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INTEGRATING MORPHOLOGY AND MOLECULAR DIAGNOSTICS IN HPV-ASSOCIATED CUTANEOUS AND ANOGENITAL LESIONS: A SYSTEMATIC REVIEW

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ABSTRACT

Background: The pathological spectrum of human papillomavirus (HPV) extends from productive cutaneous warts to high-grade anogenital squamous neoplasia and invasive carcinoma. Morphology identifies the lesion phenotype, but etiologic assignment increasingly requires molecular evidence that distinguishes viral presence from transforming infection.

Objective: To determine how histomorphology, HPV DNA/genotyping, p16, Ki-67, E4, p53, viral methylation, integration, and genomic assays can be combined to improve diagnostic classification of HPV-associated cutaneous and anogenital lesions.

Methods: A PRISMA 2020-guided systematic review was undertaken for literature published from January 2008 through January 2026. Fourteen structured evidence searches identified 140 records; 18 duplicates were removed, 122 records were screened, 53 full texts were sought, one was not retrieved, 52 reports were assessed, and 36 original human studies were included. Because of major heterogeneity in anatomical site, assay platform, and diagnostic endpoint, meta-analysis was not performed. Risk of bias was assessed using five adapted Joanna Briggs Institute domains.

Results: Across cutaneous lesions, assay-based studies detected HPV in 95% of wart preparations in one series, while multiple HPV infections occurred in 35% of biopsies in another. High-risk alpha-HPV was reported in 57.9% of cutaneous SCC and 38.2% of Bowen disease in one cohort, and HPV16 integration was demonstrated in 8 of 9 HPV16-positive cutaneous SCC samples in a 2025 study. In anal lesions, diffuse p16 discriminated high-grade AIN with 100% sensitivity and 84% specificity in one study; among 1,000 anal biopsies, block p16 occurred in 89% of AIN2 and 95% of AIN3. Vulvar data showed that morphology alone incompletely separated HPV-associated from HPV-independent SCC: in 1,594 tumors, 23.0% were HPV DNA+/p16+, whereas 66.5% were HPV DNA−/p16−. Newer studies demonstrated strong reproducibility of binary p53 interpretation and distinct HPV/p53 genomic groups. Penile studies identified frequent p16 pathway disruption, viral methylation/integration, and prognostic host methylation signatures. Overall risk of bias was low in 11 studies (30.6%), showed some concerns in 22 (61.1%), and was high in 3 (8.3%).

Conclusion: No single test is sufficient across all anatomical sites. The most reliable diagnostic model begins with morphology and site, uses HPV DNA/genotyping for viral attribution, applies p16/Ki-67/E4/p53 to define biological pathway, and reserves E6/E7 RNA, integration, methylation, or genomic profiling for discordant lesions and risk stratification.

KEYWORDS: human papillomavirus; histopathology; molecular diagnostics; p16; p53; Ki-67; E4; HPV DNA; methylation; integration; vulvar carcinoma; anal intraepithelial neoplasia; penile carcinoma; cutaneous squamous cell carcinoma

INTRODUCTION

Human papillomaviruses comprise a large family of epitheliotropic DNA viruses whose pathological effects depend on viral type, anatomical site, host immunity, and the molecular state of infection. The diagnostic challenge is not simply to detect HPV, but to determine whether a lesion is a productive viral proliferation, a transforming high-risk HPV lesion, an HPV-independent neoplasm, or a tumor in which HPV is present without being the dominant oncogenic driver [1–8].

Conventional histopathology remains the first diagnostic layer. Papillomatosis, hypergranulosis, koilocytosis, dyskeratosis, loss of epithelial maturation, basaloid growth, abnormal mitoses, and invasive keratinizing architecture each provide important biological clues. However, the same morphological pattern can arise through different molecular pathways. This is particularly evident in vulvar and penile squamous carcinomas, where HPV-associated and HPV-independent lesions overlap morphologically, and in cutaneous tumors, where HPV DNA may coexist with ultraviolet-associated carcinogenesis [4–8,17–33].

Molecular testing has therefore shifted from simple HPV detection toward pathway assignment. HPV DNA and genotype assays establish viral presence and type; p16, Ki-67, E4, and p53 characterize cell-cycle disruption, proliferative activity, productive viral replication, and HPV-independent tumor pathways; E6/E7 RNA and viral integration provide stronger evidence of transforming infection; and host/viral methylation or next-generation sequencing can add prognostic information [9–33].

