

RESEARCH ARTICLE

Cancer Therapy and Prevention

High-Risk Human Papillomavirus Prevalence and Detection of Anal Intraepithelial Neoplasia in Women Undergoing Screening Colonoscopy: A Prospective Study

Carlo Vivaldi¹ | Hanna Schmitten¹ | Matthias Schmid² | Hartmut Schäfer¹ 

¹Practice of Proctology and Endoscopy, Pan Clinic, Cologne, Germany | ²University of Bonn, University Hospital Bonn, Institute for Medical Biometry, Informatics and Epidemiology, Bonn, Germany

Correspondence: Hartmut Schäfer (h.schaefer@enddarmpraxis-koeln.de)

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ABSTRACT

The incidence of anal cancer in older women has risen significantly in recent decades, yet no standard screening strategy exists. Screening aims to detect potentially precancerous lesions, anal intraepithelial neoplasia (AIN), which can be treated to prevent cancer progression. In this prospective study, we assessed the prevalence of high-risk human papillomavirus (hrHPV), abnormal anal cytology, and AIN confirmed by high-resolution anoscopy (HRA)-guided biopsy. Women aged ≥ 40 undergoing routine screening colonoscopy in Cologne, Germany, were enrolled. Before colonoscopy, anal samples were collected for hrHPV testing and cytology. Participants with positive hrHPV and/or abnormal cytology underwent HRA with biopsy and treatment if necessary. Among 483 women (November 2023–March 2025), hrHPV was detected in 78 (16.1%), while cytological abnormalities were found in only 6 (1.2%). In hrHPV-positive women, HRA identified low-grade squamous intraepithelial lesion (LSIL) in 11 (14.1%) and high-grade squamous intraepithelial lesion (HSIL) in 6 (7.7%); all lesions were treated with argon plasma coagulation. Anal symptoms (bleeding, pain, or pruritus) were associated with hrHPV positivity (adjusted PRR: 1.72; $p = 0.023$). Among hrHPV-positive participants, infection with multiple hrHPV types was linked to AIN (adjusted PRR: 2.90; $p = 0.017$). In conclusion, hrHPV prevalence was relatively high, while cytology alone showed limited sensitivity for detecting AIN. Identification and treatment of high-grade lesions, potentially precursors to anal cancer, suggest that screening could help prevent disease progression and support incorporating anal hrHPV testing into preventive strategies for women.

1 | Introduction

From an epidemiological perspective, anal cancer represents a relatively rare malignancy, with an estimated annual incidence of approximately two new cases per 100,000 individuals. Among women over the age of 45, however, the incidence has markedly

increased over the past two decades and has more than doubled during this period [1].

The pathogenesis of anal carcinoma and its precursor lesions is well established and primarily attributed to infection of the anal canal epithelium with human papillomavirus (HPV).

Abbreviations: AIN, anal intraepithelial neoplasia; ASC-US, atypical squamous cells of undetermined significance; DNA, deoxyribonucleic acid; HIV, human immunodeficiency virus; HRA, high resolution anoscopy; hrHPV, high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; IANS, International Anal Neoplasia Society; LAST, lower anogenital squamous terminology; LSIL, low-grade squamous intraepithelial lesion.

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What's New?

Anal cancer rates are rising, particularly among older women, driven largely by persistent high-risk human papillomavirus (hrHPV) infections that the aging immune system clears less effectively. This study evaluated the role of hrHPV testing and cytology in helping improve early detection of anal intraepithelial neoplasia (AIN) among women ages 40 and older undergoing routine colonoscopy. HrHPV was found in 16.1% of participants, whereas cytological abnormalities occurred in just over 1%. Lesions in hrHPV-positive women were treated using argon plasma coagulation. The findings suggest that hrHPV-based AIN screening improves anal lesion detection, facilitating anal cancer prevention for older women.

HPV is a well-recognized oncogenic virus capable of inducing malignant transformation in various epithelial tissues. High-risk HPV genotypes infect the basal epithelial cells within the transformation zone, where they can integrate into the host genome and disrupt normal cellular regulatory pathways. In the setting of immune compromise, for example, due to human immunodeficiency virus (HIV) infection or immunosuppressive therapy following solid organ transplantation, viral clearance is impaired, predisposing to persistent HPV infection. Chronic infection may consequently progress to anal intraepithelial neoplasia (AIN) and, in some cases, invasive anal carcinoma [2].

