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HPV can establish productive infection in dysplastic oral mucosa, but HPV status is poorly predicted by histological features and p16 expression.

Running title: HPV16/18 in severely dysplastic oral epithelium

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Abstract

Aims: Previous studies have reported high-risk Human Papillomavirus (HR-HPV) in a subset of dysplastic oral epithelial lesions. Many cases exhibit a histological spectrum of atypia similar to that seen in non-HPV severe epithelial dysplasia, but some studies have suggested that HPV status can be inferred based on histological features. We aimed to assess utility of such histological features and p16 as surrogate markers of HPV infection in a retrospective cohort of 33 cases of severe epithelial dysplasia, with matched clinicopathological data and histological features.

Methods and results: Tissue sections were assessed for the expression of p16, MCM2, HPV-E4 and HPV-L1 by immunohistochemistry (IHC). HPV 16/18 E6/E7 expression was assessed by RNA-ISH (RNAScope). In the cohort, 18.2% of cases (6/33) were HR-HPV positive, with no age/gender differences between the HPV+ and HPV- groups. HPV-E4 and HPV-L1 were expressed in surface keratinocytes in 4/6 (66%) HPV positive cases, indicative of productive HPV infection. Lack of p16 expression was predictive of HPV-negative status, but sensitivity and specificity varied dependent on the cut-off. Histologically, the presence of karyorrhectic cells and abnormal mitotic figures was higher in HPV+ lesions ($p<0.05$), but the predictive specificity and sensitivity was suboptimal (sensitivity 0.75; specificity 0.52).

Conclusions: This study demonstrates for the first time that a minority of severely dysplastic oral lesions harbour productive, biologically relevant HPV infection. Consideration should be given to the specific assessment of HPV status in severe epithelial dysplasia cases as both p16 status and the presence of karyorrhectic cells are poor predictive markers of HPV status.

249 words

Keywords: oral mucosa, epithelial dysplasia, HPV, koilocytic dysplasia, HPV-associated oral epithelial dysplasia, p16, In-situ hybridisation

Introduction

Our understanding of the role of Human Papilloma Virus (HPV) in diseases of the head and neck continues to evolve. In the oropharynx, the role of HPV16 and HPV18 in the development of malignant disease is established [1], and recognised in the 2017 WHO Classification of Tumours of the Head and Neck [2]. The role which HPV plays in the development of dysplastic and malignant lesions of the oral cavity is less clear, but evidence suggests a role for HPV [3].

The reported prevalence of HPV in Oral Squamous Cell Carcinoma (OSCC) varies from 4 to 95% [4–9]. A meta-analysis indicated a mean HPV prevalence of 30.1%, but there was marked variability in the cohorts and HPV testing regimen [10,11]. Recent series, with robust methods of detection, indicate that the true incidence of HPV in OSCC is likely rather low, perhaps of the order of 5%[5]. A number of studies have also reported high risk HPV (HR-HPV) subtypes in a subset of dysplastic oral lesions[12–14], some of which have identified histological features associated with HR-HPV, including koilocyte-like cells and apoptotic bodies. In most cases, marked cytological atypia extend through most of the thickness of the epithelium[13]. Nevertheless, there is significant overlap with the histological features of conventional severe dysplasia.

The natural history of dysplastic oral lesions with HPV infection is unclear. Malignant transformation of up to 15% has been reported[14], whilst others have found little such evidence[15]. Nevertheless, it is still an open question as to whether these lesions behave similar to non-HPV infected high-grade dysplasia, or whether they present increased, or even reduced risk, of malignant transformation. In most cases, including the authors' current practice, advice is to treat the lesions similar to their non-HPV infected counterpart, but evidence for this approach is lacking [16].

Nevertheless, there are significant gaps in our knowledge of the natural history of HPV in the oral cavity. A number of tools have been used to study this in the cervix and anal lesions, including the pattern of the expression of non-oncoprotein products of the HPV genome, such as HPV-E4 [17,18]. The pattern of HPV gene expression is closely linked to differentiation and expression of the late HPV proteins (HPV-L1 and HPV-L2) which are associated with productive infection in terminally differentiating epithelial cells. In the cervix, HPV E4 is expressed transiently in CIN1 and a proportion of CIN2 cases, in a pattern that is co-ordinated with the expression of MCM[19]. HPV E4 encodes a protein which aids in HPV genome amplification and virus synthesis and is a marker of productive virus infection [20].

The aim of this study was to identify the prevalence of HR-HPV infection in a cohort of high-grade dysplastic lesions of the oral mucosa and to assess the usefulness of the suggested histological parameters and expression of p16 in the prediction of HPV status. Additionally, we wished to investigate the presence of HPV-E4 and HPV-L1 in HPV-positive lesions, in an attempt to identify productive HPV infection.

