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HPV Genotyping and p16 Expression in Biopsy Specimens from Patients with Anal Squamous Cell Carcinoma: A Review

Sofia Kristensen[1*], Andrea Colombo[2] and Lucia Fernandez[3]

[1]Department of Surgical Oncology, Aarhus University, Aarhus, Denmark

[2]Department of Molecular Virology, University of Turin, Turin, Italy

[3]Department of Pathology, Complutense University of Madrid, Madrid, Spain

***Correspondence to: Sofia Kristensen, Department of Surgical Oncology, Aarhus University, Aarhus, Denmark**

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Abstract

Almost every anal squamous cell carcinoma is caused by human papillomavirus, and p16 immunohistochemistry is used as a cheap surrogate for detecting it. The two agree most of the time. The clinically interesting patients are the ones where they do not.

Objectives: *To review the published evidence on HPV genotyping and p16 expression in anal squamous cell carcinoma: how often each is positive, how well p16 substitutes for direct viral detection, what each predicts for outcome, and what happens to patients whose two results disagree.*

Material and Methods: *Narrative review of genotyping series, immunohistochemical cohorts, molecular characterisation studies and meta-analyses indexed in PubMed and the major oncology and pathology journals. Studies were eligible if they reported HPV or p16 prevalence in a defined anal carcinoma population, the concordance between the two, or outcomes stratified by either. No new patient data were generated; every estimate quoted is one published by the original investigators.*

Results: *Across the studies pooled in a meta-analysis of 398 cases, HPV prevalence ranged from 67.9 to 95.8 per cent and p16 positivity from 65.3 to 90.7 per cent. HPV16 dominates the genotype distribution, accounting for 78.9 per cent of cases as a single infection in one series of 95 tumours, with a further 9.5 per cent carrying HPV16 alongside a second type. Against direct viral detection in 116 tumours, p16 immunohistochemistry showed 100 per cent sensitivity and 85 per cent specificity. Tumours positive for both markers had markedly better outcomes, with a pooled hazard ratio of 0.30 (95% CI 0.17-0.51) for overall survival and 0.23 (0.14-0.36) for disease-free survival, while patients discordant in either direction did not share that advantage.*

Conclusion: *p16 is a highly sensitive surrogate and an imperfect specific one, so a positive result does not establish viral causation. The prognostic benefit belongs to the doubly positive group rather than to either marker alone, which is an argument for testing both rather than substituting one for the other.*

Abbreviations: *ASCC – Anal Squamous Cell Carcinoma, HPV – Human Papillomavirus, p16 – p16INK4a, IHC – Immunohistochemistry, PCR – Polymerase Chain Reaction, CISH – Chromogenic In Situ Hybridisation, FFPE – Formalin-Fixed Paraffin-Embedded, CRT – Chemoradiotherapy, OS – Overall Survival, DFS – Disease-Free Survival, LRR – Locoregional Recurrence, HR – Hazard Ratio, pRb – Retinoblastoma Protein.*

Keywords: *Anal Squamous Cell Carcinoma; Human Papillomavirus; p16INK4a; Genotyping; Prognostic Biomarkers*

Introduction

Anal squamous cell carcinoma is uncommon, with reported incidence between one and two and a half cases per hundred thousand in developed countries, but the rate has been rising by roughly two per cent annually and the increase is most marked in women [1]. Almost all cases are attributable to human papillomavirus, which places the disease alongside cervical and oropharyngeal carcinoma as a virally driven malignancy [1,2].

The mechanistic link explains why p16 is used as a marker. The viral E7 oncoprotein inactivates the retinoblastoma protein, and because that protein normally suppresses p16, its loss produces p16 overexpression [3]. Detecting p16 by immunohistochemistry is therefore a cheap, widely available proxy for a viral infection that would otherwise require molecular testing [2,5].

That substitution was imported from head and neck oncology, where concordance between p16 immunohistochemistry and direct viral detection is high [2,5]. Whether it holds equally well in anal carcinoma, where viral prevalence is higher still, is a separate question, and the discordant groups are where the answer matters [1,4].

This review sets out reported prevalence of each marker, the genotype distribution, the concordance between the two tests, and what each predicts for outcome after chemoradiotherapy.

Material and Methods

Search strategy: PubMed and the archives of the major oncology and pathology journals were searched for studies of viral and immunohistochemical markers in anal carcinoma. Search terms combined anal squamous cell carcinoma with human papillomavirus, HPV16, genotyping, p16INK4a, immunohistochemistry, in situ hybridisation, chemoradiotherapy and prognosis. Reference lists of retrieved meta-analyses and cohorts were screened by hand.

Eligibility: Studies were included if they reported HPV or p16 prevalence in a defined anal carcinoma population, concordance between the two methods, genotype distribution, or clinical outcomes stratified by marker status. Studies in other HPV-associated sites are cited where they establish the method or provide a comparison and are identified as such.

