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RESEARCH PAPER

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Human papillomavirus genotype-specific risks for cervical intraepithelial lesions

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ABSTRACT

Prevalence of different HPV genotypes is changing after HPV vaccination. The associated risks are needed for optimizing cervical cancer screening.

To estimate HPV type-specific prevalence, odds ratio (OR), and positive predictive value (PPV) for cervical cytological abnormalities, we determined 41 different HPV genotypes in cervical samples from a population-based sample of 8351 women aged 18–51 years before HPV vaccination era (V501-033; NCT01077856).

Prevalence of HPV16 was 4.9% (95% CI: 4.4–5.5) with the PPV for high-grade cytology 11.2%, and OR 11.9 (95% CI: 8.5–16.5). Carcinogenic HPVs included in the nonavalent vaccine (HPV16,18,31,33,45,52,58) had a population prevalence of 14.4% (95% CI: 13.5–15.4), with PPV of 8.0% (95% CI: 6.8–9.3) and OR 23.7 (95% CI: 16.0–63.5) for high-grade cytology. HPV types currently included in most screening tests, but not vaccinated against (HPV35,39,51,56,59,66,68) had a joint prevalence of 8.5% (95% CI: 7.8–9.2) with PPV of 4.4% (95% CI: 3.3–5.7) and OR of 2.9 (95% CI: 2.0–4.0) for high-grade cytology. The other 27 non-carcinogenic genotypes had a prevalence of 11.8%, PPV of 2.9% (95% CI: 2.1–3.9), and OR 1.5 (95% CI: 1.1–2.2.) for high-grade cytology.

These results suggest that HPV screening tests in the post-vaccination era might perform better if restricted to the HPV types in the nonavalent vaccine and screening for all 14 HPV types might result in suboptimal balance of harms and benefits.

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Background

The high-risk types of human papillomavirus (HPV) cause several human cancers, particularly cervical cancer.¹ The established practice of cervical cancer prevention is transforming from using cytology-based screening with HPV-follow-up to HPV-based screening with cytology follow-up,^{2–5} and prophylactic vaccines that are highly effective against the targeted HPV types and related cervical abnormalities.^{6–8} Large meta-analyses of case series have described the type-specific epidemiology of HPV.^{9–11} Longitudinal, population-based, and multi-country studies may provide additional data on which HPV types are more prevalent and contribute the most to cervical disease in organized screening programs.

Over the course of years research has identified that HPV16 has the highest oncogenic potential of the over 200 HPV genotypes identified,^{12–15} while HPV18,31,33,35,39,45,51,52,56,58,59 are also classified as carcinogenic to humans, HPV68 as probably carcinogenic, and HPV26,30,34,53,66,67,69,70,73,82,85,97 as possibly carcinogenic.¹⁶ Consequently, almost all commercially


available HPV DNA detection methods that are used in screening have been developed to target HPV16,18,31,33,35,39,45, 51,52,56,58,59,66,68.¹⁷ For use in screening programs with the aim to identify women who might be at elevated risk for cervical cancer. Coupled with appropriate clinical action(s), diagnostic verification and treatment, cervical cancer can be prevented. Prophylactic HPV vaccines, however, eliminate the risks related to the viral infection by targeting either HPV16,18 (2-valent [2 v] HPV vaccine), HPV6,11,16,18 (4 vHPV vaccine), or HPV6,11,16,18,31,33,45,52,58 (9 vHPV vaccine).⁸ As the prevalence of vaccine types in populations with high vaccine coverage is reported to be very low when the vaccine is administered at an early age, methods and strategies developed for screening-based pre-vaccination HPV burden are likely to perform differently. For HPV-vaccinated populations, several mathematical models suggest less frequent screening with HPV testing rather than with cytology.^{18,19} Similar conclusions have been drawn from recent clinical studies reporting a decline in the predictive value of cytology for precancers in populations vaccinated at early ages.²

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Abbreviations CI = confidence interval; HPV = human papillomavirus; MSD = Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA; OR = Odds ratio; PCR = Polymerase chain reaction; PPV = Positive predictive value; WHO = World Health Organization

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Cervical cancer control programs in the 4 Nordic countries (Denmark, Iceland, Norway, and Sweden) are provided through organized healthcare programs that include both organized screening,²⁰ and mass-vaccination against HPV using either the 2 v or 4 vHPV vaccine.^{21–24} The objective of this study (V501-033; NCT01077856) is to estimate the HPV type-specific prevalence by age and by cytological abnormality, as well as HPV type specific risks for cytological abnormalities in the general female population in Denmark, Iceland, Norway, and Sweden prior to large-scale use of HPV vaccines. Our study was conducted within the rigorous framework of the population-based cervical screening programs in the Nordic region, with all HPV testing performed in the WHO reference lab, and provides a critical reference of background data to design optimal organized screening strategies for HPV-vaccinated birth cohorts.

Methods

Overall design

To perform surveillance and ensure both quality of the program and high attendance among the target population, mandatory reporting of all cervical cancer screening-related activities and diagnoses has been introduced in Denmark, Iceland, Norway, and Sweden.^{20,25} A comprehensive surveillance infrastructure allows screening program providers to track the individual screening history of each female resident and intervene when deviations from the recommended screening guidelines are observed. Information is collected from all private and public healthcare providers, regardless of age and including all screening-related events performed within and outside the organized program.

During the period of 2006–2009, we collected the residual liquid-based cytology (LBC) samples from routine Pap smear screening to estimate HPV type-specific prevalence in the female population. Assuming variation in baseline HPV prevalence from 5% to 25% in the general population, we intended to enroll 1000 consecutive screening attendees aged 18–26 years and 1000 women aged 27–50 years from each country in order to estimate HPV type-specific prevalence and 95% confidence interval (CI) with 80% power at a 0.05% significance level.