Materials and methods

A cohort of 49 consecutive severe epithelial dysplasia cases were identified from the Oral Pathology Diagnostic Archive, Sheffield from 2014-16. Cases from the oropharynx, vermillion of lip and patients who

had previous HNSCC or immunocompromise were excluded. After exclusions, 33 cases were independently reviewed by 3 pathologists (KDH, PMS and SAK), according to the standard WHO 3-grade dysplasia scheme. Demographic data were collected including age, gender, site, clinical description and tobacco/alcohol habits (where recorded). HR-HPV DNA in situ hybridisation (ISH) slides for seven cases were obtained from the diagnostic archive as these had been generated as part of the original diagnostic work-up.

RNAscope Assay

HR-HPV (16, 18) E6/E7 RNA-ISH was performed using the RNAscope assay (Advanced Cell Diagnostics, CA, USA), according to the manufacturer's instructions. The following scoring system was followed for HPV RNA-ISH slides assessment:

- 0: No staining or <1 dot every 10 cells; 40X magnification.
- 1: 1-3 dots/cell: 20-40X magnification.
- 2: 4-10 dots/cell. Very few dot clusters at 20-40X magnification.
- 3: >10 dots/cells. Less than 10% have dot clusters at 20X magnification
- 4: >10 dots/cell. More than 10% positive cells have dot clusters at 20X magnification

Immunohistochemistry

Immunohistochemistry for p16, MCM2, HPV-E4 and HPV-L1 was conducted on sequential 4µm FFPE sections. An HPV-positive oropharyngeal carcinoma and tissue-engineered HPV-positive cervical epithelium were used as positive controls. Antigen retrieval was conducted using heat induced epitope retrieval in 0.01M citrate buffer. After secondary antibody application, staining was visualised using the Vectastain ABC Kit (Burlingame, USA) with DAB substrate and haematoxylin counterstain.

The primary antibodies used were: Mouse monoclonal anti-human p16 (SC-56330, Santa Cruz Biotechnology, 1:200); Rabbit monoclonal anti-human MCM2 (Abcam ab108935, 1:400); HPV E4 (F1.1) from Prof John Doorbar (1:100 dilution); Mouse monoclonal anti HPV-L1 (CAMVIR, Abcam ab69, 1:500).

Expression of p16 staining was assessed by H-score, giving a score out of 300 [21]. As the intensity of expression of MCM2 did not vary much, only extent of expression was assessed: Negative: 0-5% of cells, 1: 5-25% of cells, 2: 26-50% of cells, 3: 51-75% of cells, 4: >75% of cells. Expression of HPV E4 and HPV-L1 were present or absent.

Assessment of Histopathological features

H&E stained slides were evaluated for features in conventional oral epithelial dysplasia and HPV associated OD features, using the descriptors and parameters as described in the literature [12,13]. The following parameters were assessed:

1. The pattern of surface keratin.
2. The presence of eosinophilic bodies with associated karyorrhexis or pyknosis.
3. Koilocyte-like cells.
4. Keratinocyte multinucleation.
5. Presence of suprabasal mitoses.
6. Presence of abnormal mitoses.
7. Dyskeratosis.
8. Basal cell hyperplasia.
9. Extent of cellular and nuclear pleomorphism.
10. Nuclear hyperchromatism.

Each feature was graded subjectively from 0 to 3, 0 = absent; 1 = present to a limited extent; 2 = present through most of the epithelium; 3 = prominent throughout all the epithelium. The immunohistochemistry, ISH and histological features were assessed independently by three pathologists (KDH, SAK and NH) blinded to the HPV status and after calibration on a number of cases which were not included in the cohort. Consensus scores were used in these analyses.

Ethics

The study was approved after ethical review: 08/S0709/70 (West Glasgow Research Ethics Committee).
All the cases were pseudoanonymised before analysis.

Statistical analysis

Data are expressed as mean ± standard error. Comparisons of age, p16 and MCM2 expression used Student's t-test; Histological and gender comparisons used the Fisher exact test. p16 IHC scores were analysed using the sensitivity and specificity test, at three thresholds, (1) p16 H-score > 0, (2) p16 H-score >50, and (3) p16 H-score >210. $p<0.05$ was considered statistically significant. The small size of the HPV+ cohort precludes the use of multivariate analyses.

Results

A minority of severe dysplasia cases contain transcriptionally active HR-HPV.

RNA-ISH for HPV16/18 E6 and E7 was used as the reference test for HR-HPV. Examples of the staining are shown in Figure 1. Six of the 33 cases (18.2%) contained HR-HPV and were judged to be HPV positive (Table 1 and Table 1), all with RNA ISH score of ≥2. A further four cases (13, 19, 27 and 30) had a RNA ISH score of 1, in which expression was equivocal and other factors, such as p16 staining and DNA ISH were also taken into account in overall assessment of the case. After consensus review, all were judged to be HPV negative. Of the seven cases where DNA ISH had been undertaken as part of the original diagnostic process, the DNA ISH was in agreement with the RNA ISH (100%; Table 1).

