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ORIGINAL RESEARCH ARTICLE

Adjunctive use of p16 immunohistochemistry for optimizing management of CIN lesions in a high-risk human papillomavirus-positive population

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Abstract

Introduction: Immunostaining with p16^{INK4a} (p16), a tumor-suppressor surrogate protein biomarker for high-risk human papillomavirus (hrHPV) oncogenic activity, may complement standard hematoxylin and eosin (H&E) histology review, and provide more objective criteria to support the cervical intraepithelial neoplasia (CIN) diagnosis. With this study we assessed the impact of p16 immunohistochemistry on CIN grading in an hrHPV-based screening setting.

Material and methods: In this post-hoc analysis, 326 histology follow-up samples from a group of hrHPV-positive women were stained with p16 immunohistochemistry. All H&E samples were centrally revised. The pathologists reported their level of confidence in classifying the CIN lesion.

Results: Combining H&E and p16 staining resulted in a change of diagnosis in 27.3% ($n = 89$) of cases compared with the revised H&E samples, with a decrease of 34.5% ($n = 18$) in CIN1 and 22.7% ($n = 15$) in CIN2 classifications, and an increase of 18.3% ($n = 19$) in no CIN and 20.7% ($n = 19$) in CIN3 diagnoses. The level of confidence in CIN grading by the pathologist increased with adjunctive use of p16 immunohistochemistry to standard H&E.

Conclusions: This study shows that adjunctive use of p16 immunohistochemistry to H&E morphology reduces the number of CIN1 and CIN2 classifications with a proportional increase in no CIN and CIN3 diagnoses, compared with standard H&E-based CIN diagnosis alone. The pathologists felt more confident in classifying the material

Abbreviations: CIN, cervical intraepithelial neoplasia; H&E, hematoxylin and eosin; hrHPV, high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesions; LLETZ, large loop excisions of the transformation zone; LSIL, low-grade squamous intraepithelial lesions; p16, p16^{INK4a}.

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with H&E and p16 immunohistochemistry than by using H&E alone, particularly during assessment of small biopsies. Adjunctive use of p16 immunohistochemistry to standard H&E assessment of CIN would be valuable for the diagnostic accuracy, thereby optimizing CIN management and possibly decreasing overtreatment.

KEYWORDS
cervical cancer, cervical intraepithelial neoplasia, human papillomavirus, immunohistochemistry, p16

1 | INTRODUCTION

A persistent infection with high-risk human papillomavirus (hrHPV) can lead to cervical intraepithelial neoplasia (CIN), and cervical cancer. hrHPV-based cervical cancer screening achieves greater sensitivity than cytology in the detection of cervical intraepithelial neoplasia and greater protection against cervical cancer.[1] The main concern surrounding the use of hrHPV testing for primary screening is its relatively low specificity, a direct result of the assay's inability to distinguish transient from persistent, transforming hrHPV infections.[2] The lower specificity of HPV testing is counteracted by cytological triage. However, besides an increase in detected CIN3 lesions and cervical cancer, the first year of hrHPV-based screening in the Netherlands also resulted in a strong increase of colposcopy referrals for women with CIN1 and CIN2 lesions, from respectively 90 to 587 per 100 000 women and 174 to 415 per 100 000 women, which is expected to level off at the second screening round.[3,4] This increase in colposcopy referral rates and biopsies implicates a larger number of women in need of long-term follow up, with an increased risk of overtreatment.

Clinical management of CIN is highly dependent on the accurate histological assessment of colposcopically obtained biopsies and large loop excisions of the transformation zone (LLETZ). Cervical histology is currently classified by the three-tiered CIN terminology differentiating CIN1, CIN2, and CIN3, or the two-tiered Lower Anogenital Squamous Terminology, differentiating low-grade (LSIL) and high-grade (HSIL) squamous intraepithelial lesions.[5] Both subjective scoring systems show limited reproducibility with only moderate inter- and intra-observer agreement in the morphological grading, which affects clinical management of women with cervical lesions. Obtaining an accurate pathological diagnosis can be challenging with only hematoxylin & eosin-based (H&E) morphology. Molecular biomarkers are therefore increasingly studied as a potential addition to morphological interpretation in the histological diagnosis of CIN.[6,7]

The tumor-suppressor protein $p16^{INK4a}$ (p16) is a surrogate marker of HPV oncogenic activity. Expression of the HPV E7 oncoprotein triggers de-methylation of the p16 promoter, which, as a result, causes upregulation of p16 expression.[8] A p16 biomarker might increase objectivity, and therefore accuracy, in diagnosing not only cervical smear test cytology, but also CIN lesions.[6,9–11] With this study we aim to assess the impact of p16 immunohistochemistry on CIN grading by a pathologist in an hrHPV-based screening setting.

Key message

Adjunctive use of p16 immunohistochemistry to hematoxylin and eosin morphology may optimize CIN management and it may decrease overtreatment by reducing the number of CIN1 and CIN2 classifications with a proportional increase in no CIN and CIN3 diagnoses.