The Research Ethics Committee/Data Protection Agency approved the study in each of the participating countries and decided the requirements for informed consent. In Denmark, the requirement for informed consent was waived. In Norway and Sweden, information about the study, a form for declining participation (opt-out), and a pre-paid return envelope was sent to the registered addresses of all women. In Iceland, potential participants received information about the study and an opt-out form when they attended the Cancer Detection Clinic.

Participants

A total of 16,550 consecutive residual specimens were obtained from women attending the 2006–2008 cervical cancer screening in 1) Copenhagen, Denmark, 2) Reykjavik, Iceland, 3) South- and North-Trondheim County in Norway, and 4)

Stockholm and Malmö in Sweden. Altogether, 8926 participants with consecutive samples from age groups 18–26 and 27–50 years were included in this study (Denmark 2352, Iceland 2372, Norway 2019, Sweden 2183), while the rest of the samples were frozen. Concurrent cytology diagnoses by Bethesda classification for each participant were obtained from the population-based health registries operating in each country: 1) the Pathology Data Bank in Denmark, 2) the database of the Cancer Detection Clinic in Iceland, 3) the Cytology Registry in Norway, and 4) the Swedish National Cervical Screening Registry.²⁶ Cytology diagnoses were classified according to the 2001 Bethesda System.²⁷ Women with an unsatisfactory cytology (N = 461) were excluded from the statistical analyses.

Laboratory analyses

PreservCyt solution (PreservCyt® Solution, Hologic, Inc., UK) medium was used in the majority of the subjects in all countries. LBC samples were stored at +4°C for a maximum of 3 months before they were received by the WHO HPV LabNet Global Reference Laboratory in Malmö, Sweden. DNA extraction and PCR strategy have previously been defined.²⁸ HPV genotyping was performed with a Luminex system (Biorad, CA, USA) with type-specific probes for 41 individual HPV types, including 2 variants of HPV35 and HPV58 with sequence variation in the probe target sequence (HPV 6,11,16,18,26,30,31,32,33,35,35 6624:A,39,40,42,43,44,45,51,52,53,54,55,56,58,58 668A,59,61,62, 66,67,68 subtype A, 68 subtype B,69,70,73,74,81,82,83,86, 87,89,90 and 91) and two general probes broadly reactive with most HPV types. We excluded 98 participants with invalid or missing HPV results.

We categorized cytology diagnoses as “normal“, i.e., those negative for intraepithelial lesions or malignancy, and all remaining diagnoses were grouped as “abnormal.” Atypical squamous cells of undetermined significance and low-grade squamous intraepithelial lesions were grouped as “low-grade,” and atypical squamous cells that cannot exclude high-grade, atypical glandular cells of undetermined significance, high-grade squamous intraepithelial lesions, adenocarcinoma in situ, or cervical cancer were grouped as “high-grade cervical intraepithelial lesions.”

Statistical analysis

The study was designed to enroll an equal number of participants at ages 18–26 and 27–51 years in each country. As Norway, Denmark, Sweden, and Iceland have comparable risk factors for HPV infection,^{29–32} and with minor differences between HPV positivity rates observed, we pooled countries in statistical analyses to facilitate our aim to estimate HPV type-specific prevalence by age at screening (age groups 18–23, 24–26, 27–29, 30–34, 35–39, and 40–51 years). The prevalence of HPV for types or combinations was estimated as the number of positive specimens for a given HPV type (or combinations of HPV types) divided by the total number of specimens with a valid PCR result (β -globin PCR positive by real-time PCR) for a given stratum. The prevalence estimates were weighted for unequal sampling fractions across countries, age groups, and

cytology (normal vs. abnormal). We calculated the weights as the inverse of the sampling fractions from the country and age-group sampling strata, rescaled by post-stratification on additional cytology information (normal vs. abnormal) retrieved from the national screening registries (**Supplementary Table 1**). Prevalence estimates were weighted using the R complex survey software,^{33,34} and for the HPV prevalence CIs we used methods for proportions with a small expected number of positive counts.³⁵ Kernel smoothing was applied to prevalence curves.³⁶

Genotype-specific prevalence was estimated irrespective of potential coinfections. Hence, a specimen was counted as positive for a given HPV type whether it was positive only to that specific type, or also positive to additional HPV types. HPV genotype-specific prevalence for single infections, i.e., specimens positive only to one particular HPV genotype is also provided. For prevalence referring to groups of HPV-types, a specimen was defined as positive if it included at least one of the HPV-types of the given group. “Any HPV” refer to specimens positive to at least one of the tested HPV genotypes. “Carcinogenic HPV” refer to specimens positive to 16,18,31,33,35,39,45,51,52,56,58,59 and/or 68).¹⁷ “Non-carcinogenic HPV” refer to specimens positive to 6,11,26,30,32,40,42,43,44,53,54,55,61,62, 66,67,69,70,73,74,81,83,86,87,89,90 and/or 91. “2 v”, “4 v” and “9 v” refer to specimens positive to 16 and/or 18; 6,11,16 and/or 18; and 6,11,16,18,31,33,45,52 and/or 58, respectively, based on HPV types targeted by prophylactic vaccines. To assess HPV prevalence in a hypothetical setting we assumed that vaccine-targeted HPV-types were eradicated, a highly likely situation.^{22–24,37} The combined carcinogenic HPV types then excluded carcinogenic HPVs targeted by 2 v or 4 v (i.e., specimens positive to 31,33,35,39,45,51,52,56,58,59 and/or 68); and 9 v vaccines (i.e., specimens positive to 35,39,51,56,59 and/or 68). HPV genotype-specific prevalence for single infections, i.e., specimens positive only to one particular HPV genotype is also provided.

Positive predictive value (PPV) was estimated as the number of specimens of a given cytology category positive to an HPV type (or combination of types) divided by the total number of specimens positive to the same HPV type (or combination of types), irrespective of cytology category. We estimated age-adjusted odds ratios for high-grade and low-grade cytology among women with HPV infection of the specified type compared with women negative to the specified type

and having normal cytology. Each PPV and OR point-estimates were calculated regardless of co-infections and with 95% confidence intervals to assess the precision reflecting the number of events observed.