No age or gender differences were noted between the two cohorts (Table 1). The mean age of the HPV+ cohort was 58.0±3.8 (range: 36-83 years) and HPV- was 64.5±2.64 (range: 52-70 years) (p=0.22). The M:F ratio for HPV+ was 5:3 and HPV- was 14:11 (p=0.54). A range of oral subsites were represented, and none was favoured.

Expression of HPV-E4 and HPV-L1 may indicate productive HPV infection in HPV associated dysplasia

Assessment of HPV-E4, HPV-L1 and MCM2 has been useful in understanding the lifecycle of HPV in cervical epithelium[19,20]. Four of six (66%) HPV+ cases expressed HPV-E4 in scattered superficial cells, to a variable extent (Figure 2A and 2B). Expression of HPV-L1 was detected in superficial nuclei in all HPV-E4-positive cases (Figure 2C and 2D). In dysplastic oral epithelium, the expression of MCM2 varied in its extent in both HPV+ and HPV- cases (Figure 2E and 2F). Overall, no significant difference in MCM2 expression was identified (HPV+ 2.6±1.1 vs HPV- 2.0±0.8, p=0.45). On review of the H&E stained sections, the E4 and L1 expressing cells were not obviously koilocyte-like and there was no clear relationship with changes in MCM2 expression.

p16 is an insufficiently robust biomarker for prediction of HPV status in severe OD

Assessment of p16 expression by immunohistochemistry and H-score gave a range from 0-300 (Table 1). Examples of the staining seen are shown in figure 3. All HPV+ cases expressed p16 to a variable extent, as did five HPV negative cases, (18%), in which the pattern of staining was similar to that shown in Figure 3B. Overall, the mean p16 H-score was higher in HPV+ cases compared with HPV- cases (150.0 ±46.6 vs 6.25±2.4; $p<0.05$).

Assessment of the predictive value of p16 expression in the assessment of HPV status at various thresholds showed that the test performance varied dependent on the threshold employed (Table 2). Application of the usual oropharynx threshold (intense staining in >70% of cells, H-score >210) showed poor performance as a diagnostic test (Sensitivity 0.38, specificity 1; Table 2). Other less stringent

thresholds for p16 positivity also yielded suboptimal performance, but using a lower cut off (H-score >50) resulted in better test performance (Table 2).

Apoptotic bodies are associated with HPV status but are insufficiently robust for prediction of HPV status.

Of the histological features which have been suggested in previous HPV associated OD reports, only the presence of apoptotic/karyorrhectic bodies and abnormal mitoses were associated with HPV status (Table 3: p=0.05 and p=0.04 respectively). The photomicrographs in Figure 4 demonstrate these histological features, in particular the apoptotic bodies and abnormal mitotic figures, which can be difficult to distinguish. The presence of these karyorrhectic bodies as a predictive test for HPV status is seen in table 4. This feature has a good NPV, but an insufficient PPV to warrant its use as a predictive feature.

Discussion

This study further supports the concept that HR-HPV is identifiable and transcriptionally active in a proportion of oral high-grade epithelial dysplasia. We aimed to demonstrate that the HPV identified was transcriptionally active and to assess whether HPV-E4 and HPV-L1, which is seen in productive HPV-associated lesions of the cervix, were also present in dysplastic oral lesions[20]. We restricted the cohort to severe epithelial dysplasia as the literature indicates that the histological changes seen in these HPV-associated lesions affect the majority of the thickness of the epithelium. Thus, the main histological differential diagnosis and area of interpretive difficulty is distinguishing this from non-HPV severe epithelial dysplasia. There is also a lack of clarity and agreement over nomenclature for these lesions, and this has implications for how pathologists communicate the relative risk of malignant transformation in HPV-associated and non HPV associated dysplastic lesions of the oral cavity.

In our cohort of severely dysplastic lesions, we identified six cases (6/33; 18.2%) which expressed HPV16/18 E6/E7 by RNA-ISH. This figure is in keeping with the only directly comparable study with a similar design (consecutive high grade dysplasia and assessment of HPV status using ISH) which demonstrated HPV prevalence of 17.5%[12]. Overall, the proportion of HPV positive cases reported in series and meta-analyses of high-grade dysplasia is variable and is dependent on the study design and the detection method used[8,16,23,24].

Others have reported higher proportions of severe OD cases harbouring HR-HPV, but used more sensitive techniques, such as nested PCR[9]. Key technical parameters, such as PCR cycle numbers, are often missing from the methods, hindering interpretation. Blioumi et al demonstrated HR-HPV in most lesions

(>90% of cases), but in some, this amounted to 1 HPV genome copy per 100 cells [25]. The significance of this level of HPV carriage is unclear. In our data, expression of HPV-E6 and E7 mRNA provides confirmation of transcriptionally-active HPV, avoiding the pitfalls implicit in PCR-based detection. We did encounter some difficulties in RNA-ISH based assessment of HPV-E6/E7 expression, in particular the equivocal nature of low level staining (score 1). Similar issues have been reported by other authors[26].

