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Type-specific concurrent anogenital HPV detection among young women and MSM attending Dutch sexual health clinics

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► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/sextrans-2022-055484).

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Received 17 March 2022
Accepted 25 June 2022
Published Online First 16 July 2022

To cite: Kusters JMA, Heijne JCM, van Benthem BHB, *et al*. *Sex Transm Infect* 2023;**99**:239–247.

ABSTRACT

Objectives This study aimed to investigate type-specific concurrent anogenital human papillomavirus (HPV) detection and examine associations with concurrent detection.

Methods Data from a Dutch repeated cross-sectional study among young sexual health clinic visitors (Papillomavirus Surveillance among STI clinic Youngsters in the Netherlands) between 2009 and 2019 were used. Cohen's kappa was used to assess the degree of type-specific concordance of HPV detection between anal and genital sites for 25 HPV genotypes for women and men who have sex with men (MSM) separately. Associations with type-specific concurrent HPV were identified. Receptive anal intercourse (RAI) was forced into the model to investigate its influence.

Results Among women (n=1492), type-specific concurrent anogenital detection was common; kappa was above 0.4 for 20 genotypes. Among MSM (n=614), kappa was <0.4 for all genotypes. The only significant association with type-specific concurrent anogenital detection among women was genital chlamydia (adjusted OR 1.5, 95% CI 1.1 to 2.2). RAI was not associated.

Conclusions Type-specific concurrent anogenital HPV detection was common among young women, and uncommon among MSM. For women, concurrent HPV detection was associated with genital chlamydia. Our results are suggestive of autoinoculation of HPV among women.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Type-specific concurrent anogenital human papillomavirus (HPV) infection is common for women, but yet unclear for men who have sex with men (MSM).
⇒ The role of receptive anal intercourse, autoinoculation and other associated factors in yet unclear for an additional anal HPV infection.

WHAT THIS STUDY ADDS

⇒ Type-specific concurrent anogenital HPV detection was common among young Dutch women, but not among young Dutch MSM.
⇒ Chlamydia infection increased the odds for concurrent anogenital HPV detection among young women.
⇒ Receptive anal intercourse is not related to concurrent anogenital HPV detection among young women.
⇒ Our results were suggestive for autoinoculation of HPV infection for women.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study helps in understanding the mechanisms behind HPV infection better.
⇒ Furthermore, it claims that receptive anal intercourse is not associated with an additional anal HPV infection.
⇒ It is important to know which associations are important, as anal cancers attributable to HPV is hard to treat and prevention is therefore important.

INTRODUCTION

Human papillomavirus (HPV) infection is the most common STI in the world.[1] Of the >200 genotypes that can infect humans, 12 are classified as high-risk HPV (hrHPV) genotypes, as they can cause anogenital and oropharyngeal cancers.[1–4] The attributable fraction of HPV of the total number of cervical, anal, vaginal and penile cancer cases globally is 100%, 88%, 78% and 50%, respectively.[4] Globally, 18 000 anal cancer cases for females and 17 000 for males are attributable to HPV annually.[4]

Women and men who have sex with men (MSM) are at higher risk for anal HPV than men who have sex with women (MSW).[5–7] Some studies suggest that a history of receptive anal intercourse (RAI) is an important risk factor for anal HPV infection for women,[8 9] while other studies found that a history of RAI was not a consistent risk factor for anal HPV.[10 11] For MSM, RAI could be a risk factor as well. Perhaps surprisingly, studies among MSW still showed anal HPV among 12.0%–24.8% of MSW,[12–14] although MSW do not have RAI. This suggests that other risk factors than RAI might be important. Both in women and in men, co-occurrence of anal and genital HPV is observed,[6 13] which suggests that genital infection might be an important risk factor for anal infection. For women type-specific concurrent anogenital infection is found often, whereas for MSM, few studies found a level of concordance between HPV infection in anogenital sites. Unfortunately, research on concurrent anogenital infection for MSM is scarce, and most studies are based on small numbers of participants, making it hard to draw conclusions.[15 16]

A large collaborative pooled analysis found a strong association between the presence of hrHPV in cervical and anal specimens, at type-specific level.[17] This suggests either the same source of infection (ie, sexual partner), or autoinoculation between the genital and anal site. The theory of autoinoculation is supported by a recent study on sequential type-specific HPV infection, which found increased risk for sequential HPV infection at the anal site among participants who were positive for the same genotype at the genital site compared with those who were negative at the genital site, both for women and men.[18] All men in this study were heterosexual men, making it hard to draw conclusions for MSM. Additionally, no data on anal sex were collected and therefore, the role of anal sex in concordant HPV infection remains unclear.

This study aims to investigate the prevalence of type-specific concurrent HPV detection at the genital and anal sites, both for women and for MSM in the Netherlands, and to examine associations with type-specific concurrent anogenital HPV detection.

