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Full length article

Prevalence of high-grade anal intraepithelial neoplasia in immunocompetent women treated for high-grade cervical intraepithelial neoplasia

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ABSTRACT

Background: The aim of the study was to evaluate the prevalence of high-grade anal intraepithelial neoplasia (AIN2-3) among immunocompetent women treated for high-grade cervical intraepithelial neoplasia (CIN2-3). Such knowledge is strongly needed to establish whether a screening program should be recommended in this group of patients.

Methods: This prospective study included a cohort of consecutive women with no known causes of immunosuppression treated with LEEP (loop electrosurgical excision procedure) for a histopathological diagnosis of CIN2-3 in our center between 2019 and 2021. Following the procedure, all patients were invited to undergo anal cytology and anal high-risk HPV-DNA testing (aHPV-DNA). In cases in which one or both tests resulted positive, a high-resolution anoscopy with a biopsy of suspicious lesions was performed. All women also completed a questionnaire on sexual habits.

Results: At total of 100 women were enrolled in the study. Among these, eight patients had a concomitant or past diagnosis of anogenital warts, while one patient had received a previous diagnosis of high-grade vaginal intraepithelial neoplasia. Anal Pap smears were positive for low-grade lesions in three patients, while 73 women tested positive for aHPV-DNA. Histological examinations revealed the presence of AIN2-3 lesions in four patients (6.5%; 95% C.I., 1.8 to 15.7%), who subsequently underwent excisional treatment.

Conclusions: Women with a history of high-grade cervical intraepithelial neoplasia have an intermediate risk of developing high-grade anal intraepithelial neoplasia. Future studies are needed in order to assess an ideal screening approach for this condition.

Introduction

Anal squamous cell carcinoma (ASCC) is an uncommon cancer, accounting for 1–2 % of all gastrointestinal neoplasms. However, its incidence has risen in the past decades and currently its annual incidence is of approximately 1–2 cases per 100.000 people worldwide [1].

Noticeably, the risk of developing ASCC is higher in women, the incidence ratio being 4 per 100 000 in males ≥ 60 years and 6 per 100 000 in females ≥ 60 years. [2].

According to recent data, between 2001 and 2017 in the United States the incidence of ASCC has increased among women by 2.8 % each year, while the incidence of cervical carcinoma has decreased, due to an extension of screening programs [3].

ASCC is the most common histologic subtype of anal cancer, the second most common being adenocarcinoma [1]. It arises from the squamous epithelium of the anus and extends to the dentate line, where the squamous epithelium intersects with the cylindrical epithelium of the rectum [1].

Around 90 % of ASCCs are HPV-related, especially to oncogenic types HPV-16 and HPV-18 [4]. Similarly to the cervix, anal

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intraepithelial neoplasia (AIN) is an HPV-related precancerous lesion and is classified in low-grade (AIN1) and high-grade (AIN2 and AIN 3) [5,6]. Despite the natural history of AIN is still uncertain, its progression rate to carcinoma is estimated to be 5–10 % [7,8].

The prevalence of AIN and ASCC is higher among smokers, men who have sex with men (MSM), immunocompromised subjects, and women with a history of HPV-related cancer or pre-cancer [9], while the prevalence in the general population is still unknown. Among women with a negative HIV status and no history of genital squamous intraepithelial lesions (SIL), the prevalence of AIN is estimated to be lower than 3 % [10].

To date, no guidelines for anal screening are available for people at high risk of anal cancer, or for immunocompetent women with a history of cervical intraepithelial lesions (CIN).

The AIDS Institute of the New York Department of Health recommends anal screening with cytology for individuals with HIV, men who have sex with men (MSM), cisgender or transgender women, and transgender men with HIV aged $\geq$ 35 years [11]. On the other hand, the 2021 CDC Guidelines state that data are insufficient to recommend routine anal screening with cytology in individuals with HIV, MSM without an HIV infection, and in the general population [12]. However, the American Society for Colposcopy and Cervical Pathology suggests that women living with HIV and women with neoplasia of the lower genital tract may be considered for screening with anal cytology. Similarly, the International Anal Neoplasia Society recommends anal screening with digital anal rectal examination in people living with HIV and in women with a history of high-grade cervical, vulvar or vaginal intraepithelial lesions or cancer [13].