A significant issue in the interpretation of these data is the lack of knowledge of the life cycle of HPV in oral epithelial cells. Much of the data on which we base our assumptions has been generated in cervical epithelial cells and it is not clear how readily applicable these are to oral mucosa. In CIN1 and some CIN2 cases, productive HPV infection is seen, with expression of HPV-E4 and HPV-L1 and HPV-L2. Higher grade (CIN2 and CIN3 lesions) progressively lose expression of late proteins and HPV-E4, indicative of an abortive infection [27]. We have demonstrated that HPV-E4 and HPV-L1 are expressed in the superficial keratinocytes of 4/6 HR-HPV containing severe dysplasia cases. Whilst this may indicate productive infection in the oral mucosa (perhaps somewhat analogous to the restricted expression of HPV late proteins seen in CIN2), the close relationship with MCM2 seen in CIN1 and CIN2 in the cervix was not seen. Indeed, this expression may represent residual cells of an earlier low grade lesion: thus, further investigation of the life cycle and parameters for clearance of HPV infection in normal mucosa and in a cohort of mild/moderate oral epithelial dysplasia is required[19,20], to allow more detailed comparison with cervical epithelium. However, the potential significance of the demonstration of productive HPV infection is clear: HPV infection in the context of HPV-associated oropharyngeal carcinoma corresponds to an abortive/transforming pattern of HPV gene expression, thus, it is possible that HPV-infected oral epithelial cells can act as the reservoir of productive HPV infection.

We also used HPV-E6/E7 expression to assess the performance of other surrogate markers of HPV infection in dysplastic oral mucosa, including p16 and a number of histological features. The gold standard is the demonstration of transcriptionally active HPV in fresh tissue. RNA-ISH alone performs well in comparison to the gold standard, outperforming DNA-ISH and DNA qPCR, even when combined with p16 immunohistochemistry [26].

Expression of p16 in oral epithelium is more variable than in the oropharynx and thus is a less robust biomarker for the presence of HR-HPV in the oral cavity[28,29]. Applying the standard used in the assessment of oropharyngeal lesions, we demonstrated that p16 is an insufficiently robust biomarker for the prediction of HPV-status in severely dysplastic oral lesions. However, lowering the cut-off improved performance (table 2). Similar to the variability in HPV testing, the literature presents a variable assessment of the usefulness of p16 staining. A range of p16 antibody clones have been used in

oropharyngeal lesions, which results in important differences in test performance [30], but it is not clear if this is also so in the oral cavity. We used a mouse monoclonal p16 antibody, which has similar staining characteristics to the E6H4 clone routinely used in the oropharynx: nevertheless this is a limitation of the present study, as it precludes direct comparison. There are also wide variations in what constitutes p16 positivity: some accept p16 expression in 10% of cells[31]; others accept nuclear or cytoplasmic staining alone, in addition to both[32]. Some authors have demonstrated (in oral SCC) that p16 expression assessment is dependent on the level of stringency used in scoring[33]. The answer to this issue is not clear, as in most cases, there is extrapolation from the experience in the oropharynx and such assumptions may not be valid. In a similar context to our cohort, Cunningham et al demonstrated 100% concordance of p16 expression with HPV status by assessment of nuclear staining only[34]. Whilst we did not see as clear a correlation, more investigation into the pattern of expression of p16 in HPV-associated oral lesions is warranted.

In some of the initial descriptions of koilocytic dysplasia, a range of histological features were identified as associated with HPV presence[35]. Many of these features are subjective and overlap with those in severe epithelial dysplasia. The interpretation of some of these features has been a source of controversy [36,37]. In our cohort, we found that abnormal nuclear morphology (karyorrhexis and abnormal mitoses) was associated with HR-HPV detection, similar to others [13,14,16]. However, these features performed poorly as a test, indicating that the presence of particular histological features on their own is insufficiently robust. Combining features in a more sophisticated diagnostic algorithm may be much more useful.

In conclusion, we have demonstrated that a minority of a cross-sectional cohort of severely dysplastic oral mucosa harbours transcriptionally active HR-HPV with evidence of productive HPV infection. In this context, there is association with p16 expression and certain histologic features, but these are insufficiently robust to function as surrogate tests. Further investigation is required to develop a robust, perhaps multi feature algorithm to identify these cases. We recommend consideration of a HPV-specific test if these features are present.

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Figure legends

Figure 1. Expression of HPV E6/E7 mRNA in oral epithelium by RNA ISH (RNAScope). A: Case 2, score = 0 (negative). B: Case 13, score = 0/1 (negative: occasional nuclear dots highlighted). C: Case 20, Score = 2 (positive). D: Case 26, Score = 4 (positive). Magnification: All x200, except B x400.

