Gynecologic Oncology 154 (2019) 354–359

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Age-specific HPV type distribution in high-grade cervical disease in screened and unvaccinated women

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HIGHLIGHTS

- HPV distribution was markedly polarised by age in women referred to colposcopy.
- HPV16/18 was twice as common in women <30 years than in women ≥45 with HSIL lesions.
- In women ≥45 years other high-risk HPVs were more common in HSIL lesions.
- In women ≥45 years approximately 10% of HSIL lesions were negative for high-risk HPV.

ARTICLE INFO

Article history:
Received 8 April 2019
Received in revised form 11 May 2019
Accepted 28 May 2019
Available online 5 June 2019

Keywords:
Squamous intraepithelial lesions of the cervix
Prevalence
HPV human papillomavirus
Genotype

ABSTRACT

Background and aim. Age-specific type-distribution of high-risk human papillomavirus (hrHPV) in cervical precancerous lesions is subject to change in the HPV vaccination era. Knowing the pre-vaccination type-distribution helps to anticipate changes induced by mass vaccination and optimize screening.

Methods. We recruited 1279 women referred to colposcopy for abnormal cytology into a population-based study on HPV type distribution in diagnostic cervical samples (ISRCTN10933736). The HPV genotyping findings were grouped as: HPV16/18+, other hrHPV+ (HPV31/33/35/39/45/51/52/56/58/59/66/68), non-vaccine targeted hrHPV+ (HPV35/39/51/56/59/66/68), low-risk HPV, and HPV negative. We estimated the HPV group-specific prevalence rates according to diagnostic histopathological findings in the age groups of <30 (n = 339), 30–44.9 (n = 614), and ≥45 (n = 326).

Results. Altogether 503 cases with high grade squamous intraepithelial lesion or worse (HSIL+) were diagnosed. More than half, 285 (56.7%) of HSIL+ cases were associated with HPV16/18: 64.3% (101/157) in women <30 years (reference group), 58.4% (157/269) in women 30–44.9 years (risk ratio (RR) 0.91, 95% confidence interval (95% CI) 0.78–1.06), and 35.1% (27/77) in women ≥45 years of age (RR 0.55, 95% CI 0.39–0.75). Conversely, other hrHPV's were associated with 191 (38.0%) of HSIL+: 31.9% (50/157) in women <30, 36.8% (99/269) in women 30–44.9 years, 54.6% (42/77) and in women ≥45 (RR 1.71, 95% CI 1.26–2.33). The proportion of non-vaccine targeted hrHPV and HPV negative HSIL+ increased with advancing age.

Conclusions. Pre-vaccination HPV type distribution in HSIL+ was distinctly polarised by age with HPV16/18 attributed disease being markedly more prevalent in women aged <30. In the older women the other hrHPV types, however, dominated suggesting a need for more age-dependent screening strategies.

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1. Introduction

The prevalence of high-risk human papillomavirus (hrHPV) peaks in young women, with HPV16 being globally the most common type [1,2]. Approximately 70% of cervical cancers are associated with HPV16 and HPV18, the types targeted by all three prophylactic vaccines [3–6]. The latest 9-valent vaccine directly also targets five other hrHPVs (HPV31/33/45/52/58), and the bivalent vaccine is reported to have moderate to high cross-protective efficacy against a number of other hrHPV types (HPV31/33/35/45/52) [7–11]. Prophylactic HPV vaccines have

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https://doi.org/10.1016/j.ygyno.2019.05.024
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high efficacy against high-grade squamous intraepithelial lesions (HSIL), especially in young HPV naïve women [12,13]. Evidence on protection against invasive cervical cancer from a randomised setting has been reported but long-term efficacy is yet to be confirmed [14]. Comprehensive data of a dramatic decrease in hrHPV infections and HSIL approximately a decade after vaccination are very promising [15–22].

Histological HSIL is most prevalent in women in their late twenties or early thirties [23]. The peak incidence of cervical cancer in well-screened populations is among 30–40-year-olds, but remains elevated with advancing age when cervical cancer mortality also increases [24]. An age-specific pattern of hrHPV type distribution with larger proportions of cervical cancer in older women attributed to other hrHPV types than HPV16/18 has been observed, but type distribution in HSIL has conflicting results [25–29]. At the same time with the initiation of HPV vaccination programs a shift from cytological screening to hrHPV-based screening has taken place [30–32], and HPV16/18 genotyping has been advocated as a triage test on the urgency of colposcopy after a positive hrHPV test regardless of age [33].