The practical problem is deciding which test should be used, at which anatomical site, and for what question. A highly sensitive HPV DNA assay can overcall biologically irrelevant infection, whereas p16 may be highly informative in lower-anogenital disease yet less specific in selected cutaneous contexts. Similarly, genomic and methylation assays may be valuable for progression or prognosis but unnecessary for routine benign warts. This systematic review therefore focuses on diagnostic integration rather than a simple catalogue of HPV-associated lesions.

MATERIALS AND METHODS

Review design and reporting framework

This systematic review was prepared according to PRISMA 2020 principles. The protocol was not prospectively registered. Because the objective was diagnostic integration across heterogeneous cutaneous and anogenital lesions, a qualitative synthesis was prespecified rather than a pooled meta-analysis.

Search strategy

Fourteen structured academic searches were performed for original human studies published from January 2008 through January 2026. Search concepts combined HPV/human papillomavirus with cutaneous wart, plantar wart, Bowen disease, cutaneous squamous cell carcinoma, nail-unit carcinoma, anal intraepithelial neoplasia, anal squamous cell carcinoma, vulvar intraepithelial neoplasia, vulvar squamous cell carcinoma, penile intraepithelial neoplasia, penile squamous cell carcinoma, morphology, histopathology, genotype, p16, p53, Ki-67, E4, E6/E7, RNA in situ hybridization, methylation, integration, laser-capture microdissection, and genomic sequencing. Candidate titles and DOIs were cross-checked during full-text selection.

Eligibility criteria

Eligible studies were original human investigations that linked a histopathological diagnosis or defined lesion phenotype with at least one molecular diagnostic variable: HPV DNA/genotype, viral RNA, p16, p53, Ki-67, E4, viral methylation, integration, host methylation, copy-number alteration, or genomic sequencing. Cervical-only, oral-only, and oropharyngeal-only studies were excluded unless separately analyzable non-cervical anogenital or cutaneous data were provided. Reviews, meta-analyses, isolated case reports, animal studies, in-vitro-only studies, and studies without interpretable morphology-molecular pairing were excluded.

Study selection

The searches yielded 140 records. After removal of 18 duplicates, 122 unique records underwent title and abstract screening. Sixty-nine were excluded, leaving 53 reports for retrieval. One report could not be retrieved in sufficient detail. Of 52 full-text reports assessed, 16 were excluded: secondary/review publications (n=5), anatomical sites outside the prespecified scope (n=3), absence of integrated morphology-molecular data (n=3), case reports or very small descriptive series (n=2), non-human/in-vitro work (n=1), insufficient extractable data (n=1), and an overlapping population (n=1). Thirty-six studies were included in the qualitative synthesis.

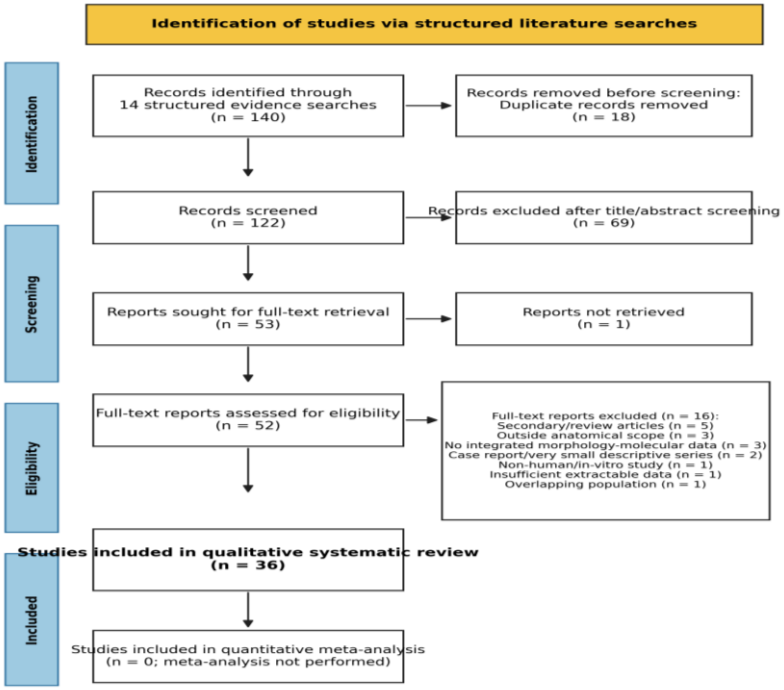

Figure 1. PRISMA 2020 flow diagram of study identification, screening, eligibility, and inclusion.