Figure 2. A and B: Expression of HPV E4 in HPV positive OD, cases 20 and 26. Four of the six HPV positive cases expressed HPV E4 in the cytoplasm of cells in the superficial layers of the epithelium. C and D. Expression of HPV L1 in HPV positive OD, cases 20 and 26. All of the E4 expressing cases also expressed nuclear HPV L1 in cells in the superficial layers of the epithelium. E and F: Expression of MCM2 in exemplar cases of HPV negative (A: case 2) and HPV negative (B: case 26) dysplastic oral epithelium Magnification: A-D x400, E&F: x200

Figure 3. Expression of p16 by immunohistochemistry in dysplastic oral epithelium with examples of H scores. A Case 13 (HPV-; H-score = 0) B: Case 17 (HPV-; H-score = 75). C: Case 15 (HPV+; H-score = 150). D Case 26 (HPV +: H-score = 270). Magnification: all x200

Figure 4. H&E stained sections from case 20 (A) and 26 (B), showing a number of histological features assessed as predictors of HPV status, including abnormal keratinisation and a number karyorrhectic/apoptotic bodies. Both cases were p16 and RNA ISH positive. Magnification: x200

Tables

Case number	Age	Gender	Site	Clinical description	Tobacco	Alcohol	HPV status	RNA ISH score	DNA ISH (+/-)	p16: H score	MCM2 score	HPV E4 (+/-)	HPV L1 (+/-)
1	72	M	–	white patch	Yes	Yes	N	0	NT	25	1	-	NT
2	78	F	Lower lip mucosa	Mixed crusted lesion	-	–	N	0	NT	0	2	-	NT
3	36	M	Lateral tongue	–	–	–	N	0	NT	25	2	-	NT
4	50	F	Ventral Tongue/FOM	–	No	Yes	N	0	NT	0	3	-	NT
6	60	M	Buccal mucosa	Homogeneous white	–	–	N	0	NT	0	3	-	NT
7	56	M	Lower lip mucosa	Ulcer	Yes	Yes	N	0	NT	0	3	-	NT
8	87	M	Lateral tongue	Ulcer	Ex	Ex	N	0	NT	0	2	-	NT
9	78	M	Floor of mouth	Mixed leukoplakia with ulceration	Yes	–	N	0	NT	0	3	-	NT
10	65	M	Lateral tongue	White patch	Yes	–	N	0	NT	0	1	-	NT
11	67	M	Palate/fauces	White area with ulceration	–	–	N	0	NT	25	3	-	NT
12	62	F	Buccal mucosa	Exophytic white lesion	Yes	–	N	0	NT	0	3	-	NT
13	79	F	Lower lip mucosa	Indurated mucosa	–	–	N	1	NT	10	3	-	NT
14	43	F	Lateral tongue	White patch	–	–	N	0	NT	0	2	-	NT
17	52	M	Ventro-lateral tongue	White patch	Yes	Yes	N	0	NT	75	2	-	NT
18	45	M	Ventral tongue	White patch	Yes	Yes	N	0	NT	0	1	-	NT
19	61	M	Lateral tongue	Reticular white lesion	–	–	N	1	NT	25	2	-	NT
21	67	F	Lateral tongue	–	–	–	N	0	NT	0	3	-	NT
22	82	F	Lateral tongue	White lesion	–	–	N	0	NT	0	2	-	NT
24	74	M	Palatoglossal fold	White patch	–	–	N	0	negative	0	4	-	NT
25	56	F	Ventral tongue	Irregular white patch	–	–	N	0	NT	0	2	-	NT

27	61	M	Palatoglossal fold	Speckled mixed leukoplakia	–	–	N	1	NT	0	2	-	NT
28	74	F	Lateral tongue	White patch	–	–	N	0	NT	0	3	-	NT
29	76	M	Lateral tongue	Mixed red-white lesion	–	–	N	0	NT	0	3	-	NT
30	47	F	Ventral tongue	Corrugated leukoplakia	–	–	N	1	negative	0	1	-	NT
31	72	M	Buccal mucosa	Erosive patch	–	–	N	0	NT	0	3	-	NT
32	65	F	Lateral tongue	–	–	–	N	0	NT	0	3	-	NT
33	74	M	Soft palate	White patch	–	–	N	0	negative	0	2	-	NT
5	60	M	Lateral Tongue/FOM	Papillary exophytic mass	–	–	P	2	NT	50	3	-	-
15	65	M	Commissure	White patch	Yes	–	P	3	positive	50	2	+	+
16	70	F	Lateral tongue	–	No	–	P	2	NT	225	2	-	-
20	50	F	Buccal mucosa	Exophytic white lesion	Yes	–	P	3	positive	50	1	+	+
23	47	M	Floor of mouth	White patch	–	–	P	3	positive	300	3	+	+
26	46	M	Buccal mucosa	White area	No	–	P	4	positive	270	4	+	+

Table 1. The clinical details of the study cohort. HPV status is as determined by RNA ISH. See Table 1 for full details of individual tests. N = negative, P = positive.