Our objective was to study the current age-specific hrHPV type distribution in cervical HSIL lesions in unvaccinated highly screened women. These data are essential in optimizing current and future screening programs.

2. Material and methods

Women 18 years of age or older referred to Helsinki University Hospital Colposcopy Unit for abnormal cytology between January 2014 and May 2016 were recruited to participate in a prospective cohort study (HELICOPTER study: ISRCTN10933736). The unit is the single referral center in the Helsinki metropolitan area with 4500 annual colposcopies serving a population base of over one million. For this study, we only included women with abnormal cytology without symptoms or clinical pathological findings as the reason of referral. Clinical data was collected at the first colposcopy, and if large loop excision of the transformation zone (LLETZ) was performed based on punch biopsy results, data on that visit was collected as well. All women were examined and treated according to Finnish Current Care guidelines [34]. The protocol was approved by Helsinki University Hospital's Ethical Committee (130/13/03/03/2013, 24.4.2013), and written informed consent was acquired from all participants.

2.1. Screening and colposcopy

During the study period organised screening was conducted by conventional cervical cytology at five-year intervals. The age of onset and finish of screening varied between municipalities, from 25–30 up to 60–65 years. Opportunistic screening outside the nationwide program was also mainly conducted by conventional cytology. After referral for abnormal cytology colposcopy was performed within time frames set by Finnish Current Care guidelines (Table A.1) [34].

A senior colposcopist with national certification or >100 annual colposcopies was always present at colposcopy. In addition to routine samples, endocervical cells were obtained with a brush for HPV genotyping. Decision to treat was mostly based on colposcopically directed punch biopsies. The threshold for treatment was histopathologically confirmed cervical intraepithelial neoplasia grade 2 or worse (CIN2+). The method of treatment for preinvasive disease was always LLETZ, performed usually within one month of the initial visit. Women referred for cytological HSIL with inadequate colposcopy or suspicion of high-grade lesion at colposcopy were treated with LLETZ at first colposcopy. LLETZ was performed at first visit also for atypical glandular cells, favor neoplasia (AGC-FN).

2.2. Clinical data

Clinical data was obtained from the electronic hospital records including a dedicated colposcopy database and histo- and cytopathology reports. Cytology was reported according to the Bethesda system. Histopathological diagnosis was regarded as negative for intraepithelial lesion or malignancy (NILM) when no abnormalities with evidence of HPV were detected. Histopathological low-grade squamous intraepithelial lesion (LSIL) was defined as either HPV atypia/atypia condylomatosa or cervical intraepithelial neoplasia grade 1 (CIN1). Histopathological HSIL was stratified between cervical intraepithelial neoplasia grades 2 and 3 (CIN2 and CIN3). Preinvasive glandular dysplasia was reported as adenocarcinoma in situ (AIS). The most severe histopathological diagnosis from punch biopsies or LLETZ specimen was included in the analysis. The colposcopists, cytologists, and histopathologists were unaware of the HPV genotyping results.

2.3. HPV genotyping

The endocervical cells in Sample Transport Medium (STM, Qiagen GMBH, Hilden, Germany) were stored at −20 °C and later divided into three aliquots without adding any medium and then stored at −80 °C. One aliquot from each sample was sent frozen to Karolinska Institute, Stockholm, Sweden, for genotyping which was performed with the Luminex assay as previously described [35].

2.4. Statistical analyses

The main outcome measures were the age-group specific prevalence of HPV types and their association with cervical histopathological diagnosis. For all analyses the women were divided into three age-strata: <30, 30–44.9, and ≥45 years of age. We grouped the HPV data as follows: HPV16/18+, other hrHPV+ (HPV31/33/35/39/45/51/52/56/58/59/66/68), hrHPV not directly targeted by prophylactic HPV vaccines (non-vaccine hrHPV+: HPV35/39/51/56/59/66/68), other HPV than high-risk (only low-risk HPV: HPV6/11/30/40/42/43/53/61/67/69/70/73/74/81/83/86/87/89/90/91), and HPV negative. When multiple HPV types were detected, a hierarchical model was used. Other hrHPV and non-vaccine hrHPV were considered positive only if HPV16 and/or HPV 18 were not present, and only low-risk HPV was positive only if hrHPVs (HPV16/18/31/33/35/39/45/51/52/56/58/59/66/68) were not present. The individual HPV groups were positive if any or multiple of the included types in the individual groups were present.