Moreover, the natural clearance of HPV infection from the anal canal declines with age, leading to a higher likelihood of persistent infection. This persistence can promote the development of dysplastic cellular changes or precancerous lesions, even in individuals without identifiable risk factors [3, 4].

As with anal HPV infection, most investigations of AIN have focused on women at increased risk for anal cancer, including those with HIV infection, other immunosuppressive conditions, or HPV-associated lesions of the cervix or vulva [4, 5].

In contrast, women without known risk factors, typically younger cohorts examined as control groups, have shown abnormal anal cytology findings, such as atypical squamous cells of undetermined significance (ASC-US) or low-grade lesions, in only 1%–6% of cases, while high-risk HPV infection has been detected in 5%–22% of women without genital pathology [5]. High-resolution anoscopy (HRA) was not performed in these studies.

Screening strategies for the early detection of AIN are essential to prevent the development of anal cancer, which carries a poor prognosis, particularly in advanced stages. The effectiveness of such screening combined with appropriate treatment has been demonstrated in a randomized trial among men who have sex with men living with HIV [6].

Compared with the extensive research on the detection of high-risk human papillomavirus (hrHPV) in the gynecologic setting,

data on the prevalence, incidence, and risk factors of anal cancer among women without recognized risk factors remain scarce [5, 7]. To our knowledge, no prospective studies have investigated these associations among older women.

The objective of this study was to identify pathological findings in anal cytology and/or hrHPV testing and to determine the prevalence of high grade intraepithelial lesions (HSIL) in a population-based cohort. We analyzed a consecutive series of women undergoing preventive colonoscopy in a specialized endoscopy and proctology practice. Anal swabs were collected for hrHPV testing and cytology, complemented by patient medical history including age, smoking status, HIV infection, and gynecologic neoplasia or precancerous lesions.

2 | Materials and Methods

2.1 | Study Design and Participants

Women who presented for preventive colonoscopy between November 2023 and February 2025 were invited to participate. Before colonoscopy, an anal brush smear was obtained for cytology and hrHPV testing.

Clinical data recorded included patient age, smoking status, HIV serostatus, and history of cervical or vaginal intraepithelial neoplasia.

2.2 | Cytopathologic Examination and hrHPV Determination

Anal cytology was classified as low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), ASC-US, or carcinoma.

Detection of hrHPV DNA was performed using real-time polymerase chain reaction (GeneXpert IV, Cepheid). The assay identified HPV 16, HPV 18/45, and 11 additional high-risk genotypes (31, 33, 35, 39, 51, 52, 56, 58, 59, 66, 68).

2.3 | High Resolution Anoscopy and Histologic Examination

Patients with abnormal cytology and/or hrHPV positivity underwent HRA. The procedure was performed by three trained surgeons in accordance with International Anal Neoplasia Society (IANS) criteria using the Proctostation system (THD, Italy) [8, 9]. Mucosal changes suspicious for dysplasia were biopsied and after histopathologic evaluation treatment was performed, if necessary, in a subsequent session.

Lesions were graded as AIN 1–3 according to the depth of epithelial dysplasia. P16 immunohistochemistry was applied to AIN 2 samples. Based on Lower Anogenital Squamous Terminology (LAST), LSIL was defined as AIN 1 or p16-negative AIN 2, whereas HSIL included p16-positive AIN 2 and AIN 3 [10]. HSIL lesions were treated with argon plasma coagulation after application of 5% acetic acid and Lugol's solution to delineate dysplastic