Data extraction and synthesis

Data were extracted on anatomical site, lesion type, sample size when available, morphological classification, HPV assay, genotype distribution, immunohistochemical or epigenetic marker, diagnostic performance, and major clinicopathological conclusion. Evidence was organized according to the diagnostic function of each modality rather than solely by lesion site.

Risk-of-bias assessment

Risk of bias was assessed at study level using five domains adapted from Joanna Briggs Institute appraisal principles: selection/representativeness; reliability of histopathological diagnosis or reference standard; validity of molecular testing; independence/blinding of index and reference assessments where relevant; and completeness of analysis/confounder handling. Each domain was categorized as low risk, some concerns, or high risk. Overall judgments were low risk when no material limitation was present, some concerns when limitations were unlikely to overturn the main conclusion, and high risk when major design or analytical limitations could materially alter interpretation.

RESULTS

Overview of the evidence

Thirty-six studies were included: eight focused primarily on cutaneous lesions, eight on anal neoplasia and anal biomarkers, nine on vulvar disease, eight on penile carcinoma, and three on broader anogenital lesions or high-risk populations. Publication years ranged from 2008 to 2025. Study designs included assay-validation studies, retrospective pathology cohorts, prospective screening cohorts, genotype–morphology studies, methylation analyses, laser-capture microdissection studies, and genomic profiling investigations.

Table 1. Representative characteristics and diagnostic contribution of the included studies

Study	Site/lesion	Integrated method	Key diagnostic finding
Redzic et al., 2021	Cutaneous warts	Genotyping assay validation	100% analytical sensitivity/specificity in validation; multiple HPV types in 35% of biopsies.
Schmitt et al., 2011	Skin warts	Multiplex HPV genotyping	HPV detected in 95%; HPV1 most frequent; strong inter-assay agreement (κ=0.71).
King et al., 2014	Plantar verrucae	HPV typing in HIV+/HIV− patients	39 lesions from 17 patients; HPV27 predominated in HIV− and HPV2 in HIV+ patients.
Dorfer et al., 2019	Hand SCC/Bowen disease	HR-HPV genotyping/viral load	HR-HPV in 56.3% overall; 83.3% of nail-unit tumors; HPV16 most frequent.
Aoki et al., 2019	Cutaneous SCC/Bowen/AK	HR alpha-HPV genotyping	HR alpha-HPV in 57.9% cSCC, 38.2% Bowen disease, 0% AK.
Brunet-Possenti et al., 2025	Cutaneous SCC	Alpha-HPV + integration markers	HPV DNA in 56.6%; alpha-HPV 19.4%; HPV16 integration in 8/9 positive samples.
Idriss et al., 2016	Bowen disease	Histology + IHC + HPV	38 cases; orthokeratotic variant identified; HPV11/16/58 detected in subset.
Maghiar et al., 2024	Cutaneous HPV lesions	p16/p53/E-cadherin profiling	Marker-expression differences supported distinct benign versus malignant molecular patterns.
Leeman et al., 2018	Anal AIN	p16/Ki-67/E4 + LCM-PCR	Diffuse p16: sensitivity 1.00, specificity 0.84 for high-grade AIN; E4 marked productive subgroups.
Wong et al., 2025	Anal screening in PLWH	HR-HPV + p16/Ki-67 dual stain	71.4% HR-HPV at baseline; 25.9% composite HSIL at 12 months.
Liu et al., 2021	Anal biopsies	Morphology + p16	1,000 biopsies; block p16 in 89% AIN2 and 95% AIN3.
Bala et al., 2013	Anal HSIL	p16 vs ProEx C	p16 outperformed ProEx C for identification of HSIL.
Hernandez et al., 2012	Anal neoplasia	Host DNA methylation	20 CpG loci increased across progression; six loci hypermethylated in invasive SCC.