MATERIALS AND METHODS

Study setting

In February 2009, the Papillomavirus Surveillance among STI clinic Youngsters in the Netherlands (PASSYON) study started. In this repeated cross-sectional study with biennial study rounds, visitors aged 16–24 years from 10 to 12 Dutch sexual health clinics (SHCs), throughout the Netherlands, covering both urban and rural areas, were asked to participate.[19] Per round, the inclusion period was approximately 2 months. In addition to routine STI testing, all participants were asked to provide a self-collected genital swab for HPV testing. Additionally, all MSM and a randomly selected subset of women were asked to provide an additional self-collected anal swab. The size of the subset that was invited for anal examination varied across the years. In 2009, few women were asked for an anal swab. In 2011 and 2013, the aim was 10% of all women, in the following rounds the aim was 30%. Selection for the subset was regardless of sexual behaviour, vaccination status or other characteristics. Participants were allowed to refuse anal examination if they did not feel comfortable with collecting an anal swab. If the aimed number of anal samples was achieved, SHCs stopped inviting for anal examination. MSW were not asked to provide anal samples. All participants were asked to complete a written questionnaire on demographics, sexual behaviour and vaccination status.

Sampling technique

Women were asked to self-collect a vaginal swab, by inserting a swab (Copan Diagnostics, Italy) about 4 cm into the vagina, until resistance was felt, and to turn the swab around along the walls of the vagina for about 15 s. Men were asked to self-collect a penile swab, by firmly moving the swab up and down the entire shaft of the penis, the glans, the coronal sulcus and under the foreskin of the penis if possible. Finally, for the anal swab, participants were asked to insert a swab about 3 cm into the anus and circle it around for about 5–10 s. Swabs were placed in a tube with 1 mL universal transport medium (Copan Diagnostics) immediately after swabs were taken.

Laboratory methods

All swabs were stored at −20°C until processing. After thawing and vortexing, 200 µL of the material was used for DNA extraction using the MagnaPure platform (Total Nucleic Acid Isolation Kit, Roche, the Netherlands). Total DNA was eluted in 100 µL elution buffer and 10 µL was used to amplify HPV-DNA with the SPF_{10} primer set. HPV-specific amplicons were detected using ELISA (HPV-DEIA, DDL Diagnostic Laboratory, the Netherlands). Positive samples were subsequently genotyped with the Line probe assay (HPV-LiPA, DDL Diagnostic Laboratory), which is able to identify 25 genotypes (6, 11, 16, 18, 31, 33–35, 39, 40, 42–45, 51–54, 56, 58, 59, 66, 68, 70, 74).

Statistical analyses

Eligible for inclusion in the current analyses were data from female and MSM participants who provided both anal and genital samples as part of any of the study rounds between 2009 and 2019. A genital or anal sample was considered positive if at least one of the 25 genotypes could be detected. All analyses were conducted for women and MSM separately. The study population characteristics were described by using descriptive statistics.

The main purpose of the analysis was to assess the prevalence of type-specific concurrent HPV at the genital and anal sites for women and MSM. To determine the level of concordance between genital and anal sites, Cohen's kappa was calculated per genotype. Concordance was considered low if the kappa value was <0.20, fair between 0.20 and 0.40, moderate between 0.40 and 0.60, high between 0.60 and 0.80 and very high if kappa values would be >0.80.[20]

Provided that at least moderate concordance for anogenital HPV detection was observed for at least one genotype for either women or for MSM, associations between potential risk factors and type-specific concurrent anogenital HPV detection were assessed for that group. This was done using multivariable logistic regression. As participants could be infected with multiple genotypes, they could contribute multiple records; one for each detected genital type-specific HPV type. In the model, concurrent HPV detection (ie, detection with the same HPV type at both genital and anal sites) was compared with genital-only detection. Generalised estimated equations were used with binomial distribution and an exchangeable correlation structure, because of the correlated nature of the observations. The model was built with a stepwise backward selection, with p value cut-off for exit of 0.20. This p value cut-off was chosen, as a lower p value may lead to selection bias and optimism as a result of overfitting, meaning that the model is too closely adapted to the data.[21] Potential risk factors included demographics, RAI, other sexual behaviour, other STIs and history of HPV vaccination. The potential risk factor 'RAI ever' was forced into the final multivariable model a priori, as this could directly explain anal detection with the same genotype.

All analyses were performed using SAS V.9.4 (SAS Institute, Cary, North Carolina, USA). If for a variable in the logistic regression models, >5% of records had a missing value, a separate category of 'unknown' was added. For the variable 'year of participation', the first three rounds were combined, in view of low numbers in the first three rounds. The chosen level of significance was p<0.05.

RESULTS

Study population

A total of 1492 women and 614 MSM provided both anal and genital samples in 2009–2019, with a median age of 21 years (IQR 20–23) and 22 years (IQR 20–23), respectively. More on the demographic characteristics can be found in table 1.

