

Anal HPV prevalence in individuals with and without other concomitant sexually transmitted infections

Alberto Rizzo¹  | Davide Moschese² | Federica Salari¹ | Andrea Giacomelli^{3,4} |
Andrea Cavallo¹ | Maria Vittoria Cossu² | Lorian Morelli¹ | Chiara Fusetti² |
Giorgia Carrozzo³ | Serena Reato³ | Valeria Micheli¹  | Spinello Antinori^{3,4} |
Alessandra Lombardi¹ | Andrea Gori^{3,4,5} | Maria Rita Gismondo^{1,4}

¹Laboratory of Clinical Microbiology, Virology and Bioemergencies, Luigi Sacco Hospital, ASST Fatebenefratelli Sacco, Milan, Italy

²I Infectious Diseases Unit, Luigi Sacco Hospital, ASST Fatebenefratelli Sacco, Milan, Italy

³III Infectious Diseases Unit, Luigi Sacco Hospital, ASST Fatebenefratelli Sacco, Milan, Italy

⁴Department of Biomedical and Clinical Sciences, Università degli Studi di Milano, Milan, Italy

⁵Centre for Multidisciplinary Research in Health Science (MACH), University of Milan, Milan, Italy

Correspondence

Alberto Rizzo, Laboratory of Clinical Microbiology, Virology and Bioemergencies, Luigi Sacco Hospital, ASST Fatebenefratelli Sacco, Via G.B. Grassi 74, 20157 Milan, Italy.
Email: alberto.rizzo@asst-fbf-sacco.it

Abstract

The association between human papillomavirus (HPV) and other sexually transmitted infections (STIs) in anal lesions still remains unclear. Aim of the study was to evaluate the prevalence of simultaneous infection of HPV and *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis* in individuals screened for HPV anal infection. A total of 507 anal samples were tested for both anal HPV and STIs: 16% resulted positive for one or more non-HPV STIs. Specifically, *C. trachomatis*, *M. genitalium*, and *N. gonorrhoeae* were detected in 8%, 5%, and 4% of cases, respectively. Two groups were considered, including a positive STI group and a negative STI group. The prevalence of HPV was similar in patients in both groups: high risk (HR)-HPV and low risk (LR)-HPV were 67% and 53% versus 62% ($p = 0.361$) and 54% ($p = 0.864$) of patients, respectively. However, HPV 16, 18, 35, 51, 59, and 69 were significantly more frequent in patients tested positive for other STIs versus HPV infection alone ($p < 0.05$). No significant differences between the two groups were observed in vaccination coverage, 28% versus 32% ($p = 0.463$), and HIV status, 86% versus 84% ($p = 0.658$). The study shows that the overall HPV status is not directly correlated to other STIs in the investigated population, except for certain HPV types, including HR-HPV 16, reinforcing the urge for a greater vaccination coverage.

KEYWORDS

anus, chlamydia, gonorrhea, mycoplasma, papillomavirus, trichomoniasis

1 | INTRODUCTION

Human papillomavirus (HPV), a non-enveloped, oncogenic virus, is the most common sexually transmitted pathogen.¹ The virus can be transmitted through skin-to-skin contact, oral, vaginal or anal sex and also with shared fomites. The same routes of transmission are shared

with other pathogens, including *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis*.² Along with HPV, they are the most common cause of sexually transmitted diseases. Globally, an estimated 300 million women and 1 in 3 men are infected with HPV; in 2020, WHO estimated 129 million new cases of chlamydia, 82 million of gonorrhea and 156 million of trichomoniasis.^{3,4} HPV,

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Author(s). *Journal of Medical Virology* published by Wiley Periodicals LLC.



specifically high-risk (HR-HPV) types, can cause cancerous and noncancerous lesions, including oropharyngeal and anogenital cancers, anogenital warts, and recurrent respiratory papillomatosis.⁵ Six vaccines have been licensed for the prevention of HPV-linked conditions: bivalent, quadrivalent and 9-valent formulations. Specifically, they all protect against HPV 16 and 18, main responsible for most of HPV-related cancer cases. In addition, quadrivalent and 9-valent can also prevent noncancerous lesions.⁶ On the other hand, other sexually transmitted infections (non-HPV STIs), including *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, and *T. vaginalis*, that cannot be prevented with vaccines, may have consequences beyond the immediate impact of acute infection too. Complications of common STIs include pelvic inflammatory disease, infertility, adverse pregnancy outcomes, epididymitis, and proctitis.⁷ Many studies investigated the association between STIs and HPV cervical abnormalities.⁸ Bacterial vaginosis has been associated with HPV infection.⁹ de Abreu et al. observed an association with *C. trachomatis* and *N. gonorrhoeae* with HR-HPV in cervical abnormalities.¹⁰ *T. vaginalis* and *M. genitalium*, two other common curable STIs might be associated with a higher prevalence of HR-HPV infections, as observed in women.^{10,11} Masiá et al., in 2020, evaluated the relationship between STIs, including HPV genotypes, and high-grade anal intraepithelial neoplasia in PWH (people with HIV) men who have sex with men (MSM): they observed that *C. trachomatis* might potentiate the oncogenic capability of HPV 16.¹²

There are limited data on the prevalence and distribution of HPV and non-HPV STIs coinfections, especially in different populations and regions. Nevertheless, this information is pivotal to identifying patterns of coinfection which may have implications for diagnosis, treatment and prevention strategies, improving public health programs to better manage and prevent the spread of STIs.