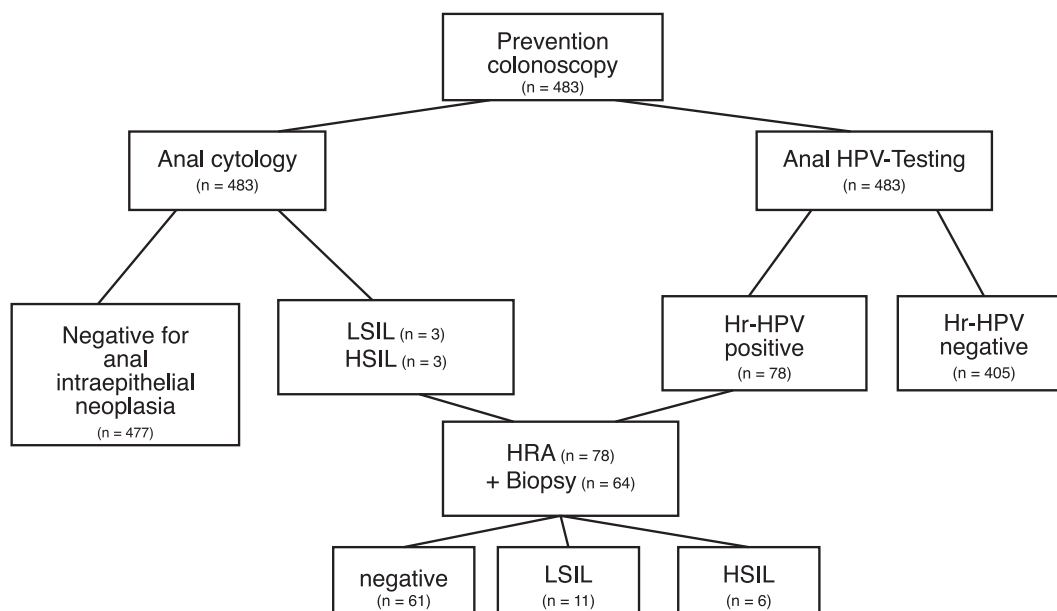


FIGURE 1 | Examination flow: 4 of 6 intraanal neoplasia in cytology were also positive in hrHPV; 1/3 LSL and 1/3 HSIL form cytology were not available for high resolution anoscopy, (patients with LSIL were routinely followed up and patients with HSIL were ablated using argon plasma coagulation).

TABLE 1 | Frequency of anal symptoms at study inclusion.

Symptom	n (%)
None	409 (84.7)
Itching	16 (3.3)
Bleeding	34 (7.0)
Weeping/pain/foreign body sensation	16 (3.3)
More than one symptom	8 (1.7)

areas. Follow-up was performed according to patient risk profiles following both IANS and German-Austrian anal neoplasia guidelines [8, 11] (Figure 1).

2.4 | Statistical Analysis

All analyses were performed using R software, version 4.5.2 [12]. Continuous variables were summarized as means with standard deviations (SDs), and categorical variables as absolute frequencies with percentages.

Associations between hrHPV infection and patient characteristics were explored using univariable Poisson regression models to estimate prevalence risk ratios (PRRs) with 95% confidence intervals (CIs). Multivariable Poisson regression models including age, cervical or vaginal neoplasia, smoking status, and presence of anal symptoms were used to calculate adjusted PRRs [13].

The relationship between biopsy-proven AIN (LSIL and HSIL) and multiple hrHPV infections (HPV 16 plus other or HPV 18/45 plus other genotypes) was examined using a separate multivariable Poisson model adjusted for age, smoking, and

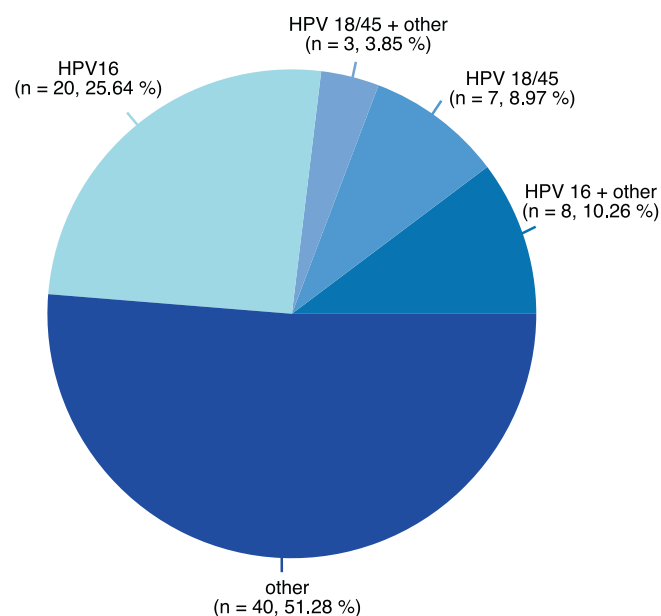


FIGURE 2 | HrHPV (n = 78); other subtypes = 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, 68. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/ijc.7077)]

anal symptoms. Robust standard errors were used to compute 95% CIs. A two-sided p value <0.05 was considered statistically significant.