2 | MATERIAL AND METHODS

2.1 | Study population

This study is a post-hoc analysis on histology samples from the PROHTECT-3B trial (Protection by Offering HPV Testing on self-sampled cervicovaginal specimens Trial-3B).[12] In the PROHTECT-3B trial, former non-responders to the cervical cancer screening program, aged 30–60 years, were invited to participate in cervical cancer screening, by offering self-sampling for hrHPV DNA testing (GP5+/6+ polymerase chain reaction; EIA HPV GP HR kit; LBP). Three laboratories performed the hrHPV tests. Women who tested hrHPV positive on their self-sample were advised to have a cervical smear taken by a physician for Papanicolaou-cytology triage testing. Women with abnormal cytology results (defined as atypical cells of undetermined significance or worse) were referred for a colposcopy-directed biopsy, whereas women with a normal cytology result (defined by negative for intraepithelial lesion or malignancy cytology result) were re-invited for an exit test with Papanicolaou cytology and hrHPV co-testing 6 months later.

Women with a positive exit test, defined as atypical cells of undetermined significance or worse cytology and/or high-risk HPV-positive test results, were in the second instance referred for histological examination by taking a colposcopy-directed biopsy, endocervical curettage, or LLETZ. Colposcopists were aware of the hrHPV-positive status and colposcopy was performed according to the Dutch national guidelines. If no abnormalities were seen at colposcopy, it was advised to take two random biopsies according to the study protocol. The database was closed with a mean follow up of 15 months (range: 6–18 months). Further details of the PROHTECT-3B trial design are reported elsewhere.[12]

2.2 | H&E, immunohistochemistry and centralized revision

Original H&E diagnoses were retrieved from the Dutch nationwide computerized registry of histopathology and cytopathology (PALGA). The histology outcomes were classified as no CIN, CIN1, CIN2, CIN3, and invasive carcinoma.

For revision, all original H&E slides from the worst histology samples collected during colposcopy procedures were obtained and subjected to blinded central review by a general pathologist. If the review diagnosis of the general pathologist was not consistent with the original H&E diagnosis, a second specialized gynecopathologist, with extensive experience in gynecopathology blindly adjudicated the case, resulting in a consensus diagnosis. Revised H&E diagnoses were assessed for all available cervical tissue specimens. Pathologists were blinded to all previous study results. Pathologists were asked to score the confidence in their diagnosis differentiating between confident in their diagnosis, rather confident and unconfident.

2.3 | p16 immunohistochemistry and interpretation

Paraffin-embedded tissue blocks were obtained from all histology samples. In the case of multiple blocks, the tissue block containing the CIN lesion that corresponded with the worst H&E assessment was selected. From this tissue block, a 4-µm-thick slice was cut and its slide was stained according to the instructions of the manufacturer with the p16 Histology kit (Roche mtm Laboratories AG). Staining was performed on a Ventana benchmark ultra (Roche mtm Laboratories AG), and each run included one control specimen.

All p16 slides were examined by a trained general pathologist. p16 slides were scored as no CIN when either no p16 positivity or focally scattered positive cells, or small positive clusters were seen. Slides were scored as CIN1 when diffuse positivity for p16 was seen in dysplastic cells in the lower third of the cervical epithelium and koilocytotic atypia were present as a sign of hrHPV infection. A CIN2 diagnosis was given when p16 positivity was evenly distributed across the lower two-thirds of the epithelium. Slides were scored as CIN3 when diffuse strong nuclear and cytoplasmic p16 block staining was seen in from two-thirds up to the whole width of the epithelium,[5] (Figure 1). The combined H&E and p16 staining score was obtained according to the opinion of the pathologist, taking into account the H&E staining, and p16 staining as described above. Pathologists were asked to score the confidence in their diagnosis differentiating between confident in their diagnosis, rather confident, and unconfident.

2.4 | Statistical analyses

Different scoring strategies including original H&E diagnosis, revised H&E diagnosis, sole p16 diagnosis, and combined revised H&E and p16 diagnosis, were compared. Descriptive statistics were used to calculate numbers and percentages of confidence. Sensitivity and specificity, with 90% confidence intervals (90% CI) were calculated for the original H&E diagnosis, the revised H&E diagnosis, and the stand-alone p16 diagnosis. The combined revised H&E and p16 diagnosis was used as reference standard. McNemar's test was used to calculate *p* values. Statistical analyses were conducted using SPSS version 20.0.1 for Windows (IBM).

