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Anal squamous cell carcinoma is predominantly driven by human papillomavirus infection across different geographic regions

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Abstract

Human papillomavirus (HPV) is considered the main etiological factor of anal squamous cell carcinoma (ASCC). However, it is not known whether there are geographical differences in terms of epidemiological features or prevalence of HPV infection. In addition, the existence and the contribution of other mechanisms to the carcinogenesis of this tumor is not clear. This study aimed at comparing the etiopathogenic and epidemiological characteristics of ASCC in Mozambique and Spain, two countries with markedly different epidemiological backgrounds regarding HPV and human immunodeficiency virus infection. All ASCCs diagnosed in two tertiary referral hospitals from Mozambique and Spain between 2019–2023 were included. HPV DNA detection and genotyping, as well as immunohistochemical staining for p16 and p53 were performed in all cases. 43 tumors were identified in Mozambique and 29 in Spain. No differences were identified in the percentage of HPV-associated tumors: 41/43 (95.3%) in Mozambique and 28/29 (96.6%) in Spain. HPV16 was the most frequently identified type in both countries, but other HPV types were more frequently found in the absence of HPV16 in Mozambique than in Spain (16/36, 44.4% vs. 2/28, 7.1%; p = 0.0016). In Mozambique, ASCC was less frequent in men (7/43; 16.3%, vs. 14/29; 48.3%; p = 0.007) and occurred in younger patients (44.1 ± 9.4 vs. 60.3 ± 12.6; p < 0.001). A small proportion of ASCC probably developed following HPV-independent mechanisms, with evidence suggesting two distinct pathways for these tumors, one with *TP53* mutation and a second with wild type *TP53*.

Introduction

Anal cancer accounts for less than 1% of all malignant neoplasms (1). According to Globocan, 27,000 new cases were estimated to occur in 2022 globally, with an age-standardized incidence rate of 0.66 per 100,000, and minimal variation between geographical regions: 0.72 per 100,000 in Africa and in Europe (2, 3). Squamous cell carcinoma (SCC) accounts for up to 80–90% of all anal cancers, while adenocarcinoma, neuroendocrine tumors, and melanoma make up the remaining 10–20% (1).

High-risk human papillomavirus (HPV) is established as a primary etiological factor in the development of anal SCC (ASCC) (3, 4). Conditions associated with an increased risk of HPV infection such as multiple sexual partners, anal intercourse, a history of other HPV-associated neoplasms or immunosuppression including human immunodeficiency virus (HIV) infection, are also recognized risk factors (1, 5, 6). Notably, the incidence of ASCC is particularly high among people living with HIV (7), especially men who have sex with men (6–8), and women with history of genital HPV-related premalignant lesions or cancer. In response to these rising incidence rates, targeted screening strategies for high-risk subgroups have been proposed in recent years (8, 9). Remarkably, although men who have sex with men have been a main focus of interest in terms of strategies to prevent ASCC, the epidemiological burden of ASCC is significantly higher in women, and the incidence in females has increased in recent decades. This rise has been particularly pronounced among immunocompromised women and immunocompetent women with HPV-associated lesions in the lower genital tract (7, 10).

Although approximately 10–15% of ASCCs have been reported to lack detectable HPV DNA, the molecular mechanisms underlying HPV-independent anal carcinogenesis remain poorly characterized (11). Evidence from other genital SCC reporting an HPV-independent oncogenic pathway suggests this might be the case (12–15). The prevalence of these dual pathways varies significantly by anatomical site; while HPV-independent mechanisms predominate in vulvar (13, 14) and penile cancers in immunocompetent individuals (12, 15), they are rare in the uterine cervix (16). In addition, marked geographic differences in the proportion of HPV-associated and HPV-independent tumors for several of these anatomical sites have been observed. In Europe, the majority of the vulvar and the penile SCC arise via HPV-independent pathways, whereas in sub-Saharan Africa most of these malignancies are HPV-associated (14, 15). Remarkably, HPV-independent SCCs consistently show a significant poorer prognosis compared with HPV-associated counterparts. This clinical behavior presents a notable paradox, as HPV-independent tumors tend to be well-differentiated and highly keratinizing, whereas HPV-associated SCCs typically display a poorly differentiated basaloid morphology (12, 13). These etiopathogenic and clinical features have led the World Health Organization (WHO) to formally recognize two separate entities for SCCs across nearly all anogenital sites: HPV-associated and HPV-independent. Notably, the anal canal remains the sole exception to this classification. Furthermore, recent evidence indicates that HPV-independent SCCs in these areas can be subdivided into two clinicopathological subtypes based on the presence or absence of *TP53* gene mutations, with the mutation-positive subtype showing significantly worse prognosis (12–15).

Unfortunately, despite the well-established association between ASCC and HPV, comparable to that observed in cervical cancer, the existence of an HPV-independent oncogenic pathway in the anal canal remains uncertain. Although such pathways are recognized at other lower anogenital sites, including the uterine cervix (17, 18), the current WHO classification acknowledges that approximately 90% of ASCCs are HPV-associated but does not formally define an HPV-independent category (1).

In this study, we aimed to characterize and compare the differential etiopathogenic landscape of ASCC in two geographical sites with marked cultural and epidemiological differences: Mozambique, a sub-Saharan African country with a high burden of HPV and HIV, and Spain, a European country with low rates of these two infections. We aimed to determine the existence of a dual-pathway model (HPV-associated vs. HPV-independent) in the anal canal and the contribution of ASCC to the overall burden of HPV-associated cancers (cervix, vulva, vagina, and penis) in the two sites (19).