For analyses, we grouped histopathological CIN3 and squamous cell carcinoma together as CIN3+, and AIS and invasive adenocarcinoma as AIS+. All high grade cervical histopathological findings were combined as HSIL+ (including CIN2, CIN3, AIS, squamous cell carcinoma and adenocarcinoma). NILM and LSIL were combined as less than HSIL (<HSIL).

We calculated the proportions of different HPV types and HPV groups according to age and histopathological category. We estimated the risk ratios (RR) of being HPV group positive between different age groups according to histopathological findings using binomial logistic regression with the women <30 years of age set as the referent group. All statistical analyses were done with STATA 15 (STATA Corp., College Station, TX).

3. Results

The study comprised of 1302 women (mean age 37.5) referred for cytological abnormality and HPV typing results were available in 1279 women (Table 1). For six cases (0.5%) the DNA sample was not taken, and in 17 (1.3%) cases the DNA extraction failed. Of the 23 cases with no HPV typing data available, 16 had histopathological NILM or LSIL, four had CIN2, two had CIN3, and one had invasive cervical cancer (adenocarcinoma). Of the 1279 women 1058 (82.7%) were HPV positive

and 221 (17.3%) were HPV negative (Table 2). The vast majority were of Caucasian ancestry and only two were HIV positive.

The most prevalent HPV type was HPV16, found in 362 cases (28.3%). The prevalence of HPV16 decreased with increasing age (Table 2). Conversely, the proportion of HPV negative cases increased with age, being 32.2% (105/326) in women over 45. Multiple HPV types were found in 340 women (26.6%) and were most common in women <30 years of age. The proportion of HPV16/18 decreased with advancing age irrespective of histopathological findings (Table 2).

Cervical histopathology results were available for 1261 (98.6%) women (Table 1). The remaining women (n = 18) had normal colposcopy of the cervix and histopathological sampling was not performed. Histopathological HSIL+ was found in 503 (39.3%) cases and low-grade or normal histopathological findings (<HSIL) in 776 (60.7%) cases.

3.1. Association of HPV types with high-grade histology

HPV16/18 positivity increased with increasing severity of histopathological findings, whereas HPV negativity decreased with advancing histopathological grade of squamous lesions (Fig. 1). The oldest age-group had the greatest proportions of disease attributed to other hrHPV types than HPV16/18 or HPV negative lesions.

In total 285 of the 503 cases of HSIL+ (56.7%) were associated with HPV16/18, with HPV16 being the most common genotype found in HSIL + in all age-groups (Table 3, Fig. 1, Table A.2). Other hrHPV types (HPV31/33/35/39/45/51/52/56/58/59/66/68) accounted for 191/503 (38.0%) cases of HSIL+ and non-vaccine targeted hrHPV types (HPV35/39/51/56/59/66/68) for 35/503 (7.0%). In women <30 years 64.3% (101/157) of the HSIL+ cases were associated with HPV16/18. The corresponding figures were 58.4% (157/269, RR 0.91, 95% confidence interval (95% CI) 0.78–1.06) in women 30–44.9 years, and 35.1% (27/77, RR 0.55, 95% CI 0.39–0.75) in women ≥45 years. Other hrHPV types than HPV16/18 on the other hand covered 31.9% (50/157) of HSIL+ in women <30, 36.8% (99/269) in women 30–44.9 (RR 1.16, 95% CI 0.88–1.52), and 54.6% (42/77) in women ≥45 (RR 1.71, 95% CI 1.26–2.33). A similar increase with age was seen in the proportion of HSIL+ associated with non-vaccine hrHPV types. The proportion of HPV negative HSIL+ cases increased with advancing age, reaching up to 6.5% in women ≥45 with RR 5.10 (95% CI 1.01–25.68) compared to women <30 (Table 3). When assuming total cross-protective efficacy for the bivalent vaccine, in HSIL+ nearly 90% of cases were attributed to hrHPV types covered by or implicated in bivalent vaccine cross-protection (HPV16/18/31/33/35/45/52) or coverage of the 9-valent vaccine (HPV16/18/31/33/45/52/58) (Table A.2).