Table 1 Characteristics of the study population, young women and MSM, recruited at sexual health clinics in 2009–2019 in the Netherlands

Characteristic	Women N (%) or median (IQR)	MSM N (%) or median (IQR)
Total	1492 (100.0)	614 (100.0)
Year of participation		
2009	12 (0.8)	51 (8.3)
2011	133 (8.9)	79 (12.9)
2013	153 (10.3)	112 (18.2)
2015	367 (24.6)	103 (16.8)
2017	409 (27.4)	110 (17.9)
2019	418 (28.0)	159 (25.9)
Median age (years)	21 (20–23)	22 (20–23)
Education level*		
Low/Middle	316 (21.3)	190 (31.2)
High	1171 (78.8)	420 (68.9)
Self-defined country of birth		
The Netherlands	1278 (85.7)	498 (81.1)
Smoking†		
(Almost) never	633 (42.4)	200 (32.6)
Previously	58 (3.9)	18 (2.9)
Currently	466 (31.2)	148 (24.1)
Unknown	335 (22.5)	248 (40.4)
Received at least one dose of HPV vaccine (self-reported)		
No	725 (48.6)	505 (82.3)
Yes	630 (42.2)	19 (3.1)
Unknown	137 (9.2)	90 (14.7)
Median age of sexual debut (years)‡	16 (15–17)	17 (15–18)
No. of sex partners previous 6 months		
0–1	384 (25.8)	101 (16.5)
2–3	727 (48.8)	185 (30.1)
≥4	380 (25.5)	328 (53.4)
No. of sex partners lifetime		
0–4	344 (23.6)	79 (13.0)
5–9	528 (35.2)	119 (19.6)
≥10	587 (39.2)	408 (67.3)
Condom use steady partner previous 6 months		
Mostly not using condom	763 (51.5)	191 (31.5)
Sometimes using condom	261 (17.6)	102 (16.8)
Always using condom	73 (4.9)	99 (16.3)
No steady partner	385 (26.0)	215 (35.4)
Condom use casual partner previous 6 months		
Mostly not using condom	344 (28.5)	73 (13.5)
Sometimes using condom	701 (58.0)	240 (44.4)
Always using condom	163 (13.5)	227 (42.0)
No casual partner	269 (18.2)	71 (11.6)
STI-related symptoms	413 (27.9)	132 (21.5)
History of STI		
No	751 (50.6)	269 (44.0)
Yes	467 (31.4)	268 (43.8)
Never tested before	267 (18.0)	75 (12.3)
Receptive anal intercourse ever§¶	751 (51.3)	512 (91.4)
Current chlamydia, genital**	221 (14.8)	21 (3.4)
HIV status		
Negative	692 (56.9)	474 (95.8)
Positive	2 (0.2)	20 (4.0)
Unknown/never tested	523 (43.0)	1 (0.2)
Genital HPV detection		
No	343 (23.0)	420 (68.4)
Single	377 (25.3)	121 (19.7)
Multiple	772 (51.7)	73 (11.9)
Anal HPV detection		
No	799 (53.6)	313 (51.0)
Single	270 (18.1)	136 (22.1)
Multiple	423 (28.4)	165 (26.9)
Type-specific concurrent detection, for at least one genotype	607 (40.7)	64 (10.4)

Totals may vary, because of missings. Total percentages may not add up to 100 due to rounding.
*High education level is defined as higher general secondary education, preuniversity education, university for applied sciences and university. Low/Middle is defined as all other levels of education.
†No information was available for those participating in 2009–2013, as the relevant question was not part of the questionnaire in that year.
‡Minimum-maximum range was 11–24 years for women and 7–22 years for MSM.
§Some MSM had exclusive receptive or insertive anal intercourse, some had a combination of receptive and insertive anal intercourse.
¶No information was available for those participating in 2009, as the relevant question was not part of the questionnaire in that year.
**Diagnosis made at the same visit to the sexual health clinic at which the participant was included in this study.
HPV, human papillomavirus; MSM, men who have sex with men.;

HPV detection

Of the women, 1149 (77.0%) had at least one genital HPV-type detection and 693 (46.5%) at least one anal HPV-type detection. For MSM this was 194 (31.6%), and 301 (49.0%) for genital and anal HPV detection, respectively (online supplemental table 1). The most common detected genital and anal HPV type for women was HPV-51 (genital: 23.8%, anal: 13.3% of all women). For MSM the most common detected genital and anal HPV type was HPV-6 (genital: 7.0%, anal: 13.0% of all MSM).

Concordance

Type-specific concurrent anogenital HPV detection was found in 40.7% of women, and in 10.4% of MSM. Type-specific and site-specific HPV prevalences are shown in figure 1 (more details in online supplemental file): among women, genital-only and concurrent anogenital detections were much more common than anal-only detections. For MSM, anal-only detection was more common than genital-only detection, concurrent anogenital detection was least common. Genital-only and concurrent anogenital detection were more common among women than among MSM, and anal-only HPV detection was more common among MSM than among women.

Among women, concurrent anogenital detection for HPV-51 was seen most often; 10.7% of all women had concurrent HPV-51 detection, followed by HPV-52 (8.8%) (table 2). Out of the 25 tested genotypes, 20 showed at least moderate concordance between anal and genital sites ($\kappa>0.4$). HPV-6 showed highest concordance ($\kappa=0.60$), followed by low-risk HPV