3 | Results

3.1 | Patient Characteristics

A total of 483 women were included between November 2023 and March 2025. The mean age was 58.7 ± 8.97 years. Forty-four (9.1%) participants reported a history of cervical intraepithelial

neoplasia grade 3 and one (0.2%) had previous cervical carcinoma. One woman (0.2%) was HIV-positive and on antiviral therapy, and 41 (8.5%) were current smokers.

Most participants (409; 84.7%) reported no anal symptoms, while 74 (15.3%) described one or more, including bleeding (7.0%), itching (3.3%), pain, weeping, or a foreign-body sensation (3.3%) (see Table 1).

3.2 | HrHPV, Anal Cytology and Histology

High-risk HPV was detected in 78 women (16.1%). Among these, HPV 16 alone was present in 20 (25.6%), other hrHPV types in 40 (51.3%), HPV 16 plus additional types in 8 (10.3%), and HPV 18/45 plus additional types in 7 (9.0%) (see Figure 2).

Anal cytology identified 6 (1.2%) abnormal results (3 LSIL, 3 HSIL). HRA was performed in 78 of 80 women (97.5%) with positive hrHPV and/or abnormal cytology; two declined the procedure. Mucosal changes suspicious for dysplasia were visualized in 45 (57.7%) cases, with biopsies obtained. Histopathology revealed LSIL in 11 (14.1%), HSIL in 6 (7.7%), intra-anal condyloma in 3 (3.9%), and normal mucosa in 33 (42.3%, see Figure 3).

3.3 | Cytology

Across the entire cohort, 477 (98.8%) cytology findings were normal. Among women with abnormal cytology ($n=6$), four (66.7%) had concurrent hrHPV infection. In two cases, AIN I

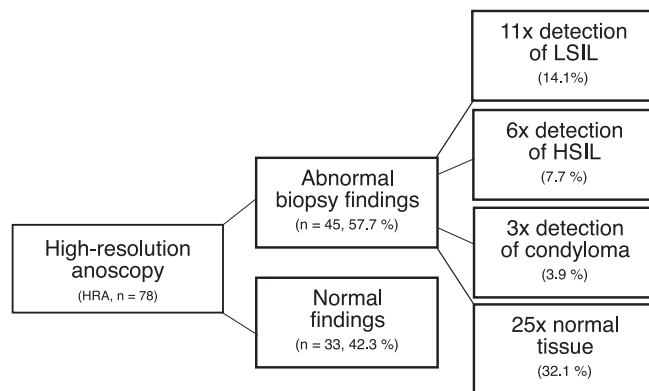


FIGURE 3 | Distribution of patients with high-resolution anoscopy ($n=78$).

TABLE 2 | Abnormal anal cytology, hrHPV, and histologic results.

Cytopathology	HrHPV	HRA	Histology
LSIL	18 and other+	Abnormal	AIN I (LSIL)
HSIL	16 and other+	Abnormal	Normal Mucosa
HSIL	16+	Abnormal	AIN I (LSIL)
LSIL	HPV neg.	No HRA	—
HSIL	18+	Abnormal	Normal mucosa
LSIL	HPV neg.	Normal	—

was confirmed histologically following HRA (see Table 2). In addition, all patients with a positive cytology result (three cases of HSIL) and concurrent detection of high-risk HPV, but without HSIL histology, were referred for repeat HRA in 1 year.

3.4 | Correlation Between hrHPV and Clinical Parameters

Univariable analyses demonstrated no associations between hrHPV infection and age, smoking, or prior cervical pathology. In contrast, anal symptoms were positively associated with hrHPV positivity (PRR: 1.66; 95% CI: 1.04–2.64) (Table 3).

Multivariable Poisson regression confirmed a significant association between anal symptoms and hrHPV infection (adjusted

TABLE 3 | Univariable associations with hrHPV infection.