CIN3+ was associated with HPV16/18 in 64.2% (154/240) with decreasing proportions along increasing age groups as with HSIL+ (Table 3). Other hrHPV types than HPV16/18 accounted for 19.3% (11/57) of CIN3+ in women <30 and 44.7% (17/38) in women ≥45. For women ≥45 the risk ratios of having HPV16/18+ CIN3+ was 0.61 (95% CI 0.43–0.88) compared to women under 30. In the 25 AIS+ cases, 72.0% (18/25) were positive for HPV16/18+ and 20.0% (5/25) were positive for other hrHPV types. All cases of AIS+ in the <30 age group (5/5) were associated with HPV16/18 whereas in women ≥45 only 33% (2/6). No cases of AIS+ were associated with non-vaccine targeted hrHPV's (HPV35/39/51/56/59/66/68).

Table 1
Characteristics of 1279 women referred to colposcopy for abnormal cytology with HPV typing results by age strata.

	All n = 1279		<30 n = 339		30–44.9 n = 614		≥45 n = 326	
Age median (range)	35.1 (19.2–83.7)		26.1 (19.2–29.9)		35.2 (30.0–44.9)		51.4 (45.0–83.7)	
Referral reason	%	n	%	n	%	n	%	n
ASC-US (repeated)	10.6	135	14.5	49	6.0	37	15.0	49
LSIL	39.3	502	26.6	90	44.6	274	42.3	138
ASC-H	24.9	318	31.6	107	23.6	145	20.3	66
HSIL	19.8	253	25.1	85	21.2	130	11.7	38
AGC-NOS	3.4	43	1.5	5	2.6	16	6.8	22
AGC-FN	2.2	28	0.9	3	2.0	12	4.0	13
Repeated abnormal cytology[a]	28.9	370	41.0	139	23.3	143	27.0	88
Baseline cervical histology								
NILM	30.0	383	20.9	71	24.6	151	49.4	161
LSIL	29.3	375	31.3	106	30.3	186	25.5	83
CIN2	18.6	238	28.0	95	17.9	110	10.1	33
CIN3	17.7	226	15.9	54	22.8	140	9.8	32
AIS	1.5	19	1.5	5	1.8	11	0.9	3
Cervical cancer	1.6	20	0.9	3	1.3	8	2.8	9[b]
No cervical sample	1.4	18	1.5	5	1.3	8	1.5	5
LLETZ in baseline	37.2	476	24.2	82	43.8	269	38.3	125

Abbreviations: ASC-US: atypical squamous cells of undetermined significance; LSIL: low grade squamous intraepithelial lesion; ASC-H: atypical squamous cells cannot exclude HSIL; HSIL: high grade squamous intraepithelial lesion; AGC-NOS: atypical glandular cells not otherwise specified; AGC-FN: atypical glandular cells favor neoplasia; NILM: negative for intraepithelial lesion or malignancy; CIN2: cervical intraepithelial neoplasia grade 2; CIN3: cervical intraepithelial neoplasia grade 3; AIS: adenocarcinoma in situ; LLETZ: large loop excision of the transformation zone.

[a] Repeated low-grade cytological abnormality (ASC-US, LSIL, AGC-NOS) in also another smear six months to two years prior to colposcopy.

[b] In addition to nine cervical cancers seven endometrial carcinomas were diagnosed in women ≥45.

4. Discussion

In this population-based cohort of unvaccinated women referred to colposcopy for abnormal cytology we found distinct age-specific patterns of hrHPV type distribution in high-grade cervical disease. The proportions of HSIL+ attributable to HPV16/18 decreased with increasing age from 64.3% in women under 30 years of age to only 35.1% in women 45 years or above with the group's median age being only 51.4 years. Conversely, other hrHPV types than HPV16/18 were more common in HSIL+ in the oldest age group. Furthermore, approximately 10% of HSIL+ cases in the oldest age group were hrHPV negative.

