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Grading immunohistochemical markers p16^{INK4a} and HPV E4 identifies productive and transforming lesions caused by low- and high-risk HPV within high-grade anal squamous intraepithelial lesions

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Summary

Objectives Because current guidelines recognise high-grade anal squamous intraepithelial lesions (HSILs) and low-grade SILs (LSILs), and recommend treatment of all HSILs although not all progress to cancer, this study aims to distinguish transforming and productive HSILs by grading immunohistochemical (IHC) biomarkers p16^{INK4a} (p16) and E4 in low-risk human papillomavirus (lrHPV) and high-risk (hr)HPV-associated SILs as a potential basis for more selective treatment.
Methods Immunostaining for p16 and HPV E4 was performed and graded in 183 biopsies from 108 HIV-positive men who have sex with men. The causative HPV genotype of the worst lesion was identified using the HPV SPF10-PCR-DEIA-LiPA25 version 1 system, with laser capture microdissection for multiple infections. The worst lesions were scored for p16 (0–4) to identify activity of the hrHPV E7 gene, and panHPV E4 (0–2) to mark HPV production and life cycle completion.
Results There were 37 normal biopsies, 60 LSILs and 86 HSILs, with 85% of LSILs caused by lrHPV and 93% of HSILs by hrHPV. No normal biopsy showed E4, but 43% of LSILs and 37% of HSILs were E4 positive. No differences in E4 positivity rates were found between lrHPV and hrHPV lesions. Most of the lesions caused by lrHPV (90%) showed very extensive patchy p16 staining; p16 grade in HSILs was variable, with frequency of productive HPV infection dropping with increasing p16 grade.
Conclusions Combined p16/E4 IHC identifies productive and nonproductive HSILs associated with hrHPV within the group of HSILs defined by the Lower Anogenital Squamous Terminology recommendations. This opens the possibility of investigating selective treatment of advanced transforming HSILs caused by hrHPV, and a 'wait and see' policy for productive HSILs.

What's already known about this topic?

- For preventing anal cancer in high-risk populations, all patients with high-grade squamous intraepithelial lesions (HSILs) are treated, even though this group of lesions is heterogeneous, the histology is variable and regression is frequent.

What does this study add?

- By adding human papillomavirus (HPV) E4 immunohistochemistry to p16 ^{INK4a} (p16), and grading expression of both markers, different biomarker expression patterns that reflect the heterogeneity of HSILs can be identified.
- Moreover, p16/E4 staining can separate high-risk HPV-associated HSILs into productive and more advanced transforming lesions, providing a potential basis for selective treatment.

The incidence of squamous cell carcinoma of the anal canal and anal intraepithelial neoplasia (AIN), also called squamous intraepithelial lesions (SILs), is increasing, especially in high-risk populations such as men who have sex with men (MSM), HIV-infected (HIV+) patients, and women with a history of a vulvar or cervical human papillomavirus (HPV)-related malignancy.[1–3] High-risk (hr)HPV is detected in over 80% of anal cancers,[4–8] while carcinoma associated with low-risk (lr)HPV is rare.[9] Because of the similarities in aetiology and pathology between cervical intraepithelial neoplasias (CINs) and AINs, the clinical approaches to these lesions are similar. In the case of suspected anal high-grade SILs (HSILs), patients are subjected to a high-resolution anoscopy (HRA) during which biopsies are taken of abnormal appearing regions; treatment follows after confirming diagnosis of HSILs by histopathology, which has low inter- and intraobserver agreement.[10,11]

The Lower Anogenital Squamous Terminology (LAST) recommendations recognise only HSILs and low-grade SILs (LSILs).[12] This separation is based on the assumption that LSILs represent a productive HPV infection that will regress, whereas HSILs are considered to represent a transforming HPV infection that has a high chance of progression to cancer and is in need of treatment. However, it is estimated that only 10% of anal HSILs ultimately progress to cancer[7] if left untreated, and about 47% show regression.[13,14] The LAST recommendations for pathological diagnosis make only limited use of immunohistochemical (IHC) biomarkers. The recommendations state that diagnosis of HSILs should be made using haematoxylin and eosin (HE) histopathology, supported by the use of p16^{INK4a} (p16) as a surrogate marker for hrHPV E7 transforming gene activity only to confirm HSIL diagnosis in case of uncertainty or disagreement about LSIL vs. HSIL to show presence of diffuse p16 positivity.[12]

