respectively. When cytology was interpreted knowing the HPV positivity status, the sensitivity was higher (58.9%, 95% CI = 54.2 to 63.5; $P < .0001$) and the specificity was lower (78.9%, 95% CI = 77.4 to 80.4, $P < .0001$), whereas the PPV decreased to 30.0% (95% CI = 27.0 to 33.1) and the referral rate increased to 26.1% (95% CI = 24.6 to 27.6). In age/stratified analysis, similar results were observed for women younger than 50 and 50 years and older. Nevertheless, among women 50+ years, sensitivity was slightly higher when knowing HPV positivity (43.3%, 95% CI = 31.6 to 55.9 vs 48.2%, 95% CI = 35.7 to 61.0). Similar results were observed for CIN2+ detection (Table 2).

When excluding center 7 (without reinterpretation), overall sensitivity was 45.2% (95% CI = 40.2 to 50.3) when cytology was interpreted without HPV positivity knowledge, and 57.3% (95% CI = 52.1 to 62.2) when interpreted with that knowledge. Similarly, there was a decrease in specificity from 89.7% (95% CI = 88.4 to 90.9) to 77.5% (95% CI = 75.7 to 79.1).

Sensitivity varied across study centers, ranging from 32.8% (95% CI = 21.3 to 46.0) to 61.5% (95% CI = 49.8 to 72.3) when cytology was interpreted without knowledge of HPV positivity, and from 36.1% (95% CI = 24.2 to 49.4) to 93.4% (95% CI = 85.3 to 97.8) when HPV positivity was known (Figure 2). Knowledge of HPV positivity led to an increase in sensitivity in 6 centers (56.6% vs 93.4%, 33.3% vs 37.5%, 35.4% vs 43.4%, 32.8% vs 36.1%, 61.5% vs 66.7%, and 59.3% vs 68.2) with a slight decrease in specificity in each center. Notably, center 1 had a marked increase in sensitivity (from 56.6% to 93.4%) and a prominent loss in specificity (from 88.5% to 39.6%). No impact was observed in center 6 (Figure 2); a similar pattern was observed for CIN2+ detection (Figure S1).

In center 1, the evaluation process was replicated by having the same pathologist re-examine the HSIL+ slides, unaware that the slides were part of the study, but knowing only that the slides were from HPV-positive women; the rereading once again showed higher sensitivity, but slightly lower than in the previous report (86.8%, 95% CI = 77.1 to 93.5), with also a decrease in specificity (59.9%, 95% CI = 55.0 to 64.7) for CIN3+.

When considering results only from center 7, where results were not paired, the sensitivity was 59.3% (95% CI = 45.7 to 71.9) without HPV positivity knowledge and 68.2% (95% CI = 55.6 to 79.1) with such knowledge, whereas the specificity was 88.0% (95% CI = 84.9 to 90.6) and 84.7% (95% CI = 81.5 to 87.6), respectively.

The overall improvement in sensitivity for CIN3+ detection with HPV positivity knowledge was 10% (pooled proportion of difference in sensitivity was 0.10, 95% CI = 0.03 to 0.18). A significant improvement in sensitivity was observed for center 1, center 3, and center 5 (Figure S2). When center 1 was excluded, the overall improvement was 5% (pooled proportion of difference was 0.05, 95% CI = 0.03 to 0.08), which remained significant ($P < .001$).

## Discussion

An increasing number of countries are transitioning from cytology to HPV test for primary cervical cancer screening,[1,3,8,11] following WHO recommendations. However, given HPV's lower specificity, triage is needed to identify women at higher risk of disease among the HPV-positives to restrict colposcopy and treatment to those in real need.[12] WHO has suggested using HPV16/18, colposcopy, VIA, or cytology to triage HPV-positive women, depending on feasibility and available resources.[4] Cytology triage appears to be the immediate option due to the pre-existing infrastructure in place in LA and other low and middle-income countries (LMICs).[8]

It has been suggested that cytology interpreted with HPV positivity knowledge may exhibit higher sensitivity compared with cytology interpreted without awareness of HPV positivity,[5,6,13-17] resulting from increased attention during cytology interpretation and more accurate identification of abnormalities that might otherwise be considered irrelevant.[5,15]

This is the first multicenter study across LA evaluating the impact of HPV positivity knowledge on cervical cytology performance. The scale and scope of this study provide crucial evidence for countries in the region that are in the process of transitioning to HPV-based screening and may also inform other LMICs undergoing similar transitions. We analyzed data from 7 ESTAMPA study centers, including more than 4000 HPV-positive women, among whom 743 CIN2+ cases were detected (comprising 253 CIN2, 455 CIN3, and 35 cancer).