Study	Site/lesion	Integrated method	Key diagnostic finding
Molano et al., 2016	Anal HSIL	HPV16 methylation + LCM	LCM provided higher, more lesion-specific methylation estimates than whole tissue.
Chaiwongkot et al., 2021	Anal AIN	HPV16 L1 methylation	L1 hypermethylation at CpG 5600/5609 enriched in AIN2-3.
Phillips et al., 2022	Anal HSIL	CADM1/MAL methylation	CADM1 methylation distinguished HSIL; MAL particularly informative in HIV-positive participants.
Rakislova et al., 2017	Vulvar SCC	Morphology + HPV DNA + p16	1,594 tumors; 23.0% HPV DNA+/p16+ and 66.5% DNA−/p16−; morphology alone insufficient.
Griesinger et al., 2021	Vulvar HSIL mimicking dVIN	Morphology + p16/p53 + HPV	38 lesions; block p16 and HPV evidence despite dVIN-like morphology.
Jeffus et al., 2024	Vulvar SCC	p16/p53 interobserver study	p16 interpretation highly reproducible; p53/pathway assignment more variable but improved with training.
Rasmussen et al., 2025	Vulvar SCC	Six-pattern p53 interpretation	1,293 tumors; 86.9% agreement for binary wild-type vs mutated p53 classification.
Davidson, 2023	Vulvar SCC	NGS genomic landscape	443 tumors profiled; 37% HPV+; HPV− group separated into p53-mutant and p53-wild-type genomic subsets.
Thuijs et al., 2021	VIN/VSCC risk	Host DNA methylation	192 vulvar samples; methylation increased in VIN adjacent to VSCC and in VSCC.
Kjær et al., 2025	Vulvar SCC prognosis	p16 + hrHPV DNA	1,277 women; 5-y survival 67% p16+ vs 45% p16−; hrHPV added prognostic information.
Pakharukova & Yushkov, 2025	Vulvar SCC	Morphometry + p16/p53 + HPV DNA	IHC specificity for HPV pathway 96.36% vs 80.65% histology and 75.47% viral DNA.
Jones et al., 2017	VIN3	HPV E2 methylation	E2 methylation >4% predicted cidofovir response with 88.2% sensitivity and 84.6% specificity.
Poetsch et al., 2011	Penile SCC	p16 IHC/LOH/methylation	p16 positive in 50%; LOH 62%; promoter hypermethylation 42%; p16 loss linked to nodal metastasis.
Hojný et al., 2024	Penile SCC	Targeted/genomic profiling	TP53/CDKN2A and other alterations associated with shorter survival; 160 potentially actionable alterations.
Sharma et al., 2025	Penile carcinoma	HPV genotype + clinicopathology	HPV prevalence 74.1%; HPV16 in 75%; multiple types in 75% of HPV-positive patients.
Busso-Lopes et al., 2015	Penile carcinoma	Copy-number genomic profiling	HPV in 34.8%; 3p/8p alterations associated with adverse outcomes.
Kalantari et al., 2008	Penile carcinoma	HPV methylation/integration	HPV16 integration confirmed in 11/15 lesions; extensive L1/LCR methylation.
Yanagawa et al., 2008	Penile SCC	HPV DNA + p53 + methylation	26 tumors; HPV DNA 11.5%, p53 overexpression 50%; methylation more frequent in HPV-negative disease.
Gu et al., 2021	Node-positive penile SCC	Prognostic methylation panel	3-CpG score HR 5.8 in development and 9.9 in validation; improved pN-stage C-index by 0.09.
Kuasne et al., 2015	Penile carcinoma	Genome-wide methylation/transcriptome	171 hypermethylated probes; 54 inverse methylation-expression genes; BDNF methylation linked to prognosis.

Study	Site/lesion	Integrated method	Key diagnostic finding
Tso et al., 2015	Anogenital HPV in women	Cyto-colpo-histology + typing	98 women; anal HSIL in 29% of HIV-positive vs 0% of HIV-negative women.
Ingles et al., 2015	External genital lesions in men	Pathology + HPV attribution	2,754 men; 377 pathologically confirmed lesions, including condyloma and PeIN.
Lisboa et al., 2019	External anogenital warts	Multisite HPV DNA	Anal HPV prevalence 59.9%; 92.3% in patients with perianal warts.

Risk of bias

Eleven studies (30.6%) were judged to have low overall risk of bias, 22 (61.1%) had some concerns, and three (8.3%) were high risk. The most frequent concerns were retrospective or convenience-based case selection, restriction to high-risk populations, limited reporting of blinding between morphology and molecular assays, and small cohorts in rare tumors. Molecular assay validity was generally strong, particularly in studies using laser-capture microdissection, validated genotyping platforms, methylation assays, or next-generation sequencing. The highest-risk studies were limited principally by very small samples or incomplete control of case selection and analytical confounding.