Threshold	Description	Sensitivity	Specificity	PPV	NPV
1	Any p16 staining	0.88 (0.47-0.99)	0.84 (0.63-0.95)	0.64	0.95
2	p16 H-score >50	0.63 (0.25-0.9)	1 (0.83-1.0)	1	0.89
3	p16 H-score >210	0.38 (0.1-0.74)	1 (0.83-1.0)	1	0.83

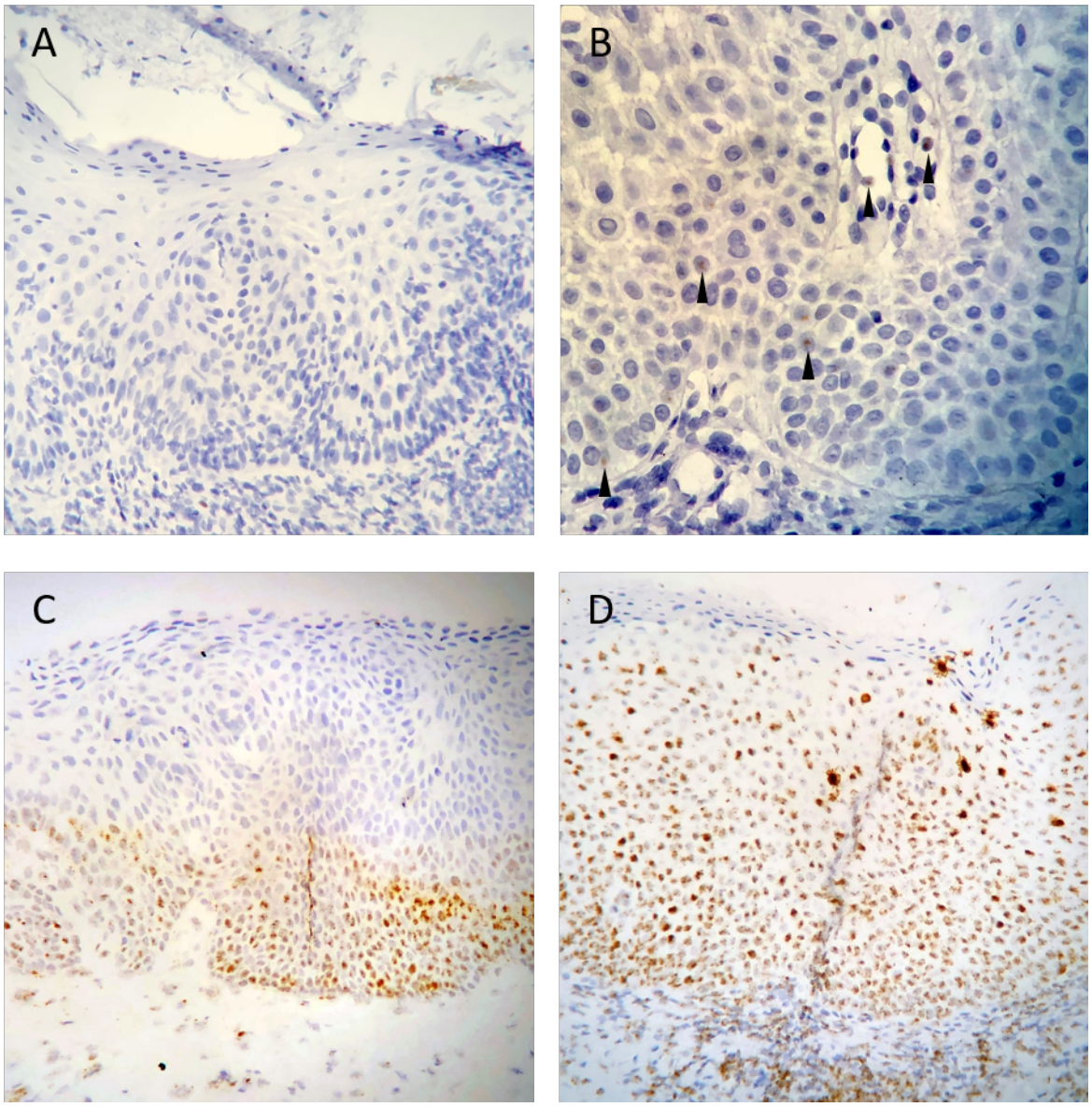
Table 2. Performance of p16 as a predictive test for HPV status, at three different threshold cut offs. Sensitivity and specificity are expressed with their relevant 95% confidence interval.

Histopathological feature	P-value
Apoptosis & karyorrhexis	0.05
Koilocyte-like cells	0.11
Keratinocyte multinucleation	0.51
Supra-basal mitosis	0.37
Abnormal mitotic figures	0.04
Dyskeratosis	0.65
Nuclear and cellular pleomorphism	0.19
Nuclear hyperchromatism	0.29
Basal cell hyperplasia	0.61

Table 3. Assessment of the overall differences in the extent of a number of scored histological features on comparison of HPV+ and HPV cohorts. Assessment was by Fishers exact test.