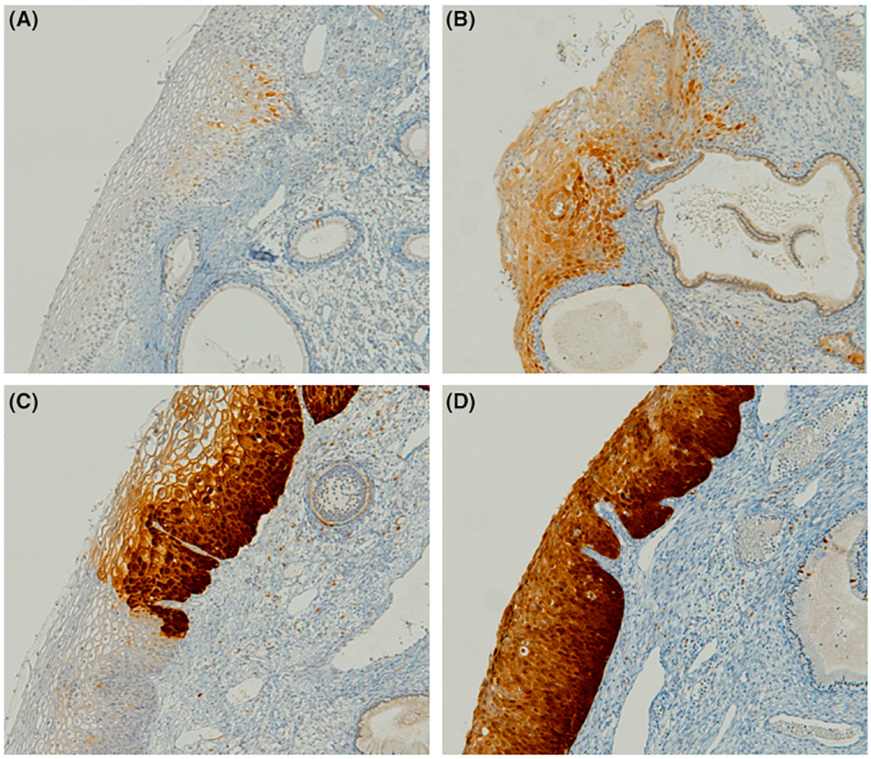

FIGURE 1 Representative examples are shown for the $p16^{INK4A}$ (p16) immunostaining patterns. Scored as no cervical intraepithelial neoplasia (CIN) when no p16 positivity or focally scattered positive cells, or small positive clusters were seen (A). Scored as CIN1 when diffuse positivity for p16 was seen in dysplastic cells in the lower third of the cervical epithelium and koilocytotic atypia was present as a sign of a high-risk human papillomavirus infection (B). Scored as CIN2 when p16 positivity was evenly distributed across the lower two-thirds of the epithelium (C). Scored as CIN3 when diffuse strong nuclear and cytoplasmic p16 block staining was seen in from two-thirds up to the whole width of the epithelium (D).

2.5 | Ethics statement

All women provided written informed consent. The Ministry of Health gave ethical approval for the PROHTECT3b study on August 31, 2010 (No. 2010/WBO04), and the regional institutional review board approved the protocol for this post-hoc analysis on March 16, 2012.

3 | RESULTS

Of the 405 eligible women from the PROHTECT-3B trial, 48 were excluded because of the unavailability of histological material. In addition, in 31 cases the lesions were not present in the p16-stained slides, resulting in a study population of 326 samples. From these 326 samples, the original H&E diagnosis included 101 samples without a CIN lesion, 65 were scored as CIN1, 62 as CIN2, 85 as CIN3, and 13 as cervical carcinoma. Of the 326 samples, 166 were biopsies, four samples were from an endocervical curettage, 145 were from LLETZ, and 11 were scored as unknown.

Table 1 shows an overview of the original H&E diagnosis compared with the centralized revised H&E diagnosis (without p16). Centralized revision of the samples led to an upgrade of 37 cases (11.3%), a downgrade of 24 cases (7.4%), and in 265 cases (81.3%) the diagnosis was identical to the original H&E diagnosis. The largest shift in diagnoses, both upgraded and downgraded, was found in samples initially diagnosed as CIN1, with a total shift of 35.4%, and CIN2 with a total shift of 32.3%. One carcinoma was downgraded to CIN3, which was a sample from a small fragmented cervical biopsy (Table 1).

Table 2 shows an overview of the revised H&E diagnosis compared with the p16-only diagnosis. Six cases that were initially scored as carcinoma were downgraded to CIN3 based on the p16 slide alone, eight cases were missing because material was no longer available. Table 3 shows an overview of the original H&E diagnosis compared with the diagnosis based on H&E and p16 staining combined.

Table 4 and the Sankey diagram in Figure 2 show an overview of the revised H&E diagnosis compared with the diagnosis based on the combined H&E and p16 diagnosis. Combining H&E with p16 led to an upgrade of 44 cases (13.5%), a downgrade of 45 cases (13.8%), and in 237 (72.7%) the diagnosis was similar to the revised H&E diagnosis. The largest shift of diagnosis was found in samples diagnosed as CIN1, with a shift of 59.6%, and CIN2 with a shift of 51.5%. The shift in diagnoses was low with 8.3% and 8.7% when respectively a carcinoma or CIN3 was diagnosed, or 14.4% in case of no CIN. Of all 52 CIN1 cases, 22 (42.3%) cases were re-diagnosed as no CIN when p16 was added to the revised H&E diagnosis. Of all 66 CIN2 cases, 20 cases (30.3%) were re-diagnosed as CIN3, and 14 cases (21.2%) were re-diagnosed as no CIN or CIN1. One microinvasive carcinoma was re-diagnosed as CIN3, which was based on a LLETZ. It is possible that this was caused by sampling error (Table 4).

TABLE 1 Original H&E diagnosis compared with the revised H&E diagnosis

	Revised H&E diagnosis						Shift	Upgrade	Downgrade
Original H&E diagnosis	No CIN	CIN1	CIN2	CIN3	Carcinoma	Total	%	%	%
No CIN	91	7	3	0	0	101	9.9	9.9	0.0
CIN1	8	42	14	1	0	65	35.4	23.1	12.3
CIN2	5	3	42	12	0	62	32.3	19.4	12.9
CIN3	0	0	7	78	0	85	8.2	0.0	8.2
Carcinoma	0	0	0	1	12	13	7.7	0.0	7.7
Total	104	52	66	92	12	326	18.7	11.3	7.4

Abbreviations: CIN, cervical intraepithelial neoplasia; H&E hematoxylin & eosin.