Figure 2. Traffic-light risk-of-bias assessment of included studies

Green (+) = low risk; Yellow (?) = some concerns; Red (−) = high risk

Figure 2. Traffic-light risk-of-bias assessment of the 36 included studies.

Diagnostic performance of morphology as the first layer

Morphology remained indispensable for defining lesion type but was not uniformly sufficient for assigning HPV causality. In cutaneous warts, cytopathic morphology can strongly suggest productive infection, but molecular assays demonstrated mixed infections and genotype diversity that cannot be reliably inferred from H&E alone [1–3]. In hand and nail-region squamous lesions, high-risk mucosal HPV was concentrated in anatomically specific tumors, demonstrating that site modifies the meaning of an otherwise similar squamous morphology [4–7].

The limitations of morphology were most clearly demonstrated in vulvar disease. In the 1,594-case study by Rakislova et al., HPV-associated cancers were more often basaloid or warty and HPV-independent tumors more often keratinizing, yet no individual histological feature separated the two pathways with sufficient accuracy [17]. Griesinger et al. further showed that HPV-associated HSIL can mimic differentiated VIN or lichen sclerosus morphologically, making p16 and HPV testing decisive in selected cases [18].

The 2025 morphometric study of vulvar SCC quantified these differences: HPV-associated tumors had smaller cells and a higher nuclear-to-cytoplasmic ratio, but immunohistochemical classification still provided substantially higher specificity for HPV pathway assignment than morphology alone [24]. These results support a practical rule: morphology should determine what question to ask, but not always provide the final etiological answer.

HPV DNA and genotyping: sensitive viral attribution without proof of transformation

HPV DNA testing was highly effective for identifying viral type in productive lesions. The BSwart multiplex assay detected HPV in 95% of skin-wart preparations, and a later wart-associated assay reported excellent analytical performance while detecting multiple infections in 35% of biopsies [1,2]. These findings are useful for etiological attribution but also illustrate why a positive DNA result must be interpreted in context when more than one type is present.

In cutaneous carcinoma, DNA positivity did not behave uniformly. Dorfer et al. detected high-risk HPV in 56.3% of SCC/Bowen lesions of the hand, with enrichment in nail-unit tumors and HPV16 dominance [4]. Aoki et al. reported high-risk alpha-HPV in 57.9% of cutaneous SCC and 38.2% of Bowen disease but none of the actinic keratoses studied [5]. In 2025, Brunet-Possenti et al. found HPV DNA in 56.6% of cSCC samples, but only 19.4% were alpha-HPV; importantly, viral integration was demonstrated in most HPV16-positive tumors [6]. Thus, simple DNA detection and mechanistic evidence of viral participation are not equivalent.

Anogenital epidemiological studies similarly showed that high HPV prevalence may reduce specificity. In patients with external warts, anal HPV DNA was detected in 59.9% overall and 92.3% among those with perianal warts [36]. This background prevalence strengthens the need for morphology, biomarker pattern, and—when clinically relevant—transcriptional or epigenetic evidence.

p16, Ki-67, E4 and p53: mapping biological state

Among tissue biomarkers, p16 was most useful when interpreted as a pattern rather than a simple positive/negative stain. Leeman et al. found that null/patchy versus diffuse p16 discriminated high-grade anal intraepithelial neoplasia with 100% sensitivity and 84% specificity, while Ki-67 distinguished normal epithelium from AIN with 92% sensitivity and 100% specificity [9]. E4 added a different biological dimension: 70% of AIN2 lesions were E4-positive whereas AIN3 was uniformly E4-negative, supporting a transition from productive to transforming infection.

The 1,000-biopsy anal study by Liu et al. demonstrated the scale of this relationship. Block-type p16 was present in 89% of AIN2 and 95% of AIN3 but in only 37% of AIN1, while normal mucosa showed no block positivity [11]. This makes p16 most valuable as an adjunct to morphology in intermediate/high-grade lesions rather than as a general screening marker.

p53 assumes greater importance when the question is HPV independence. In vulvar SCC, binary p53 interpretation achieved substantially better agreement than six-pattern classification across 1,293 tumors, and most p16-positive tumors resolved to a wild-type p53 pattern [20]. The interobserver study by Jeffus et al. similarly found near-perfect p16 reproducibility but more variable p53/pathway assignment, particularly outside gynecologic pathology practice [19]. These findings argue for standardized pattern-based p53 interpretation and molecular backup for discordant cases.