Previous studies have found comparable age-specific patterns of decreasing HPV16/18 positivity with increasing age in invasive cancer and to a lesser extent in HSIL [25–28]. Our study is to date the largest population-based study on histopathological HSIL to confirm this age-specific type distribution. The reasons for the observed polarisation of hrHPV types by age remains unclear, but a longer latency to high-grade disease with other types than HPV16/18 has been suggested, as well as the reactivation of latent infections after immune senescence [25,36]. A birth cohort effect, i.e. women born in a certain calendar time-period having been exposed to different hrHPV types and screened differently, might also play a role. In Finland the overall coverage of organised and opportunistic screening smears (at least one in five years) has, however, been over 90% for decades [37]. However, according to studies on sexual behavior patterns in industrialized countries HPV exposures (new sexual partners) are becoming more common in middle-aged women [38].

HPV16 and 18 antibody levels have been found to be significantly lower for the quadrivalent vaccine compared to the bivalent vaccine after up to 12 years of follow-up [39]. It is not yet established how long beyond a decade vaccine immune response lasts, or moreover, how permanent the moderate to high cross-protection is. When comparing the hrHPV type distribution assuming total cross-protective efficacy for the bivalent vaccine there were no major differences between the bivalent and 9-valent vaccine-covered HPV types in any histopathological categories by age. However, in light of the confirmed age-specific polarisation of hrHPV type distribution, the incidence of HSIL + might not be reduced to the same extent over time following prophylactic vaccination especially if it relies on cross-protection [12,13,20,21]. These data cannot confirm when the older women have acquired the HPV infections and thus how prophylactic HPV vaccination in adolescence would have altered this. Moreover, larger proportions of high-grade disease were associated with hrHPV types not targeted by the

Table 2
HPV types of 1279 women by cervical histopathological findings in age strata.

	All		<30		30–44.9		≥45	
	<HSIL n = 776	HSIL+ n = 503	<HSIL n = 182	HSIL+ n = 157	<HSIL n = 345	HSIL+ n = 269	<HSIL n = 249	HSIL+ n = 77
Number of HPV types	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)
HPV negative	26.3 (204)	3.4 (17)	17.0 (31)	1.3 (2)	21.5 (74)	3.7 (10)	39.8 (99)	6.5 (5)
Single type	49.0 (380)	67.2 (338)	48.9 (89)	59.2 (93)	53.6 (185)	70.6 (190)	42.6 (106)	71.4 (55)
2 types	17.9 (139)	22.3 (112)	24.2 (44)	26.8 (42)	18.6 (64)	20.8 (56)	12.4 (31)	18.2(14)
≥3 types	6.8 (53)	7.2 (36)	9.9 (18)	12.7 (20)	6.4 (22)	4.8 (13)	5.2 (13)	3.9 (3)
HPV type[a]								
HPV16	13.5 (105)	51.1 (257)	20.9 (38)	59.2 (93)	13.6 (47)	53.2 (143)	8.0 (20)	27.3 (21)
HPV18	5.0 (39)	7.0 (35)	7.1 (13)	7.0 (11)	4.9 (17)	6.7 (18)	3.6 (9)	7.8 (6)
HPV31	6.8 (53)	14.5 (73)	9.9 (18)	14.0 (22)	7.0 (24)	16.4 (44)	4.4 (11)	9.1 (7)
HPV33	2.1 (16)	6.8 (34)	3.3 (6)	8.3 (13)	1.7 (6)	6.0 (16)	1.6 (4)	6.5 (5)
HPV45	4.5 (35)	4.4 (22)	5.0 (9)	3.8 (6)	6.7 (23)	3.7 (10)	1.2 (3)	7.8 (6)
HPV52	5.2 (40)	11.5 (58)	7.1 (13)	10.8 (17)	4.6 (16)	11.5 (31)	4.4 (11)	13.0 (10)
HPV58	2.7 (21)	3.6 (18)	1.1 (2)	3.8 (6)	3.8 (13)	2.6 (7)	2.4 (6)	6.5 (5)
HPV groups								
HPV16/18+	18.0 (140)	56.7 (285)	26.4 (48)	64.3 (101)	18.3 (63)	58.4 (157)	11.7 (29)	35.1 (27)
Other hrHPV+[b]	38.1 (296)	38.0 (191)	39.6 (72)	31.9 (50)	42.9 (148)	36.8 (99)	30.5 (76)	54.6 (42)
Non-vaccine hrHPV+[c]	20.6 (160)	7.0 (35)	21.4 (39)	5.1 (8)	22.3 (77)	5.6 (15)	17.7 (44)	15.6 (12)
Only low-risk HPV+[d]	17.5 (136)	2.0 (10)	17.0 (31)	2.6 (4)	17.4 (60)	1.1 (3)	18.1 (45)	3.9 (3)