We aimed to evaluate the prevalence and distribution of HPV by STIs status in anal samples of patients attending a sexual health clinic.

2 | MATERIALS AND METHODS

2.1 | Study design

This was a retrospective observational study conducted between January 1, 2022 and December 31, 2023.

2.2 | Setting

The sexual health clinic of a large Infectious Diseases Department (Luigi Sacco Hospital, Milan, Italy).

2.3 | Study population

Individuals who were sexually active (with at least one partner in the past 3 months) and reported unprotected anal intercourse, or who had a history of previous sexually transmitted infections, regardless

of their current sexual activity, were included. All patients who underwent HPV testing were informed about the procedure and its implications through a consultation with an Infectious Diseases specialist and all were simultaneously tested in the anal canal for *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, and *T. vaginalis* DNA. Testing anal samples for HPV is not a standard in STIs screening programs. However, screening for other STIs during HPV evaluation should be performed to rule out possible different confounding causes of anal inflammation.

2.4 | Data collection

Data about laboratory examinations, including cytology and histology, and demographic characteristics like age, biological sex, HIV status, and 9-valent HPV vaccination status were included in the study.

2.5 | Laboratory procedures

From January 2022 to March 2023, the presence of *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium* and *T. vaginalis* DNA was investigated by means of STI PLUS ELITE MGB® Kit (ELITechGroup) on the sample-to-result system ELITE InGenius (ELITechGroup). Because of hospital policies change, since March 2023 the test was performed using the RT-PCR Allplex™ STI Essential Assay (Seegene), on the Microlab NIMBUS (Seegene) and the CFX96 RT-PCR instrument (Bio-Rad). The specimens were collected using the Universal Transport Medium (UTM-RT®) system paired with flocked swab FLOQSwabs® (Copan Diagnostics).

Anal sample collection for HPV DNA test was performed using the ThinPrep™ Pap Test PreservCyt™ (Hologic) system. Nucleic acid extraction was performed through the STARMag kit (Seegene) on Microlab NIMBUS (Hamilton) platform. HPV genotyping was performed immediately after DNA extraction by means of Anyplex™ II HPV28 Detection RT-PCR commercial kit (Seegene), which detect 12 carcinogenic (HR-HPV) (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59), one probable carcinogenic (HPV 68) and six possibly carcinogenic genotypes (HPV 26, 53, 66, 69, 73, 82) (probable/possible HR-HPV), plus nine low-risk (LR-HPV) types (HPV 6, 11, 40, 42, 43, 44, 54, 61, 70). The assay targets human beta-globin as an internal control to monitor DNA purification efficiency, PCR inhibition, and cell adequacy. Cytology results were classified following the Bethesda system: inadequate, negative for intraepithelial lesion or malignancy, atypical squamous cells (ASC), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells cannot rule out high-grade squamous intraepithelial lesion (ASC-H), high-grade squamous intraepithelial lesion (HSIL) and squamous cells carcinoma (SCC). Anal tissue samples were classified as normal (negative), anal intraepithelial neoplasia-1 (AIN-1), anal intraepithelial neoplasia-2 (AIN-2), anal intraepithelial neoplasia-3 (AIN-3), squamous cell carcinoma (SCC).

2.6 | Statistical analysis

Categorical variables were represented by proportions; median and interquartile range (IQR) values were calculated for continuous variables. Chi-square (χ^2) and Fisher exact test were used as significance tests. Statistical significance was set at $p < 0.05$. STATA v18 was used for statistical analysis.

3 | RESULTS

3.1 | Study population

A total of 507 individuals were tested. The median age was 48 years (IQR 40–54), and they were male in 97% (493/507) of cases, mostly men who have sex with men (MSM), and PWH in 84% (428/507) of cases. Of the cases investigated, 86% (437 out of 507) tested positive for one or more pathogens, while 14% (70 out of 507) tested negative for any infection. One or more STIs were detected in 16% (79/507) of patients, mostly males (77/79; 97%) with a median age of 45 years (IQR 36–51); 86% (68/79) of them were PWH. Specifically, including both single and multiple infections, *C. trachomatis* was detected in 8% (41/507) of individuals, *M. genitalium* in 5% (24/507), *N. gonorrhoeae* in 4% (19/507), and *T. vaginalis* in 0.4% (2/507). STIs coinfections represented 1.4% (7/507) of cases: *C. trachomatis*–*N. gonorrhoeae* ($n = 4$), *C. trachomatis*–*T. vaginalis* ($n = 1$), *C. trachomatis*–*M. genitalium* ($n = 1$), *M. genitalium*–*T. vaginalis* ($n = 1$).