Despite these recommendations, screening with cytology remains controversial since, to date, no trial has proven that screening and subsequent treatment of anal lesions decrease anal cancer rates in these populations [14,15]. In fact, there is no consensus as to which screening method would be the most adequate, although anal cytology is thought to be the best candidate [16,17,18]. According to a recent review, the sensitivity of anal cytology for the detection of any-grade AIN ranges from 47 % to 90 %, and is greater for high-grade disease. Specificity is lower and ranges between 32 % and 60 % [19]. High-resolution anoscopy (HRA) has a higher sensitivity (90 %) but a lower specificity (19 %) and is more invasive, compared to cytology, which is cost-effective and less uncomfortable than HRA. Moreover, HRA is characterized by a significant learning curve, is operator-dependent and more costly [20].

The main objective of this study was to evaluate the prevalence of AIN2-3 among immunocompetent women with CIN2-3. Secondary outcomes included an analysis of possible risk factors associated with anal HPV infection including age, tobacco use, number of sexual partners in the last 12 months, engaging in anal intercourse and routine use of barrier methods during vaginal or anal intercourse.

Materials and methods

Consecutive women aged > 25 years who underwent surgical treatment with a Loop Electrosurgical Excision Procedure (LEEP) because of a histologically diagnosed CIN2-3 between September 2019 and December 2021 were included in this prospective monocentric study. All the procedures were performed at the "Regional Referral Centre for Prevention, Diagnosis and Treatment of HPV-related Genital Disorders", Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. Local institutional review board approval was obtained before data collection (Comitato Etico Milano Area 2; 525_2019bis; approved on July 4th, 2019). All patients provided written informed consent before taking part in the study.

Patients with a concomitant or previous diagnosis of vaginal or vulvar preneoplastic disease or anogenital warts or both of them were included, whereas those with a history of pelvic irradiation or anogenital cancer as well as pregnant women were excluded. Other exclusion criteria were known immunosuppressive diseases, current use of immunosuppressive therapies, HIV infection, past or current HPV vaccination.

Enrolled patients were invited to undergo anal cytology and anal high-risk HPV-DNA testing (aHPV-DNA). When one or both tests were positive, a high-resolution anoscopy (HRA) with a biopsy of suspicious lesions was performed.

Anal cytology was carried out with the aid of a brush device provided with nylon bristles and a polypropylene shaft, manufactured by Yuhuan City Shengbo Mould Manufacturing Co.,Ltd (China). The procedure followed established standards [7]: a brush was inserted four centimeters into the anal canal and rotated while being withdrawn in order to sample the transformation zone.

Cytological results were reported according to the latest 2014 Bethesda System classification [11], while HPV DNA testing was aimed at detecting the high-risk genotypes, specifically "carcinogenic types" HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and "probably or possibly carcinogenic types" HPV 68, 73, 82, 26, 53, 66, 69 [21]. No specific test for the detection of anal high-risk HPV is currently recommended, therefore, we chose Anyplex II HPV-DNA test which is FDA approved for cervical cancer screening [22] and is currently used in our center.

High-resolution anoscopy consists in the examination of the anal canal and of the perineal area using a colposcope for lighting and magnification, after application of 5 % acetic acid and Lugol iodine solution [23]. This procedure was carried out by one trained colposcopist who also performed targeted biopsies of suspicious lesions. Histological samples were classified as low-grade lesions (AIN 1), high-grade lesions (AIN 2 or AIN 3) or as other lesions. Women with a histological diagnosis of AIN 2–3 were referred to the colorectal surgery department in order to schedule a surgical excision of the lesion, which was performed to evaluate surgical margins and to confirm the diagnosis. Definitive treatment was performed by a colorectal surgeon in collaboration with a gynecologist and was carried out under spinal anesthesia and colposcopic guidance. All patients were subsequently referred for HRA, anal cytology and HPV DNA testing 6 and 12 months after treatment.

Participants were also asked to complete a questionnaire regarding their medical history and sexual habits. In particular, questions regarding medical history focused on tobacco use, allergies, obstetric history, chronic diseases, medications and surgical history. The sexual behavior section investigated items such as number of sexual partners, history of sexually transmitted diseases (STDs), engagement in anal intercourse and routine use of barrier methods.

Data was organized in a Microsoft Excel spreadsheet (version 15.33; Microsoft, Redmond, WA). Descriptive statistics were used for demographic and medical data; continuous variables are presented as means with standard deviations (M $\pm$ SD), while categorical data as percentages and frequencies. The usual Chi square test and Fisher's Exact test were used, as appropriate, to analyze factors associated with anal HPV-infection, setting statistical significance at the usual 5 % probability value. The sample size (n = 100) was calculated considering a prevalence of HG-AIN of less than 5 % of the width of the Confidence Interval.