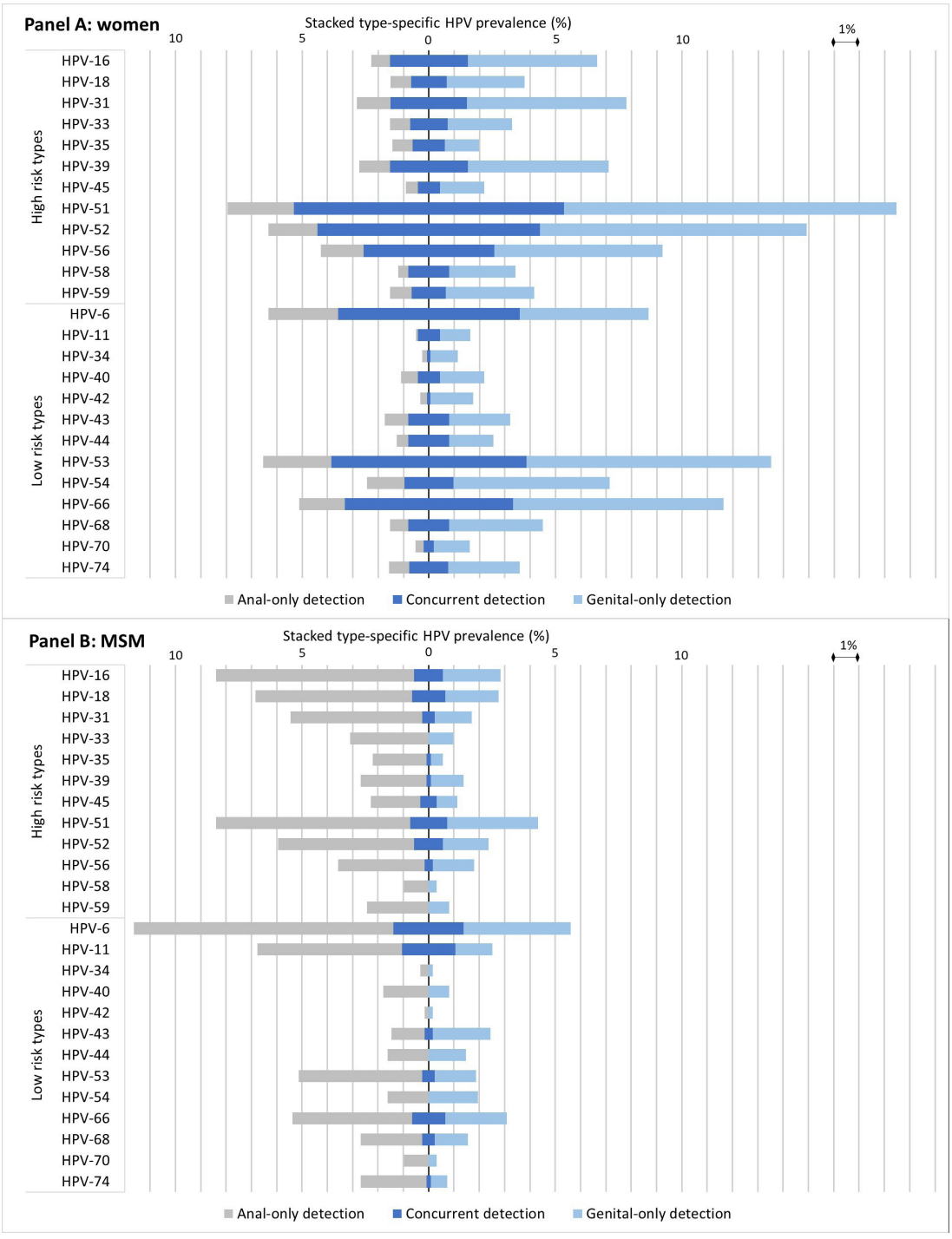

Figure 1 Type-specific anal, genital and concurrent HPV detection for clients of sexual health clinics in the Netherlands in 2009-2019. Presented as stacked prevalences, centered around concurrent anogenital infections. Concurrent anogenital detections are symmetrically distributed on both sides of the zero-axis. Panel A: women, panel B: MSM.HPV, human papillomavirus.

(lrHPV) type HPV-44 (κ=0.58). Of the hrHPV types, HPV-35 had the highest level of concordance (κ=0.53).

Among MSM, highest concordance was detected for HPV-6 (2.8%). None of the HPV types had a kappa above 0.40. lrHPV-11 had the highest kappa (κ=0.34), followed by hrHPV-45 (κ=0.30).

Associations

As only among women at least moderate concordance was detected between genital and anal sites, associations with concurrent anogenital detection were determined for women only. In this analysis, 1149 women with at least one detected genital HPV type were included, contributing 2731 genital type-specific detections. Due to missing values 2677 detections were included in the analysis, contributing 1622 genital-only and 1055 type-specific concurrent anogenital HPV detections (online supplemental figure 2).