	Est. prev. ratio	95% confidence interval
Cervical pathology y/n	0.81	[0.37; 1.76]
Anal symptoms y/n	1.66	[1.04; 2.64]
Smoking y/n	1.41	[0.76; 2.60]
Age < 50 vs. ≥ 50 years	1.00	[0.57; 1.76]
Age < 60 vs. ≥ 60 years	0.90	[0.60; 1.35]
Age < 70 vs. ≥ 70 years	0.74	[0.44; 1.24]
Age < 80 vs. ≥ 80 years	0.48	[0.15; 1.51]

TABLE 4 | Multivariable analysis of factors associated with hrHPV infection (adjusted prevalence risk ratios from Poisson regression).

	Est. prev. ratio	95% confidence interval
Intercept	0.06	[0.02; 0.26]
Age [years]	1.01	[0.99; 1.04]
Symptoms y/n	1.72	[1.08; 2.73]
Cervical pathology y/n	0.83	[0.38; 1.80]
Smoking y/n	1.56	[0.84; 2.88]

PRR: 1.72; 95% CI: 1.08–2.73; $p=0.023$) after adjusting for age, smoking, and cervical pathology (Table 4).

Among HRA-examined women ($n=78$), multiple hrHPV infections were independently associated with biopsy-confirmed AIN (adjusted PRR: 2.90; 95% CI: 1.21–6.96; $p=0.017$), while no other factors were statistically significant.

4 | Discussion

Anal cancer incidence among women has increased markedly, yet population-based screening strategies remain absent. Incorporating AIN screening into preventive colonoscopy targets the age group at highest risk and leverages an existing, well-established screening framework. This approach also complements gynecologic screening, where anal assessment is not routinely implemented [14]. To our knowledge, this is the first study to assess routine anal cytology and anal hrHPV testing during screening colonoscopy in an older female population. The aim was to determine the prevalence of hrHPV positivity, abnormal anal cytology, and AIN in this cohort of 483 women (mean age 58.7 years).

hrHPV was detected in 78 of 483 women (16.1%), whereas abnormal anal cytology was identified in only 6 cases (1.2%; 3 LSIL and 3 HSIL). Because data on anal HPV infection in older women are limited, comparisons must largely rely on studies conducted in younger populations. In the Hawaii Cohort Study, a large cross-sectional analysis of 1363 healthy women with a mean age of 38 years, anal HPV infection was detected in 26% of participants, including oncogenic HPV types in 13.1% [15]. In the same study, HPV 16 was detected in 26 participants (7.0%). A similarly high prevalence of anal hrHPV infection was reported in another study of partially vaccinated individuals ($n=645$; mean age 18 years), in which 42% tested positive [16]. However, the proportion of oncogenic HPV infections in that study was low (2.0%). In our cohort, HPV 16 accounted for 35.8% of hrHPV infections, meaning that more than one-third of hrHPV-positive women carried this genotype. This high prevalence of HPV 16 highlights the need for further diagnostic evaluation to rule out anal neoplasia. Anal hrHPV infection and anal neoplasia occur more frequently in established risk groups, including women with prior lower genital tract neoplasia, transplant recipients, individuals living with HIV, smokers, and patients with other immunocompromising conditions. These groups are therefore included in current screening recommendations issued by scientific societies [8, 17].

In our cohort, neither univariable nor multivariable analyses demonstrated significant associations between the investigated risk factors and hrHPV infection.

This may be attributable to the limited number of women in the respective risk groups, particularly those with a smoking history or prior lower genital tract neoplasia.

By contrast, anal symptoms were significantly associated with hrHPV positivity in both analyses. This observation is not easily explained, as HPV infection is generally asymptomatic, although

concurrent conditions such as genital warts, hemorrhoids, or other benign anal disorders may account for symptoms such as bleeding, pruritus, or pain. On the other hand, the anal conditions described can lead to disruption of the mucosal barrier and thus facilitate infection with high-risk papillomaviruses.

HrHPV infection was also more frequent in women aged 70–80 years and 80–90 years (Table 3). Other established risk factors, including prior lower genital tract neoplasia, HIV infection, and immunosuppression, were too infrequent in our cohort to permit meaningful statistical evaluation.

In a pooled analysis of 26 studies, Wei et al. showed that anal HPV 16 infection persists longer and later in life than cervical HPV infection in women. In line with this observation, hrHPV infection in our cohort was more common among women aged 70–90 years than among younger age groups [18].