HPV DNA was detected in 84% (426/507) of individuals, mostly males (98%, 416/426), with a median age of 48 (IQR 39–54) years; 84% (358/426) of HPV-positive patients were PWH. HR-HPV, probable/possible HR-HPV and LR-HPV types had a prevalence of 63% (317/507), 39% (197/507), and 54% (274/507), respectively. The overall RT-PCR results for HPV types and other STIs are resumed in Table 1.

Data about anal cytology were available for 501 out of 507 patients: 0.6% (3/501) were diagnosed with HSIL, 0.8% (4/501) with ASC-H, 12% (61/501) ASC, 17% (85/501) with LSIL; 2% (11/501) were considered inadequate. Biopsy was performed on 136 out of 507 patients, with the following results: 0.7% (1/136) SCC, 8% (11/136) AIN-3, 8% (11/136) AIN-2, 45% (61/136) AIN-1, 3% (4/136) inadequate. Inadequate samples were excluded from further analyses. High-grade lesions were detected in patients with HPV, as compared to HPV-negative individuals, as follows: 0.7% (3/416) HSIL and 17.5% (22/126) AIN-2/AIN-3/SCC versus 0% (0/74) HSIL and 16.6% (1/6) AIN-2/AIN-3/SCC, respectively. Specifically, AIN-3 ($n = 11$) and SCC ($n = 1$) were observed only in HPV-positive patients.

All individuals were divided into 2 groups: patients tested positive for non-HPV STIs (*C. trachomatis* and/or *N. gonorrhoeae* and/or *M. genitalium* and/or *T. vaginalis*) and patients tested negative for non-HPV STIs. Both groups included patients tested positive or negative for HPV DNA. Socio-demographic characteristics are shown in Supplementary 1.

TABLE 1 Detection of single and multiple sexually transmitted infections (non-HPV STIs: *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis*) and high-risk (HR), probable/possible HR and low-risk (LR) HPV types.

Sexually transmitted infections	Total, $n = 507$
Non-HPV STIs positive, n (%)	79 (16%)
<i>C. trachomatis</i>	35 (7%)
<i>M. genitalium</i>	22 (4%)
<i>N. gonorrhoeae</i>	15 (3%)
<i>C. trachomatis</i> and <i>N. gonorrhoeae</i>	4 (1%)
<i>C. trachomatis</i> and <i>T. vaginalis</i>	1 (<1%)
<i>C. trachomatis</i> and <i>M. genitalium</i>	1 (<1%)
<i>M. genitalium</i> and <i>T. vaginalis</i>	1 (<1%)
HPV positive, n (%)	426 (84%)
HR-HPV	317 (63%)
Probable/possible HR-HPV	197 (39%)
LR-HPV	274 (54%)
Concomitant HPV and non-HPV STIs positive, n (%)	68 (13%)

3.2 | HPV coinfection

The most common HPV types were HPV 42 (22%, 111/507) and 16 (21%, 108/507), followed by HPV 58 (17%, 88/507) and 53 (17%, 85/507). The presence of HPV DNA was 86% (68/79) in the group of patients with a non-HPV concomitant STI and 84% (358/428) in those without it ($p = 0.587$). Specifically, in the two groups considered, a positive STI group and a negative STI group, the prevalence of HPV was similar: HR-HPV and LR-HPV were 67% (53/79) and 53% (42/79) versus 62% (264/428) ($p = 0.361$) and 54% (232/428) ($p = 0.864$) of patients, respectively. Probable/possible types were detected in 47% (37/79) versus 37% (160/428) ($p = 0.113$) of groups patients.

Among those with a concomitant non-HPV STIs, 51% (27/53) of HR-HPV cases, 51% (19/37) of probable/possible HR-HPV and 55% (23/42) of LR-HPV were detected in patients tested positive for *C. trachomatis* while 34% (18/53) of HR-HPV, 35% (13/37) of probable/possible HR-HPV and 31% (13/42) of LR-HPV were positive for *M. genitalium*.

The prevalence of genotypes in individuals with and without a concomitant non-HPV STIs is shown in Table 2. HPV 16 was the most frequent HPV type in individuals with a concomitant non-HPV STIs, 32% (25/79), followed by HPV 42, 25% (20/79), and 53, 20% (16/79). On the other hand, in individuals without a concomitant non-HPV STIs, HPV 42 was the most common genotype, 21% (91/428), followed by HPV 16, 19% (83/428), and 58, 17% (74/428).