A biomarker specific for productive HPV infection, such as HPV E4, in combination with patterns of diffuse p16 expression as a marker of the transforming activity of the hrHPV E7 gene, might help to classify more objectively AINs, both LSILs and HSILs, and provide a basis for more selective treatment, avoiding unnecessary intervention for self-limiting lesions. Currently, the LAST recommendations recommend that all HSILs be treated. There are several treatment modalities for anal HSILs, including infrared coagulation, electrocautery, surgical excision and topical application of trichloroacetic acid or imiquimod. Currently, electrocautery is the treatment of choice for intra-anal HSILs in many centres.[15] However, there is no international consensus guideline, recurrence rates are high for all modalities, and all can cause side-effects such as pain and anal blood loss.[16] Selective treatment of only those HSILs with a higher chance of progression to cancer could prevent overtreatment and negative side effects.

E4 is a marker of completion of the HPV life cycle seen in productive HPV infection associated with low-grade CIN or AIN.[17,18] Expression of E4 in HPV-infected differentiated squamous cells results in disruption of the cell's keratin filamentous network, inhibits formation of the cornified envelope and plays a role in virus release and transmission.[19]

p16 is diffusely overexpressed in high-grade intraepithelial lesions and carcinomas driven by hrHPV, and is induced by HPV E7 of hrHPV types.[12,20,21] It is a protein regulating the G1 to S phase checkpoint of the cell cycle.

Previously we have shown that the high-grade AIN2 and AIN3 differ from each other in expression of abundant E4, and no HPV E4 was found in AIN3 lesions.[22] In this study we demonstrate the heterogeneity of HSILs using a combination of IHC biomarkers p16 and E4 within the category of HSILs defined by current practice using LAST recommendations.[12] We determine the causative HPV genotype of SILs and relate the HPV genotype to the p16 and HPV E4 biomarker expression pattern to show that, based on patterns of biomarker expression of p16/E4, we can identify productive HSILs associated with hrHPV and SILs associated with lrHPV. This provides a potential basis for selection of cases currently treated as HSILs that could be appropriate for a 'wait and see' policy and not require immediate treatment.

Materials and methods

Study population

For the present study, 183 biopsies from two studies were used. For the first group, biopsies from the H2M2 cohort study were selected.[23] H2M2 is a multicentre prospective cohort study of HIV+ MSM aged ≥ 18 years, conducted at several clinics in Amsterdam. Men had anal HPV testing every 6 months for 2 years, and in the course of regular care they were offered anal screening using HRA. At the initial HRA, biopsies were taken from

HSIL-suspected areas, detecting a HSIL in at least one biopsy in 50 men. All other biopsies from these 50 men were also included, resulting in a total number of 116 biopsies.

A second group was selected from the pathology files of the New York Presbyterian Hospital. This group consisted of 67 biopsies from 53 MSM of whom 47 were HIV+. Of the 67 biopsies, 60 were from HIV+ MSM. Biopsies from this second group had been previously stained with biomarkers p16, Ki-67 and E4 for description of expression patterns in different grades of AIN.[22]

Histology processing and review

The formalin-fixed paraffin-embedded (FFPE) material of all included biopsies was cut at DDL Diagnostic Laboratory, Rijswijk, the Netherlands. Subsequent slides were used for 4-μm slides for haematoxylin and eosin (HE) staining (before and after), three 4-μm slides for IHC staining (p16 and E4), one membrane slide for laser capture microdissection (LCM), and one tube (3 × 8 μm) for HPV detection.

Two specialized pathologists reviewed the HE slide, first individually and then together, to make a consensus diagnosis. Then, the p16 slide was used to confirm detection of HSILs in a set of AIN1 and all AIN2, in an approach based on the LAST recommendations: histologically normal: normal; histologically AIN1 (no suspicion of AIN2): LSIL; histologically AIN1 (suspicion of AIN2 by at least one pathologist) or AIN2, p16 negative: LSIL; histologically AIN1 (suspicion of AIN2 by at least one pathologist) or AIN2 and p16 diffusely positive: HSIL; consensus diagnosis AIN3: HSIL.