TABLE 2 Revised H&E diagnosis compared with the p16 diagnosis

	p16 diagnosis							Shift	Upgrade	Downgrade
Revised H&E diagnosis	No CIN	CIN1	CIN2	CIN3	Carcinoma	Missing	Total	%	%	%
No CIN	90	2	3	4	0	5	104	9.1	9.1	0.0
CIN1	37	5	5	5	0	0	52	90.4	19.2	71.2
CIN2	17	5	12	31	0	1	66	81.5	47.7	33.8
CIN3	3	2	6	79	0	2	92	12.2	0.0	12.2
Carcinoma	0	0	0	6	6	0	12	50.0	0.0	50.0
Total	147	14	26	125	6	8	326	39.6	15.7	23.9

Abbreviations: CIN, cervical intraepithelial neoplasia; H&E hematoxylin & eosin; p16, $p16^{INK4A}$.

TABLE 3 Original H&E diagnosis compared with the combined revised H&E and p16 diagnosis

	Combined revised H&E and p16 diagnosis						Shift	Upgrade	Downgrade
Original H&E diagnosis	No CIN	CIN1	CIN2	CIN3	Carcinoma	Total	%	%	%
No CIN	87	7	6	1	0	101	13.9	13.9	0.0
CIN1	22	21	16	6	0	65	67.7	33.8	33.8
CIN2	10	6	20	26	0	62	67.7	41.9	25.8
CIN3	0	0	9	76	0	85	10.6	0.0	10.6
Carcinoma	0	0	0	2	11	13	15.4	0.0	15.4
Total	119	34	51	111	11	326	34.0	19.0	15.0

Abbreviations: CIN, cervical intraepithelial neoplasia; H&E hematoxylin & eosin; p16, $p16^{INK4A}$.

TABLE 4 Revised H&E diagnosis compared with the combined revised H&E and p16 diagnosis

	Combined revised H&E and p16 diagnosis						Shift	Upgrade	Downgrade
Revised H&E diagnosis	No CIN	CIN1	CIN2	CIN3	Carcinoma	Total	%	%	%
No CIN	89	7	5	3	0	104	14.4	14.4	0.0
CIN1	22	21	6	3	0	52	59.6	17.3	42.3
CIN2	8	6	32	20	0	66	51.5	30.3	21.2
CIN3	0	0	8	84	0	92	8.7	0.0	8.7
Carcinoma	0	0	0	1	11	12	8.3	0.0	8.3
Total	119	34	51	111	11	326	27.3	13.5	13.8

Abbreviations: CIN, cervical intraepithelial neoplasia; H&E hematoxylin & eosin; p16, $p16^{INK4A}$.