Results

One hundred immunocompetent women treated for HG-CIN were consecutively enrolled in the study. Baseline characteristics of the included patients are shown in Table 1. The mean age was 37.7 years (SD 7.7). A total of 33 patients suffered from chronic diseases and 32 were active smokers.

The most common cervical lesion in this group was CIN 3 (74 %). Nine patients had been previously diagnosed with other HPV-related conditions: eight had a history of genital warts, while one patient had a history of high-grade vaginal intraepithelial neoplasia.

The results of the questionnaire on sexual behavior are shown in

Table 1
Baseline characteristics of the study population.

	N (%) or mean ± SD
Mean age	37.7 ± 7.7 years
Chronic diseases	33
Autoimmune conditions*	13 (39 %)
Other	20 (61 %)
Chronic medications	28
Combined oral contraceptives	11 (40 %)
Other drugs	14 (60 %)
Allergies	25
Smoking habit	32
Less than 10 cigarettes/day	19 (59 %)
10–20 cigarettes/day	12 (38 %)
More than 20 cigarettes/day	1 (3 %)
Cervical Intraepithelial Neoplasia (CIN)	100
CIN 2	26
CIN 3	74
History of HPV-related diseases (apart from CIN 2-CIN 3)	9
Genital warts only	8 (90 %)
VaIN only	1 (10 %)
Partners with a history of genital warts	2

*The autoimmune conditions we observed in our study group (e. g. Hashimoto's disease, vitiligo) were not causing immunosuppression.

Table 2
Results of the questionnaire on medical history and sexual habits.

Number of sexual partners in the last 2 months	N
0	23
1	70
1–5	6
>5	1
Number of sexual partners in the last 12 months	
0	9
1	73
1–5	16
>5	2
Patients using contraceptive methods	42
Combined oral contraceptives	15
Condom	21
A combination of methods	6
Patients reporting having had anal sex at least once in their lives	53
Patients using methods of barrier during anal intercourse	22
Patients with a history of sexually-transmitted diseases	7
C. trachomatis	3
N. gonorrhoeae	1
HSV-2	1
T. vaginalis	2
Partners with a history of sexually-transmitted diseases	2
C. trachomatis	2

Table 2. A total of 73 women stated they had had a single sexual partner over the previous year, while 18 had had multiple partners and nine had had none. A total of 53 women stated they had engaged in anal intercourse at least once during their life and half of these had never used barrier methods. Only 7 % of the patients had a history of STDs, the most frequent being Chlamydia trachomatis.

The study findings are summarized in Fig. 1. All patients underwent both anal HPV-DNA testing and anal cytology. The distribution of high-risk HPV genotypes is shown in Fig. 2**.** The most common genotypes detected were HPV-16 (36 %) and HPV-53 (19 %). In 29 women more than one genotype was detected.

A total of 73 women tested positive for anal HPV-DNA and among these three also had an abnormal anal cytology, which was classified as low-grade in all cases (ASCUS or LSIL). All 73 patients were subsequently referred to HRA, which however was declined by 11 women. Four out of 62 patients received a histological diagnosis of HG-AIN (6.5 %; 95 % C.I., 1.8 to 15.7 %). Three patients were referred to the colorectal surgery department for surgical excision of the lesion, while one did not undergo surgery since the lesion, whose maximum diameter was of a few millimeters, had been completely removed while performing an excisional punch biopsy.

The histological diagnoses obtained from surgical excisions were the following: one case of AIN1 (1.6 %), two of AIN2 (3.2 %) and one of AIN3 (1.6 %). At a six and twelve-month follow-up anal cytology, HPV-DNA testing and HRA were repeated and none of the patients presented with a recurrence of HG-AIN.

The association between anal HPV infection and potential risk factors for HPV transmission and infection is shown in Table 3. No statistically significant association was found.

Cervical high-risk HPV-DNA testing was available for only 34 patients. Among these, 33 tested positive, and 18 were positive for both cervical and anal HPV testing. A concordance between the cervical and the anal of HPV genotype was found in 50 % of women. The most frequent concordant HPV type was 16, that was found in six women.