Among HR-HPV and probable/possible HR-HPV types, HPV 16, 18, 35, 51, 59, and 69 were significantly more prevalent in individuals with a concomitant non-HPV STIs ($p < 0.05$). There were no statistically significant differences for LR-HPV genotypes.

TABLE 2 Prevalence of high risk-human papillomavirus (HR-HPV) (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59), probable/possible HR-HPV (HPV 68, 26, 53, 66, 69, 73, 82) and LR-HPV (HPV 6, 11, 40, 42, 43, 44, 54, 61, 70) types in patients tested positive and negative for non-HPV STIs (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis*).

HPV types	Non-HPV STIs positive (n = 79)		Non-HPV STIs negative (n = 428)		p Value
HPV 16	25	32%	83	19%	0.023
HPV 18	13	16%	25	6%	0.000
HPV 31	8	10%	45	11%	1
HPV 33	9	11%	49	11%	1
HPV 35	12	15%	29	7%	0.021
HPV 39	9	11%	29	7%	0.162
HPV 45	1	1%	23	5%	0.151
HPV 51	13	16%	37	9%	0.040
HPV 52	6	8%	29	7%	0.808
HPV 56	4	5%	31	7%	0.631
HPV 58	14	18%	74	17%	0.873
HPV 59	11	14%	29	7%	0.039
HPV 26	1	1%	2	0.5%	0.399
HPV 53	16	20%	69	16%	0.411
HPV 66	10	13%	29	7%	0.103
HPV 68	10	13%	49	11%	0.706
HPV 69	6	8%	9	2%	0.018
HPV 73	9	11%	25	6%	0.084
HPV 82	6	8%	21	5%	0.409
HPV 6	14	18%	56	13%	0.287
HPV 11	7	9%	31	7%	0.641
HPV 40	6	8%	13	3%	0.096
HPV 42	20	25%	91	21%	0.459
HPV 43	5	6%	22	5%	0.592
HPV 44	4	5%	48	11%	0.108
HPV 54	3	4%	38	9%	0.176
HPV 61	3	4%	26	6%	0.599
HPV 70	11	14%	36	8%	0.138

3.3 | Anal cytology and histology

Cytology results were available for 99% (78/79) and 96% (412/428) of patients positive and negative for non-HPV STIs, respectively. HSIL was detected in 1.3% (1/78) and 0.5% (2/412) of cases ($p = 0.408$), respectively. In the group of patients negative for STIs, 11 additional specimens were considered inadequate. On the other hand, histologic examination was performed on 29% (23/79) and 25% (113/428) of

TABLE 3 Classification of anal samples collected from patients tested positive for non-HPV STIs (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis*) and patients negative for non-HPV STIs, according to cytologic (n = 78 vs. n = 412) and histologic (n = 23 vs. n = 109) examination.

	Non-HPV STIs positive	Non-HPV STIs negative	p Value
Cytology (n)			
neg	78	412	
neg	51 (65.4%)	286 (69%)	0.480
ASC	9 (11%)	52 (13%)	0.790
LSIL	17 (22.3%)	68 (16.5%)	0.257
ASC-H	0 (-)	4 (1%)	-
HSIL	1 (1.3%)	2 (0.5%)	0.408
Histology (n)			
neg	23	109	
neg	6 (26.1%)	42 (38.5%)	0.374
AIN-1	13 (56.5%)	48 (44.1%)	0.275
AIN-2	0 (-)	11 (10.1%)	-
AIN-3	3 (13.1%)	8 (7.3%)	0.368
SCC	1 (4.3%)	0 (-)	-

Abbreviations: AIN-1, anal intraepithelial neoplasia-1; AIN-2, anal intraepithelial neoplasia-2; AIN-3, anal intraepithelial neoplasia-3; ASC, atypical squamous cells; ASC-H, atypical squamous cells cannot exclude HSIL; HPV, HPV, human papillomavirus vaccine; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; neg, patient tested negative for cytology/histology; SCC, anal squamous cell carcinoma; STI, sexually transmitted infection.

patients positive and negative for non-HPV STIs, respectively. In the group negative for non-HPV STIs, 3% (4/113) of specimens resulted in inadequate histologic examination. The overall prevalence of AIN-2/AIN-3/SCC was similar in the two groups: 17% (4/23) versus 17% (19/109) ($p = 0.996$) (Table 3).

Among patients tested positive for at least one non-HPV STI, high-grade lesions were more frequently detected in those coinfecting with HPV, as compared to those tested positive for non-HPV STIs but negative for HPV DNA: all cases of AIN-3 (n = 3) and the only case of SCC were reported in this group (Table 4).

Given the hypothesis that *C. trachomatis* may potentiate the oncogenic capability of HPV¹², we divided patients with available histology in *C. trachomatis* positive-HPV positive (n = 12) and *C. trachomatis* negative-HPV positive (n = 114) groups. However, we did not observe a significant difference in the prevalence of high-grade lesions, including AIN-2, AIN-3, SCC, among the two groups: 16.7% (2/12) versus 17.5% (20/114) ($p = 0.939$).