Methods

General characteristics of the two geographical settings

This is a retrospective and descriptive study performed in two international sentinel sites: the Pathology Department of the Maputo Central Hospital (MCH) in Mozambique, and the Pathology Department of the Hospital Clinic of Barcelona (HCB) in Spain. The MCH is a 1500-bed hospital that serves as the primary

quaternary referral health care facility in Mozambique. The HCB is a 700-bed high-complexity tertiary referral center in Barcelona, Spain, serving as a regional hub for oncological and infectious diseases.

This was a hospital-based, retrospective series from two tertiary referral centers with different catchment areas and referral pathways; therefore, the comparisons presented are descriptive.

All methods were performed in accordance with the relevant guidelines and regulations.

Identification of tumors of the anogenital area

Data from the Pathology departments of the MCH and the HCB were comprehensively reviewed looking for all carcinomas arising in any anogenital area in which HPV has been shown to be associated with a proportion of cancers (uterine cervix, vagina, vulva, penis, anal canal), from January 2019 to December 2023. The search included all histological specimens, including biopsies and surgical specimens. Metastatic lesions from non-anogenital primary sites were excluded. While the primary focus was on histologically confirmed malignancies, cases with a presumptive clinical diagnosis recorded within the pathology registry were also reviewed to ensure maximum capture of the disease burden.

Clinicopathological data, including age, gender, date and method of diagnosis, anatomical topography, and microscopic morphology, were recorded in a Microsoft Access database (Microsoft Co, Redmond, WA, USA) which, upon data entry, prevented the use of non-existent codes and performed checks for internal consistency among variables. A rigorous de-duplication process was performed by cross-referencing patient names, ages, and medical record numbers. Patient records and information were anonymized and de-identified prior to analysis.

The study included all cancers fulfilling the following inclusion criteria: a) histological diagnosis of ASCC at either MCH or HCB and b) availability of sufficient formalin-fixed paraffin-embedded (FFPE) material for histological evaluation, immunohistochemical (IHC) staining and HPV DNA detection and genotyping. Cases with histological diagnosis other than SCC and those with insufficient material for IHC and/or HPV analysis were excluded.

To ensure diagnostic standardization, after identification, the FFPE blocks from the Mozambique cohort were transferred to the HCB. From all cases identified in the two sites 3 μm sections were obtained and stained with hematoxylin & eosin, p16 and p53. All cases were carefully reevaluated by three experts in the pathology of HPV-associated lesions (N.R., J.O., A.S.).

p16 immunohistochemical stain

p16 IHC stain was performed in all samples using the CINtec Histology Kit (clone E6H4; Roche, Heidelberg, Germany). Tumors with strong and diffuse block-like type of staining were considered as positive, whereas tumors with patchy or completely negative staining were considered as negative.

HPV detection

DNA extraction was performed on whole sections of FFPE tissue from surgical specimens. SPF10 PCR and the LiPA25 system (Labo Biomedical Products, Rijswijk, The Netherlands) were used for HPV DNA detection and genotyping. A volume of 10 µL of isolated DNA was used for PCR amplification through the SPF10-LiPA system (Fujirebio, Belgium). HPV genotyping was conducted using INNO-LiPA HPV Genotyping Extra II kit (Fujirebio, Belgium). The test allows for the detection of high-risk HPV types 16, 18, 31, 33,35,39, 45, 51, 52, 56, 58, 59 and 68; of the probable high-risk HPV types 26, 53, 66, 70, 73 and 82; and of the low-risk HPV types 6, 11, 40, 42, 43, 44, 54, 61, 62, 67, 81, 83 and 89.

HPV-based tumor classification

Cases showing either positive p16 IHC and/or positivity for HPV were classified as HPV-associated. The categorization of a tumor as HPV-independent required both negative p16 IHC staining and absence of high-risk HPV DNA (15, 20, 21).

p53 immunohistochemical stain

p53 IHC was conducted in all cases with a monoclonal antibody (clone DO-7; Dako, Carpinteria, CA, USA). The IHC staining was evaluated in the invasive component of the SCC according to the recent p53 pattern-based interpretation framework. Staining patterns were stratified into two clinical categories: wild type, which includes two patterns (scattered and mid-epithelial) and mutant type, which correlates with a mutant protein and includes four patterns (diffuse overexpression, basal overexpression, cytoplasmic and null) (22).

Statistical analysis

The statistical package SPSS 28.0 (SPSS, Chicago, IL, USA) was used to perform data analyses. Categorical variables are presented as absolute numbers and percentages and were analyzed using the chi-square or Fisher's exact test. Quantitative variables are shown as mean ± standard deviation (SD) and were compared using the student’s t-test or wilcoxon test depending on data distribution or analysis of variance (ANOVA) when appropriated. All p-values were two-sided, and a value of $p < 0.05$ was considered statistically significant.

Results

Anal carcinomas in Mozambique

Fifty-nine tumors suspected of ASCCs were identified in the files of the MCH in the reference period (2019–2023). Three cases were excluded after histological review due to diagnostic reclassification (two plasmablastic lymphomas and one poorly differentiated colonic adenocarcinoma). Thirteen additional cases were excluded due to insufficient material for additional testing. Thus, 43 tumors met the inclusion

criteria and were finally included in the study, 36 (83.7%) occurred in women, and 7 (16.3%) in men. The average age of the patients was 44.1 ± 9.4 (range 30 to 70 years). No differences in terms of age at diagnosis were observed between women and men (43.7 ± 9.3 vs. 46.1 ± 10.4; p = 0.66). The demographic, the histological and IHC features as well as the results of HPV detection of the Mozambican cases are shown in Table 1.