Our findings indicate that knowledge of HPV positivity significantly affected overall sensitivity for CIN3+ detection, increasing it by 10%. However, it also led to more false-positive results, resulting in a decrease in specificity, which is consistent with previous findings.[14,18] This loss in specificity can lead to a higher number of referrals for colposcopy, potentially overburdening colposcopy services, particularly in low-resource settings, leading to delays in care and increased costs. Moreover, the potential harms of unnecessary diagnostic and therapeutic procedures such as overtreatment are noted.[19] In our study, we observed that knowledge of HPV positivity resulted in increased referral of low-risk women, from 15.4% to 26.1%, along with a decrease in the PPV from 40.5% to 30.0%; these results were similar across age groups and highlight the need for careful consideration when selecting triage strategies.

The increased number of cytology abnormalities we observed when HPV positivity was known is consistent with previous findings.[13,15,20] Interestingly, we observed an important increase in cytologies initially reported as NILM, which were subsequently upgraded to HSIL+ following interpretation with knowledge of

**Table 2.** Overall and age-stratified performance of cytology to detect CIN3+ and CIN2+ among HPV-positive women.

| | CIN3+ | | | | CIN2+ | | | |
|---|---|---|---|---|---|---|---|---|
| **Performance measures** | **All women** | **Women <50 y** | **Women 50+ y** | **P[a]** | **All women** | **Women <50 y** | **Women 50+ y** | **P[a]** |
| ***Without knowing HPV positivity*** | | | | | | | | |
| Sensitivity (%) | 47.2 (42.5-51.9) | 47.8 (42.7-52.9) | 43.3 (31.6-55.9) | .615 | 38.9 (35.1-42.8) | 39.7 (35.6-44.0) | 34.1 (25.2-44.3) | .364 |
| Specificity (%) | 89.4 (88.2-90.5) | 87.5 (85.9-88.8) | 94.7 (92.9-96.1) | <.001 | 89.4 (88.2-90.5) | 87.5 (85.9-88.8) | 94.7 (92.9-96.1) | <.001 |
| PPV (%) | 40.5 (36.2-44.9) | 40.6 (36.0-45.3) | 40.0 (29.0-52.1) | 1 | 44.9 (40.8-49.2) | 45.0 (40.6-49.6) | 44.3 (33.2-55.9) | 1 |
| cNPV (%) | 8.3 (7.3-9.4) | 9.7 (8.4-11.0) | 4.6 (3.3-6.4) | <.001 | 13.2 (12.0-14.5) | 15.1 (13.7-16.7) | 7.9 (6.2-10.0) | <.001 |
| Referral rate (%) | 15.4 (14.2-16.7) | 17.9 (16.4-19.5) | 8.1 (6.4-10.2) | <.001 | 15.7 (14.6-17.0) | 18.1 (16.7-19.7) | 8.4 (6.7-10.5) | <.001 |
| TP | 200 | 174 | 26 | | 240 | 209 | 31 | |
| FP | 294 | 255 | 39 | | 294 | 255 | 39 | |
| FN | 224 | 190 | 34 | | 377 | 317 | 60 | |
| TN | 2480 | 1777 | 703 | | 2480 | 1777 | 703 | |
| ***Knowing HPV positivity*** | | | | | | | | |
| Sensitivity (%) | 58.9 (54.2-63.5) | 60.5 (55.5-65.3) | 48.2 (35.7-61.0) | .109 | 50.6 (46.7-54.6) | 51.8 (47.5-55.9) | 43.2 (33.0-54.1) | .188 |
| Specificity (%) | 78.9 (77.4-80.4) | 78.9 (77.0-80.6) | 79.2 (76.1-81.9) | .904 | 78.9 (77.4-80.4) | 78.9 (77.0-80.6) | 79.2 (76.1-81.9) | .904 |
| PPV (%) | 30.0 (27.0-33.1) | 34.2 (30.7-37.9) | 14.6 (10.2-20.4) | <.001 | 34.7 (31.6-37.8) | 39.1 (35.6-42.7) | 18.1 (13.3-24.2) | <.001 |
| cNPV (%) | 7.4 (6.4-8.5) | 8.3 (7.1-9.7) | 4.6 (3.2-6.5) | .003 | 12.1 (10.9-13.4) | 13.8 (12.3-15.5) | 7.1 (5.4-9.4) | <.001 |
| Referral rate (%) | 26.1 (24.6-27.6) | 27.2 (25.5-29.0) | 22.7 (20.0-25.7) | .013 | 26.4 (25.0-27.9) | 27.5 (25.8-29.3) | 23.0 (20.3-26.0) | .011 |
| TP | 254 | 227 | 27 | | 315 | 280 | 35 | |
| FP | 594 | 436 | 158 | | 594 | 436 | 158 | |
| FN | 177 | 148 | 29 | | 307 | 261 | 46 | |
| TN | 2226 | 1626 | 600 | | 2226 | 1626 | 600 | |