In further analyses, the consensus diagnosis and the LAST diagnosis were used for comparison.

Human papillomavirus genotyping and laser capture microdissection

HPV genotyping of whole tissue sections (WTS) was done using the analytically sensitive SPF10-PCR-DEIA-LiPA25 version 1 system, genotyping 25 lr- and hrHPV types.[24] The causative genotype of the highest graded lesion present on biopsy was attributed to the genotype found in the WTS in case of a single infection in ≥ AIN1 biopsies. From biopsies in which multiple HPV genotypes were found, the worst lesion was selected for LCM to identify the causative type of this worst lesion. Laser-captured worst lesions were analysed according to the same HPV testing algorithm (SPF10).[25]

Immunohistochemistry

FFPE sections 4-μm thick were used for IHC staining with $p16^{INK4a}$ and panHPV E4 using heat-induced epitope retrieval with citrate buffer (Dako/Agilent Technologies, Santa Clara, CA, U.S.A.) and a primary mouse monoclonal antibody anti-$p16^{INK4a}$ clone E6H4 [Ventana Medical Systems Inc. (Roche Diagnostics), Mannheim, Germany], and XR-E4-1 (Labo Biomedical Products BV, Rijswijk, the Netherlands). The panHPV E4 antibody has been found to be reactive against at least HPV genotypes 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67 and 70.[17,22] Reactivity was visualized using the EnVision Detection System (Dako/Agilent Technologies).

All slides were scored jointly by two expert pathologists for p16 grade and E4 positivity, resulting in an immunoscore for each marker.

$p16^{INK4a}$ immunohistochemistry

p16 immunostaining was classified as no staining (grade 0), patchy p16 positivity (grade 1), diffuse p16 positivity restricted to the lower one-third of the epithelium (grade 2), diffuse p16 positivity restricted to the lower two-thirds of the epithelium (grade 3), or diffuse staining above the lower two-thirds up to full-thickness staining (grade 4). In the evaluation of p16 scores in relation to LAST classification of HSILs, diffuse p16 staining (grade 2, 3 or 4) was considered positive.

Human papillomavirus E4 immunohistochemistry

PanHPV E4 immunoreactivity in the worst lesion was scored as negative (score 0); focal: restricted to groups of a few cells in the upper layers of the epithelium (score 1); and extensive: upper half of the epithelium or more (score 2).[17] Any E4 positivity (score 1 or 2) in the highest-grade lesion identified was considered E4 positive. E4 positivity at the edge of a high-grade lesion adjacent to a low-grade lesion or normal epithelium was considered negative as in previous studies.[22]

Statistical analyses

Results were analysed using IBM SPSS Statistics version 22·0 for Windows (IBM Corp., Armonk, NY, U.S.A.). Data were presented as absolute numbers and percentages. Percentages were compared using the χ^2-test, and the level of statistical significance was set at $P < 0{\cdot}05$.

Results

In 183 biopsies from 108 patients, classified by LAST recommendations applied by two expert pathologists, there were 37 normal, 60 LSILs and 86 HSILs as the worst lesions seen in the biopsies. Expert HE consensus diagnosis using the AIN classification was negative in 37 biopsies, AIN1 in 67 biopsies, AIN2 in 43 biopsies and AIN3 in 36 biopsies. Table 1 compares consensus AIN diagnoses with LAST diagnoses.

Immunohistochemical marker scoring

Results of p16 and E4 scoring of the worst areas of lesions in different grades of AIN and SILs are shown in Tables 2 and 3. The p16 score increased with lesion severity, with 83% of all negative/AIN1 lesions (86 of 104) and 93% of negative/LSILs (90 of 97) by LAST criteria showing no or patchy p16 staining. Of all ≥ AIN2 lesions, 89% (70 of 79) showed diffuse

Table 1 Consensus diagnosis based on haematoxylin and eosin staining using anal intraepithelial neoplasia (AIN) classification compared with $p16^{INK4a}$ -supported Lower Anogenital Squamous Terminology (LAST) diagnosis