Table 1
Anal squamous cell carcinomas from Mozambique in the period 2019–2023

Year	Age	Gender	Hist	HPV	p16	p53
HPV-associated tumors						
2019	35	F	Warty	Negative	Positive	Scattered cells
2019	30	F	Non-keratinizing	16	Positive	Scattered cells
2019	53	F	Keratinizing	Negative	Positive	Scattered cells
2019	33	F	Keratinizing	16–52	Positive	Scattered cells
2020	44	F	Basaloid	18	Positive	Scattered cells
2021	39	F	Basaloid	16	Positive	Scattered cells
2021	40	F	Basaloid	58 – 53	Positive	Scattered cells
2021	42	F	Keratinizing	16	Positive	Scattered cells
2021	30	F	Keratinizing	26	Positive	Scattered cells
2021	39	M	Keratinizing	45	Positive	Scattered cells
2021	34	F	Basaloid	16	Positive	Scattered cells
2021	42	F	Keratinizing	18	Positive	Scattered cells
2021	41	F	Non-keratinizing	16–51	Positive	Scattered cells
2021	68	M	Basaloid	16	Positive	Scattered cells
2021	32	F	Keratinizing	16	Positive	Scattered cells
2021	44	F	Basaloid	16	Positive	Scattered cells
2021	55	F	Basaloid	16	Positive	Scattered cells
2021	48	M	Basaloid	Negative	Positive	Scattered cells
2022	70	F	Non-keratinizing	Negative	Positive	Scattered cells
2022	41	M	Keratinizing	16	Positive	Scattered cells
2022	50	F	Non-keratinizing	16	Positive	Scattered cells
2022	43	F	Keratinizing	16-39-51	Positive	Scattered cells
2022	47	M	Keratinizing	Negative	Positive	Scattered cells
2022	38	F	Keratinizing	18	Positive	Scattered cells

Year	Age	Gender	Hist	HPV	p16	p53
2022	39	F	Keratinizing	16–18	Positive	Scattered cells
2022	43	F	Keratinizing	16–18	Positive	Scattered cells
2022	NR	F	Basaloid	18	Positive	Scattered cells
2022	38	M	Keratinizing	16-45-51-68-70	Positive	Mid-epithelial
2023	60	F	Keratinizing	18	Positive	Mid-epithelial
2023	44	F	Keratinizing	16	Positive	Mid-epithelial
2023	39	F	Basaloid	18	Positive	Scattered cells
2023	55	F	Non-keratinizing	6	Positive	Scattered cells
2023	42	F	Keratinizing	68 – 6	Positive	Scattered cells
2023	40	F	Keratinizing	59 – 6	Positive	Scattered cells
2023	63	F	Non-keratinizing	16	Positive	Scattered cells
2023	48	F	Keratinizing	16–45	Positive	Mid-epithelial
2023	45	F	Basaloid	45	Positive	Scattered cells
2023	35	F	Keratinizing	16	Positive	Scattered cells
2023	44	F	Keratinizing	35	Positive	Scattered cells
2023	42	M	Non-keratinizing	18	Positive	Scattered cells
2023	38	F	Non-keratinizing	33	Positive	Scattered cells
HPV-independent tumors						
2021	56	F	Keratinizing	Negative	Negative	Diffuse overexpression
2022	44	F	Keratinizing	Negative	Negative	Diffuse overexpression

Among 43 ASCC, 41 tumors (95.3%) showed diffuse, block type positive IHC staining for p16 and were, consequently considered as HPV-associated. Of these, 36 cases (83.7% of all tumors; 87.8% of HPV-associated tumors) showed concordant p16 positivity and HPV DNA detection, whereas 5 tumors (11.6% of all tumors; 12.2% of HPV-associated tumors) were p16-positive but high-risk HPV DNA-negative and were considered HPV-associated based on p16 overexpression, despite negative high-risk

HPV DNA testing. All HPV-associated cases showed wild type p53 IHC. The remaining 2 tumors (4.7%) were negative for both p16 and high-risk HPV DNA and were classified as HPV-independent. These two tumors showed a mutant type of p53 IHC expression (diffuse overexpression), and showed histologically a keratinizing pattern (Fig. 1, panel C-C'-C").

Genotype distributions and multiple infection rates were evaluated among the 36 HPV DNA-positive tumors. HPV16 was the most frequent genotype, detected in 20 cases (55.6%), occurring as a single infection in 13 tumors and in association with other HPV types in 7 cases. HPV types other than HPV16 were exclusively involved in 16 of 36 tumors (44.4%). HPV18 was identified in nine tumors (25.0%), including 7 single infections and 2 cases in association with HPV16. Multiple HPV infections were observed in 10 of 36 tumors (27.8%): eight tumors harbored two HPV types, and one tumor each harbored three and five HPV types.

Anal carcinomas in Spain

Twenty-nine ASCC were diagnosed in the HCB in the reference period. All of them were confirmed in the histological revision and all of them had available material for ancillary testing. Fifteen tumors (51.7%) occurred in women and 14 (48.3%) in men. The average age was 60.3 ± 12.9 (range 37 to 89 years). No differences in terms of age at diagnosis were observed between women and men (62.3 ± 11.0 vs. 58.3 ± 14.8; $p = 0.421$). The demographic, the histological and IHC features as well as the results of HPV detection of the cases from Spain are shown in Table 2.