Discussion

The overall prevalence of HG-AIN in our series was 6.5 % (4/62; 95 % C.I., 1.8 to 15.7 %) although we found only one case of AIN3 (1.6 %; 95 % C.I., 0.04 to 8.7 %). Few studies have analyzed the prevalence of HG-AIN in immunocompetent women with a history of HG-CIN.

Jacyntho and co-workers found a 17.4 % prevalence of AIN of any grade and a 3 % of high-grade AIN among 184 HIV-negative immunocompetent women with genital squamous intraepithelial lesions (cervical, vaginal, vulvar or perianal). Among healthy controls, 2.6 % presented with low-grade AIN and no cases of high-grade AIN were detected [24]. Also Koppe *et al* compared a group of 106 immunocompetent women with a history of genital intraepithelial neoplasia or cancer with 74 healthy controls: in the former group, the overall prevalence of AIN was 10.4 %, while high-grade AIN was found in 4.7 % of patients. Conversely, among healthy controls only 1.4 % had low-grade AIN and no cases of high-grade AIN were identified. All patients underwent HRA with biopsy of suspicious areas. Exclusion criteria were comparable to the ones we adopted in our study and this may explain the similarity between our data and Koppe and co-workers' findings [25].

While studying 324 women with CIN of any grade or cervical cancer by the means of anal cytology, HR-HPV, HRA and anal biopsy, which was performed on suspicious lesions or in cases of abnormal anal Pap smears, Heraclio *et al* found anal intraepithelial neoplasia in 11 % of patients and HG-AIN in 4 %. Patients with HIV and those undergoing radiotherapy or chemotherapy for gynecological cancer were excluded [26].

Similarly, Wohlmuth *et al* performed anal cytology and HRA on 317 women with a prior diagnosis of CIN 2–3 or cervical cancer: AIN of any grade was found in 9.5 % of participants and HG-AIN was diagnosed in 6.5 % of cases. Conversely to our study, all patients were aged $\geq$ 40 years, HRA was performed in patients with abnormal cytologic findings, regardless of HPV testing results and immunosuppression or previous HPV vaccination were not exclusion criteria. Given the natural history of squamous intraepithelial lesions, it is plausible that a higher prevalence of AIN can be found in older patients and in women with a history of immunosuppressive diseases [27]. Other relevant studies are summarized in Table 4 [28,29,30,31].

Compared to our study, in almost all the above-mentioned studies, patients underwent HRA regardless of cytology or HPV-DNA testing. Furthermore, most studies included HIV positive or immunocompromised patients and/or patients with cervical cancer. This could explain the different findings regarding the prevalence of HG-AIN.

The prevalence of anal HPV infection was significant in our population, as 73 % tested positive for HR-HPV DNA. HPV-16 was the most frequent genotype, followed by HPV-53. Similar results have been reported in other studies [26,29,32,33].

Finally, analyzing those considered as risk factors for anal HPV infection, according to the most recent evidence [4,19,27], we found no significant association between age, tobacco use, multiple sexual