[a] $\chi^2$ test. 95% confidence intervals (CI) are shown in brackets.
Abbreviations: y = years; TP = true positives; FP = false positives; TN = true negatives; FN = false negatives.

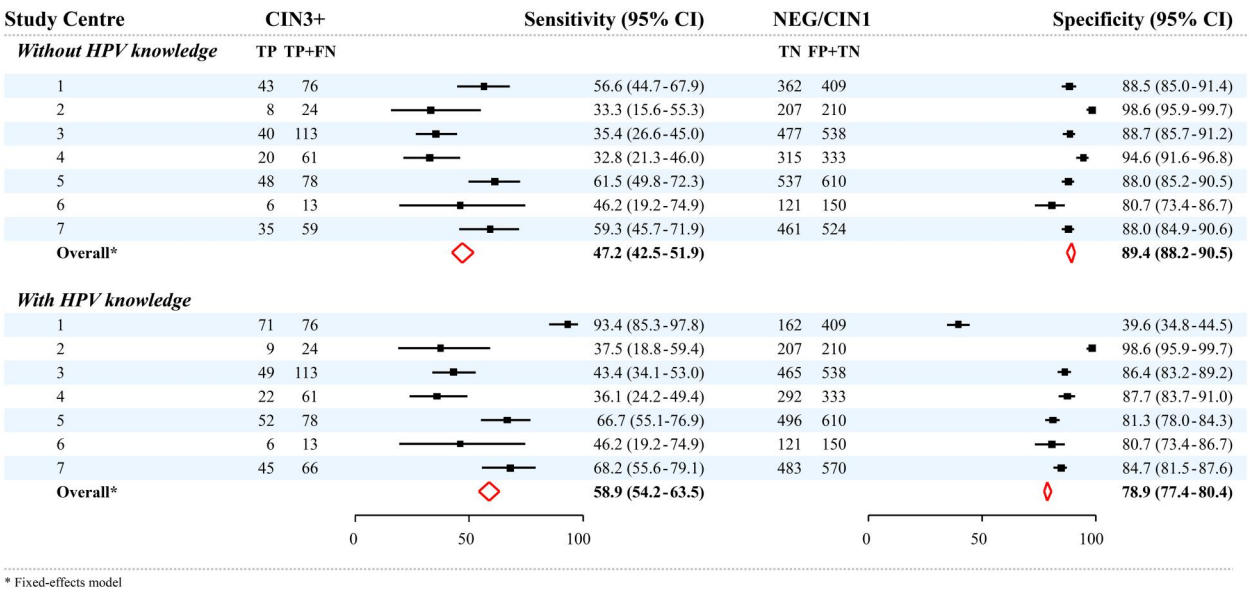


**Figure 2.** Forest plots for the performance of cytology without and with knowledge of HPV positivity for CIN3+ detection at 7 study centers.