3.4 | HPV vaccination and vaccine-targeted types

HPV complete vaccination cycle with 9-valent formulation, protective against genotypes 6, 11, 16, 18, 31, 33, 45, 52, and 58, was

TABLE 4 Cytology and histology results in patients presenting HPV coinfection with at least one among *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis* (non-HPV STIs), versus patients tested positive for non-HPV STIs but negative for HPV DNA.

	STIs-HPV coinfections	STIs-only patients
Cytology (n)	67	11
neg	41 (61.2%)	10 (90.9%)
ASC	9 (13.4%)	0
LSIL	16 (23.9%)	1 (9.1%)
ASC-H	0	0
HSIL	1 (1.5%)	0
Histology (n)	21	2
neg	5 (24%)	1 (50%)
AIN-1	12 (57%)	1 (50%)
AIN-2	0	0
AIN-3	3 (14%)	0
SCC	1 (5%)	0

Abbreviations: AIN-1, anal intraepithelial neoplasia-1; AIN-2, anal intraepithelial neoplasia-2; AIN-3, anal intraepithelial neoplasia-3; ASC, atypical squamous cells; ASC-H, atypical squamous cells cannot exclude HSIL; HPV, human papillomavirus vaccine; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; neg, patient tested negative for cytology/histology; SCC, anal squamous cell carcinoma; STI, sexually transmitted infection.

reported in 28% (22/79) of individuals with a concomitant non-HPV STIs and in 32% (137/428) of those without it ($p = 0.463$). Among the vaccinated participants with concomitant non-HPV STIs, the detection rates of HR-HPV, probable/possible HR-HPV and LR-HPV were 59% (13/22), 50% (11/22), 54% (12/22), respectively; 77% (17/22) of individuals were tested for HPV > 1 year after vaccination. Specifically, the prevalence of vaccine-targeted HPV types was 68% (15/22). On the other hand, among vaccinated individuals without a concomitant non-HPV STIs, HR-HPV, probable/possible HR-HPV, and LR-HPV were detected in 58% (79/137), 39% (54/137) and 59% (81/137) of cases; 82% (112/137) of patients were tested > 1 year after vaccine administration. Specifically, the prevalence of vaccine-targeted HPV types was 53% (73/137); no significant difference in vaccine-targeted HPV types was observed in the two groups ($p = 0.192$).

4 | DISCUSSION

According to the WHO, more than 1 million STIs are acquired every day in the world. Specifically, each year the number of chlamydia, gonorrhea, syphilis, and trichomoniasis new cases is estimated to be 374 million. On the other hand, HPV is the leading STI and is also associated with different types of cancers, both in men and

women.^{3,4} In a recent study we observed a prevalence of 84% for any type of HPV and of 65%–73% for HR-HPV among sexually active individuals, particularly in men older than 45 years.¹³ The present study evaluated the coinfection rate of HPV with *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, and *T. vaginalis*. Specifically, *C. trachomatis* was detected in 8% of cases, *M. genitalium* 5%, *N. gonorrhoeae* 4% and *T. vaginalis* 0.4%, falling within the ranges observed from USA CDC and other studies on MSMs that reported a prevalence up to 24% for gonorrhea, 23% for chlamydia and 6.4% for *M. genitalium*.^{14–16} In the group of individuals tested negative for *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, and *T. vaginalis*, we detected HR-HPV types and HPV 16 in 62% and 19% of cases; the prevalence increased to 67% and 32% in the group of patients tested positive for non-HPV STIs, in accordance with a collaborative pooled analysis of 64 studies published in 2021.¹⁷ On the other hand, lower detection rates of STIs, including HPV, were shown in a recent study by Fasciana et al. that enrolled individuals attending an STIs Unit: the authors reported HPV infection in 55.1% of patients and less cases of multiple STIs (6.4%). However, that study included more women (30%) and less PWH (33%).¹⁸

In our study, both individuals with and without concomitant non-HPV STIs showed a high prevalence of any type of HPV without statistically significant differences, except for HPV 16, 18, 35, 51, 59, and 69 that were significantly more frequent in patients tested positive for other STIs: HPV 16 and 18 are among the most common types found in the genital area, both in men and women.^{19,20} Similarly, a study published in 2020 regarding 353 sexually active women reported that HR-HPV types were more prevalent among women who had coinfection with another STI.²¹ Specifically, some studies showed an association between HR-HPV and other STIs in cervical carcinogenesis,^{22,23} hypothesizing similar pathways in both the infection and lesion progression of the anal canal. In fact, the proposed synergism might be exerted through several mechanisms: *C. trachomatis*, *N. gonorrhoeae*, and *M. genitalium* may induce chronic inflammation, alter the expression of cellular receptors and coreceptors for HPV and interfere with natural clearance of HPV through immune suppression, facilitating HPV infection and persistence.^{24,25} The most abundant expression of the oncogenes E6 and E7 among HR-HPV types has been reported in HPV 16 and Olivera et al. showed a synergism between HPV 16 E6/E7 and *C. trachomatis*. These observations corroborate the hypothesis that a mutual screening for these infections may help to intervene and prevent severe complications like HPV-related cancer.^{26,27} Strong evidence that STIs, and in particular HPV, are associated with anal cancer was already provided in 1997.²⁸