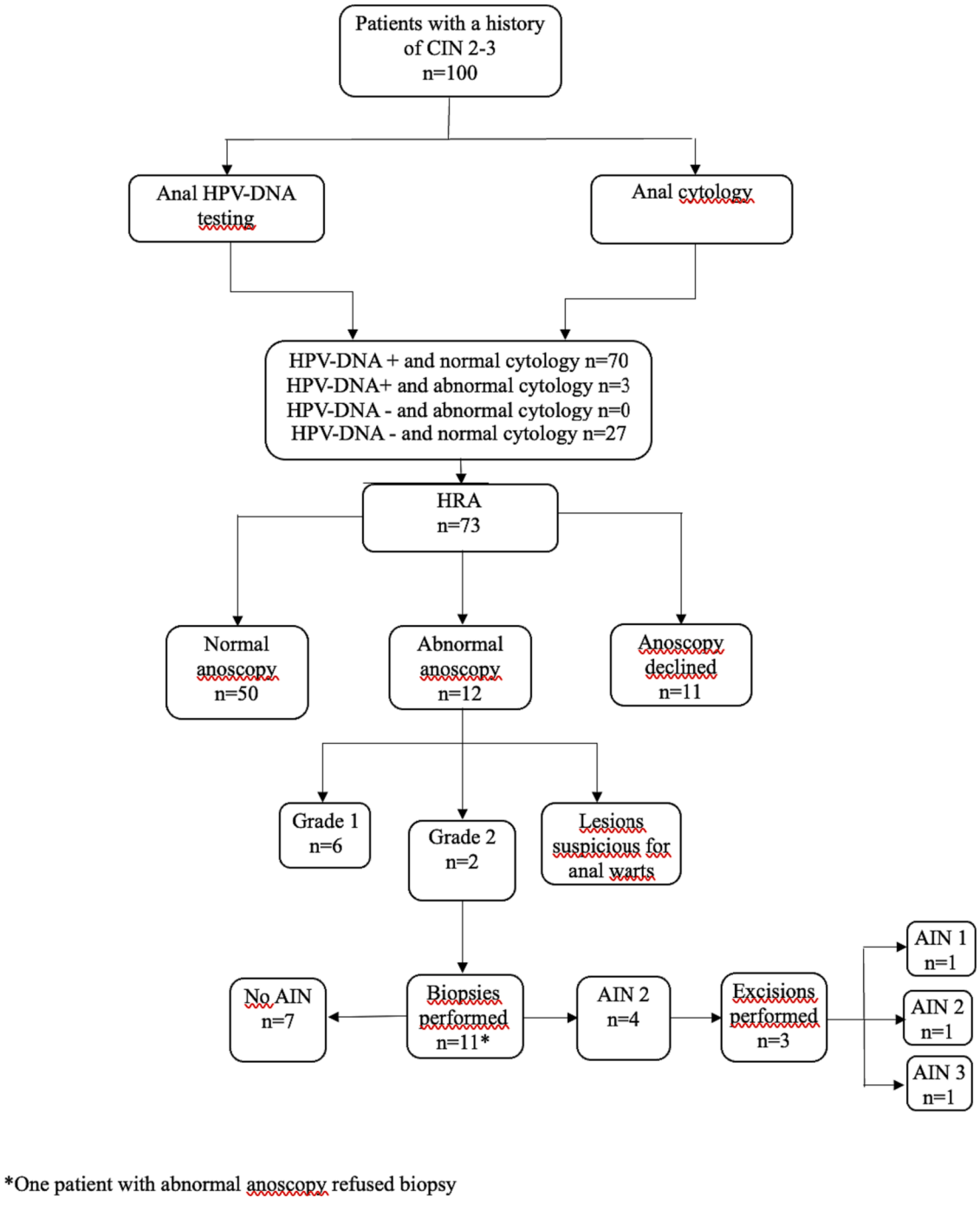

Fig. 1. Study results.

partners, routine use of barrier methods, engaging in anal intercourse and the detection of anal HR-HPV. However, the small sample size does not allow to draw firm conclusions regarding the abovementioned associations.

Notably, in our study, only 50 % of women testing positive for anal HR-HPV stated that they had engaged in anal intercourse. This could be due to different reasons. Firstly, many women may not be comfortable with sharing information regarding sexual behavior for cultural or personal reasons, thus there may be a reporting bias. According to Gana and co-workers, [34] anal sex has become part of the routine sexual repertoire of many young women. Secondly, anal sex doesn't seem to be a precondition for anal infection, and different studies support this assumption [4,30,35], as cervical HPV infection may serve as a viral reservoir for anal infection. On the other hand, some studies confirmed that anal intercourse is significantly associated with anal HPV infection [36,37], especially in MSM. In our series, among HPV-positive women who engaged in anal intercourse, only 45 % declared using condoms. Therefore, the use of condoms does not appear to be protective for anal HPV transmission, as for vaginal infection [38]. Wong *et al* reported results similar to our findings [39].

The limitations of our study include a small sample size, its mono-centric setting and the absence of a control group. Nonetheless, its strengths include the establishment of a multidisciplinary team with the colorectal surgery and pathology departments, allowing patients to receive continuous care and support to throughout every step of the protocol; and a negligible inter-observer variability since only one