Results

HPV results and cytology diagnoses were available for 8367 women: 2319 in Denmark, 2310 in Iceland, 1972 in Norway, and 1766 in Sweden (**Table 1**). Altogether, there were 3528 women 18–26 years old (827, 861, 1031, and 809 from Denmark, Iceland, Norway, and Sweden, respectively). More than half (58%) of samples were from women older than age 26 (27–51 years). For 7524 (90%) subjects, the concurrent cytology was normal while 661 (8%) had low-grade and 182 (2%) had high-grade diagnoses.

In order to provide a comprehensive overview that would allow comparing all carcinogenic and non-carcinogenic HPV types, we performed comprehensive HPV genotyping. 40 different HPV types were detected, with the overall prevalence of 25.3% (95% CI: 24.1–26.5) (**Table 2**). The combined prevalence of 13 different carcinogenic HPV types was 18.1% (95% CI: 17.1–19.1) and 11.8% (95% CI: 11.0–12.7) for the 26 different non-carcinogenic HPV types. The combined prevalence of HPV types targeted by each of the 2 v, 4 v, and 9 v HPV vaccines was 6.6% (95% CI: 5.9–7.2), 7.8% (95% CI: 7.1–8.5), and 14.4% (95% CI: 13.5–15.4), respectively (**Table 2**). When we ignored HPV16,18 or HPV16,18,31,33,45,52,58, a hypothetical scenario after successful immunization with 2 v and 4 v or 9 v vaccine, the overall carcinogenic HPV prevalence of 18% was reduced to 14.2% (95% CI: 13.3–15.1) or 7.5% (95% CI: 6.8–8.2), respectively. The overall type-specific prevalence varied, with HPV16 being the most prevalent type at 4.9% (95% CI: 4.4–5.5). In contrast, thirteen non-carcinogenic types (HPV26,30,32,40,44,55,61,62,69, 74,83,87,90) had a type-specific prevalence of 0.1% or less. HPV31 and HPV42 were the second most prevalent infections, with 2.9% for each (**Supplementary Table 2**).

The combined prevalence of carcinogenic and non-carcinogenic HPVs was highest among the youngest age groups (**Table 2**). In the 18–23 age group, nearly half of the population, 46.2% (95% CI: 43.3–49.0) was positive for at least one carcinogenic HPV type and about a third, 30.0% (95% CI: 27.5–32.7) was positive for non-carcinogenic HPV types. A higher prevalence of carcinogenic HPV than non-carcinogenic HPV became gradually less apparent by increasing age until after 38 years of age, when

Table 1. Distribution of participants by age at screening, country, and corresponding cytology abnormalities in Denmark, Iceland, Norway, and Sweden, in 2006–2008.

Age group	Nordic Countries										Cervical intraepithelial lesion		
	Denmark		Iceland		Norway		Sweden		Total		Normal ^a	Low-grade ^b	High-grade ^c
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)			
18–23	271	(11.7)	498	(21.6)	649	(32.9)	330	(18.7)	1748	(20.9)	1498	221	29
24–26	556	(24.0)	363	(15.7)	382	(19.4)	479	(27.1)	1780	(21.3)	1531	190	59
27–29	353	(15.2)	251	(10.9)	130	(6.6)	114	(6.5)	848	(10.1)	749	73	26
30–34	394	(17.0)	393	(17.0)	242	(12.3)	180	(10.2)	1209	(14.5)	1118	65	26
35–39	259	(11.2)	381	(16.5)	248	(12.6)	218	(12.3)	1106	(13.2)	1030	52	24
40–51	486	(21.0)	424	(18.4)	321	(16.3)	445	(25.2)	1676	(20.0)	1598	60	18
Total	2319		2310		1972		1766		8367		7524	661	182

^aNegative for intraepithelial lesions or malignancy.

^bAtypical squamous intraepithelial lesion or low-grade squamous intraepithelial lesion.

^cAtypical squamous cells cannot rule out high-grade lesion, high-grade squamous intraepithelial lesion, adenocarcinoma in situ, or cervical cancer.

Table 2. Weighted prevalence^a of 40 HPV types measured, carcinogenic HPV^b, non-carcinogenic HPV^c, the 5 most common HPV types in declining order, vaccine-targeted carcinogenic HPV types in combination^{d,ef}, and a hypothetical prevalence of remaining carcinogenic HPVs if vaccine-targeted HPVs would be eliminated^{g,h}, by age group.