In the univariable logistic regression analyses, type-specific concurrent HPV was significantly associated with current genital *Chlamydia trachomatis* (chlamydia), with an OR 1.5 (95% CI

Table 2 Type-specific concordance of detected HPV infection at genital and anal sites, young women and MSM recruited at sexual health clinics in 2009–2019 in the Netherlands

	Women (genital/anal)					MSM (genital/anal)				
HPV type	(+/+) %	(+/–) %	(–/+) %	(–/–) %	Kappa (95% CI)	(+/+) %	(+/–) %	(–/+) %	(–/–) %	Kappa (95% CI)
High-risk types										
HPV-16	3.1	5.1	0.7	91.1	0.49* (0.38 to 0.59)	1.1	2.3	7.8	88.8	0.14 (–0.06 to 0.34)
HPV-18	1.4	5.1	0.8	94.7	0.40*(0.25 to 0.55)	1.3	2.1	6.2	90.4	0.20 (–0.01 to 0.41)
HPV-31	3.0	6.3	1.2	90.2	0.41* (0.30 to 0.51)	0.5	1.5	5.2	92.8	0.10 (–0.16 to 0.37)
HPV-33	1.5	2.6	0.8	95.2	0.45* (0.30 to 0.60)	0.0	1.0	3.1	96.0	–0.02 (–0.40 to 0.37)
HPV-35	1.3	1.3	0.8	96.6	0.53* (0.37 to 0.69)	0.2	0.5	2.1	97.2	0.10 (–0.33 to 0.54)
HPV-39	3.1	5.6	1.2	90.2	0.44* (0.34 to 0.55)	0.2	1.3	2.6	95.9	0.06 (–0.31 to 0.43)
HPV-45	0.9	1.7	0.5	96.9	0.43* (0.24 to 0.62)	0.7	0.8	2.0	96.6	0.30 (–0.02 to 0.63)
HPV-51	10.7	13.1	2.6	73.6	0.49* (0.45 to 0.55)	1.5	3.6	7.7	87.3	0.15 (–0.04 to 0.34)
HPV-52	8.8	10.5	1.9	78.8	0.52* (0.45 to 0.58)	1.1	1.8	5.4	91.7	0.21 (–0.02 to 0.43)
HPV-56	5.2	6.6	1.7	86.5	0.51* (0.43 to 0.59)	0.3	1.6	3.4	94.6	0.09 (–0.22 to 0.40)
HPV-58	1.6	2.6	0.4	95.4	0.50* (0.36 to 0.65)	0.0	0.3	1.0	98.7	0.00 (–0.70 to 0.69)
HPV-59	1.3	3.4	0.9	94.3	0.36 (0.21 to 0.51)	0.0	0.8	2.4	98.7	–0.01 (–0.45 to 0.42)
Low-risk types										
HPV-6	7.2	5.1	2.8	85.0	0.60† (0.53 to 0.67)	2.8	4.2	10.3	82.7	0.20 (0.05 to 0.36)
HPV-11	0.9	1.2	0.1	97.9	0.57* (0.38 to 0.76)	2.1	1.5	5.7	90.7	0.34 (0.15 to 0.53)
HPV-34	0.1	1.1	0.2	98.6	0.17 (-0.20 to 0.54)	0.0	0.2	0.3	99.5	0.00 (-1.13 to 1.13)
HPV-40	0.9	1.7	0.7	96.7	0.41* (0.22 to 0.60)	0.0	0.8	1.8	97.4	–0.01 (–0.50 to 0.48)
HPV-42	0.1	1.7	0.3	97.9	0.12 (-0.20 to 0.43)	0.0	0.2	0.2	99.7	0.00 (–1.39 to 1.38)
HPV-43	1.6	2.4	0.9	95.0	0.47* (0.33 to 0.62)	0.3	2.3	1.3	96.1	0.14 (–0.22 to 0.49)
HPV-44	1.6	1.7	0.5	96.2	0.58* (0.44 to 0.72)	0.0	1.5	1.6	96.9	–0.02 (–0.47 to 0.43)
HPV-53	7.7	9.7	2.7	80.0	0.49* (0.42 to 0.56)	0.5	1.6	4.9	93.0	0.10 (–0.17 to 0.37)
HPV-54	1.9	6.2	1.5	90.4	0.30 (0.18 to 0.43)	0.0	2.0	1.6	96.4	–0.02 (–0.44 to 0.40)
HPV-66	6.6	8.3	1.9	83.2	0.51* (0.44 to 0.59)	1.3	2.4	4.7	91.5	0.23 (0.01 to 0.45)
HPV-68	1.6	3.7	0.7	94.0	0.40* (0.26 to 0.54)	0.5	1.3	2.4	95.8	0.19 (–0.14 to 0.51)
HPV-70	0.4	1.4	0.3	97.9	0.31 (0.45 to 0.57)	0.0	0.3	1.0	98.7	0.00 (–0.70 to 0.69)
HPV-74	1.5	2.8	0.8	94.8	0.44* (0.30 to 0.59)	0.2	0.7	2.6	96.6	0.08 (–0.32 to 0.48)

The analyses are based on 1492 women and 614 MSM with genital and anal samples collected in 2009, 2011, 2013, 2015, 2017 or 2019. Samples positive for HPV-DNA but with untypable genotypes were excluded from these analyses; +, presence of corresponding HPV genotype; –, absence of corresponding genotype. Total percentages may not add up to 100% due to rounding.
*Moderate concordance.
†High concordance.
HPV, human papillomavirus; MSM, men who have sex with men.

1.2 to 1.9) (table 3). Ever having had RAI was not significantly associated (OR 1.1, 95% CI 0.9 to 1.3).