	LAST diagnosis		
Consensus diagnosis	Normal (n = 37)	LSIL (n = 60)	HSIL (n = 86)
Normal (n = 37)	37 (100)	0	0
AIN1 (n = 67)	0	56 (84)	11 (16)
AIN2 (n = 43)	0	4 (9)	39 (91)
AIN3 (n = 36)	0	0	36 (100)

Values are presented as n (%). LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade SIL.

p16 staining above the lower one-third of the epithelium, with 58% of AIN2 showing diffuse staining above the lower two-thirds of the epithelium and 78% of AIN3 showing this pattern. Using the LAST diagnosis of HSIL, only 1% (one of 86) showed patchy staining and 67% showed staining of more than two-thirds of the epithelium.

E4 was negative in 100% of the normal biopsies, while 49% of the AIN1 lesions (33 of 67) scored positive for E4. In AIN2, 56% of the lesions (24 of 43) were E4 positive and only one of the 36 AIN3 lesions was E4 positive (3%) ($P < 0{\cdot}001$). When using the LAST diagnosis, 26 of 60 LSILs (43%) and 32 of 86 HSILs (37%) were E4 positive ($P = 0{\cdot}46$).

Human papillomavirus genotyping of the worst lesion

Single human papillomavirus infections

WTS (n = 183) were tested for HPV positivity and genotyped, resulting in nine HPV-negative biopsies, 120 with a single HPV genotype, of which 11 could not be genotyped (type X), and 54 with multiple genotypes present. The most frequently found HPV genotype as a single infection was HPV6 (22 biopsies from 18 patients), followed by HPV16 (17 biopsies from 16 patients).

The causative genotype of the worst lesion present on biopsy was attributed to the genotype found in the WTS in case of a single infection in ≥ LSIL biopsies. Biopsies in which no abnormal epithelium was found were excluded when identifying the causative type (19 of 120 single infections).

Multiple human papillomavirus infections

The causative type of the worst lesion in the 45 of 54 biopsies with multiple infections was identified using LCM: nine biopsies contained normal anal epithelium only and were not further analysed. The worst diagnosis of the remaining 45 biopsies was LSIL in 10 and HSIL in 35.

In total, a causative genotype was identified for 139 of 146 worst lesions and seven worst lesions were HPV positive but could not be genotyped (type X). Table S1 (see Supporting Information) shows the causative genotype in LSILs and HSILs according to LAST diagnosis. Most LSILs were caused by lrHPV (51 of 60, 85%) and most HSILs were caused by hrHPV (80 of 86, 93%, $P < 0{\cdot}001$), making an important distinction between disease caused by lrHPV and by hrHPV.

Immunohistochemical staining patterns in lesions caused by low- and high-risk human papillomavirus infections

The relationship between expression patterns of p16 and E4, and causative HPV infection (lr- or hrHPV) was explored in relation to the LAST classification. Of the 57 lesions caused by lrHPV, most showed an extensive patchy p16 staining pattern (51 of 57, 90%) as shown in Figure 1, but six of 57 (11%) showed diffuse p16 staining and were called HSILs. Lesions caused by hrHPV showed a diffuse p16 staining pattern in 96% of cases (85 of 89) (example in Fig. 2), and the remaining four lesions showed a less extensive patchy staining pattern that was restricted to the lower one-third of the epithelium. There was no significant difference in E4 positivity between lesions caused by lr- or hrHPV (E4 positivity: 22 of 57, 38·6% of lesions and 36 of 89, 40·4% of lesions, respectively, $P = 0{\cdot}82$).

Table 2 $p16^{INK4a}$ scores in biopsies with different grades of anal intraepithelial neoplasia (AIN) by (a) consensus diagnosis and (b) Lower Anogenital Squamous Terminology (LAST) diagnosis

	p16 score				
(a) Consensus diagnosis	Negative (grade 0)	Patchy (grade 1)	≤ Lower 1/3 (grade 2)	≤ Lower 2/3 (grade 3)	> Lower 2/3 (grade 4)
Normal (n = 37)	20 (54)	16 (43)	1 (3)	0	0
AIN1 (n = 67)	1 (2)	49 (73)	4 (6)	7 (10)	6 (9)
AIN2 (n = 43)	0	4 (9)	3 (7)	11 (26)	25 (58)
AIN3 (n = 36)	0	1 (3)	1 (3)	6 (16)	28 (78)