Abbreviations: <HSIL: normal histopathological findings or cervical intraepithelial neoplasia grade 1 (CIN1)/histopathological low-grade squamous intraepithelial lesion (LSIL); HSIL+: histopathological CIN2, CIN3, adenocarcinoma in situ (AIS), squamous cell carcinoma, adenocarcinoma.
[a] Irrespective of multiple infections.
[b] HPV31/33/35/39/45/51/52/56/58/59/66/68-positive.
[c] HPV35/39/51/56/59/66/68-positive.
[d] HPV6/11/30/40/42/43/53/61/67/69/70/73/74/81/83/86/87/89/90/91-positive.

prophylactic vaccines with advancing age (for example 0/57 (0.0%) of cases of CIN3+ in women <30 and 5/38 (13.2%) in women ≥45).

When assessing and modelling the efficacy of vaccination with different screening and reflex-testing strategies, the distinct age-specific hrHPV type distribution should not be overlooked. HPV16/18 genotyping has been considered beneficial as an adjunctive triage test, regardless of age [33,40]. Our findings should be carefully considered when applying HPV16/18 genotyping as a triage test — only approximately a third of HSIL+ in the older women was attributed to these types. Furthermore, in a large retrospective study approximately 20%

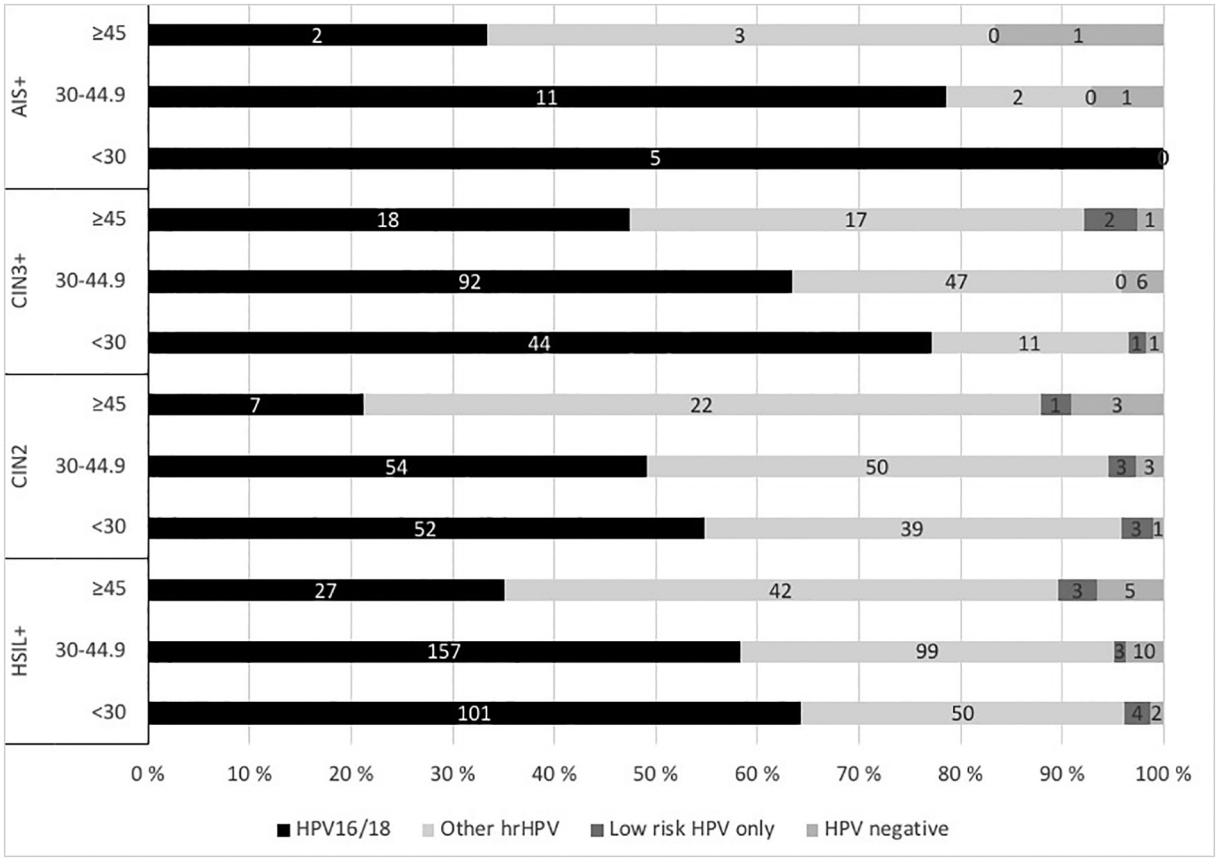

Fig. 1. HPV type distribution in 503 high grade cervical lesions in different age strata.

Table 3
Histopathological CIN2, CIN3+, and HSIL+ according to HPV groups in age strata and risk ratios (RR) for HPV group positive high-grade histology with women <30 set as the referent group.