Epigenetic and genomic diagnostics: from lesion classification to risk stratification

Epigenetic markers were especially informative when morphology and HPV detection could not answer questions of progression risk. Hernandez et al. identified a panel of host CpG loci that became increasingly methylated across the spectrum from normal anal mucosa and in-situ neoplasia to invasive SCC [13]. Molano et al. demonstrated that laser-capture microdissection yielded more lesion-specific HPV16 methylation estimates than whole-tissue sections [14], illustrating how spatial sampling can materially change molecular interpretation.

Additional anal studies refined this approach. HPV16 L1 methylation at specific CpG sites increased in AIN2-3 [15], while CADM1 and MAL methylation showed potential for detecting high-grade lesions, particularly in high-risk populations [16]. These assays remain investigational, but they address a question that p16 and HPV DNA cannot fully answer: which HPV-positive lesions are biologically closer to progression?

In vulvar disease, DNA methylation likewise distinguished VIN adjacent to carcinoma from VIN unaccompanied by cancer [22]. At the genomic level, a 443-tumor series identified three broad vulvar molecular

contexts—HPV-positive, HPV-negative/p53-mutant, and HPV-negative/p53-wild-type—with distinct mutation profiles [21]. This expands etiological classification beyond a binary HPV-positive/negative model.

Penile studies showed the same principle. p16 inactivation through allelic loss or promoter methylation was linked to aggressive behavior [26]. Copy-number alterations in 3p/8p predicted adverse outcomes [29], viral methylation and integration were frequent in HPV16-associated tumors [30], and genome-wide methylation/transcriptome analysis identified prognostic epigenetic candidates [33]. A validated three-CpG panel substantially improved cancer-specific survival prediction beyond nodal stage in node-positive disease [32].

Site-specific synthesis of integrated diagnostics

Setting	Primary morphological question	Most useful viral test	Key tissue biomarkers	Main caveat
Cutaneous warts	Confirm productive viral morphology; type attribution if needed	HPV DNA/genotype	p16 generally not required	Mixed infection and background cutaneous HPV can complicate attribution
Hand/nail/Bowen lesions	Separate viral-type morphology from UV-related squamous neoplasia	Genotype + viral load/integration	p16/p53 only with site-specific caution	High-risk alpha-HPV has strongest evidence in nail/finger subsets
Anal LSIL/AIN1	Identify productive infection	HPV typing; E4 if research question	Ki-67; p16 usually non-block/variable	High HPV prevalence limits specificity of DNA alone
Anal HSIL/AIN2-3	Confirm transforming lesion and grade	HR-HPV DNA; RNA when discordant	Block p16 + Ki-67; E4 helps identify productive component	Methylation may refine progression risk
Vulvar SCC/HSIL	Assign HPV-associated vs HPV-independent pathway	HPV DNA/RNA as needed	p16 first-line pathway marker; p53 for HPV-independent classification	Morphology overlaps; binary p53 more reproducible than detailed patterns
Penile SCC/PeIN	Assign HPV-related pathway and prognosis	HPV genotype/integration	p16 + p53/pathway context	Host methylation/genomics may refine prognostic risk

Integrated diagnostic pathway

The evidence supports a staged diagnostic strategy. The first step is an anatomically informed morphological diagnosis. The second is clarification of the question: detection of HPV, proof of a transforming HPV pathway, resolution of a discordant phenotype, or prediction of progression/prognosis. DNA/genotyping is best suited to viral attribution; p16/Ki-67/E4/p53 to biological pathway mapping; E6/E7 RNA or integration to stronger causal inference; and methylation/genomic profiling to risk stratification. This hierarchy reduces unnecessary molecular testing while improving interpretation of discordant lesions.

DISCUSSION

This review differs from prevalence-focused HPV reviews by asking how morphological and molecular data should be combined in diagnostic practice. The evidence shows that the hierarchy of tests must be anatomical-site specific. A result that is highly informative in vulvar or anal disease may have much lower etiologic specificity in cutaneous SCC, and a highly sensitive DNA assay can create diagnostic uncertainty if viral presence is equated with viral causation.