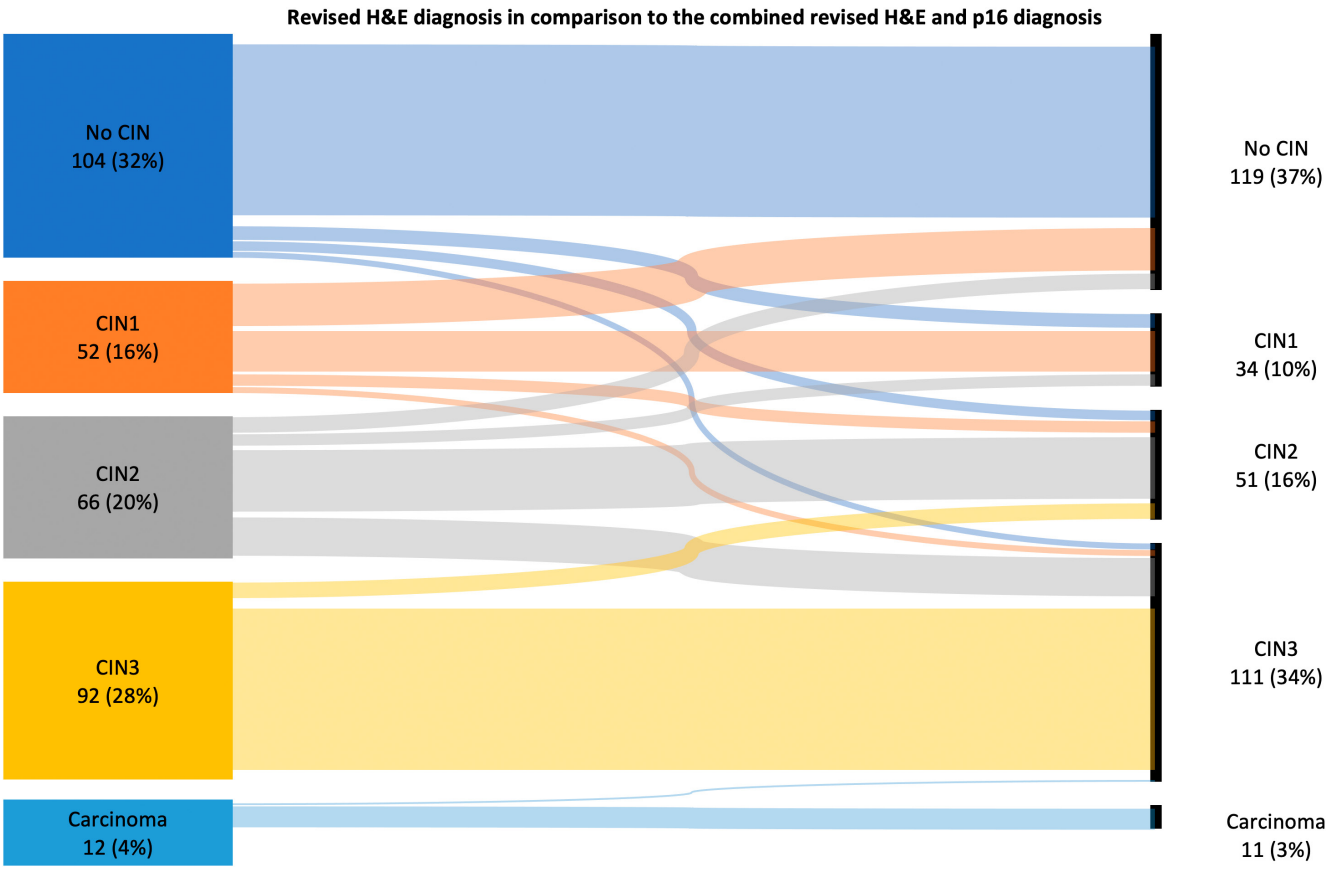

FIGURE 2 Sankey diagram comparing the revised H&E diagnosis in comparison to the combined revised H&E and p16 diagnosis. CIN, cervical intraepithelial neoplasia; H&E, hematoxylin and eosin.

Figure 3 shows an overview of the outcome of different scoring strategies. This showed a decrease of CIN1 and CIN2 diagnoses, with an increase in no CIN and CIN3 diagnoses when p16 was combined with the revised H&E diagnosis.

Centralized revision results in a non-significant increased sensitivity and specificity for CIN2+ ($p = 0.08$) and CIN3+ ($p = 0.26$), compared with the original H&E diagnosis, when the combined revised H&E with p16 diagnosis was used as reference standard, indicating a possible additional value of centralized revision. The p16 diagnosis alone resulted in a significantly higher sensitivity for CIN3+, but at the cost of a decreased specificity, compared with the original H&E diagnosis ($p = 0.17$ for CIN2+ and $p = 0.0001$ for CIN3+) (Table 5). As the combined revised H&E and p16 diagnosis was used as reference standard, sensitivity and specificity could not be estimated.

The general pathologist had a higher level of confidence on the diagnosis in 39% of the cases with adjunctive use of p16 immunohistochemistry to the H&E staining. On the other hand, adjunctive use of p16 immunohistochemistry with H&E, decreased the level of confidence for 18% of the cases. The specialized gynecopathologist was more confident in 35% of the cases when p16 immunohistochemistry was added to the H&E staining, and reported a similar level of confidence in 48% of cases. The general and specialized pathologists felt a higher level of confidence on diagnosing CIN1 and CIN2, in respectively 51% and 50% of all cases with adjunctive use of p16 immunohistochemistry and H&E staining. The general and specialized pathologists especially described a higher level of confidence when grading biopsies compared with LLETZ, with an increase of confidence in diagnosing of 45% and 34%, respectively.

4 | DISCUSSION

Reproducibility of an accurate pathological diagnosis in CIN lesions can be challenging with only H&E morphology. Molecular biomarker p16 is therefore increasingly used as an addition to morphological interpretation in the histological diagnosis of CIN. In this post hoc study, we evaluate grading of CIN lesions using H&E staining and p16 immunohistochemistry, either alone or in combination. Adjunctive use of p16 immunohistochemistry to all H&E staining of CIN lesions reduced the number of CIN1 and CIN2 cases, with a shift to increased numbers of no CIN and CIN3. Adjunctive use of p16 immunohistochemistry would therefore improve reproducibility and would result in a decrease of women in need of follow up, and possibly decrease the risk of overtreatment.