	HPV16/18+ n = 425		Other hrHPV+ n = 487		Non-vaccine hrHPV+ n = 195		Only low risk HPV+ n = 146		HPV negative n = 221	
	n (%)	RR (95% CI)	n (%)	RR (95% CI)	n (%)	RR (95% CI)	n (%)	RR (95% CI)	n (%)	RR (95% CI)
HSIL+ <30 N = 157	101 (64.3)	Ref	50 (31.9)	Ref	8 (5.1)	Ref	4 (2.6)	Ref	2 (1.3)	Ref
HSIL+ 30–44.9 N = 269	157 (58.4)	0.91 (0.78–1.06)	99 (36.8)	1.16 (0.88–1.52)	15 (5.6)	1.09 (0.47–2.52)	3 (1.1)	0.44 (0.10–1.93)	10 (3.7)	2.9 (0.65–13.15)
HSIL+ ≥45 N = 77	27 (35.1)	0.55 (0.39–0.75)	42 (54.6)	1.71 (1.26–2.33)	12 (15.6)	3.06 (1.30–7.17)	3 (3.9)	1.53 (0.35–6.66)	5 (6.5)	5.10 (1.01–25.68)
HSIL+ total N = 503	285 (56.7)		191 (38.0)		35 (7.0)		10 (2.0)		17 (3.4)	
CIN2 <30 N = 95	52 (54.7)	Ref	39 (41.1)	Ref	8 (8.4)	Ref	3 (3.2)	Ref	1 (1.1)	Ref
CIN2 30–44.9 N = 110	54 (49.1)	0.90 (0.69–1.17)	50 (45.5)	1.11 (0.81–1.52)	9 (8.2)	0.97 (0.39–2.42)	3 (2.7)	0.86 (0.18–4.18)	3 (2.7)	2.59 (0.27–24.49)
CIN2 ≥45 N = 33	7 (21.2)	0.39 (0.20–0.77)	22 (66.7)	1.62 (1.15–2.28)	7 (21.2)	2.52 (0.99–6.41)	1 (3.0)	0.96 (0.10–8-91	3 (9.1)	8.63 (0.93–80.17)
CIN2 total N = 238	113 (47.5)		111 (46.6)		24 (10.1)		7 (2.9)		7 (2.9)	
CIN3+ <30 N = 57	44 (77.2)	Ref	11 (19.3)	Ref	0 (0.0)	Ref	1 (1.8)	Ref	1 (1.8)	Ref
CIN3+ 30–44.9 N = 145	92 (63.5)	0.82 (0.68–0.99)	47 (32.4)	1.68 (0.94–3.00)	6 (4.1)	NA	0 (0.0)	NA	6 (4.1)	2.36 (0.29–19.16)
CIN3+ ≥45 N = 38	18 (47.4)	0.61 (0.43–0.88)	17 (44.7)	2.32 (1.23–4.39)	5 (13.2)	NA	2 (5.3)	NA	1 (2.6)	1.50 (0.10–23.26)
CIN3+ total N = 240	154 (64.2)		75 (31.3)		11 (4.6)		3 (1.3)		8 (3.3)	