Threshold	Sensitivity	Specificity	PPV	NPV
Score of 2 or 3	0.75 (0.36-0.96)	0.52 (0.32-0.72)	0.33	0.87

Table 4. Performance of score 2 or 3 apoptotic/karyorrhectic cell as a predictive test for HPV status. Sensitivity and specificity are expressed with their relevant 95% confidence interval



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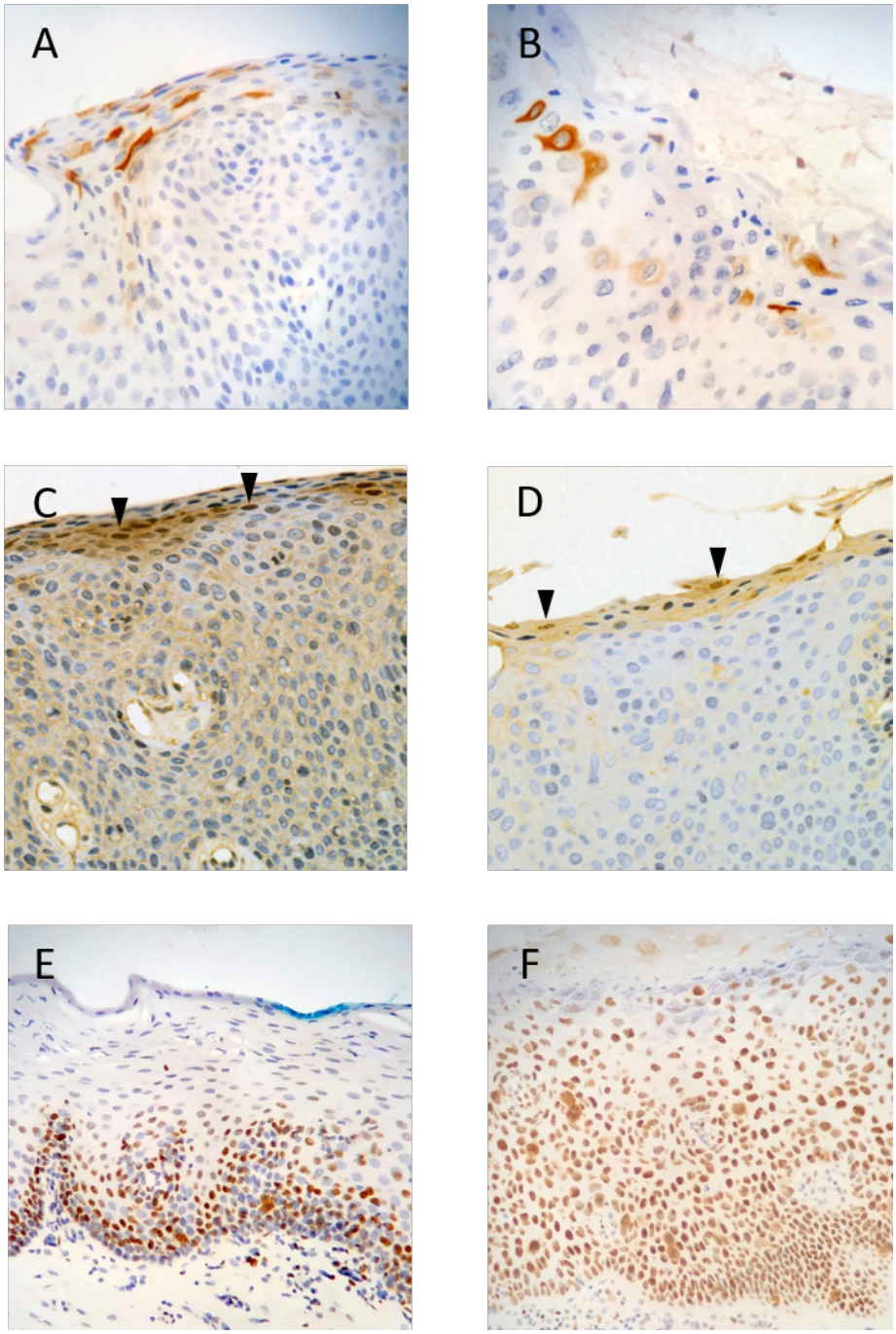