The strongest recurring observation was that morphology is necessary but not always sufficient. In vulvar disease, large series demonstrated meaningful but incomplete associations between warty/basaloid morphology and HPV positivity, while some HPV-associated lesions displayed differentiated or keratinizing patterns. Conversely, high-grade HPV-associated vulvar lesions can mimic dVIN. These findings justify p16 as a pathway-defining adjunct and p53 as a second-layer classifier when HPV-independent disease is suspected.

Anal pathology provides the clearest example of a biomarker continuum. Ki-67 identifies abnormal proliferation, diffuse block p16 tracks transforming activity, and E4 identifies productive viral replication. Their combination explains why lesions with a similar grade can be biologically heterogeneous. Methylation assays may eventually add a progression-risk dimension, but current evidence remains insufficient for universal routine adoption.

Cutaneous lesions require a more cautious framework. High-risk alpha-HPV has been repeatedly demonstrated in hand, nail-unit, Bowen, and selected cutaneous SCC cohorts, but its prevalence varies widely by site, age, cohort, and assay. The 2025 integration study strengthens causal inference for a subset of HPV16-positive cutaneous SCC, yet this should not be generalized to all cutaneous SCC. For routine practice, histological phenotype, anatomical location, and molecular evidence of integration or viral activity should be considered together.

Penile carcinoma illustrates the transition from etiologic classification to precision risk assessment. HPV and p16 define one pathway, but p16 loss, host methylation, copy-number alteration, and gene mutation patterns carry independent prognostic information. Molecular diagnostics in this setting therefore serve two separate purposes: identifying an HPV-associated pathway and refining risk within or beyond that pathway.

The review also highlights an important methodological lesson. Spatially precise methods such as laser-capture microdissection can change methylation estimates and clarify which component of a heterogeneous lesion carries a molecular alteration. As tissue sequencing and RNA-based methods become more accessible, spatially resolved molecular pathology is likely to become increasingly relevant for HPV-associated neoplasia.

Clinical and pathology implications

- Use morphology and anatomical site to define the initial lesion category before ordering HPV-related tests.
- Use HPV DNA/genotype assays when the clinical question is viral detection or type attribution; do not interpret DNA positivity alone as proof of transforming infection.
- Use block-pattern p16, supported by Ki-67 and morphology, for diagnostically difficult lower-anogenital HSIL; E4 can help distinguish productive from transforming components in research or specialized practice.
- In vulvar carcinoma, interpret p16 and p53 together when assigning HPV-associated versus HPV-independent pathways, and use HPV RNA or other molecular testing for discordant cases.
- In cutaneous and nail-unit SCC, require stronger site-specific evidence before labeling a tumor HPV-driven; viral integration or transcription adds more causal weight than p16 alone.
- Reserve methylation and genomic profiling for risk stratification, prognostication, unresolved pathway assignment, or research rather than routine benign wart diagnosis.

Strengths and limitations

Strengths of this review include a diagnostic rather than purely epidemiological framework, incorporation of morphology, immunohistochemistry, viral assays, epigenetics, integration, and genomic profiling, and inclusion of large pathology cohorts alongside mechanistic studies. The 36-study evidence set also spans benign productive lesions, precursors, and invasive tumors, allowing comparison of diagnostic questions across the disease spectrum.

The main limitation is heterogeneity. Included studies differed in tissue preparation, genotype panels, p16 thresholds, reference standards, patient populations, and molecular endpoints. Several studies were retrospective, some involved small numbers of rare tumors, and high-risk populations such as people living with HIV were overrepresented in anal studies. These differences precluded pooled meta-analysis. In addition, methylation and genomic signatures remain insufficiently standardized for routine cross-laboratory application. Risk-of-bias assessment showed that most evidence was of low risk or had only some concerns, but three small or methodologically limited studies were judged high risk.

CONCLUSION

The diagnosis of HPV-associated cutaneous and anogenital disease is most accurate when morphology and molecular testing are treated as complementary layers rather than competing alternatives. Morphology defines the lesion and anatomical context; HPV DNA/genotyping establishes viral presence and type; p16, Ki-67, E4, and p53 define biological pathway; E6/E7 RNA or viral integration strengthen causal attribution; and methylation or genomic assays provide emerging information on progression and prognosis. The optimal sequence is therefore question-driven and site-specific. This integrated strategy reduces both under-recognition of transforming HPV disease and over-attribution of incidental HPV positivity.

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