Definitions: CIN2: cervical intraepithelial neoplasia grade 2; CIN3+: cervical intraepithelial neoplasia grade 3 and squamous cell carcinoma; AIS+: adenocarcinoma in situ and adenocarcinoma; HSIL+: CIN2, CIN3, AIS, squamous cell carcinoma, adenocarcinoma; other hrHPV+: HPV31/33/35/39/45/51/52/56/58/59/66/68-positive; non-vaccine hrHPV+: HPV35/39/51/56/59/66/68-positive; only low-risk HPV+: HPV6/11/30/40/42/43/53/61/67/69/70/73/74/81/83/86/87/89/90/91-positive; Ref: reference; NA: not applicable.

of invasive cervical cancers were HPV negative [41], while 10% HSIL+ lesions in the oldest age group were also hrHPV negative here. These findings should be taken into consideration when shifting to HPV-based screening with the majority of screening-age population still being unvaccinated. Overall approximately 95% of HSIL lesions here were positive for hrHPV types included in most HPV tests approved for screening: up to 96% in women under 30 and 90% in women over 45. Detection of the hrHPV negative lesions would perhaps warrant co-testing with cytology when screening older women who previously have not attended HPV-based screening. This type of strategy has been implemented in Sweden where women approximately 41 years of age are co-tested [42]. However, in women under the age of 30 nearly 90% of CIN3+ and all cases AIS+ were associated with HPV16/18 in comparison to only approximately half of CIN2 cases and a fourth of <HSIL cases. HPV16/18 positivity in this age group can be considered to be strongly associated with true high-grade disease. Screening for all hrHPV types in young women would not, in light of these data, result in detection of significantly more HSIL+, but rather reduce the positive predictive value of the HPV screening test. Stratification of screened HPV types according to age could be hence considered.

Strengths of our study are generalisability of findings with the cohort deriving from an unselected population of a single referral center serving a large population base with likely high prior attendance of cytology-based cervical cancer screening. Our data should accurately reflect the current hrHPV type distribution in clinically relevant disease omitting transient infections. HPV status was determined using high-quality HPV genotyping in an international HPV reference laboratory, with a very low number of unsuccessful samples. Weaknesses of the study include the small number of cases glandular disease and invasive cancers. The numbers of infections with individual HPV types were also low, excluding HPV16. Our study assessed real-life age-specific HPV type distribution in women requiring colposcopy, not a cross-section of a screening population. Due to this, we were able to include more cases of HSIL+ than studies in screening populations, but the data therefore cannot directly be used to estimate HPV burden in the whole population.

In conclusion, our results showed that HPV type distribution in high-grade cervical lesions is distinctly polarised according to age in a highly screened population with HPV16/18 attributed disease most prevalent in younger women. In women over 45, only a third of the HSIL+ findings were attributable to HPV16/18, while other hrHPV types and hrHPV negativity were more prevalent. The performance of current HPV-based screening is hence age-dependent and could be modified according to age to achieve better predictive values. Furthermore, when the majority of the screening-aged population is still unvaccinated hrHPV test only might not be optimal screening strategy, especially among older women with low prior adherence to screening.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2019.05.024.

Acknowledgments

Funding: This work was supported by Cancer Society of Finland (KA); Biomedicum Helsinki Foundation (KA); Finnish Medical Foundation (KA); Special State Funding of Medical Research (Helsinki and Uusimaa Hospital District) (PN); Eemil Aaltonen Foundation (IK); Finnish-Norwegian Medical Fund (KA, IK); Finnish Gynaecological Society (IK); Jalmari and Rauha Ahokas Foundation (IK).

Role of the funding source: The funders of the study did not participate in study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the article for publication.

Availability of data: Further data is available from the corresponding author upon request.

Declaration of competing interest: Dr. Dillner reports grants from Merck/SPMSD, during the conduct of the study.

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