HPV positivity (210 cases), indicating that, indeed, awareness of HPV-positive status significantly influences morphological interpretation, increasing sensitivity and potentially inducing overinterpretation.[13,20] This effect was particularly prominent in center 1, where sensitivity increased with marked decrease in specificity. Among 396 cases initially reported as NILM, 181 were reclassified as HSIL+ when HPV positivity was known. However, only 24 out of these 181 cases were histologically diagnosed as CIN3+. Because it appears that there was an unconscious bias due to HPV positivity knowledge in this center, we determined whether these results were maintained when the same cytopathologist re-evaluated the set of HSIL+ slides under blinded conditions, with knowledge that the slides were from HPV-positive women. The rereading resulted again in greater sensitivity but slightly lower than in the previous report (86.8%, 95% CI = 77.1 to 93.5 vs 93.4%, 95% CI = 85.3 to 97.8), with also a decrease in specificity (59.9%, 95% CI = 55.0 to 64.7 vs 39.6%, 95% CI = 34.8 to 44.5) for CIN3+ detection (data not shown). It is well known that cytology results are highly subjective and have poor reproducibility.[21] The pronounced change observed in center 1 emphasizes that cytology is inherently a subjective test, where individual interpretation can be influenced by additional clinical information. These results align with previous findings,[8,22] confirming that when cytotechnologists/cytopathologists are aware of the presence of HPV infection, they may be more inclined to intensively look for HPV-related abnormalities and report them even when they are not present. The finding of this extreme result in 1 center is important and highlights the need for strict monitoring, training, and quality assurance when using cytology.

When excluding center 1 (where the specificity was severely reduced), the performance estimates were in the same direction, with sensitivity increasing and specificity decreasing with HPV positivity knowledge, with a sensitivity without knowledge of 45.1% (95% CI = 40.0 to 50.4) and specificity of 89.6% (95% CI = 88.3 to 90.7). On the other hand, when HPV positivity was known, the sensitivity increased to 51.5% (95% CI = 46.4 to 56.7), whereas specificity decreased to 85.6% (95% CI = 84.1 to 87.0) (data not shown).

The heterogeneity between the centers is likely the result of differences in settings and specific laboratory conditions at each participating center. Preliminary work in a separate analysis within ESTAMPA, which explored whether certain characteristics of cytology laboratories could be associated with test performance, showed that all laboratories had extensive experience, with more than 15 years of reading cytology slides. Most laboratories regularly provided training updates for interpreters. In addition, all positive slides, except in 1 laboratory, are reviewed by a second reader, and a percentage of negative slides are also reevaluated as a quality control measure. Despite these practices, limited sensitivity and important performance variations are still observed across laboratories.

An important point to emphasize is that some lesions may be missed in cases where cytology results are classified as unsatisfactory. In ESTAMPA, women who tested positive for HPV were referred to colposcopy regardless of their cytology results, which allowed for the detection CIN3 cases even among women with unsatisfactory cytology. In fact, when exploring the risk of CIN3+ among women with unsatisfactory cytology results, we observed a risk of approximately of 22.7%, compared with 7.4% for women with negative cytology results and 35.5% for those with ASC-US+. These findings highlight the importance of requesting a second sample or conducting additional diagnostic procedures for women who receive an unsatisfactory cytology result, as potentially high-grade diseases could otherwise be missed. In our study, this issue was mitigated because all women also underwent HPV testing and were referred for colposcopy based on their HPV status, ensuring appropriate follow-up and management.

Furthermore, because 2 different HPV tests were used in ESTAMPA, in previous reports we explored whether the performance characteristics of the HPV tests used in the study (HC2 and COBAS 4800) were consistent across study centers. Both assays showed high sensitivity for detecting CIN3+, with minimal variation (ranging from 96.7% to 100%).[8] Importantly, no significant differences were found in the sensitivity for CIN3+ detection between the 2 tests, and the interpreters were informed only that the women were HPV-positive, without any details provided

about the specific type of test used, suggesting that the type of HPV test used did not influence the overall cytology assessment.

When a random-effects model was considered to account for variation between study centers, the overall results were similar. The sensitivity for CIN3+ detection was 45.3% (95% CI = 32.8 to 58.4) when cytology was interpreted without knowledge of HPV positivity and 58.1% (95% CI = 45.0 to 70.2) when interpreted with knowledge of HPV positivity, whereas the specificity was 92.2% (95% CI = 85.1 to 96.1) and 83.2% (95% CI = 70.7 to 91.1), respectively (data not shown).