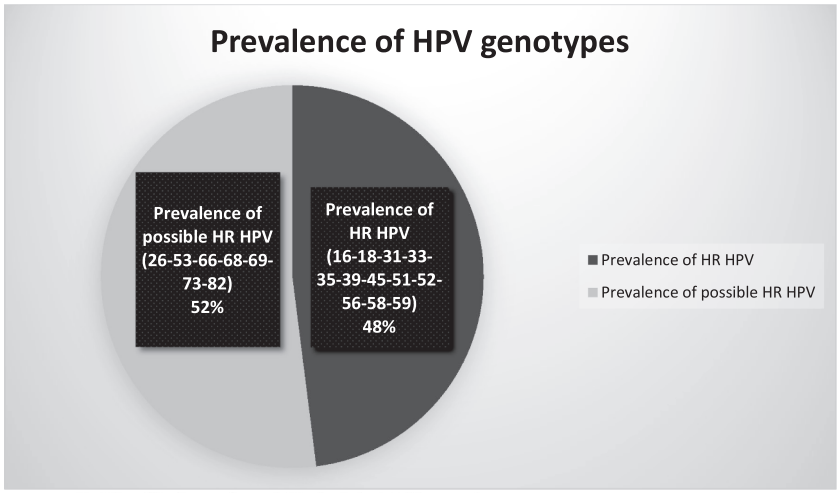

Fig. 2. Distribution of anal high-risk HPV genotype.

Table 3
Association between risk factors and prevalence of anal HPV infection.

	HPV-DNA+ (N = 73)	HPV-DNA- (N = 27)	P-value
Active smokers	24	8	0.81
History of multiple sexual partners in the last 2 months	6	1	0.67
History of multiple sexual partners in the last 12 months	16	2	0.14
History of anal intercourse	37	16	0.5
Use of methods of barrier during vaginal intercourse	33	9	0.36
Use of methods of barrier during anal intercourse	16	6	1
Age ≤ 30	11	6	0.39
Age > 30	62	21	0.39

expert physician performed the HRAs.

In conclusion, our data confirmed an overall 6.5 % prevalence of HG-AIN (AIN2-3) among immunocompetent patients with a history of CIN2-3. Despite the prevalence of AIN 3, which is the true precursor of anal cancer, was only 1.6 %, a screening program for HG-AIN may be useful in a selected high-risk population such as women with high-grade cervical intraepithelial neoplasia. Therefore, considering the results of our study and considering the available literature on the topic, additional research on greater cohorts of patients would be needed to evaluate the effectiveness of a screening program and the most adequate screening method.

Furthermore, the efficacy of the currently available vaccines in the prevention of anal HPV infections and anal intraepithelial neoplasia must not be overlooked [40]. Indeed, the FDA Vaccines and Related Biological Products Advisory Committee (VRBPAC) recommends the quadrivalent and nonavalent HPV vaccines for the prevention of anal cancer related to HPV types 16, 18 in males and females aged 9 to 26 years [41].

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

Table 4
Comparison among relevant studies.

Author and publication year	Study design	Population size	Population characteristics	Screening tools	AIN prevalence
Heraclio et al, 2011	Cross-sectional	324 cases	Women with cervical intraepithelial neoplasia or cervical cancer	Anal cytology, HRA ± punch biopsy	11 % (**4 % HG-AIN**)
Koppe et al, 2011	Cross-sectional	106 cases, 74 controls	HIV-negative women with genital intraepithelial neoplasia	HRA ± punch biopsy	10.4 % in the study group (**4.7 % HG-AIN**), 1.4 % in the control group
Jacyntho et al, 2011	Cross-sectional	184 cases, 76 controls	HIV-negative women with genital intraepithelial neoplasia	Colposcopy, HRA ± punch biopsy	17.4 % nel gruppo di studio (**3 % HG-AIN**), 2–6 % nei controlli
ElNaggar et al, 2013	Cohort study	327 cases	Women with genital intraepithelial neoplasia	Anal cytology and HRA ± punch biopsy	19.6 % (**8.6 % HG-AIN**)
Park et al, 2009	Cross-sectional	102 cases	Women with genital intraepitelial neoplasia or cervical cancer who were never vaccinated against HPV	HPV-DNA test, anal cytology, HRA if positive cytology	6.9 % (AIN 1), **no HG-AIN**
Tatti et al, 2012	Cross-sectional	481 cases	Women with genital intraepithelial neoplasia	Anal cytology, HRA ± punch biopsy	27.8 % **(5.8 % HG-AIN)**
Wohlmuth et al, 2021	Cohort study	317 cases	Women > 40 years old with CIN2-3 or cervical cancer	HPV-DNA test + anal cytology; HRA if positive cytology	9.5 % (**AIN-HSIL 6.5 %**)
Albuquerque et al, 2021	Retrospective study	253 cases	Women with a history of anogenital neoplasia and multizonal anogenital disease during assessment	Colposcopy, HRA ± punch biopsy	**13.4 % AIN-HSIL**

the work reported in this paper.

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