Data extraction and handling: Design, sample size, detection method, scoring threshold and reported prevalence or effect estimates with confidence intervals were extracted as published. Detection method is reported alongside every prevalence figure, because polymerase chain reaction, in situ hybridisation and immunohistochemistry do not yield equivalent results. No pooling or re-analysis was performed and no patient-level data were obtained. Figures 1 to 4 and Tables 1 to 3 reproduce published values only. This is a narrative synthesis and was not registered as a systematic review.

Results

Almost every anal cancer carries the virus: The largest genotyping series analysed 496 invasive anal cancers and 43 high-grade intraepithelial lesions from 24 countries using broad-spectrum amplification with genotyping. HPV DNA was detected in 88.3 per cent of invasive cancers (95% CI 85.1-91.0) and in 95.4 per cent of grade 2 or 3 intraepithelial neoplasia (95% CI 84.2-99.4), with HPV16 the most frequent type (Figure 1) [3]. HPV16 predominates in anal carcinoma as it does at other anogenital sites, where worldwide type-distribution surveys have established the same hierarchy [12,13].

Higher-sensitivity genotyping raises the figure further, approaching universal positivity, which the investigators noted carries different implications for vaccine prevention than for prognosis [4]. The distinction matters: a method sensitive enough to detect virus in nearly every tumour necessarily loses the ability to separate patients by viral status, and prognostic stratification depends on that separation [4,5].

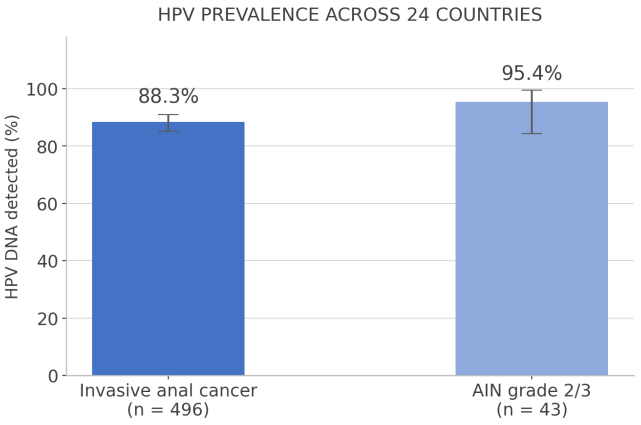

Figure 1: HPV DNA prevalence in invasive anal cancer and in high-grade intraepithelial neoplasia across 24 countries [3]

Reported positivity varies more than the biology does: Across the four studies pooled in a meta-analysis of 398 patients, HPV prevalence ranged from 67.9 to 95.8 per cent and p16 positivity from 65.3 to 90.7 per cent (Figure 2) [1]. For a disease in which the virus is present in close to nine tumours in ten under standardised testing [3], a reported range spanning nearly thirty percentage points reflects methodology rather than genuine differences between populations.

Three sources of that variation are identifiable. Detection platforms differ in analytical sensitivity, with high-sensitivity genotyping recovering virus that conventional assays miss [4]. Immunohistochemical thresholds for calling p16 positive are not standardised between studies [1,5]. And archival material of differing age and fixation yields differing amounts of amplifiable DNA [3,4].

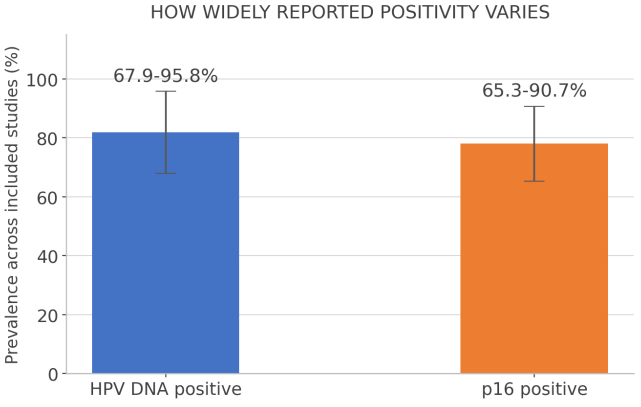

Figure 2: Range of reported HPV and p16 positivity across the studies pooled in a meta-analysis of 398 cases [1]

The prognostic benefit belongs to the concordant group: The pooled analysis addressed the question that discordance raises. Tumours positive for both HPV and p16 had markedly better outcomes, with a hazard ratio of 0.30 (95% CI 0.17-0.51) for overall survival and 0.23 (95% CI 0.14-0.36) for the composite of disease-free, disease-specific, relapse-free and progression-free survival (Figure 3). Patients who were HPV-negative but p16-positive, or HPV-positive but p16-negative, did not share that favourable prognosis [1].