Age groups	18–23 n = 1748	24–26 n = 1780	27–29 n = 848	30–34 n = 1209	35–39 n = 1106	40–51 n = 1676	Total n = 8367
Prevalence of HPV types							
Any HPV	56.7%	47.1%	36.2%	26.4%	17.7%	12.6%	25.3%
95% CI	53.8–59.6	44.4–9.8	32.2–40.4	23.3–29.7	14.9–20.7	10.8–14.6	24.1–26.5
Carcinogenic HPV ^b	46.2%	36.6%	25.8%	19.5%	11.8%	7.3%	18.1%
95% CI	43.3–49.0	34.1–39.2	22.3–29.6	16.8–22.5	9.5–14.4	5.9–8.9	17.1–19.1
non-carcinogenic HPV ^c	30.0%	22.5%	16.9%	10.6%	7.7%	6.4%	11.8%
	27.5–32.7	20.4–24.8	13.8–20.3	8.6–13.0	5.8–9.9	5.1–7.9	11.0–12.7
The five most common HPV types in declining order							
1 st most prevalent HPV type	HPV 16	HPV 16	HPV 16	HPV 16	HPV 16	HPV 70	HPV 16
% (95% CI)	14.2 (12.3–16.2)	11.4 (9.8–13.1)	8.3 (6.1–10.9)	4.6 (3.3–6.4)	3.2 (2.0–4.8)	1.5 (0.9–2.4)	4.9 (4.4–5.5)
2 nd most prevalent HPV type	HPV 31	HPV 31	HPV 42	HPV 52	HPV 48	HPV 16	HPV 31
% (95% CI)	9.3 (7.8–11.1)	6.6 (5.4–8.0)	5.3 (3.7–7.4)	3.6 (2.4–5.0)	2.0 (1.0–3.5)	1.1 (0.6–1.9)	2.9 (2.5–3.3)
3 rd most prevalent HPV type	HPV 42	HPV 52	HPV 31	HPV 31	HPV 52	HPV 42	HPV 42
% (95% CI)	8.5 (7.0–10.1)	6.0 (4.8–7.3)	4.1 (2.8–5.9)	3.0 (1.9–4.5)	1.8 (1.0–2.9)	0.9 (0.5–1.6)	2.9 (2.4–3.3)
4 th most prevalent HPV type	HPV 51	HPV 42	HPV 52	HPV 39	HPV 31	HPV 52	HPV 52
% (95% CI)	8.5 (7.1–10.2)	5.8 (4.6–7.2)	3.9 (2.4–5.8)	2.2 (1.2–3.5)	1.8 (1.0–3.1)	0.9 (0.5–1.6)	2.8 (2.4–3.3)
5 th most prevalent HPV type	HPV 56	HPV 18	HPV 45	HPV 42	HPV 18	HPV 39	HPV 56
% (95% CI)	7.5 (6.1–9.1)	4.6 (3.6–5.9)	3.3 (2.0–5.2)	2.1 (1.2–3.4)	1.6 (0.8–3.0)	0.9 (0.4–1.6)	2.0 (1.7–2.4)
HPV types targeted by HPV vaccines							
2-valent ^d	19.7%	15.6%	10.0%	6.3%	4.6%	1.5%	6.6%
16, 18	17.6–22.0	13.7–17.5	7.6–12.8	4.7–8.2	3.1–6.5	0.9–2.4	5.9–7.2
4-valent ^e	23.8%	17.4%	11.9%	6.8%	5.4%	2.3%	7.8%
6,11,16,18	21.5–26.2	15.5–19.5	9.2–15.0	5.2–8.8	3.8–7.4	1.6–3.3	7.1–8.5
9-valent ^f	36.6%	29.6%	22.3%	14.9%	10.0%	5.1%	14.4%
6,11,16,18,31,33,45,52,58	33.9–39.4	27.3–32.1	18.9–26.0	12.5–17.6	7.9–12.5	3.9–6.5	13.5–15.4
Prevalence of the carcinogenic HPV types after removing those targeted by HPV vaccines							
2 v and 4 v vaccine ^g	37.1%	28.5%	20.4%	14.8%	8.4%	6.2%	14.2%
31,33,35,39,45,51,52,56,58,59,68	34.4–39.9	26.2–31.0	17.2–23.9	12.3–17.5	6.5–10.6	4.9–7.7	13.3–15.1
9 v vaccine ^h	23.2%	16.0%	10.0%	7.1%	3.5%	3.4%	7.5%
35,39,51,56,59,68	20.9–25.6	14.2–18.0	7.7–12.7	5.4–9.2	2.3–5.1	2.5–4.6	6.8–8.2

^aThe prevalence of HPV is estimated as the number of positive specimens for a given HPV type (or combination of HPV types) divided by the total number of specimens with a valid PCR result, for each stratum. Weighting was performed for the sampling fractions (Supplementary Table 1).

^bCarcinogenic HPV is defined as positive to one of the HPV types 16,18,31,33,35,39,45,51,52,56,58,59,68.

^cNon-carcinogenic HPV is defined as positive to one of the HPV types 6,11,26,30,32,40,42,43,44,53,54,55,61,62,66,67,69,70,73,74,81,83,86,87,89,90,91.

^dHPV types included in 2 valent vaccine HPV16, 18

^eHPV types included in 4 valent vaccine, HPV6,11,16,18

^fHPV types included in 9 valent vaccine, HPV6,11,16,18,31,33,45,52,58

^gPrevalence of the carcinogenic HPV types after removing those targeted by HPV vaccines, HPV31,33,35,39,45,51,52,56,58,59,68

^hHPV 35,39,51,56,59,68

Abbreviations: CI = confidence interval; HPV = human papillomavirus.

carcinogenic and non-carcinogenic HPV types were equally common in women with normal cytology (Figure 1). The 46% prevalence of carcinogenic HPVs in the 18–23 age group was reduced to 37.1% (95% CI: 34.4–39.9) when we assumed no positivity to HPV16,18 and to 23.2% (95% CI: 20.9–25.6) when we assumed no positivity to HPV16,18,31,33,45,52,58. Carcinogenic HPV prevalence of 36.6% in the 24–26 age group was similarly reduced to 28.5% (95% CI: 26.2–31.0) and 16.0% (95% CI:14.2–18.0), respectively.

Overall, HPV16,31,42,52,56,51,18,39,45,70 (in declining order) were the ten most common HPV types (Supplementary Table 2). HPV16 and HPV42 were among the five most common HPV types in all age groups, with HPV16 being the most common in all age groups except those 40–51 years (Table 2). HPV31 was the second most common in age groups 18–23 and 24–26 years, at 9.3% and 6.6%, respectively. In age groups 18–23 and 24–26 years, 5.2% and 2.3% were positive to HPV6, respectively. Of carcinogenic HPV types, HPV35 and HPV58 always ranked lower than ten of the most common HPVs in all age groups.

In total, we detected 40, 36, and 23 different HPV types among those with normal, low-grade, and high-grade cytology

(Supplementary Table 3). Prevalence of six carcinogenic HPVs (16,18,31,45,52,58) and HPV66 increased with the increasing severity of cytology, while HPV53 was equally prevalent in low- and high-grade cytology (Supplementary Table 3). Prevalence of carcinogenic HPV33,35,39,51,59,68 and non-carcinogenic HPV42,43,67,70,73,81,89 was highest in low-grade cytology; however, HPV53 and 56 were highest in high-grade cytology as a single infection.