We frequently observed high-grade cytology and histology lesions, especially AIN-3 and SCC, in HPV patients, but we did not observe significant differences in patients coinfecting with HPV and other STIs. Specifically, our results did not show significantly different prevalence of high-grade lesions among *C. trachomatis* patients, in contrast with Masiá et al.¹² The low number of cases with simultaneous infections and available histology might be another partial explanation for discordant observations with other studies. However, we found that HR HPV 16, 18, 35, 51, 59 and 69 were significantly more frequent in the STI-positive group. This finding is consistent

with and may corroborate the observed association between HPV and concomitant STIs proposed by other studies.^{25,29} It must be noted that other authors observed a correlation between simultaneous infections and cervical abnormalities.¹⁰ A possible explanation is that they evaluated the relationship in a population of women attending basic health units for cervical cancer screening, which is different from ours. In addition, pathogens interaction in anal site might result in different consequences from cervical ones. The aforementioned synergistic mechanisms, including increased inflammation, immune evasion, and interference with natural viral clearance, might partially explain the frequent detection of HPV, specifically HR-HPV types, with other concomitant STIs. However, in the absence of more detailed information about sexual activity and habits of the individuals, we cannot exclude that the association of these very frequent STIs might partially derive from an increased sexual activity as compared to the general population.

A recent Canadian study, in sexually active individuals aged 16–30 years, observed that vaccination was effective at preventing HPV infection when vaccine was administered within 5 years after the first anal sex, with national guidelines recommending HPV vaccination at a younger age, before the begin of sexual activity, where possible.³⁰ WHO recommend HPV vaccination in individuals aged 9–26 years, providing protection against persistent HPV infection and HPV-related lesions.^{31,32} In our study, results showed no significant differences in HPV prevalence in vaccinated and unvaccinated individuals aged 23–78 years. However, we did not collect follow-up data on persistent HPV infection and anogenital lesions. Moreover, since most of the population enrolled in this study was aged 40 years or higher, we consider the likeliness of vaccination within 5 years or before sexual debut improbable.

This study presents several limitations: (1) the retrospective, cross-sectional nature of the study limited the collection and analysis of data, including specific data about sexual behavior; (2) results were collected only from individuals considered worth of HPV evaluation by their physicians, thus they not be truly representative of the general population; (3) we were not able to collect data regarding HPV infection over time. Our results show that the overall HPV status is not correlated to other non-HPV STIs. Nevertheless, it correlates with certain HR HPV types, including HR-HPV 16, justifying a careful HPV evaluation by a specialist and the potential association with HPV-related anal lesions progression. Nevertheless, the high prevalence of HPV among these individuals underlines the importance of prompt HPV vaccination and prevention strategies implementation. Further studies should investigate the role of coinfections in HPV infection and related anal lesions pathogenesis.

AUTHOR CONTRIBUTIONS

Alberto Rizzo: writing—original draft; experimental design; laboratory analysis. **Davide Moschese:** Conceptualization; followed patients; revision. **Federica Salari:** Writing—original draft; revision. **Andrea Giacomelli:** Experimental design; revision. **Andrea Cavallo:** Laboratory analysis. **Maria Vittoria Cossu:** Followed patients and revision. **Loriana**

Morelli: Laboratory analysis. **Chiara Fusetti:** Followed patients and revision. **Giorgia Carrozzo:** Followed patients and revision. **Serena Reato:** Followed patients and revision. **Valeria Micheli:** Laboratory analysis and revision. **Spinello Antinori:** Revision and coordination. **Alessandra Lombardi:** Laboratory analysis and revision. **Andrea Gori:** Revision and coordination. **Maria Rita Gismondo:** Revision and coordination.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

The study was conducted as part of Registro per il monitoraggio, lo studio e la promozione dell'assistenza dei pazienti afferenti al Dipartimento di Malattie Infettive dell'ASST-FBF-SACCO P.O. Luigi Sacco—Experimental registry n. 2022/ST/238. Protocol N.0011903/2023, 16/03/2023. The procedures being performed were part of the routine care.