Table 2
Anal squamous cell carcinomas from Spain in the period 2019–2023

Year	Age	Gender	Hist	HPV	p16	p53
HPV-associated tumors						
2019	85	F	Basaloid	16	Positive	Scattered cells
2019	49	M	Basaloid	16	Positive	Scattered cells
2019	51	F	Keratinizing	16	Positive	Scattered cells
2019	51	M	Warty	16–58	Positive	Scattered cells
2019	57	M	Keratinizing	16	Positive	Scattered cells
2019	47	F	Warty	16	Positive	Scattered cells
2019	81	M	Keratinizing	16	Positive	Scattered cells
2019	68	F	Keratinizing	16	Positive	Scattered cells
2020	48	F	Warty	52	Positive	Scattered cells
2020	69	M	Warty	16-52-66	Positive	Mid-epithelial
2020	65	F	Keratinizing	16	Positive	Diffuse overexpression
2020	56	F	Keratinizing	16	Positive	Scattered cells
2020	41	M	Non-keratinizing	16	Positive	Scattered cells
2020	60	F	Basaloid	16	Positive	Scattered cells
2021	89	M	Basaloid	51	Positive	Scattered cells
2021	64	F	Basaloid	16–33	Positive	Scattered cells
2021	61	M	Basaloid	16-45-53-11-44	Positive	Scattered cells
2021	70	F	Basaloid	16	Positive	Scattered cells
2022	50	M	Basaloid	16	Positive	Scattered cells
2022	54	M	Warty	16	Positive	Scattered cells
2022	77	F	Basaloid	16	Positive	Scattered cells
2022	68	F	Non-keratinizing	16–53	Positive	Scattered cells
2022	66	F	Basaloid	16	Positive	Scattered cells
2023	48	F	Basaloid	16	Positive	Scattered cells
2023	61	F	Keratinizing	16–66	Positive	Scattered cells
2023	48	M	Keratinizing	16–45	Positive	Scattered cells

Year	Age	Gender	Hist	HPV	p16	p53
2023	37	M	Warty	16–53	Positive	Scattered cells
2023	71	M	Basaloid	16 – 6	Positive	Scattered cells
HPV-independent tumors						
2019	58	M	Verrucous	Negative	Negative	Scattered cells

Among the 29 ASCC, 28 tumors (96.6%) showed diffuse, block-type p16 immunoreactivity and were classified as HPV-associated. All 28 p16-positive tumors demonstrated concordant HPV DNA detection (100% concordance). Almost all HPV-associated tumors showed a wild type p53 immunohistochemical staining pattern (28/29 cases, 96.6%), except for one case that exhibited diffuse p53 overexpression. One tumor (3.4%) was negative for both p16 and HPV DNA and was classified as HPV-independent; this tumor showed a wild type p53 staining pattern and displayed a keratinizing histological pattern (Fig. 1, B-B'-B").

Genotype distributions and multiple infection rates were evaluated among the 28 high-risk HPV DNA-positive tumors. HPV16 was the predominant genotype, detected in 26 cases (92.9%), occurring as a single infection in 17 tumors and in association with other HPV types in 9 cases. HPV types other than HPV16 were exclusively involved in 2 of 28 tumors (7.1%). Multiple HPV infections were identified in 9 of 28 tumors (32.1%): seven tumors harbored two HPV types, and one tumor each harbored three and five HPV types.

Differences between Mozambique and Spain

ASCC were less frequent among men in Mozambique than in Spain (7/43; 16.3%, vs. 14/29; 48.3%; p = 0.007). Patients in Mozambique were younger at diagnosis than those in Spain (44.1 ± 9.4 years old vs. 60.3 ± 12.6 years old; p < 0.001).

No significant differences were identified in the proportion of HPV-associated tumors (41/43, 95.3% vs. 28/29, 96.6%; p = 1.000), and or in the proportion of tumors harboring multiple HPV types among high-risk HPV-positive cases (10/36, 27.8% vs. 9/28, 32.1%; p = 0.7858).

HPV genotypes showed higher diversity in Mozambique compared with Spain. HPV types other than HPV16 were more frequently found as exclusively involved in ASCC in Mozambique than in Spain (16/36, 44.4% vs. 2/28, 7.1%, p = 0.0016).

The histological patterns of the HPV-associated tumors differed between Mozambique and Spain (p = 0.0118). In Mozambique, the tumors were predominantly keratinizing (21/41, 51.2%), followed by basaloid (11/41, 26.8%;), non-keratinizing (8/41, 19.5%), and warty subtypes (1/41, 2.4%). In contrast, basaloid tumors were the most frequent subtype in Spain (12/28, 42.9%), followed by keratinizing (8/28, 28.6%), warty (6/28, 21.4%), and non-keratinizing tumors (2/28, 7.1%).

Contribution of anal cancer to the burden of cancers of the anogenital tract

The distribution of malignancies across the different anatomical sites of the anogenital tract in Mozambique and Spain during the period 2019–2023 is summarized in Table 3. A total of 3,030 anogenital carcinomas were diagnosed at MCH in Mozambique compared with 356 at HCB in Spain. In absolute terms, all tumor types were more frequently recorded in Mozambique. However, the magnitude of these differences varied substantially by anatomical site. While cancers of the vulva, vagina, penis, and anal canal were approximately twofold higher in Mozambique, cervical cancer cases were more than thirteen times more frequent than those diagnosed in Spain. Importantly, when considering proportional distribution within each hospital-based series, cervical cancer accounted for the vast majority of anogenital malignancies in Mozambique (89.3%), whereas in Spain the distribution was more balanced, with cervical cancer representing 55.1% of cases. Consequently, the relative contribution of non-cervical anogenital cancers—including anal carcinoma—to the overall anogenital cancer burden was proportionally lower in Mozambique than in Spain. Accordingly, ASCC represented only a small fraction of the overall HPV-associated cancer burden in both sites, with a lower relative contribution in the series from Mozambique (8.1% vs. 1.8%), due to the overwhelming predominance of cervical cancer. The overall distribution of tumor sites differed significantly between countries (χ^2 test, $p < 0.001$).

Table 3
Burden of carcinomas of the anogenital tract in Mozambique and Spain in the period 2019–2023

Anatomical site	**Mozambique**		**Spain**	
	Absolute number	**(%)**	**Absolute number**	**(%)**
Uterine Cervix	2705	(89.3%)	196	(55.1%)
Vulva	161	(5.3%)	85	(23.9%)
Vagina	35	(1.2%)	19	(5.3%)
Penis	73	(2.4%)	27	(7.6%)
Anal Canal	56	(1.8%)	29	(8.1%)
Overall	**3030**	**(100%)**	**356**	**(100%)**

The distribution of anogenital carcinoma sites differed significantly between countries (χ^2 test, $p < 0.001$).