In screening, excessive testing of women with typically self-resolving low-grade lesions would be regarded as harms while exams yielding in high proportion of women who need to be treated for cervical disease would be regarded as benefits. Hence, the high PPV for high-grade cytology and low PPV for low-grade cytology would likely provide a favorable balance between harms and benefits. We observed large variation by age when detecting high-grade cytology in those positive to carcinogenic HPVs, with PPVs ranging between 14.3% in the age-group of 35–39 years and 3% in the age-group of 18–23 years. However, only for 35–39 years old HPV16,18 positives had PPV point estimates for high-grade cytology higher than the PPV for low-grade cytology (Table 3). After ignoring positivity to the carcinogenic HPVs targeted by 2- or 4-valent HPV vaccine and

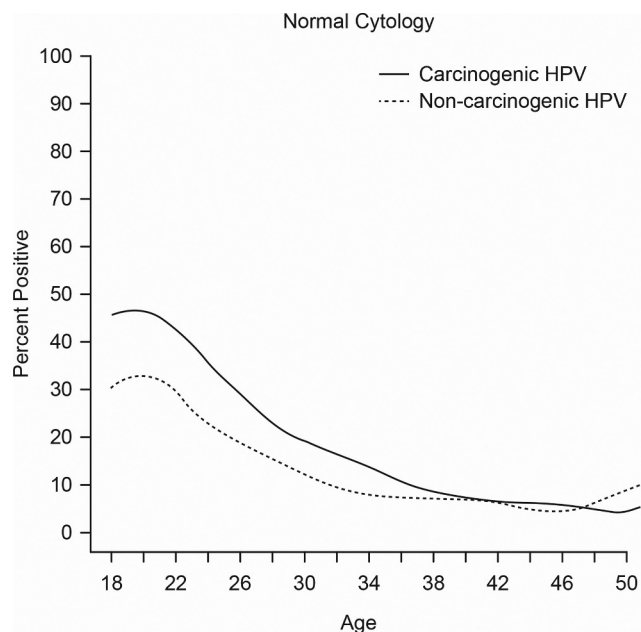


Figure 1. The age-specific prevalence of carcinogenic HPV types and non-carcinogenic HPV types among those with normal cytology. Abbreviation: HPV = human papillomavirus.

9-valent HPV vaccine, the age-specific PPVs for low-grade cytology ranged between 5.7% (95% CI: 0.7–19.2) and 23.5% (95% CI: 12.8–37.5) for both categories, i.e. those positive to HPV31,33,35,39,45,51,52,56,58,59,68 and those positive to HPV35,39,51,56,59,68. The PPVs for high-grade cytology were low for both groups, with only 4.3% (95% CI: 3.3 – 5.5) among those positive to the HPV31,33,35,39,45,51,52,56,58,59,68 and 2.3% (95% CI: 1.2 – 4.0) of those positive to HPV35,39,51,56,59,68. In contrast, for the non-carcinogenic HPVs the PPV for the concomitant low-grade and high-grade cytology was 19.1 (95% CI: 17.2 – 21.2) and 2.9 (95% CI: 2.1 – 3.9), respectively. We observed large differences in PPVs between carcinogenic HPV types: the PPV for low-grade cytology was 23.4% for HPV16 and 30.6% for HPV39 (the highest PPV for low-grade cytology) while the PPV for high-grade cytology was 11.2 for HPV16 and 3.7% for HPV39 (Supplementary Table 4).

While PPV is a useful metric of the predictive value of the screening test and is commonly used in public health, odd ratio provides an insight to the etiological fraction of the infection which can be related to the lesion. We, therefore, present both metrics which complement each other with 95% confidence intervals to illustrate the precision of the point estimates which is determined by the number of events observed. Of 13 HPV genotypes significantly associated with high-grade cytology, 11 were classified as carcinogenic, with the highest OR for HPV16 (11.9 [95% CI: 8.5–16.5]) (Figure 2A). Among those positive to carcinogenic HPV53,35,39, the observed increased risk was at borderline significance only. Significantly elevated risk for low-grade cytology was observed for 23 genotypes, including all carcinogenic HPV genotypes, and the highest OR was observed for HPV39 (4.6 [95% CI: 3.4–6.1]) (Figure 2B).

Compared to normal cytology, for those positive to at least one of the carcinogenic HPVs, we observed OR 28.2

(95% CI: 17.9–46.2) for high-grade cytology and 9.3 (95% CI: 7.6–11.3) for low-grade cytology. Similarly, for non-carcinogenic HPV, we observed OR 1.5 (95% CI: 1.1–2.2) for high-grade cytology and 3.3 (95% CI: 2.8–4.0) for low-grade cytology. For those positive to at least one of the carcinogenic HPVs included in the 9 v vaccine, we observed OR 23.7 (95% CI: 16.0–36.5) for high-grade cytology. In contrast, the overall weighted prevalence was 8.5% (95% CI: 7.80–9.2) for the types included in most HPV screening tests, but excluded from the vaccines (i.e., HPV35,39,51,56,59,66,68), with the PPV and OR for: 1) high-grade cytology of 4.4% (95% CI 3.3–5.7) and 2.9 (95% CI: 2.0–4.0), respectively; 2) low-grade cytology 22.6% (95% CI: 20.3–25.1) and 4.3 (95% CI: 3.6–5.1), respectively. For the five most common non-carcinogenic genotypes, HPV42,53,66,70,73, the OR for high-grade cytology was 1.9 (95% CI: 1.3–2.8) and for low-grade cytology the OR was 3.4 (95% CI: 2.8–4.1).