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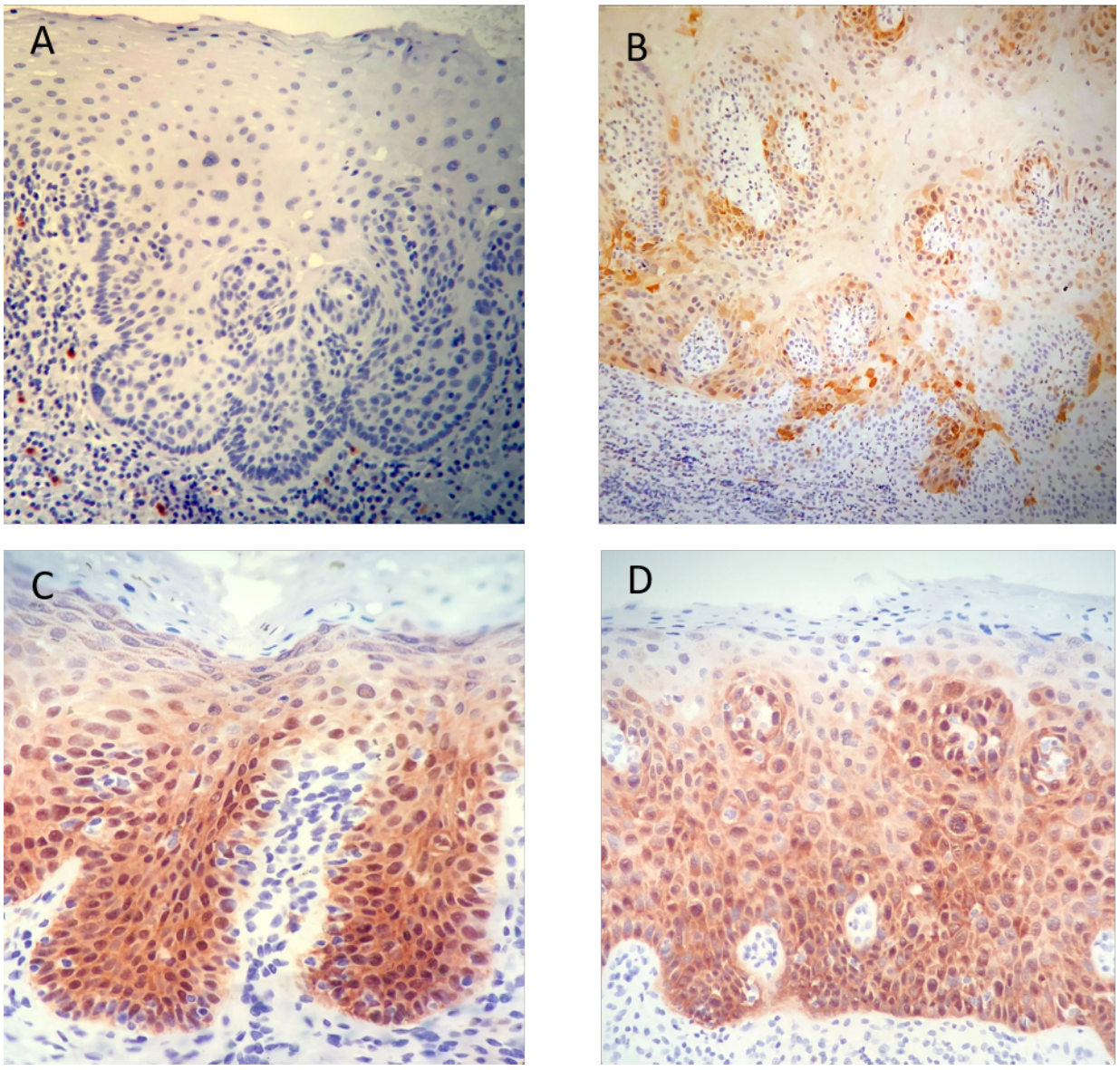

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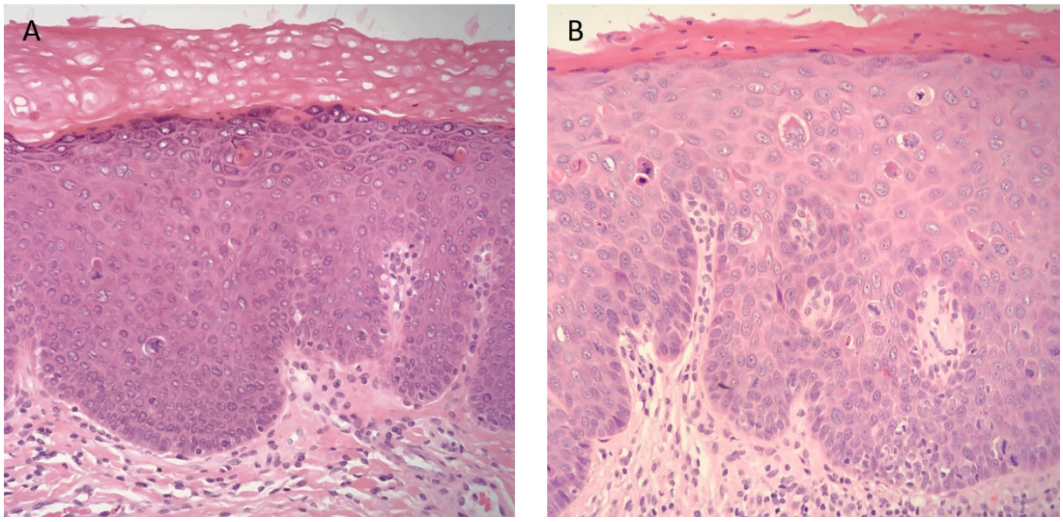

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