That result argues against substituting one test for the other. If p16 alone carried the prognostic information, the p16-positive discordant group would behave like the doubly positive one, and it does not [1]. Reviews of biomarker stratification in this disease reach the same position, treating viral status and its immunohistochemical surrogate as related but non-equivalent [5]. An independent

genotyping cohort staged I to III reported the same direction of effect for both markers [10], and a systematic review of virus-related survival reached compatible conclusions, with HPV16 in particular associated with improved overall survival [11].

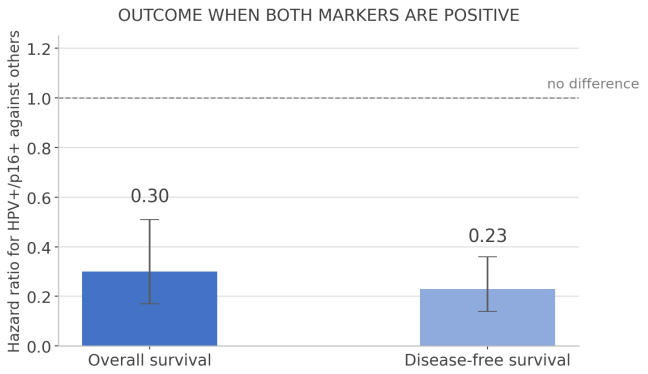

Figure 3: Pooled hazard ratios for tumours positive for both markers against all others [1]

Study	n	Focus	Principal reported finding
Grabenbauer et al. 2006 [6]	—	Immune	Cytotoxic T cell infiltrate predicts outcome
Williams et al. 2013 [7]	—	Serum	Squamous cell carcinoma antigen is prognostic
Alemany et al. 2015 [3]	539	Genotyping	HPV DNA in 88.3% of invasive cancers, 24 countries
Baricevic et al. 2015 [4]	—	Genotyping	Near universal positivity with sensitive methods
Gilbert et al. 2016 [8]	—	Immune	Lymphocyte score stratifies outcome beyond p16
Sun et al. 2018 [1]	398	Meta-analysis	HPV+/p16+ overall survival hazard ratio 0.30

Table 1: Principal studies reviewed, with focus and the finding as reported by the original investigators

Viral presence is not the same as viral drive: Detecting HPV DNA establishes that the virus is present, not that it is transcriptionally active and driving the tumour. Integration analysis found HPV16 DNA integrated in 25 of 35 anal carcinomas, roughly 71 per cent, with recurrent insertion at a single genomic locus [9], which implies that a meaningful minority of virus-positive tumours carry the genome in episomal form.

A single 107-patient cohort put numbers on the concordance directly. High-risk HPV DNA was detected in 93 of 107 tumours, 87 per cent, and every one of those overexpressed p16. Of the 14 HPV-negative tumours, ten were also p16-negative and four were p16-positive (Figure 4). The HPV-negative group responded poorly to standard chemoradiotherapy and frequently carried disruptive TP53 mutations [14], which identifies the non-viral pathway that the discordant results partly reflect. Wild-type p53 with AKT activation has been described in the same disease [20], and p53 overexpression was linked to outcome in a randomised trial cohort [19].

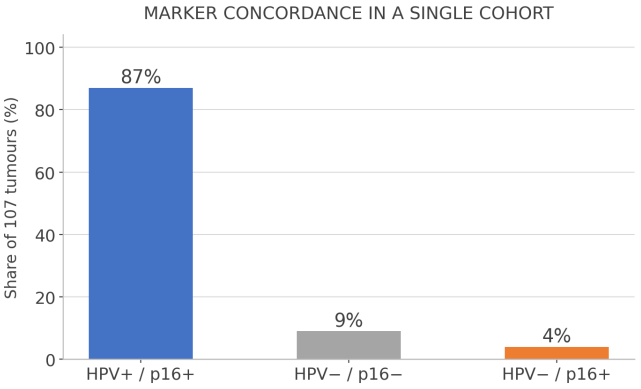

Figure 4: Concordance between HPV status and p16 expression in 107 consecutive tumours [14]

Viral load and radiosensitivity: Beyond presence and absence, viral DNA load together with p16 expression has been shown to predict local control after chemoradiotherapy [15], and an independent cohort reported prognostic value for viral status in the same setting [17]. The mechanistic explanation offered is that HPV- and p16-positive cells carry impaired double-strand break repair and are therefore more radiosensitive [21], which would account for why the marker predicts response to radiotherapy rather than merely indexing tumour biology.