The main strength of our study apart from the large sample size and the design of the ESTAMPA study is the high adherence to the protocol and retention to colposcopy and treatment and to the 18-months follow-up visit for HPV-positive women with no evident disease at enrollment, which allowed for adequate disease ascertainment.[7,8,23] Another strength was that for the reinterpretation with HPV positivity knowledge, cytology smears were routed through the same pathway as in the ESTAMPA study and in many centers, as in routine practice, reflecting real-life conditions. Furthermore, at center 7, a group of smears were processed and interpreted with HPV positivity knowledge in real life, which provided a comparison group that simulates reflex testing.

One limitation was the absence of an external review for cytology smears, which could have enhanced the quality of the performance evaluation. However, this issue was mitigated by the reinterpretation exercise and by rerouting the smears through the same pathway. Additionally, these results were based on local diagnoses and not on histology reviewed by an international panel of experts using the LAST nomenclature.[9] However, among the included women, 77% have been fully reviewed and 452 HSIL+ have been confirmed, and preliminary performance estimates for HSIL+ detection yielded similar results to those based on local diagnoses. Cytology had higher sensitivity in detecting HSIL+ when the HPV positivity status was known (42.4%, 95% CI = 37.4 to 47.6 vs 54.6%, 95% CI = 49.5 to 59.6) with a lower specificity (76.8%, 95% CI = 74.9 to 78.5 vs 88.79%, 95% CI = 87.4 to 90.1).

With the implementation of primary HPV-based screening with cytology triage, the number of cytologies will decrease, which could be an advantage because it allows interpreters to have more time to read each slide due to reduced workload. However, it also brings the challenge of maintaining high-quality training for cytology interpreters.

As previously reported,[8] in the ESTAMPA study, all procedures, from cervical sample collection to cytology reading and reporting, adhered to quality assurance protocols. Before the study was launched, all clinicians received standardized training for cervical samples collection, and although no specific training was provided for cytology processing and interpretation, the professionals involved were experienced and the cytology laboratories were fully accredited, maintaining local quality assurance controls.[8] Despite this, the study found limited sensitivity in most laboratories, which is consistent with previous research indicating that cytology has a limited performance even under optimal conditions.[24,25] Additional improvements in the quality assurance systems could produce better results.

Another challenge in using cytology as a triage test arises when self-sampling is used for HPV testing as part of screening. In such cases, HPV-positive women may require an additional clinician-collected sample, because cytology cannot be reliably performed on self-collected specimens.[26]

The current installed capacity, available resources, and the experience with cytology in LA may support its use as a triage method. However, it is important to note that despite an increase in sensitivity when HPV positivity is known, cytology still exhibits limitations in effectively detecting high-grade cervical lesions. The variability in performance and subjectivity in the interpretation further reinforces the urgent need for new triage methods to mitigate these challenges. Several alternative triage methods, such as molecular biomarkers, including HPV genotyping, extended genotyping, host and viral methylation markers, and p16/Ki67 staining, have shown promising accuracy in predicting the presence of precancerous lesions.[12,27-30] An added advantage of molecular tests such as HPV genotyping and methylation is that they do not rely on assessment of cellular morphology,[30] can be performed on self-collected samples, and are more objective and reproducible. Some of these markers are currently under evaluation in the ESTAMPA study, with results expected to be published in the near future. We are also evaluating the performance of liquid-based cytology in some of the centers participating in ESTAMPA.

In conclusion, although there was an increase in the sensitivity of cytology when considering HPV positivity, this improvement was limited and varied across study centers, which reinforces the urgent need for alternative triage strategies to support progress toward global cervical cancer elimination goals in the region.

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## Supplementary material

Supplementary material is available at *JNCI: Journal of the National Cancer Institute* online.

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## Conflicts of interest

All authors declare no competing interests.

## Data availability

The data used in this study selected several variables (age at recruitment, screening history, cytology and HPV testing results, colposcopy, histology, and results at the 18-month visit) from the EStudio multicéntrico de TAMizaje y triaje de cáncer de cuello uterino con pruebas del virus del PApiloma humano (ESTAMPA) study database, which is stored securely at the International Agency for Research on Cancer. Anonymized individual participant data or aggregated data would be available upon reasonable request to the corresponding author (ramirezt@iarc.who.int), after signing a contract, and with approval from the principal and local investigators.

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