Discussion

This study provides valuable information on the epidemiology of ASCC in sub-Saharan Africa, a region where such data are particularly scarce. Our data reveal notable similarities in the proportion of ASCCs associated with HPV, independently of the geographical region (95.3% in Mozambique, 96.6% in Spain)

(4, 20, 21, 24). This contrasts with the wider geographic variability reported for other lower anogenital sites, such as the vulva and the penis (12–15), where the proportion of HPV-associated tumors was extremely high in Mozambique (97% in the vulva, 89% in the penis), but remarkably low in Spain (19% in the vulva, 29% in the penis). Despite this similarity in terms of percentage of HPV-associated tumors, Mozambican ASCCs showed significant differences: they occurred at a younger age, predominantly affected women, and showed greater HPV genotype diversity than those from Spain.

Indeed, in spite of the existing similarities, ASCC in Mozambique and Spain showed significant epidemiological differences. ASCC were less frequent in men in Mozambique compared with Spain (16.3% vs. 48.3%, $p = 0.0019$). The tumors occurred in younger patients in Mozambique than in Spain (44.1 ± 9.4 years old vs. 60.3 ± 12.6; $p < 0.001$). These differences may reflect underlying social and behavioral habits in the populations of the two countries: poor acceptance of homosexual behavior (25), early onset of sexual activity (26), and differences in HIV burden (27–29). Indeed, in many European settings, ASCC disproportionately affects men who have sex with men, who are at increased risk for persistent anal high-risk HPV infection and subsequent ASCC, potentially contributing to the higher proportion of male cases observed in Spain (30).

In keeping with previous data, HPV16 was the HPV type most frequently involved in ASCC in both countries (31). However, HPV types other than HPV16 were frequently found as exclusively involved in ASCC in Mozambique, with statistically significant differences compared with Spain (44.4% vs. 7.1%, $p = 0.0016$), a finding previously reported in other studies, which have shown higher HPV type variability in sub-Saharan Africa compared with other regions (22). This finding may be attributable to differences in the prevalence of HIV infection between Spain and Mozambique. Although the individual HIV status was not available for the Mozambican cases, HIV prevalence in Mozambique is reported to be as high as 12.5%, being particularly high in the Maputo urban area, where this study was conducted (28). These findings may have implications for vaccination strategies and post-vaccination surveillance in regions with high HIV burden. Finally, previous studies have shown an increased incidence of multiple HPV infections in women living with HIV (32–34). In our series, however, the frequency of multiple HPV infections did not differ significantly between Mozambique and Spain (27.8%, vs. 32.1%).

Although uncommon and represented by only a small number of cases, HPV-independent ASCC were identified in both cohorts (two in Mozambique and one in Barcelona) and showed two distinct p53 IHC patterns, which strongly correlate with the mutational status of the *TP53* gene (35, 36): diffuse overexpression consistent with *TP53* mutation and a wild type staining pattern. This supports emerging evidence that HPV-independent SCC may arise through heterogeneous molecular pathways rather than a single uniform mechanism. A similar dichotomy in SCC of the uterine cervix has recently been described by Stolnicu et al (37) and mirrors observations in vulvar and penile SCC (12–15). Although numbers are small, this distinction may be clinically relevant given the poorer prognosis reported for *TP53*-mutant HPV-independent tumors in other anogenital sites (16, 17), and warrants confirmation in larger series.

All cancers of the lower anogenital region were more frequent in absolute numbers in Mozambique than in Spain. However, the magnitude of this difference varied considerably by anatomical site. While cancers of the vulva, vagina, penis, and anal canal were approximately two- to threefold more frequent in Mozambique, cervical carcinoma was more than thirteen times more common, underscoring the disproportionate burden of HPV-related disease at this anatomical site in the African setting (2, 3). As a result of this extraordinary high frequency of cervical cancer, the percentual contribution of malignancies other than cancer of the uterine cervix to the overall number of tumors of the lower anogenital tract was significantly lower in Mozambique than in Spain. Indeed, the proportion of anal cancer in Mozambique was 1.8% compared to 8.1% in Spain, with similar patterns observed for other sites, ranging from 1.2% of vaginal tumors to 5.3% for vulvar cancer in Mozambique, compared with 5.3% and 23.9%, respectively, in Spain. This distribution reflects the dominant impact of cervical cancer on the anogenital oncologic landscape in Mozambique.

In Spain, p16 IHC and HPV DNA testing were fully concordant, whereas in Mozambique, five p16-positive tumors (12.2%) tested negative for HPV DNA. Although additional assays to further assess these discordant cases could have been used such as HPV E6/E7 mRNA testing, *in situ* hybridization, they were not performed, following the pragmatic approach used in our prior Mozambique–Spain comparative series of vulvar and penile SCC based on p16 IHC and SPF10/LiPA HPV DNA testing on FFPE tissue (14, 15). Moreover, p16 has specifically been evaluated as a surrogate marker of transcriptionally active HPV infection in ASCC. In a study comparing p16 immunohistochemistry with HPV E6/E7 mRNA expression in ASCC, Frankart et al. reported 96% concordance, with a sensitivity of 96%, specificity of 100%, and positive predictive value of 100% for p16 immunohistochemistry. Thus, although the evidence in the anal canal is more limited than for cervical carcinoma, available data support a strong association between block-type p16 expression and transcriptionally active HPV infection in ASCC (38). Indeed, similar discordances have been previously reported in other anogenital tumors, which have been attributed to poor HPV DNA preservation due to fixation- or processing-related degradation frequently detected in FFPE specimens rather than true HPV negativity (24). The predominance of HPV-associated histologic subtypes (basaloid, warty, non-keratinizing) and the wild type p53 pattern in these cases (12–15, 25) further support this interpretation. Indeed, as observed in other studies, virtually all HPV-associated tumors had a wild type pattern of expression of p53, which is consistent with the functional inactivation of p53 mediated by the HPV E6 protein (39).