Six cases of AIN were identified by brush cytology, including three LSIL and three HSIL lesions; in two cases, the diagnosis was confirmed by HRA. The low number of abnormal cytologic findings despite a comparatively high prevalence of hrHPV infection is consistent with previous reports showing substantial variability in the sensitivity, specificity, positive predictive value, and negative predictive value of anal cytology. This variability has been attributed in part to differences in sampling technique and to the level of expertise of cytopathologists in the interpretation of anal cytology [19, 20].

In our study, anal cytology was performed as routine clinical care rather than in a specialized center for anal cytopathology. It seems unlikely that bowel preparation before colonoscopy adversely affected sample adequacy, particularly as the smears showed high cellularity. Nevertheless, the very low proportion of intraepithelial neoplasia detected by cytology, even in comparison with previous reports, suggests that methodological limitations cannot be excluded.

This finding is consistent with data on anal cytology, which showed that the rate of technically unsuitable samples was significantly higher among HIV-negative women (15%) than among men living with HIV (5%) [9].

A major strength of our study is that HRA was performed in all women who tested positive for hrHPV. Patients who tested negative for high-risk HPV did not undergo HRA; consequently, we might have underestimated the prevalence of HSIL if women who had false negative HPV testing for hrHPV and did not undergo HRA. Among the 78 women who underwent HRA, HSIL was detected in 6 (7.7%) with a median age of 58.7 years. Given that HSIL represents a direct precursor of malignant transformation, its detection and removal are clinically warranted [6].

As no directly comparable population has been described in the literature, reference must be made to screening cohorts with established risk profiles for anal neoplasia. A recent meta-analysis reported an overall HSIL prevalence of 20%, ranging from 22% in men who have sex with men living with HIV to 13% in women and 12% in men who have sex with men without HIV [21]. In another study of 327 women with a history of lower genital tract

neoplasia, the prevalence of HSIL (AIN 2/3) was 8.5%, compared with 1.2% in our cohort [22].

It appears clear that the stand-alone HPV screening method has equivalent sensitivity (92%) and specificity (42%) for the detection of anal premalignant lesions to combined screening using cytology and HPV typing (sensitivity 93%, specificity 33%) [21, 23, 24]. This trend is also reflected in the comments in the current guidelines on AIN [8, 17]. Whether anal cytology can be dispensed with in favor of HPV typing as a screening method will ultimately depend on the availability of the methods.

Our study demonstrated a significant association between multiple hrHPV infections and the detection of HSIL/LSIL. These findings raise the question of whether AIN screening, preferably using hrHPV PCR testing, could be meaningfully integrated into preventive colonoscopy.

Notably, high-grade colorectal polyps, established precursors of colorectal cancer, are detected in 2.4% of routine colonoscopies, compared with 1.2% for high-grade intraepithelial neoplasia in our cohort [25]. When hrHPV testing was performed before screening colonoscopy, the prevalence of HSIL in our cohort increased to 7.7%. The addition of anal swab sampling for hrHPV to routine colonoscopy has the potential to identify women at risk for HSIL and possible anal cancer.

5 | Conclusion

These findings support our hypothesis that AIN screening integrated into colonoscopy, using hrHPV pretesting, may facilitate the detection and treatment of anal cancer precursor lesions and, thereby, contribute to the prevention of anal carcinoma.

Author Contributions

Carlo Vivaldi: writing – original draft, investigation, visualization, formal analysis, project administration. **Hanna Schmitten:** investigation, writing – review and editing. **Matthias Schmid:** conceptualization, formal analysis, data curation, visualization, writing – review and editing. **Hartmut Schäfer:** conceptualization, investigation, writing – original draft, visualization, formal analysis, project administration, data curation, supervision.

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Ethics Statement

The study protocol was approved by the local ethics committee (University of Cologne, approval number: 25-1114) and conducted in accordance with the revised Declaration of Helsinki (2013).

Consent

Written informed consent was obtained, and all participants received information regarding data collection and study procedures.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Pseudonymized data that support the findings of this study are available from the corresponding author upon reasonable request and subject to approval from relevant authorities. All source code is publicly available on GitHub (<https://github.com/matthiasschmid25/neoplasia>). Pseudonymized data that support the findings of this study can be available upon request subject to institutional approval under the data sharing policy. Further information is available from the corresponding author upon reasonable request.

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