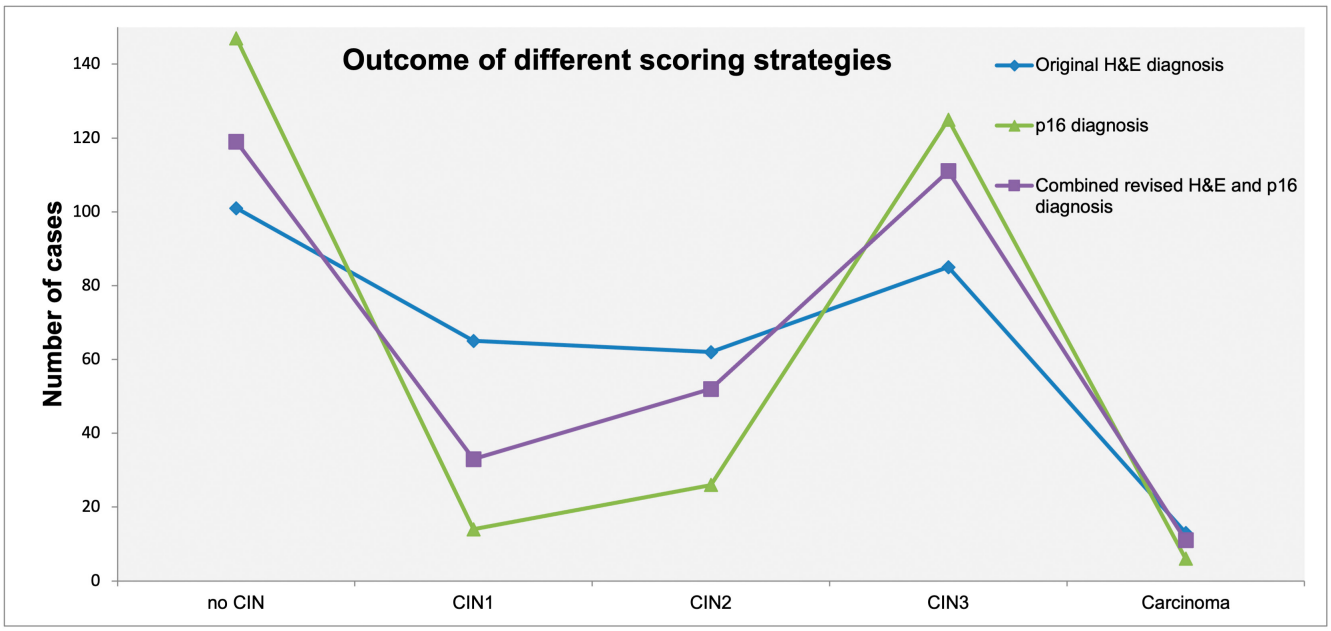

FIGURE 3 Outcome of different scoring strategies. CIN, cervical intraepithelial neoplasia; H&E, hematoxylin and eosin.

TABLE 5 Sensitivity and specificity for different scoring strategies

	Sensitivity CIN2+ (95% CI)	Specificity CIN2+ (95% CI)	Sensitivity CIN3+ (95% CI)	Specificity CIN3+ (95% CI)
Original H&E diagnosis	83.2% (76.6%–88.3%)	89.5% (83.3%–93.7%)	73.0% (64.0%–80.4%)	95.6% (91.5%–97.8%)
Revised H&E diagnosis	90.2% (84.5%–94.0%)	90.8% (84.8%–94.7%)	78.7% (70.2%–85.4%)	96.1% (92.1%–98.2%)
p16 diagnosis	87.1% (80.8%–91.5%)	93.9% (88.4%–97.0%)	92.4% (85.7%–96.3%)	89.4% (84.1%–93.2%)

Abbreviations: CI, confidence interval; CIN, cervical intraepithelial neoplasia; H&E hematoxylin & eosin; p16, $p16^{INK4A}$.

Our results are in line with the large study by Stoler et al that routinely used adjunctive p16 immunohistochemistry to cervical biopsy interpretation in eleven hundred cervical biopsies.[11] They concluded that adjunctive use of p16 immunohistochemistry ensures that more women are treated correctly without treating more women, by providing more accurate and reproducible diagnostic results in the interpretation of cervical biopsies.[11] Also a cross-sectional study by van Zummeren et al is in agreement with these results. They have shown that the grading of CIN using a p16 and Ki-67 immunoscore system shows a higher accuracy and better reproducibility compared with the classical CIN grading system, especially for CIN3 and CIN1.[7] A previously described risk of positive p16 stain in 30% of CIN1 lesions with attributable risk of overtreatment is not seen in this study.[5] Also, previous studies have performed p16 staining in only histological low-grade or only histological high-grade CIN. In a study focused on low-grade CIN lesions, the authors conclude that p16 has limited value and it is advised to only use p16 staining when a high-grade lesion is in the differential diagnosis, as p16 showed only a low value as a clinical marker of progressive low-grade lesions.[13] Miralpeix et al advised that the status of p16 staining could be considered for management of high-grade lesions.[14] However, for low-grade lesions, the study by Mills et al has shown that presence or absence of p16 staining does not predict progression or regression in CIN1 and recommends that management of CIN1 should therefore not be altered with regard to the p16 diagnosis.[15] p16 has also been studied in combination with Ki-67 or E4 immunoscoring, combined with FAM19A4/miR124-2 methylation markers in the corresponding cervical scrape, indicating that biomarker profiles, including immunoscore and methylation status could help the clinician in the decision for immediate treatment or a "wait and see" policy to reduce overtreatment of high-grade CIN lesions.[16,17]

It has been stated that biomarker p16 should be used as an addition to H&E-based diagnosis, to differentiate difficult CIN lesions, or in the case of a professional disagreement in histological specimen interpretation.[18] The Dutch CIN, AIS, and VAIN guideline advises the use of biomarkers p16 and/or MIB1 when in doubt between CIN1 and CIN2, or when there is doubt about diagnosing a reactive cervical abnormality as atrophy or immature squamous metaplasia.[19] Adjunctive use of p16 immunohistochemistry is used in a selected group of cases because in some cases costs might outweigh the additional value when a pathologist is already very certain about a diagnosis. Also, the Lower Anogenital Squamous Terminology guidelines advise the use of p16 when the pathologist is in doubt, which significantly increased the use of p16 staining worldwide.[20,21] In the Lower Anogenital Squamous Terminology guideline, cervical lesions are divided using a dual-scoring system differentiating HSIL and LSIL lesions. A block-positive p16 staining of the epithelium, indicating a continuous segment of cells positive for p16, is considered characteristic for hrHPV-associated high-grade CIN lesions, and treatment of all HSIL lesions is recommended.[5]

A limitation of this study is the lack of a reference standard for diagnosing CIN. However, previously it has been described that H&E staining combined with immunohistochemistry markers, as used in this study, shows the highest accuracy in diagnosing CIN.[22–24] Also, an example of sampling error can be the case in which a carcinoma was diagnosed in the H&E slide, but was not found in the p16 slide. It is possible that the lesion is already cut out when slicing the p16 staining in the second instance. This risk can be reduced by combining the two slides for a diagnosis. In this study, 79 samples had to be excluded from the study because no material was available for p16 staining. In this study 6-month follow up was offered with hrHPV testing and cytology combined. This differs from the current Dutch cervical cancer screening program, as hrHPV-positive women with normal cytology results are only offered a 6-month follow up cytology test without hrHPV test. This could result in different referral rates or CIN2+ compared with this study. For example, in the study by Aitken et al the CIN2+ rate of women who performed self-sampling in 2017 in the Dutch cervical cancer screening program was 57.1% in the case of immediate referral for colposcopy and 41.3% for women who underwent colposcopy after a 6-month follow-up smear.[25] In this study the CIN2+ rate of revised H&E combined with p16 was 53.1%.