The findings of this study should be interpreted with caution, as the sample size is small, a direct consequence of the rarity of ASCC in both populations, which precludes definitive conclusions. Nevertheless, despite this limitation, the results presented are methodologically rigorous, as uniform diagnostic criteria, validated molecular techniques, and quality controls in histopathological and immunohistochemical interpretation have been applied. Therefore, the differences observed between Mozambique and Spain, although preliminary, provide relevant insights into the epidemiology of ASCC in these two distinct geographic and socioeconomic contexts. In addition, this was a retrospective, hospital-based study rather than population-based incidence rates. HIV-related information was not available in the Mozambican series, precluding adjustment for HIV infection and limiting the

interpretation of clinical differences such as age, sex distribution, and HPV genotype differences. Relevant clinical data (e.g., tumor stage, treatment details, outcome/survival) were also unavailable, preventing any assessment of prognostic differences. Larger multicenter studies are warranted.

In summary, our findings show that ASCC is overwhelmingly HPV-driven both in Mozambique and Spain, regardless of the marked differences in demographic and epidemiological background between the two settings. The younger age at diagnosis and the broader HPV genotype distribution observed in Mozambique may reflect distinct epidemiological conditions, potentially influenced by differences in HIV prevalence and patterns of HPV exposure. These observations underscore the need for region-specific surveillance strategies and reinforce the importance of sustained HPV vaccination programs and HIV control efforts in reducing the burden of HPV-related malignancies worldwide.

Abbreviations

AIN
Anal intraepithelial neoplasia
ANOVA
Analysis of variance
ASCC
Anal squamous cell carcinoma
FFPE
Formalin—fixed paraffin—embedded
HIV
Human immunodeficiency virus
HPV
Human papillomavirus
HR
HPV—High—risk human papillomavirus
HSIL
High—grade squamous intraepithelial lesion
IANS
International Anal Neoplasia Society
IARC
International Agency for Research on Cancer
IHC
Immunohistochemistry
LiPA
Line probe assay
SCC
Squamous cell carcinoma
SD

Standard deviation
SPF10
Short PCR fragment 10
WHO
World Health Organization

Declarations

Disclaimer

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. The views expressed in this article are solely those of the authors.

Conflict of Interest Statement

None of the authors has any conflict of interest to declare related to the present project.

Ethics Statement

The study was approved by the ethics committees of the Faculty of Medicine of the Eduardo Mondlane University and Maputo Central Hospital (CIBS FM&HCM/071/2017) and Hospital Clínic de Barcelona (HCB/2023/0133). The requirement for informed consent was waived by the corresponding ethics committees due to the retrospective nature of the study and the use of anonymized/de-identified data and archived tissue samples.

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Author Contribution

I.B. Acquisition, analysis, and interpretation of data. Drafted the manuscript and approved the submitted version.J.L-D. Acquisition, analysis, and interpretation of data. Drafted the manuscript and approved the

submitted version.N.P. Analysis, and interpretation of data, revision of the manuscript. Approval of the submitted version.N.R. Conception and design of the work; interpretation of data, revision of the manuscript. Approval of the submitted version.L.M. HPV detection, immunohistochemical stains, revision of the manuscrit. Approval of the submitted version.I.M. Acquisition, analysis, and interpretation of data. Drafted the manuscript and approved the submitted version.L.L. Acquisition, analysis, and interpretation of data, revision of the manuscript. Approval of the submitted version.K.D. Analysis, and interpretation of data, revision of the manuscript. Approval of the submitted version.S.V. Analysis, and interpretation of data, revision of the manuscript. Approval of the submitted version.A.G. HPV detection, immunohistochemical stains, revision of the manuscript. Approval of the submitted version.F.F. Acquisition, analysis, and interpretation of data, revision of the manuscript. Approval of the submitted version.M.R.I. Acquisition, analysis, and interpretation of data, revision of the manuscript. Approval of the submitted version.C.C. Acquisition, analysis, and interpretation of data, revision of the manuscript. Approval of the submitted version.J.O. Conception and design of the work; interpretation of data, revision of the manuscript. Approval of the submitted version.M.D.P. Conception and design of the work; interpretation of data, revision of the manuscript. Approval of the submitted version.A.S. Conception and design of the work; interpretation of data, revision of the manuscript. Approval of the submitted version.

Data Availability

De-identified data are available from the corresponding author on reasonable request and subject to approval by the institutional ethics committee.

References

1. WHO Classification of Tumours Editorial Board. Digestive system tumours [Internet]. 5th ed. WHO classification of tumours series, editor. Vol. 1. Lyon (France): International Agency for Research on Cancer; 2019 [cited 2025 Dec 21]. Available from: https://tumourclassification.iarc.who.int/chapters/31
2. International Agency for Research on Cancer (IARC). GLOBOCAN 2022: Mozambique and Spain – Cancer Today. Lyon, France: World Health Organization. [Internet]. 2023. (2023). Available from: https://gco.iarc.who.int
3. Sung, H. et al. Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. [Internet]. (2024). Available from: https://gco.iarc.who.int
4. de Martel, C., Georges, D., Bray, F., Ferlay, J. & Clifford, G. M. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. *Lancet Glob Health*. **8** (2), e180–e190 (2020).
5. Clifford, G. M. et al. A meta-analysis of anal cancer incidence by risk group: Toward a unified anal cancer risk scale. *Int. J. Cancer*. **148** (1), 38–47 (2021).
6. Donà, M. G. et al. Anal Cytological Lesions and HPV Infection in Individuals at Increased Risk for Anal Cancer. *Cancer Cytopathol.* **126** (7), 461–470 (2018).