ORCID

Alberto Rizzo  <http://orcid.org/0000-0001-5999-006X>

Valeria Micheli  <http://orcid.org/0000-0001-5629-3875>

REFERENCES

1. Genital HPV Infection—Basic Fact Sheet. Centers for Disease Control and Prevention, CDC. Accessed July 5, 2024. <https://www.cdc.gov/std/hpv/stdfact-hpv.htm>
2. Van Gerwen OT, Muzny CA, Marrazzo JM. Sexually transmitted infections and female reproductive health. *Nat Microbiol.* 2022;7(8):1116–1126. doi:10.1038/s41564-022-01177-x
3. World Health Organization. Sexually Transmitted Infections (STIs). Key facts. July 2023. Accessed October 17, 2023. [https://www.who.int/news-room/fact-sheets/detail/sexually-transmitted-infections-\(stis\)](https://www.who.int/news-room/fact-sheets/detail/sexually-transmitted-infections-(stis))
4. Bruni L, Albero G, Rowley J, et al. Global and regional estimates of genital human papillomavirus prevalence among men: a systematic review and meta-analysis. *Lancet Global Health.* 2023;11(9):e1345–e1362. doi:10.1016/S2214-109X(23)00305-4
5. Dunne EF, Park IU. HPV and HPV-associated diseases. *Infect Dis Clin North Am.* 2013;27(4):765–778. doi:10.1016/j.idc.2013.09.001
6. World Health Organization. Human papillomavirus vaccines (HPV). Immunization, Vaccines and Biologicals. December 2022. Accessed January 11, 2024. [https://www.who.int/teams/immunization-vaccines-and-biologicals/diseases/human-papillomavirus-vaccines-\(HPV\)](https://www.who.int/teams/immunization-vaccines-and-biologicals/diseases/human-papillomavirus-vaccines-(HPV))
7. Chesson HW, Mayaud P, Aral SO. Sexually transmitted infections: Impact and cost-effectiveness of prevention. In: Holmes KK, Bertozzi S, Bloom BR, et al. editors. *Major Infectious Diseases*, 3rd ed. The International Bank for Reconstruction and Development/The World Bank; 2017. November 3, Chapter 10. Available from <https://www.ncbi.nlm.nih.gov/books/NBK525195/>. doi:10.1596/978-1-4648-0524-0_ch10