Discussion

In our population-based study, we observed high prevalence, but limited risk for cytological abnormalities of HPV types not targeted by HPV vaccines in women below 30 years of age. Regardless of age, we demonstrate almost always higher probability, i.e. PPV, for simultaneous low-grade cytology than for high-grade cytology among those positive to individual HPV types. This adds further to the evidence of the distinctive oncogenic potential of individual HPV types commonly referred to as carcinogenic and as well the age dependency of these HPV types, and the overall contribution of non-carcinogenic HPVs to the large share of total HPV prevalence. Our results imply that HPV technology detecting 14 different HPV types (16,18,31,33,35,39,45,51,52,56,58,59,68 and 66) might be suboptimal with regard to detection of high-grade lesions at low population prevalence of positivity. Our data suggest that screening strategies might perform better if restricted to the HPV types targeted by the nonavalent vaccine and when applied in an age-optimized manner. Several HPV screening tests that could be used for such strategies are already commercially available.

In agreement with others, we observed an overall higher prevalence of mucosal carcinogenic compared to non-carcinogenic HPV types, specifically in younger ages.^{38,39} With HPV16 as the most prevalent and with the highest risk for simultaneous high-grade cytology. Although the HPV prevalence decreased with increasing age which most likely reflects decreased exposure to HPV in these unvaccinated birth cohorts, after age of 35 years the prevalence of non-carcinogenic and carcinogenic HPVs was comparable. HPVs targeted by the 2 v and 4 v HPV vaccines contributed about a third of the overall 18% prevalence of carcinogenic HPV types, while HPV types targeted by the 9 v vaccine contributed about 80%. The same HPV types were also associated with increased risk for high-grade cytology: compared to normal cytology, the risk of high-grade cytology was 11.9 times higher for HPV16 but only non-significantly increased for HPV68 and HPV59. Also, PPV for high-grade lesions varied from 11.2% for HPV16 to 3% for HPV59. In screening, however, guidelines

Table 3. Positive predictive value^a for concomitant low-grade and high-grade cytology, combined as carcinogenic HPV^b, 2 v and 4v HPV Vaccine vaccine-targeted carcinogenic HPV types in combination^c, HPV types targeted by 9valent HPV vaccine^d, 27 non-carcinogenic HPV types^e and age.

Age groups	18–23	24–26	27–29	30–34	35–39	40–51	Total
Carcinogenic HPV							
#	860	705	253	253	147	147	2365
PPV for Low-grade cytology	22.1%	20.6%	22.1%	18.6%	19.0%	20.4%	21.0%
95% CI	19.4–25.0	17.6–23.7	17.2–27.8	14.0–23.9	13.0–26.3	14.2–27.8	19.3–22.7
PPV for High-grade cytology	3.0%	7.5%	9.1%	9.1%	14.3%	7.5%	6.6%
95% CI	2.0–4.4	5.7–9.7	5.9–13.3	5.9–13.3	9.1–21.0	3.8–13.0	5.7–7.7
Carcinogenic HPV types targeted by 2 v HPV or 4v vaccines^c, HPV16,18							
#	372	292	94	84	46	33	921
PPV for Low-grade cytology	23.9%	20.2%	21.3%	21.4%	15.2%	24.2%	21.8%
95% CI	19.7–28.6	15.8–25.3	13.5–30.9	13.2–31.7	6.3–28.9	11.1–42.3	19.2–24.6
PPV for High-grade cytology	4.3%	12.3%	17.0%	16.7%	21.7%	9.1%	10.3%
95% CI	2.5–6.9	8.8–16.7	10.1–26.2	9.4–26.4	10.9–36.4	1.9–24.3	8.4–12.5
Carcinogenic HPV types targeted by 9v vaccines^d, HPV16, 18, 31, 33,45,52,58							
#	668	553	202	181	112	92	1808
PPV for Low-grade cytology	22.0%	21.0%	21.8%	18.8%	23.2%	20.7%	21.3%
95% CI	18.9–25.3	17.7–24.6	16.3–28.1	13.4–25.2	15.8–32.1	12.9–30.4	19.5–23.3
PPV for High-grade cytology	3.4%	9.4%	10.4%	11.0%	17.0%	9.8%	8.0%
95% CI	2.2–5.1	7.1–12.1	6.6–15.5	6.9–16.5	10.5–25.2	4.6–17.8	6.8–9.3
Remaining carcinogenic HPV types after excluding those targeted by 2v and 4v HPV vaccines^e, HPV31,33,35,39,45,51,52,56,58,59,68							
#	488	413	159	169	101	114	1444
PPV for Low-grade cytology	20.7%	20.8%	22.6%	17.2%	20.8%	19.3%	20.4%
95% CI	17.2–24.6	17.0–25.1	16.4–29.9	11.8–23.7	13.4–30.0	12.5–27.7	18.4–22.6
PPV for High-grade cytology	2.0%	4.1%	4.4%	5.3%	10.9%	7.0%	4.3%
95% CI	1.0–3.7	2.4–6.5	1.8–8.9	2.5–9.9	5.6–18.7	3.1–13.4	3.3–5.5
Remaining carcinogenic HPV types after excluding these HPV types which are targeted by 9v HPV vaccines^f, HPV35,39,51,56,59,68							
#	192	152	51	72	35	55	557
PPV for Low-grade cytology	22.4%	19.1%	23.5%	18.1%	5.7%	20.0%	19.7%
95% CI	16.7–29.0	13.2–26.2	12.8–37.5	10.0–28.9	0.7–19.2	10.4–33	16.5–23.3
PPV for High-grade cytology	1.6%	0.7%	3.9%	4.2%	5.7%	3.6%	2.3%
95% CI	0.3–4.5	0.0–3.6	0.5–13.5	0.9–11.7	0.7–19.2	0.4–12.5	1.2–4.0
27 non-carcinogenic HPV types^g							
#	551	426	157	135	95	115	1479
PPV for Low-grade cytology	20.5%	20.9%	18.5%	17.0%	16.8%	11.3%	19.1%
95% CI	17.2–24.1	17.1–25.1	12.7–25.4	11.1–24.5	9.9–25.9	6.2–18.6	17.2–21.2
PPV for High-grade cytology	1.3%	3.8%	3.8%	5.2%	2.1%	4.3%	2.9%
95% CI	0.5–2.6	2.2–6.0	1.4–8.1	2.1–10.4	0.3–7.4	1.4–9.9	2.1–3.9

^aPositive predictive value was estimated as the number of specimens of a given cytology category positive to an HPV type (or combination of types) divided by the total number of specimens positive to the same HPV type (or combination of types) irrespective of cytology category.