7. Deshmukh, A. A. et al. Global burden of HPV-attributable squamous cell carcinoma of the anus in 2020, according to sex and HIV status: A worldwide analysis. *Int. J. Cancer*. **152** (3), 417–428 (2023).
8. Dyer, C. E. F. et al. Anal cancer screening in men who have sex with men living with HIV: The pilot of anal screening study (PASS). *HIV Med.* (2025).
9. Fuertes, I. et al. A cross-sectional pilot study to define anal cancer risk factors in HIV-positive solid organ transplant recipients. Infect Dis [Internet]. ;1–11. Available from: https://www.tandfonline.com/doi/full/ (2025). 10.1080/23744235.2025.2573412
10. Bruni, L. et al. ICO HPV Information Centre ICO/IARC Information Centre on HPVand Cancer (HPV Information Centre). Human Papillomavirus and Related Diseases in the World. Summary Report 17 June 2019. (2019).
11. Alemany, L. et al. Human papillomavirus DNA prevalence and type distribution in anal carcinomas worldwide. *Int. J. Cancer*. **136** (1), 98–107 (2015).
12. Alemany, L. et al. Role of Human Papillomavirus in Penile Carcinomas Worldwide. *Eur. Urol.* **69** (5), 953–961 (2016).
13. Rakislova, N. et al. Histological characteristics of HPV-associated and -independent squamous cell carcinomas of the vulva: A study of 1,594 cases. *Int. J. Cancer*. **141** (12), 2517–2527 (2017).
14. Rakislova, N. et al. Differential etiopathogenic features of vulvar squamous cell carcinomas in sub-Saharan Africa and Europe. *Int. J. Cancer*. **152** (3), 496–503 (2023).
15. Manzotti, C. et al. Penile Squamous Cell Carcinomas in Sub-Saharan Africa and Europe: Differential Etiopathogenesis. *Cancers (Basel)*. **14**, 21 (2022).
16. de Sanjose, S. et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol.* **11** (11), 1048–1056 (2010).
17. Sethi, N. *Parallels and divergence in head and neck and cervical cancer. BJOG: An International Journal of Obstetrics and Gynaecology* p. 128 (Blackwell Publishing Ltd, 2015).
18. Nicolás, I. et al. HPV-negative tumors of the uterine cervix. *Mod. Pathol.* **32** (8), 1189–1196 (2019).
19. Pinto, A. et al. TP53 Wild-Type, Human Papillomavirus-Independent Anal Growth/(Intra)Epithelial Lesion (ANGEL): A Potential Precursor of Anal Squamous Cell Carcinoma. *Mod. Pathol.* **38** (5), 100721 (2025).
20. de Sanjosé, S. D. M. et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis. ;2007;7(7):453-9. (2007).
21. del Pino, M. et al. Value of p16INK4a as a marker of progression/regression in cervical intraepithelial neoplasia grade 1. Am J Obstet Gynecol. ;201(5):488.e1-488.e7. (2009).
22. Kaderly Rasmussen, E. L. et al. Six-pattern p53 interpretation in 1293 vulvar squamous cell carcinomas: inter-pathologist variation and pattern distribution according to p16 status. *Histopathology* **87** (6), 815–826 (2025).

23. Nicolás, I. et al. HPV-negative tumors of the uterine cervix. *Mod. Pathol.* **32** (8), 1189–1196 (2019).
24. Alemany, L. et al. Articles Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol. [Internet]*. **11**, 1048–1056 (2010). Available from: www.thelancet.com/oncology.
25. Bailey, J. M. et al. Sexual orientation, controversy, and science. *Psychol. Sci. Public. Interest.* **17** (2), 45–101 (2016).
26. Mekonnen, A. G. & Mittiku, Y. M. Early-onset of sexual activity as a potential risk of cervical cancer in Africa: A review of literature. PLOS Global Public Health. Public Library of Science; (2023).
27. Asangbeh-Kerman, S. L. et al. Cervical cancer prevention in countries with the highest HIV prevalence: a review of policies. *BMC Public. Health* ;**22**(1). (2022).
28. Gudo, E. S. et al. HIV incidence and prevalence among adults in Mozambique: estimates from the Population-based HIV Impact Assessment Survey (INSIDA 2021) and district-level modelling. *J. Int. AIDS Soc.* ;**28**(11). (2025).
29. Birdthistle, I. et al. Recent levels and trends in HIV incidence rates among adolescent girls and young women in ten high-prevalence African countries: a systematic review and meta-analysis. *Lancet Glob Health*. **7** (11), e1521–e1540 (2019).
30. Ebrahimi, F., Rasizadeh, R., Jafari, S. & Baghi, H. B. Prevalence of HPV in anal cancer: exploring the role of infection and inflammation. *Infect. Agent Cancer*. **19** (1), 63 (2024).
31. Alemany, L. et al. Human papillomavirus DNA prevalence and type distribution in anal carcinomas worldwide. *Int. J. Cancer*. **136** (1), 98–107 (2015).
32. Asangbeh-Kerman, S. L. et al. Cervical cancer prevention in countries with the highest HIV prevalence: a review of policies. *BMC Public. Health* ;**22**(1). (2022).
33. Birdthistle, I. et al. Recent levels and trends in HIV incidence rates among adolescent girls and young women in ten high-prevalence African countries: a systematic review and meta-analysis. *Lancet Glob Health*. **7** (11), e1521–e1540 (2019).
34. Levi, J. E. et al. High prevalence of human papillomavirus (HPV) infections and high frequency of multiple HPV genotypes in human immunodeficiency virus-infected women in Brazil. *J. Clin. Microbiol.* **40** (9), 3341–3345 (2002).
35. Wei, K. et al. Proficiency Testing of p53 Immunohistochemistry Pattern Read-out in Vulvar Biopsies Demonstrates Frequent Basal Overexpression Interpretation in TP53 Wild-type Cases. *Int. J. Gynecol. Pathol.* **44(6)** (Nov 1), 485–495 (2025).
36. Yang, H. et al. Classification of Vulvar Squamous Cell Carcinoma and Precursor Lesions by p16 and p53 Immunohistochemistry: Considerations, Caveats, and an Algorithmic Approach. *Mod. Pathol.* ;**36**(6). (2023).
37. Stolnicu, S. et al. Cervical Human Papillomavirus–Independent Squamous Cell Carcinoma: A Clinicopathological Review and Outcomes Analysis Compared With Human Papillomavirus–Associated Squamous Cell Carcinoma. *Mod. Pathol.* **38** (6), 100742 (2025).