In the multivariable logistic regression model only current genital chlamydia was significantly associated with concurrent HPV detection (OR 1.5; 95% CI 1.1 to 1.9). A history of HPV vaccination was not significantly associated with type-specific concurrent anogenital detection: (OR for vaccination 0.9, 95% CI 0.7 to 1.1; OR for vaccination status unknown 0.7, 95% CI 0.5 to 1.1). Finally, concurrent detection was not significantly associated with RAI ever (OR 1.1, 95% CI 0.9 to 1.3). In a sensitivity analysis, 'RAI ever' was replaced with 'RAI in the previous 6 months'. Final associated variables in the multivariable model were the same, and RAI in the previous 6 months was not associated with concurrent infection (OR 1.0, 95% CI 0.8 to 1.3).

DISCUSSION

This study assessed the level of type-specific concordance of genital and anal HPV detection among young women and MSM who visited an SHC in the Netherlands in 2009–2019, and aimed to determine associations with concurrent anogenital detection with the same HPV genotype. Type-specific concurrent HPV was common for most HPV genotypes among young women, but not for MSM. The only association with type-specific concurrent HPV among women was having genital chlamydia. Strikingly, ever having had RAI was not associated.

A strength was that a large dataset was used, comprising multiple years and geographic locations throughout the Netherlands, making it the largest study of its kind for young women and MSM. Additionally, participants were aged 16–24 years, the age span in which the incidence of HPV infection is often at a peak, especially in women.[5] Another strength was that women were randomly asked for anal testing, irrespective of sexual behaviour, which minimises selection bias. Finally, Cohen's kappa is a statistic that is often used to measure inter-rater reliability. It is also applicable for measuring concordance of detections, as it is a statistic to measure agreement, while taking chance of concordance into account. Therefore, this statistic is chosen over other statistics not accounting for chance, such as the phi-statistic. We used conventional predefined cut-off points[20]; these are arbitrary. Regardless of cut-off points, MSM have very low kappas, not significantly above zero (ie, chance concordance) and a clear difference with women is apparent.

Table 3 Risk factors for type-specific concurrent anogenital HPV detection, comparing young women with concurrent infection with women with genital-only infection, recruited at sexual health clinics in 2009–2019 in the Netherlands

Factor	n/N* (%)	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Year of participation†					
2009, 2011 or 2013	195/466 (41.9)	1	0.80		
2015	262/692 (37.9)	0.9 (0.6 to 1.2)			
2017	309/782 (39.5)	0.9 (0.7 to 1.2)			
2019	309/791 (39.1)	0.9 (0.7 to 1.2)			
Age (years)					
16–18	69/166 (41.6)	1.2 (0.8 to 1.7)			
19–21	456/1128 (40.4)	1.1 (0.9 to 1.3)			
22–24	550/1437 (38.3)	1	0.60		
Education level‡					
Low/Middle	232/569 (40.8)	1	0.45		
High		0.9 (0.7 to 1.2)			
Self-defined country of birth					
The Netherlands	932/2376 (39.2)	1	0.90		
Other	834/2146 (38.9)	1.0 (0.7 to 1.3)			
Smoking§					
(Almost) never	443/1133 (39.1)	1	0.76		
Previously	49/128 (38.3)	1.0 (0.6 to 1.6)			
Currently	357/929 (38.4)	0.9 (0.7 to 1.2)			
Unknown	226/541 (41.8)	1.1 (0.8 to 1.4)			
Received at least one dose of HPV vaccine (self-reported)					
No	579/1410 (41.1)	1	0.27	1	0.20
Yes	416/1085 (38.3)	0.9 (0.7 to 1.1)		0.9 (0.7 to 1.1)	
Unknown	80/236 (33.9)	0.8 (0.5 to 1.1)		0.7 (0.5 to 1.1)	
Age of sexual debut (years)					
≤14	144/363 (39.7)	0.9 (0.7 to 1.3)			
15–16	501/1317 (38.0)	0.9 (0.7 to 1.1)			
≥17	412/1016 (40.6)	1	0.38		
No. of sex partners past 6 months					
0–1	240/592 (40.5)	1	0.43		
2–3	522/1285 (40.6)	1.0 (0.8 to 1.3)			
≥4	312/850 (36.7)	0.9 (0.7 to 1.2)			
No. of sex partners lifetime					
0–4	168/418 (40.2)	1	0.59		
5–9	396/970 (40.8)	1.1 (0.8 to 1.4)			
≥10	487/1286 (37.9)	0.9 (0.7 to 1.3)			
Condom use with steady partner previous 6 months					
Mostly not using condom	576/1445 (39.9)	1.2 (0.8 to 2.0)			
Sometimes using condom	185/436 (42.4)	1.4 (0.8 to 2.4)			
Always using condom	36/109 (33.0)	1	0.61		
No steady partner	272/724 (37.6)	1.2 (0.7 to 2.0)			
Condom use with casual partner previous 6 months					
Mostly not using condom	272/673 (40.4)	1.2 (0.9 to 1.8)			
Sometimes using condom	537/1374 (39.1)	1.1 (0.8 to 1.6)			
Always using condom	88/255 (34.5)	1	0.60		
No casual partners	170/405 (42.0)	1.3 (0.8 to 1.9)			
STI-related symptoms					
No	762/1951 (39.1)	1	0.53		
Yes	308/766 (40.2)	1.1 (0.9 to 1.3)			
History of STI					
No	497/1259 (39.5)	1	0.91		
Yes	426/1086 (39.1)	1.0 (0.8 to 1.2)			