^bCarcinogenic HPV is defined as positive to one of the HPV types 16,18,31,33,35,39,45,51,52,56,58,59,68.

^cCarcinogenic HPV types included in 2valent or 4valent HPV vaccine, HPV16, 18

^dHPV types included in 9valent HPV vaccine, HPV16,18,31,33,45,52,58

^eRemaining of 13 carcinogenic HPV types after removing those targeted by 2valent and 4valent HPV vaccines, HPV31,33,35,39,45,51,52,56,58,59,68

^fRemaining of 13 carcinogenic HPV types after removing those targeted by 9valent HPV vaccines, HPV35,39,51,56,59,68

^g27 non-carcinogenic HPV is defined as positive to one of the HPV types 6,11,26,30,32,40,42,43,44,53,54,55,61,62,66,67,69,70,73,74,81,83,86,87,89,90,91.

Abbreviations: PPV = Positive predictive value, CI = confidence interval; HPV = human papillomavirus.

recommend detection of carcinogenic HPV as a group in (non-vaccinated) women 30 years and older.⁴⁰ We conventionally categorized 13 HPV types as carcinogenic.^{4,16} Of which oncogenic potential (i.e., risk for high-grade cytology) for individual HPVs varied. The group of 13 different carcinogenic HPVs had a 28- and 9 times higher OR for high-grade and low-grade cytology, respectively; those positive to HPV types included in most HPV screening tests, but excluded from the vaccines (HPV35,39,51,56,59,66,68) had only 2.9 times higher OR for high-grade lesions. The latter was comparable with the risks for high-grade and low-grade lesions we observed for the five most common non-carcinogenic types. Thus, the HPV types detected in screening considerably affect the harms and benefit balance of the entire program, an observation, which was supported by a recent study confirming clinical benefit of immediate colposcopy referral for all HPV16/18-positive women whereas women with other hrHPV infections are triaged with cytology in a pre-vaccinated population.⁴¹One

should notice that in Asian countries, for example, HPV58 has been reported to have a far more oncogenic profile compared with the Nordic countries,^{42,43} implying that regional/geographical differences should be considered in establishing an optimal screening program.

Now, the optimal screening scenario should be considered for the scenario when all 2 v and 4 v HPV vaccine-targeted HPV types are eliminated, with an assumed prevalence of carcinogenic HPVs reduced to 28% among those who enter the screening program. This simplified assumption does not include the effect of possible cross-protection that the HPV vaccination might confer against non-vaccine types.⁴⁴ Nor the possibility that exposure to HPVs and related cervical lesions might increase over time.⁴⁵ As expected, the potential effect of the 9 v vaccine would be higher, with a reduction in carcinogenic HPV prevalence to 16% at screening start, but with practical implications in the more distant future, because the 9 vHPV vaccine was licensed and became available in 2016. In contrast, the first birth