	p16 score				
(b) LAST diagnosis	Negative (grade 0)	Patchy (grade 1)	≤ Lower 1/3 (grade 2)	≤ Lower 2/3 (grade 3)	> Lower 2/3 (grade 4)
Normal (n = 37)	20 (54)	16 (43)	1 (3)	0	0
LSIL (n = 60)	1 (2)	53 (88)	3 (5)	2 (3)	1 (2)
HSIL (n = 86)	0	1 (1)	5 (6)	22 (26)	58 (67)

Values are presented as n (%). LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade SIL.

Table 3 E4 scores (negative vs. focal or extensive positivity) in biopsies with different grades of anal intraepithelial neoplasia (AIN) by (a) consensus diagnosis and (b) Lower Anogenital Squamous Terminology (LAST) diagnosis

	E4 score		
(a) Consensus diagnosis	Negative (grade 0)	Focal positivity (grade 1)	Extensive positivity (grade 2)
Normal (n = 37)	37 (100)	0	0
AIN1 (n = 67)	34 (51)	7 (10)	26 (39)
AIN2 (n = 43)	19 (44)	8 (19)	16 (37)
AIN3 (n = 36)	35 (97)	0	1 (3)
	E4 score		
(b) LAST diagnosis	Negative (grade 0)	Focal positivity (grade 1)	Extensive positivity (grade 2)
Normal (n = 37)	37 (100)	0	0
LSIL (n = 60)	34 (57)	5 (8)	21 (35)
HSIL (n = 86)	54 (63)	10 (12)	22 (25)

Values are presented as n (%). LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade SIL.

Table 4 shows the relation between p16 grade and E4 positivity in HSILs. There was one HSIL/AIN3 that had a patchy p16 staining pattern, E4 negativity, and that was associated with lrHPV. In the group of 85 HSILs that showed diffuse p16 positivity (≥ grade 2), there was a gradual decrease in E4 positivity from 60% (three of five) in HSILs showing p16 in the lower one-third of the epithelium, to 41% (nine of 22) of HSILs with p16 in the lower two-thirds and 35% (20 of 58) in HSILs with p16 in more than two-thirds of the epithelium.

Discussion

This study showed that anal HSILs are heterogeneous, with p16 and E4 making complementary, specific contributions to defining the nature of an anal SIL. This enabled separation of HSILs into those expressing both HPV E4 and p16, and those expressing only p16. Increasing p16 expression was associated with decreasing E4 expression. This variation was partly reflected in the HE grading of AIN2 and AIN3.

p16 is a surrogate marker for the activity of hrHPV E7,[26] which has transforming activity, but is also necessary for production of viral particles. Its grade increases with lesion severity both between LSILs and HSILs and with AIN grading. Its expression pattern can largely separate lrHPV infections from hrHPV infections. Importantly, there were two distinct extensive patterns of p16 staining, one being extensive patchy staining caused by lrHPV infection and the other being diffuse p16 staining caused by hrHPV infection. Recognizing the difference is important for accurate classification of lesions. Several studies have found similar differences in expression of p16 between lr- and hrHPV infection in cervical biopsies.[27,28] Some patchy p16 expression is also seen in certain physiological states such as metaplasia in the cervix,[29] but this is not as extensive as seen here.

E4 indicates the continued presence of completion of the life cycle of HPV,[19] which is found in both LSILs and HSILs. We showed that hrHPV-positive HSILs with evidence of transformation as defined by diffuse p16 positivity are not homogeneous: almost half show evidence of continuing completion of the HPV life cycle, and productive infection as indicated by E4 expression.

We demonstrated that as p16 expression increased there was a decrease of E4 positivity with increasing p16 grade. Scoring of p16 and E4 IHC markers has previously been shown to be reproducible and separates productive from more advanced transforming infections.[18,30] This provides a more reliable potential approach to selecting patients for treatment than does AIN/SIL diagnosis.