Our results show that adjunctive use of p16 immunohistochemistry with the H&E staining decreases the number of cases diagnosed as CIN1 and CIN2 and increases the number of no CIN or CIN3 diagnoses. This may decrease overtreatment in the case of a biopsy, and therefore the number of unnecessary LLETZ. It also increases the level of confidence in properly diagnosing CIN lesions, especially in the case of assessment of a biopsy. Studies on the accuracy of diagnosing high-grade CIN lesions show that LLETZ may be negative for high-grade CIN in 14%–24% of all biopsy-proven CIN cases.[26–28] Sam de Lazaro et al show that adjunctive use of p16 immunohistochemistry reduces the frequency of false-positive CIN2 diagnoses and unnecessary LLETZ as conservative management in case of CIN2 results in high regression rates.[29] This is especially important when women are of fertile age, because unnecessary treatment may result in an increased risk of perinatal complications.[30] Additional studies should be performed comparing the costs of adjunctive use of p16 immunohistochemistry, in comparison with the prevention of LLETZ.

5 | CONCLUSION

This study shows that adjunctive use of p16 immunohistochemistry with H&E staining in diagnosing CIN lesions reduces the number of CIN1 and CIN2 lesions with a proportional increase in diagnosing no CIN and CIN3, leading to improved differentiation between lesions in need of treatment, and lesions in which watchful waiting is a proper strategy. This may decrease unnecessary follow up and possibly the risk of overtreatment. For the pathologist, the level of confidence in diagnosing CIN lesions increased when p16 was additionally available next to standard H&E, especially during biopsy assessment. We therefore support the adjunctive use of p16 immunohistochemistry with H&E staining in diagnosing CIN lesions.

AUTHOR CONTRIBUTIONS

RE, JV, RB, LM, WM, RB, AS, and JB designed the study. RE, JV, MH, RB, CM, DH, FK, WM, RB, and AS collected the data and RE, LR, GS, JH, AS, and JB performed the data analysis. RE, RB, AS, and JB wrote the first draft of the paper and RE, LR, GS, JH, JV, MH, RB, LM, CM, DH, FK, WM, RB, AS, and JB were responsible for writing and approving the final paper.

FUNDING INFORMATION

Kits for p16 staining were kindly donated by Roche.

CONFLICT OF INTEREST

DAMH has been on the speakers' bureau of Qiagen, serves occasionally on the scientific advisory boards of Pfizer and Bristol-Myers Squibb, and is a minority shareholder of Self-screen B.V., a spin-off company of VUmc. Self-screen B.V. holds patents related to the work (HPV and methylation markers). CJLMM has served occasionally on the scientific advisory board (expert meeting) of Qiagen and SPMSD/Merck, and served on occasion as a consultant for Qiagen. He also served occasionally on the scientific board of GSK, and has received speakers' fee from SPMSD/Merck. CJLMM has been co-investigator on a Sanofi Pasteur MSD sponsored trial, for which his institute received research funding. CJLMM is minority shareholder and part-time CEO of Self-Screen B.V., which holds patents on hrHPV test and methylation marker tests in cervical screening. CJLMM has a very small number of shares in Qiagen. The other authors declare that they have no conflict of interest.