Continued

Table 3 Continued

Factor	n/N* (%)	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Never tested before	144/368 (39.1)	0.9 (0.7 to 1.3)			
Current chlamydia, genital¶					
No	860/2278 (37.8)	1	0.003	**1**	**0.003**
Yes	215/452 (47.6)	1.5 (1.2 to 1.9)		**1.5 (1.1 to 1.9)**	
Receptive anal intercourse ever**					
No	469/1243 (37.7)	1	0.56	1	0.60
Yes	586/1435 (40.8)	1.1 (0.9 to 1.3)		1.1 (0.9 to 1.3)	

The analyses are based on 1149 women with detected genital infection, of which 607 had at least one type-specific concurrent anogenital detected infection, contributing 2731 type-specific genital HPV infections. The final multivariable model is based on 2677 type-specific genital detected infections, due to some missing data, comparing 1055 concurrent anogenital infections with 1622 genital-only infections.
*N=concurrent anogenital infections.
†Due to small numbers of inclusion for anal examination in the first three rounds, these rounds are combined for the analysis.
‡High education level is defined for school of higher general secondary education, pre-university education, university for applied sciences and university. Low/Middle is defined for all other levels of education.
§No information was available for those participating in 2009–2013, as the relevant question was not part of the questionnaire in that year.
¶Diagnosis made at the same visit to the sexual health clinic at which the participant was included in this study.
**No information was available for those participating in 2009, as the relevant question was not part of the questionnaire in that year.
HPV, human papillomavirus;

This study also has limitations. First of all, participants were recruited at SHCs, a population with higher STI prevalence, including HPV. Therefore, results might not be generalisable. However, it is likely that the type-specific kappas are similar between SHC visitors and the general population. Second, no information was collected on the number, or characteristics of SHC visitors that declined participation. Potentially, the invitation for anal examination might have discouraged participation. Unfortunately, it was not possible to examine if this has led to sampling bias. Additionally, some important data, like vaccination status and sexual behaviour, were self-reported, which might have led to recall bias or social desirability bias. The resulting misclassification might lead to underestimation of the true association for these variables. A previous analysis of PASSYON data showed reliable reporting of vaccination,[22] so misclassification for vaccination status is unlikely. Finally, it would have been interesting to compare the levels of concordance of type-specific anogenital HPV of MSM with those of MSW. This way, the of role sexual behaviour would be explored, as there is no anatomical difference. Unfortunately, the study design of the PASSYON study did not include any anal sampling for MSW, and therefore this comparison was not possible.

The first finding of our study was that concurrent anogenital detection is common for young women. In previous studies, concurrent infection at the genital and anal site for women has been analysed and reported before. Only one study calculated the type-specific Cohen's kappa between genital and anal site, as was done in our study.[23] That study calculated the level of concordance between vaginal or vulva and anal infection for eight HPV genotypes; all kappas were lower than in our study. Possible reasons for this discrepancy include differences in geographical areas (China vs the Netherlands), age (18–55 years vs 16–24 years) and vaccination status (having been vaccinated was an exclusion criterion[23]). Another study among referred women for colposcopy, found that 17 HPV genotypes (out of 35) detected in the cervix, correlated with the types in the anus.[24] As this study only included referred women, and another statistic for concordance was used, comparing results is challenging.

Our study did not find substantial concordance for any genotype for MSM, which is a striking difference compared with women. There are few published studies on concurrent anogenital HPV for MSM, but our findings are in line with the few available studies.[15 25 26] One study also found a higher anal-only HPV prevalence than penile-only and concurrent HPV among MSM.[25] Major differences between that study and ours were that it reported relative distributions instead of kappas, participants had a higher median age (40.1 years vs 22 years) and a higher median number of lifetime sex partners (200 vs 15), both risk factors for HPV. A Greek study among men showed 7% type-specific concurrent infection. Like in our study, anal infection was more common than genital infection.[26] Unfortunately, the study population was a mix of MSW and MSM (30.3%) and the analysis was not stratified by sexual orientation. Finally, a small study (n=127) conducted in the USA found highest level of concurrent infection for HPV-6 (4.0%), like in our study (2.8%).[15] However, neither the American, nor the Greek study calculated kappas as measure for concordance, making comparisons on concordance impossible.

In our study, a robust positive association was observed between current genital chlamydia and type-specific concurrent anogenital HPV detection for women. An explanation could be that, although the exact relationship between chlamydia and HPV is yet unclear, they might act as mutual associations for infection, as suggested in a systematic review and meta-analysis.[27] Unfortunately, our cross-sectional study design did not allow to study the chronological sequence of infections, or whether the infections occurred simultaneously. Previous studies suggested that chlamydia might facilitate infection of multiple HPV types, as it might play a role in disturbing and modulating the immune response that is involved in HPV clearance.[28 29] A disturbed immune response could facilitate HPV to go from the genital to the anal site, or vice versa, and this autoinoculation results in a concurrent infection. Future studies are advised to investigate the role of biological mechanisms of chlamydia in relation to HPV.