In this set of biopsies (176 of 183 from HIV+ MSM, 96·2%), few LSILs (nine of 60, 15%) were associated with hrHPV, and only six of 86 HSILs (7%) were associated with lrHPV. In the case of HIV+ MSM, the clinical implications of HSILs associated with lrHPV are uncertain.[31] Such HSILs/AIN2 lesions showing extensive patchy p16 staining in the absence of E4 might represent an abortive, nonproductive lrHPV infection overexpressing HPV E7 and not completing the HPV life cycle. The mechanism for strong patchy expression of p16 in lrHPV infections is unclear.[32]

This study supports the suggestion that anal HSILs represent a highly heterogeneous group, consisting of productive lesions that are potentially self-limiting as well as transforming lesions with a risk of progression to cancer.[14] It also supports the use of p16 as a surrogate for hrHPV identification.

Scoring based on immunomarkers has a better inter- and intraobserver agreement compared with grading based on morphology,[18,33] and opens up the possibility of reproducible subclassification of the heterogeneity of HSILs. In our study, 37% of HSILs according to our LAST diagnosis showed E4 positivity as evidence of productive infections. Previous research in CIN showed that a dual biomarker approach using E4 and p16 can distinguish HPV-associated CIN1 from other pathologies and may be used to divide the CIN2 group according to the extent of life-cycle deregulation.[17] The percentage of E4-positive HSILs (37%) is in line with the percentage of E4-positive CIN2 lesions found by van Baars *et al.* (43·5%).[18]

Based on biomarker expression used in this study, E4-positive hrHPV-associated HSILs expressing p16 and lrHPV-associated SILs with extensive patchy p16 warrant investigation of a 'wait and see' management policy rather than immediate

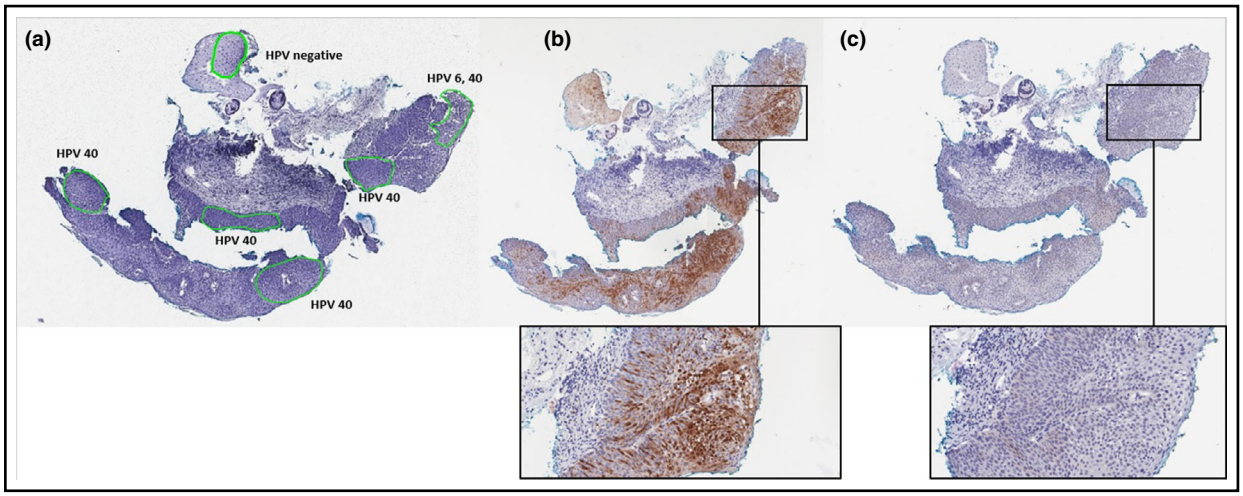

Fig 1. Consensus diagnosis AIN2/HSIL (anal intraepithelial neoplasia 2/high-grade squamous intraepithelial lesion) with human papillomavirus (HPV) genotypes 6, 31 and 40 detected in the whole tissue sections. Six regions were selected for laser capture microdissection and polymerase chain reaction, and HPV40 was identified as the causative genotype of the worst lesion present (a). The $p16^{INK4a}$ immunohistochemical stain shows an extensive patchy staining pattern (b), and there is no HPV E4 expression (c). Higher magnification × 20.