8. Alotaibi HJ, Almajhdi FN, Alsaleh AN, et al. Association of sexually transmitted infections and human papillomavirus co-infection with abnormal cervical cytology among women in Saudi Arabia. *Saudi J Biol Sci.* 2020;27(6):1587–1595. doi:10.1016/j.sjbs.2020.03.021
9. Lu Z, Zhao P, Lu H, Xiao M. Analyses of human papillomavirus, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Neisseria gonorrhoeae*, and co-infections in a gynecology outpatient clinic in Haikou area, China. *BMC Womens Health.* 2023;23(1):117. doi:10.1186/s12905-023-02259-6
10. de Abreu AL, Malaguti N, Souza RP, et al. Association of human papillomavirus, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* co-infections on the risk of high-grade squamous intraepithelial cervical lesion. *Am J Cancer Res.* 2016;6(6):1371–1383.
11. Ye H, Song T, Zeng X, Li L, Hou M, Xi M. Association between genital mycoplasmas infection and human papillomavirus infection, abnormal cervical cytopathology, and cervical cancer: a systematic review and meta-analysis. *Arch Gynecol Obstet.* 2018;297(6):1377–1387. doi:10.1007/s00404-018-4733-5
12. Masiá M, Fernández-González M, García JA, et al. Infection with *Chlamydia trachomatis* increases the risk of high-grade anal intraepithelial neoplasia in people living with human immunodeficiency virus. *Clin Infect Dis.* 2020;70(10):2161–2167. doi:10.1093/cid/ciz606
13. Rizzo A, Moschese D, Salari F, et al. High prevalence of high-risk HPV genotypes in individuals attending an infectious diseases clinic from 2018 to 2022 in Milan, Italy. *J Med Virol.* 2024;96(1):e29370. doi:10.1002/jmv.29370
14. Centers for Disease Control and Prevention. Men Who Have Sex with Men (MSM). Sexually Transmitted Infections Treatment Guidelines, 2021. Accessed July 2021. <https://www.cdc.gov/std/treatment-guidelines/msm.htm>
15. Tu W, Li YY, Kuang YQ, et al. High prevalence of sexually transmitted infections and risk factors among HIV-positive individuals in Yunnan, China. *Eur J Med Res.* 2022;27(1):9. doi:10.1186/s40001-022-00635-w
16. Jansen K, Steffen G, Potthoff A, et al. STI in times of PrEP: high prevalence of chlamydia, gonorrhea, and mycoplasma at different anatomic sites in men who have sex with men in Germany. *BMC Infect Dis.* 2020;20(1):110. doi:10.1186/s12879-020-4831-4
17. Wei F, Gaisa MM, D'Souza G, et al. Epidemiology of anal human papillomavirus infection and high-grade squamous intraepithelial lesions in 29 900 men according to HIV status, sexuality, and age: a collaborative pooled analysis of 64 studies. *Lancet HIV.* 2021;8(9):e531–e543. doi:10.1016/S2352-3018(21)00108-9
18. Fasciana T, Capra G, Di Carlo P, et al. Socio-demographic characteristics and sexual behavioral factors of patients with sexually transmitted infections attending a hospital in Southern Italy. *Int J Environ Res Public Health.* 2021;18(9):4722. doi:10.3390/ijerph18094722
19. Muresu N, Sotgiu G, Saderi L, et al. Distribution of HPV genotypes in patients with a diagnosis of anal cancer in an Italian region. *Int J Environ Res Public Health.* 2020;17(12):4516. doi:10.3390/ijerph17124516
20. Chinyowa S, Palefsky JM, Chirenje ZM, Makunike-Mutasa R, Munjoma M, Muguti GI. Anal human papillomavirus infection in HIV-positive men and women at two opportunistic infections clinics in Harare, Zimbabwe. *BMC Public Health.* 2018;18(1):1260. doi:10.1186/s12889-018-6170-6
21. Paula Almeida Cunha A, Kassandra Pereira Belfort I, Pedro Belfort Mendes F, et al. Human papillomavirus and its association with other sexually transmitted coinfection among sexually active women from the northeast of Brazil. *Interdiscip Perspect Infect Dis.* 2020;2020:8838317. doi:10.1155/2020/8838317
22. Biernat-Sudolska M, Szostek S, Rojek-Zakrzewska D, Klimek M, Kosz-Vnenchak M. Concomitant infections with human papillomavirus and various mycoplasma and ureaplasma species in women with abnormal cervical cytology. *Adv Med Sci.* 2011;56(2):299–303. doi:10.2478/v10039-011-0028-9
23. Discacciati MG, Gimenes F, Pennacchi PC, et al. MMP-9/RECK imbalance: a mechanism associated with high-grade cervical lesions and genital infection by human papillomavirus and *Chlamydia trachomatis*. *Cancer Epidemiol Biomarkers Prevent.* 2015;24(10):1539–1547. doi:10.1158/1055-9965.EPI-15-0420
24. Jiang M, Ding H, He L, et al. Association between co-infection with *Chlamydia trachomatis* or *Mycoplasma genitalium* and cervical lesions in HPV-positive population in Hunan, China: a cross-sectional study. *Infect Agent Cancer.* 2023;18(1):76. doi:10.1186/s13027-023-00544-5
25. Ma Z, Gharizadeh B, Cai X, et al. A comprehensive HPV-STI NGS assay for detection of 29 HPV types and 14 non-HPV sexually transmitted infections. *Infect Agent Cancer.* 2022;17(1):9. doi:10.1186/s13027-022-00420-8
26. Nikolic N, Basica B, Mandic A, et al. E6/E7 mRNA expression of the most prevalent high-risk HPV genotypes in cervical samples from serbian women. *Diagnostics.* 2023;13(5):917. doi:10.3390/diagnostics13050917
27. Olivera C, Mosmann JP, Anna AN, et al. Expression of HPV-16 E6 and E7 oncoproteins alters *Chlamydia trachomatis* developmental cycle and induces increased levels of immune regulatory molecules. *Front Cell Infect Microbiol.* 2023;13:1214017. doi:10.3389/fcimb.2023.1214017
28. Frisch M, Glimelius B, van den Brule AJ, et al. Sexually transmitted infection as a cause of anal cancer. *N Engl J Med.* 1997;337(19):1350–1358. doi:10.1056/NEJM199711063371904
29. Jalil EM, Wilson EC, Monteiro L, et al. High prevalence of anal high-risk HPV infection among transwomen: estimates from a Brazilian RDS study. *J Int AIDS Soc.* 2021;24(3):e25691. doi:10.1002/jia2.25691
30. Chambers C, Deeks SL, Sutradhar R, et al. Vaccine effectiveness against 12-month incident and persistent anal human papillomavirus infection among gay, bisexual, and other men who have sex with men. *J Infect Dis.* 2023;228(1):89–100. doi:10.1093/infdis/jiad005; Erratum: *J Infect Dis.* 2023;227(10):1219.
31. Pan American Health Organization. Human Papillomavirus (HPV) Vaccine. World Health Organization. Accessed March 18, 2024. <https://www.paho.org/en/human-papillomavirus-hpv-vaccine>
32. Rosado C, Fernandes AR, Rodrigues AG, Lisboa C. Impact of human papillomavirus vaccination on male disease: a systematic review. *Vaccines.* 2023;11(6):1083. doi:10.3390/vaccines11061083

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Rizzo A, Moschese D, Salari F, et al. Anal HPV prevalence in individuals with and without other concomitant sexually transmitted infections. *J Med Virol.* 2024;0:e29852. doi:10.1002/jmv.29852