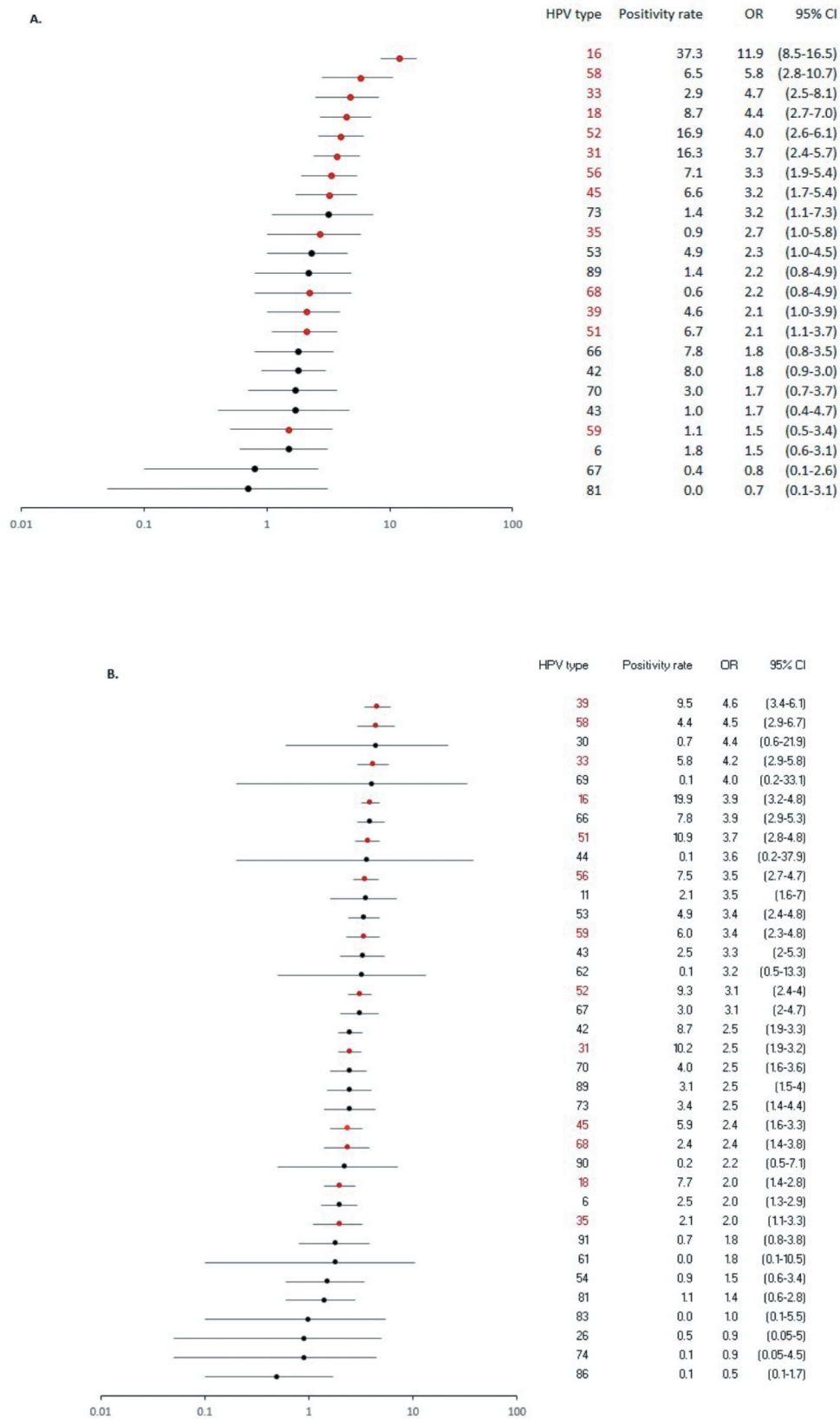


Figure 2. Adjusted odds ratios with 95% CIs for high-grade (A) and low-grade (B) cytology diagnoses among women positive to specified HPV type with HPV infection of the specified type compared with women having normal cytology. Carcinogenic HPV types are marked in red. Abbreviations: CI = confidence interval; HPV = human papillomavirus; OR = odds ratio.

cohorts of routinely vaccinated girls enter the cervical cancer screening program already in 2022 (Norway), 2020 (Denmark), and 2023 (Sweden) and we expect very low prevalence of HPV16

and HPV18, given documented high 14 years efficacy of the 4 v vaccine.⁶ And the additional benefit from herd immunity.⁴⁶ In addition, the number of high-grade lesions to be screened and

treated is expected to be reduced at least by 50% through vaccination,⁴⁴ implying that diagnostic yields of precancers requiring treatment among screen positives will likely be low. Yet, when we ignored any positivity to HPV types targeted by the 4 v vaccine, the prevalence of 11 carcinogenic types in the age at screening start, i.e. 24–26 was still 28.5%, which is likely too high to justify the switch from cytology screening to HPV screening, if the current technology based on the detection of 14 specific HPV types is used. Strategies using modern HPV tests focusing on the 9 HPV vaccine types are likely to perform better. Use of the 9 v vaccine would hypothetically reduce the combined prevalence of remaining carcinogenic HPV types (HPV35,39,51,56,59,68) to 7.5%. The clinical value, however, may be limited if the contemporary screening technology will be used to detect the remaining HPV39,51,59,68 types and precancers with lower potential for progression. Two recent studies based on mathematical models suggest implementing a longer screening interval and delayed screening start for HPV-vaccinated women.^{18,47} Immediate delay of screening age, however, would inevitably change the epidemiology of the asymptomatic precancer, which has an extremely high burden among women younger than 30 years.²⁰ Therefore, when the first vaccinated cohorts come to screening, public health providers need to consider screening tools and strategies based on updated empirical data and mathematical models.

Only one-quarter of all 40 HPV types propagate in the population and most likely maintain persistent infection. This feature is well described for carcinogenic HPV types.⁴⁸ Interestingly, the non-carcinogenic HPV42 and HPV70 types were among the most prevalent types overall, and specifically in women older than 35 years. We also document that positivity to the five most common non-carcinogenic HPVs had a significant 1.9 times elevated risk of having high-grade than normal cytology. Although the biological role of individual non-carcinogenic HPVs for progression of cervical cancer is negligible, it is possible that at least some non-carcinogenic HPVs have developed a distinctive adaptive mechanism to thrive and produce viral progenies.⁴⁹ Therefore, a post-vaccination surveillance to detect changes in the entire spectrum of HPV types affecting cervical mucosa can improve understanding of relationships between HPV types.

Our study should not be overinterpreted as we had no invasive cancer cases in the cohort, the study was cross-sectional, and disease assessment was based on concomitant cytology diagnosis and was not histologically confirmed. Histological grading is considered a golden standard for the clinical management of precancers, where self-limiting cervical intraepithelial neoplasia (CIN) grade 1 are discriminated from CIN 2/3 which require treatment. Sub-optimal inter- and intra-observer variability of the histological grading specifically for CIN2.^{50–52} is perhaps even better described than uncertainty in reproducing cytological diagnosis.⁵³ And suggest substantial interpretive variability for disease assessment regardless of cytological or histological assessment of the disease. To our knowledge, it is the largest population-based HPV prevalence study performed in the Nordic region on a large set of mucosal HPV types, representing the typical distribution of cytological abnormalities in the screening population.⁵⁴ The rigorous study design included identical HPV detection methodology

in all countries at a centralized, WHO-certified laboratory, weighted prevalence estimates, and simultaneous conduct of sample collection in all countries. These are important elements that allowed us to enhance representability of the data and comparability between the countries. Furthermore, cytology classification based on information from national registries was a useful benchmark in assessing the real-world impact needed for the translation of results into existing healthcare practices. Finally, the study design allows empirical observations of the emerging trends in HPV prevalence by repeating sample collection. Reduction in prevalence of HPV types in the screening population was observed five years later, reflecting the influx of HPV-vaccinated women in cohorts who enter screening.⁵⁵

The understanding that persistent infection with high-risk HPV is a necessary cause of cervical cancer has led to developments in new technologies and transformed cervical cancer prevention programs. As a result, the high-risk HPV test is gradually replacing cytology testing in nationwide screening programs and women with immunity against HPV16 and 18 are about to enter screening age. We detected a high prevalence of the HPV types included in common HPV testing kits but not targeted by the HPV vaccines. HPV screening tests which include all oncogenic HPV types in the post-vaccination era are likely to detect predominantly low-grade lesions. Risk profiles of high-grade and low-grade cytology in our study suggest that current screening of HPV-vaccinated cohorts might perform better if restricted to the HPV types in the nonavalent vaccine. Further research is needed for developing post-vaccination cervical cancer screening strategies with optimal balance of harms and benefits to inform public health practices and policy.

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No potential conflicts of interest were disclosed.

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