38. Frankart, A. J. et al. Assessing the Reliability and Positive Predictive Value of p16 as a Surrogate for Human Papillomavirus-Mediated E6/7 mRNA Expression in Squamous Cell Carcinoma of the Anal Canal. Dis Colon Rectum. ;64(4):459–465. (2021). 10.1097/DCR.0000000000001836. PMID: 33394778.
39. Kaufmann, A. M., Backsch, C., Schneider, A. & Dürst, M. HPV induced cervical carcinogenesis: molecular basis and vaccine development. *Zentralbl Gynakol*. **Nov;124** (11), 511–524 (2002).

Figures

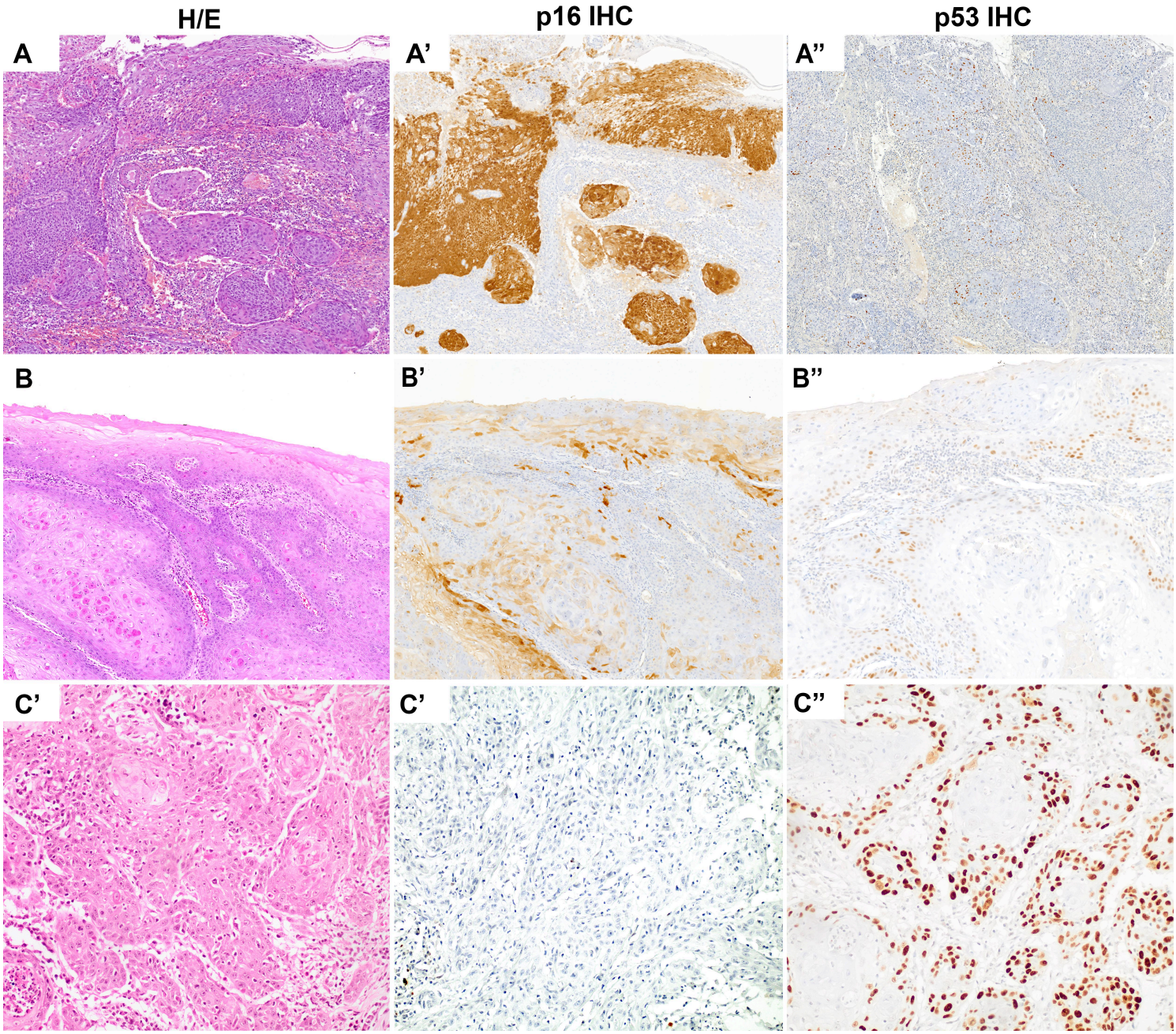


Figure 1

Histologic (hematoxylin and eosin), p16 and p53 IHC expression patterns in a case of human papillomavirus (HPV)-associated anal squamous cell carcinoma (ASCC) (A), a HPV-independent ASCC with wild type p53 (case B) and a HPV-independent ASCC with mutant type p53 (case C). A, B and C, hematoxylin and eosin. A', B', and C', p16 immunohistochemistry (IHC). A", B", and C", p53 IHC.