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REFERENCES

1. Rijkaart D, Berkhof J, Rozendaal L, et al. Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial. *Lancet Oncol*. 2012;13:78-88.
2. Cuzick J, Clavel C, Petry K, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int J Can*. 2006;119:1095-1101.
3. Landelijke monitoring Bevolkingsonderzoek Baarmoederhalskanker ErasmusMC en Palga, Bevolkingsonderzoek baarmoederhalskanker monitor 2017, eerste resultaten van het vernieuwde bevolkingsonderzoek baarmoederhalskanker. [National monitoring Cervical Cancer Population Screening ErasmusMC and Palga, Cervical Cancer Population Monitor 2017, first results of the extensive cervical cancer population screening.] In Dutch. Obtained from: RIVM.nl. Accessed 27-08-2019.
4. Veijalainen O, Kares S, Kotaniemi-Talonen L, et al. Primary HPV screening for cervical cancer: results after two screening rounds in a regional screening program in Finland. *Acta Obstet Gynecol Scand*. 2021;100:403-409.
5. Darragh T, Colgan T, Cox J, 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. *Int J Gynecol Pathol*. 2013;32:76-115.
6. Ebisch R, Siebers A, Bosgraaf R, et al. Triage of high-risk HPV positive women in cervical cancer screening. *Expert Rev Anticancer Ther*. 2016;16:1073-1085.
7. van Zummeren M, Leeman A, Kremer W, et al. Three-tiered score for Ki-67 and p16(ink4a) improves accuracy and reproducibility of grading CIN lesions. *J Clin Pathol*. 2018;71:981-988.
8. McLaughlin-Drubin M, Crum C, Munger K. Human papillomavirus E7 oncoprotein induces KDM6A and KDM6B histone demethylase expression and causes epigenetic reprogramming. *Proc Natl Acad Sci U S A*. 2011;108:2130-2135.
9. Magkana M, Mentzelopoulou P, Magkana E, et al. p16/Ki-67 dual staining is a reliable biomarker for risk statification for patients with borderline/mild cytology in cervical cancer screening. *Anticancer Res*. 2022;42:2599-2606.
10. Ebisch R, van der Horst J, Hermsen M, et al. Evaluation of p16/Ki-67 dual-stained cytology as triage test for high-risk human papillomavirus-positive women. *Mod Pathol*. 2017;30:1021-1031.
11. Stoler M, Wright T, 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;42:1001-1009.
12. Bosgraaf R, Verhoef V, Massuger L, et al. Comparative performance of novel self-sampling methods in detecting high-risk human papillomavirus in 30,130 women not attending cervical screening. *Int J Cancer*. 2015;136:646-655.
13. Sagasta A, Castillo P, Saco A, et al. p16 staining has limited value in predicting the outcome of histological low-grade squamous intraepithelial lesions of the cervix. *Mod Pathol*. 2016;29:51-59.
14. Miralpeix E, Genoves J, Maria Sole-Sedeno J, et al. Usefulness of p16(INK4a) staining for managing histological high-grade squamous intraepithelial cervical lesions. *Mod Pathol*. 2017;30:304-310.
15. Mills A, Paquette C, Castle P, et al. Risk stratification by p16 immunostaining of CIN1 biopsies. A retrospective study of patients from the quadrivalent HPV vaccine trials. *Am J Surg Pathol*. 2015;39:611-617.
16. Leeman A, Jenkins D, del Pino M, et al. Expression of p16 and HPV E4 on biopsys samples and methylation of FAM19A4 and miR124-2 on cervical cytology samples in the classification of cervical squamous intraeptihelial lesions. *Cancer Med*. 2020;9:2454-2461.
17. Vink F, Dick S, Heideman D, et al. Classification of high-grade cervical intraepithelial neoplasia by p16INK4A, LKi-67, HPV E4 and FAM19A4/miR124-2 methylation status demonstrates considerable heterogeneity with potential consequences for management. *Int J Cancer*. 2021;149:707-716.
18. Silva D, Goncalves A, Cobucci R, et al. Immunohistochemical expression of p16, Ki-67 and p53 in cervical lesions - A systematic review. *Pathol Res Pract*. 2017;213:723-729.
19. Werkgroep richtlijn CIN AEV. Richtlijn CIN, AIS en VAIN 2015 [Working group guideline CIN AEV. Guideline CIN, AIS and VAIN 2015] In Dutch. Cited 2019 04-05-2019. Available from: https://www.oncoline.nl/cin-ais-en-vain.
20. Clinton L, Miyazaki K, Ayabe A, Davis J, Tauchi-Nishi P, Shimizu D. The LAST guidelines in clinical practice: implementing recommendations for p16 use. *Am J Clin Pathol*. 2015;144:844-849.
21. Thrall M. Effect of Lower Anogenital Squamous Terminology Recommendations on the Use of p16 Immunohistochemistry and the Proportion of High-Grade Diagnoses in Cervical Biopsy Specimens. *Am J Clin Pathol*. 2016;145:524-530.
22. Horn L, Reichert A, Oster A, et al. Immunostaining for p16INK4a used as a conjunctive tool improves interobserver agreement of the histologic diagnosis of cervical intraepithelial neoplasia. *Am J Surg Pathol*. 2008;32:502-512.

23. Dijkstra M, Heideman D, de Roy S, et al. p16(INK4a) immunostaining as an alternative to histology review for reliable grading of cervical intraepithelial lesions. *J Clin Pathol*. 2010;63:972-977.
24. Bergeron C, Ronco G, Reuschenbach M, et al. The clinical impact of using p16(INK4a) immunochemistry in cervical histopathology and cytology: an update of recent developments. *Int J Cancer*. 2015;136:2741-2751.
25. Aitken C, van Agt H, Siebers A, et al. Introduction of primary screening using high-risk HPV DNA detection in the Dutch cervical cancer screening programme: a population-based cohort study. *BMC Med*. 2019;17:228.
26. Livasy C, Moore D, van Le L. The clinical significance of a negative loop electrosurgical cone biopsy for high-grade dysplasia. *Obstet Gynecol*. 2004;104:250-254.
27. Ryu A, Nam K, Chung S, et al. Absence of dysplasia in the excised cervix by a loop electrosurgical excision procedure in the treatment of cervical intraepithelial neoplasia. *J Gynecol Oncol*. 2010;21:87-92.
28. Witt B, Factor R, Jarboe E, Layfield L. Negative loop electrosurgical cone biopsy finding following a biopsy diagnosis of high-grade squamous intraepithelial lesion: frequency and clinical significance. *Arch Pathol Lab Med*. 2012;136:1259-1261.
29. de Sam LS, Newbill C, Berlin M, Morgan T. p16 Staining of Cervical Biopsies may decrease the frequency of unnecessary Loop electrosurgical excision procedures. *J Low Genit Tract Dis*. 2016;20:201-206.
30. Kyrgiou M, Mitra A, Arbyn M, et al. Fertility and early pregnancy outcomes after treatment for cervical intraepithelial neoplasia: systematic review and meta-analysis. *BMJ*. 2014;349:g6192.

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