Finally, no significant association between RAI, and type-specific concurrent HPV detection was found among women, meaning that RAI is not an important associated factor among women in this study. Two other explanations for concurrent anogenital HPV among young women might be infection during sex without penile-anal penetration, or autoinoculation from the

genital to the anorectal site, or vice versa. The cross-sectional design of our study makes it impossible to assess the sequence of infections. Fortunately, previous studies have studied sequential infections at anatomical locations. For example, one study observed a high relative risk of acquiring anal HPV infection after cervical infection with the same genotype, and vice versa.[30] Another study found an increased HR for acquiring anal infection with any HPV type after genital infection, compared with women without preceding genital infection.[18] The increased risk of anal infection after genital infection with the same type, in combination with our finding of RAI not being associated, supports the theory of autoinoculation. Autoinoculation is plausible, as HPV is a field infection and the vulva and anus are anatomically very close. This would then also explain why MSM in our study are less likely to have concurrent detection, as the penis and anus are anatomically less close. Additional longitudinal studies might unravel the potential role of autoinoculation.

In conclusion, this study found that type-specific concurrent anogenital HPV detection is common among young women, but not among MSM. The only related association with concurrent infection for women was current genital chlamydia, increasing the odds for an additional anal HPV detection with the same type. Moreover, none of the explored sexual behaviours was associated with concurrent HPV detection, including RAI. Therefore, our study is suggestive for autoinoculation as an explanation for concurrent HPV detection among women.

Handling editor Claudia S Estcourt

Acknowledgements The authors would like to thank the following for their valuable contributions to the design or execution of the study: Hein Boot (deceased), Elske van Logchem, Naomi van Marm, Suzan Leussink, Tim Severs, Rutger Schepp, Kahren van Eer, Najima Lamkaraf, Maarten Schipper and Rianne Vriend. Furthermore, the sexual health centres, including all nurses and physicians, within the Public Health Services and the hospitals are acknowledged for their permission to collect data from their patients and for their efforts. The authors acknowledge the medical microbiological laboratories and the analysts for storage and testing of the samples. Medical microbiological laboratories: Certe: D. Adema, R. Buist-Arkema, D. Luijt, S. Meijer, J. Schirm. ETZ Hospital Tilburg: A. Buiting, H. Verbakel, P. van Esch, J. Verweij. Erasmus Medical Center: A. van der Eijk, R.C. Huisman. University Medical Center Utrecht: F. Verduyn Lunel, S. Lakbiach, R. Schuurman. Gelre Hospital: A. Bollee-Karsel. Public Health Laboratory Amsterdam: D. Abma, K. Adams, S. Bruisten, I. Linde, P. Oostvogel, C. Touwen, W. Vermeulen. Maastricht University Medical Center: J. Nelissen, P. Wolffs. Jeroen Bosch Hospital: N. van Duijvendijk, P. Schneeberger. Radboud University Medical Center: M. Dinnissen-van Poppel, W. Melchers. Izore: M. Hooghiemstra, H. Huisman, J. Weel. LabMicTA: F. Bosma, F. Geeraedts, I. Polman, T. Smits. Isala: P. van Goor, M. Wolfhagen. Rijnstate: E. van Koolwijk, M. Peters, C. Swanink, R. Tiemessen, L. de Ruijter. 356 Medical Laboratory Dr Stein & Collegae: J. Janssen, M. Pelsers. Canisius Wilhelmina Hospital: W. de Waal. Public Health Services: PHS Drenthe: G. Aalfs. PHS IJsselland: H. van Buel. PHS Gelderland-Zuid: C. van Bokhoven-Rombouts, P. Cornelissen, M. Kersten, C. van Ruitenbeek, I. Molenaar. University Medical Center Utrecht: E. Doorn. PHS Rotterdam-Rijnmond: H. Götz, M. Illidge, J. Stam, E. Swaders. PHS Groningen: F. Postma. PHS Zuid Limburg: A M. Niekamp, M. Smit. PHS Fryslân: D. Bukasa, M. Chirandjilal, T. Taconis. PHS Twente: M. de Graas, I. Hondelink, C. Kampman. PHS Hart voor Brabant: M. van de Pas. PHS Amsterdam: T. Heijman, A. Hogewoning, M. van Rooijen. PHS Gelderland-Midden: F. Neienhuijsen, M. Pelgrim.

Contributors JK: conceptualisation, methodology, formal analysis, writing original draft, guarantor; JH: conceptualisation, methodology, review and editing, supervision; BvB: conceptualisation, review and editing, supervision, funding acquisition; AK: validation, analysis samples, review and editing; MSvdL: conceptualisation, methodology, review and editing, supervision.

Funding This work was supported by the Ministry of Health, Welfare and Sport, the Netherlands.

Disclaimer The funders had no role in study design, data collection and analysis, interpretation of data, decision to publish or preparation of the manuscript.

Competing interests All authors reported to have no conflicts of interest. All authors have submitted the ICMJE form for disclosure of potential conflicts of interest. Conflicts that the editors consider relevant to the context of the manuscript have been disclosed.

Patient consent for publication Not applicable.

Ethics approval This study was approved by Medical Ethics Committee of the University of Utrecht, The Netherlands (reference number: 08/397). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Data are available on reasonable request, and can be requested by email.

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