Other markers have been examined alongside. Combined p16, p53, EGFR and KRAS assessment correlated with outcomes after chemoradiotherapy [16], metabolic tumour volume on functional imaging was an independent determinant of recurrence-free survival [25], and salvage surgery outcomes after local failure have been characterised separately [22]. Trials adding targeted agents to chemoradiotherapy have been conducted in this population [24]. Incidental high-grade lesions found on haemorrhoidectomy specimens carry the same genotypes, which bears on how early disease is detected [23]. The clinical relevance of HPV-induced neoplasia differs by anatomical site, which is why findings transfer imperfectly from the oropharynx [18].

This is the biological reason p16 retains a role despite being indirect. It reflects the downstream consequence of viral E7 activity rather than the presence of viral DNA, so it reports function where genotyping reports presence [2,5]. Neither alone captures both, which fits the finding that only the concordant result carries the prognostic advantage [1].

Result	Most likely interpretation	Reported outcome
HPV positive, p16 positive	Transcriptionally active viral disease	Best prognosis; OS hazard ratio 0.30 [1]
HPV negative, p16 positive	p16 raised through a non-viral route	Not comparably favourable [1]
HPV positive, p16 negative	Virus present but not driving the tumour	Not comparably favourable [1,9]
HPV negative, p16 negative	Non-viral pathway	Poorest reported outcomes [1]

Table 2: The four possible marker combinations and what each has been reported to mean

Markers beyond the virus: Neither viral status nor p16 exhausts the prognostic information in a biopsy. Tumour-infiltrating cytotoxic T cells, but not regulatory T cells, predicted outcome in anal carcinoma [6], and a lymphocyte score was later shown to stratify outcomes over and above p16 after chemoradiotherapy [8]. Serum squamous cell carcinoma antigen has also been reported as

prognostic [7].

The practical implication is that viral markers are one component of a stratification rather than the stratification itself [5,8]. That framing also offers an explanation for why the doubly positive group does well: it is likely enriched for the immune-responsive phenotype those studies identify separately.

Discussion

Two conclusions follow. The first is that reported positivity for either marker says as much about the assay as about the tumour. Standardised genotyping across 24 countries put HPV prevalence at 88.3 per cent [3], while the studies pooled for prognosis reported anywhere between 67.9 and 95.8 per cent [1]. A prevalence figure quoted without its detection method is close to uninterpretable.

The second is that the prognostic advantage tracks concordance rather than either marker alone. Doubly positive tumours carry a hazard ratio of 0.30 for overall survival, and both discordant groups lose that benefit [1]. Because the two discordant states arise for different reasons, p16 elevation without virus in one and probable episomal or silent infection in the other [9], they are biologically distinct even where their prognosis is similarly unfavourable.

What this implies for testing

The inference runs against the economy that motivated using p16 as a surrogate in the first place. If the favourable outcome belongs to the doubly positive group, establishing that a patient is in it requires both tests [1,5]. Where only one is available the choice depends on the question: genotyping establishes presence and identifies the type, which matters for vaccine-related epidemiology, while p16 reports the functional consequence, which is what relates to outcome [2,3,5].

A second implication concerns high-sensitivity assays. Methods detecting virus in nearly every tumour are valuable for establishing attributable fraction and for vaccine policy, and unhelpful for prognostic stratification, because a marker positive in everyone separates nobody [4].

Study limitations

The limitations belong to the underlying literature. The pooled survival estimates rest on four studies and 398 patients, a small base for a four-way stratification, and the discordant subgroups within it are smaller still [1]. Detection methods differ between cohorts and are not equivalent in sensitivity [3,4]. Immunohistochemical thresholds for p16 positivity are not standardised, which alone could account for part of the reported prevalence range [1,5]. Most contributing cohorts are retrospective, single-institution and based on archival material of varying age [3,9]. Stage at diagnosis remains the dominant prognostic factor and was adjusted for inconsistently across the pooled studies [1].

Two gaps deserve naming. No study reviewed here reports outcomes for patients whose treatment was altered on the basis of marker status, so the prognostic association has not been translated into a management decision [1,5]. And the two discordant groups have not been characterised in sufficient numbers to establish whether they differ from one another, which the underlying biology would predict [1,9].

Conclusion

Anal squamous cell carcinoma is overwhelmingly virally driven, with HPV DNA present in 88.3 per cent of invasive tumours under standardised genotyping and approaching universal positivity with more sensitive methods. Reported prevalence of both HPV and p16 varies far more across the literature than the biology can explain, so any figure quoted without its method is unreliable. The favourable prognosis, a hazard ratio of 0.30 for overall survival, belongs specifically to tumours positive for both markers, and neither discordant group shares it. Genotyping reports viral presence and p16 reports viral consequence; because outcome tracks the combination, the two are complements rather than alternatives.

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Conflict of Interest

No conflicting interest is declared by any of the authors.

Ethics Statement

This is a survey of literature already in print. Since no participant was enrolled and no new record consulted, the work lay outside the remit of ethical review.

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