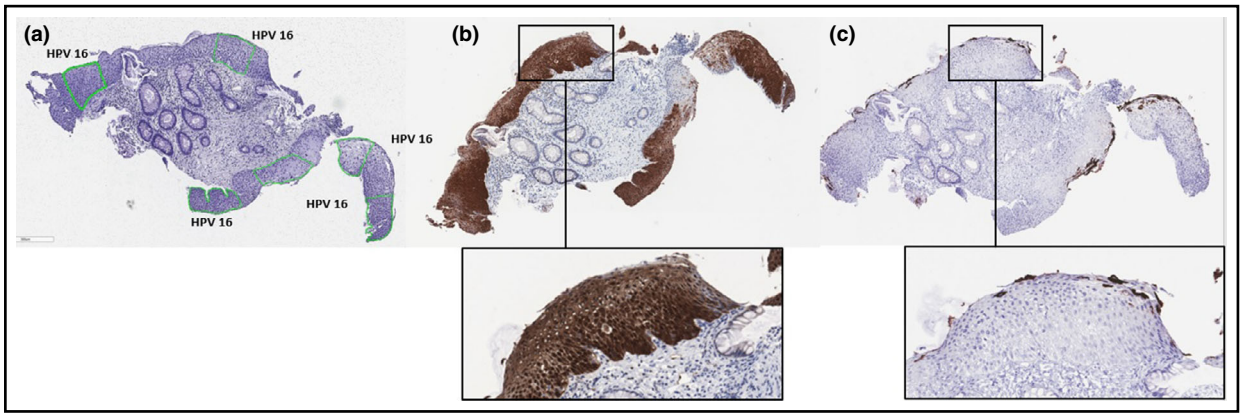

Fig 2. Consensus diagnosis AIN2/HSIL (anal intraepithelial neoplasia 2/high-grade squamous intraepithelial lesion) with human papillomavirus (HPV) genotypes 16 and 18 detected in the whole tissue sections. Six regions were selected for laser capture microdissection and polymerase chain reaction, and HPV16 was identified as the causative genotype of the worst lesion present (a). The $p16^{INK4a}$ immunohistochemical stain shows full-thickness diffuse staining (b) and there is extensive superficial HPV E4 expression (c). Higher magnification × 20.

Table 4 The relation between $p16^{INK4a}$ (p16) grade and E4 positivity in high-grade squamous intraepithelial lesions

	E4		
p16 grade	Negative	Positive	Total
1 = patchy	1 (100)	0	1
2 = ≤ lower 1/3	2 (40)	3 (60)	5
3 = ≤ lower 2/3	13 (59)	9 (41)	22
4 = > lower 2/3	38 (66)	20 (34)	58
Total	54 (63	32 (37)	86

Values are presented as n (%).

treatment. Studies of serial biopsies and well documented clinical follow-up studies are necessary to establish the optimal use of IHC markers in routine practice and to optimize patient selection for treatment.

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Appendix

Conflicts of interest

C.J.L.M.M. is a minority shareholder of Self-screen B.V., a spin-off company of VUmc, of which he has been a part-time director since September 2017. He has received speaker's fees from Qiagen and Sanofi Pasteur MSD/Merck, served occasionally on the scientific advisory board (expert meeting) of Qiagen and Sanofi Pasteur MSD/Merck and GSK, and has been an occasional consultant for Qiagen; he has a very small number of shares in Qiagen, and was minority shareholder of Diassay B.V. until April 2016. He has been coinvestigator on a Sanofi

Pasteur MSD-sponsored trial, of which his institute received research funding. W.G.V.Q. is a shareholder of Labo Bio-medical Products. The institution of M.F.S.v.d.L. received study funding from Sanofi Pasteur MSD and Janssen Infectious Diseases & Vaccines; he was a coinvestigator in a Merck-funded investigator-initiated study; he was an investigator on a Sanofi Pasteur MSD sponsored trial; he served on a vaccine advisory board of GSK; and his institution received in-kind contribution for a human papillomavirus study from Stichting Pathologie, Onderzoek en Ontwikkeling (SPOO).

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1 Causative human papillomavirus genotype of the worst lesion, composed of single infections found on whole tissue sections and laser capture microdissection results of biopsies with multiple infection.