

# The Age Distribution of Type-Specific High-Risk Human Papillomavirus Incidence in Two Population-Based Screening Trials

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## Abstract

**Background:** Age- and type-specific high-risk human papillomavirus (hrHPV) incidence estimates in screen-eligible women are relevant from a public health perspective because they provide an indication of the effect of vaccination on the occurrence of screen-positives in HPV-based screening. However, limited data from women over 25 years of age are available.

**Methods:** In 24,105 hrHPV-negative women participating in Dutch (Population-Based Screening Study Amsterdam: POBASCAM) and Italian (New Technologies for Cervical Cancer: NTCC) population-based randomized controlled screening trials the age- and type-specific distribution of incident hrHPV infections detected at the next screening round was assessed. HPV types were grouped into vaccine (bivalent: HPV16/18; polyvalent HPV16/18/31/33/45/52/58) and nonvaccine types.

**Results:** The incidence of screen-detected hrHPV among women ages 29 to 56 years was 2.54% (95% confidence interval, 2.30–2.78) in POBASCAM and 2.77% (2.36–3.19) in NTCC. In both studies, the incidence of bivalent, polyvalent,

and nonpolyvalent infections decreased with age ( $P < 0.0001$ ). Among women with incident infection(s), vaccine-type positivity changed quadratically with age, in particular for the polyvalent vaccine ( $P$  values: POBASCAM: bivalent 0.264, polyvalent 0.038; NTCC bivalent 0.039, polyvalent 0.005). However, more than 20% and 50% of women with incident hrHPV were positive for bivalent and polyvalent vaccine types, respectively, in all ages in both studies.

**Conclusions:** We observed decreasing age trends of hrHPV vaccine and nonvaccine type incidences and age-related differences in the vaccine-type positivity among women with incident infections. Most importantly, hrHPV infections continued to be detected in all ages and the contribution of vaccine types remained substantial.

**Impact:** Our results indicate a considerable reduction of new hrHPV infections in vaccinated cohorts, ensuing revision of screening guidelines. *Cancer Epidemiol Biomarkers Prev*; 24(1); 111–8. ©2014 AACR.

## Introduction

Human papillomavirus (HPV) is an ubiquitous, sexually transmitted infectious pathogen with high transmission potential. Some HPV types (high-risk, hrHPV) are oncogenic and persistent infection with hrHPV is a necessary prerequisite for the development of cervical cancer. HPV types 16 and 18 are associated with about 70% of cervical cancer and HPV16 is the most common type in HPV-positive women worldwide (1, 2). A decreasing trend of hrHPV prevalence with age has been well documented among women with normal cytology in most regions of the world where

organized screening programs have been implemented (1). Age- and type-specific HPV incidences are less well documented, especially not in women over 25 years of age, but provide information that is relevant from a public health perspective and that cannot be directly inferred from HPV prevalence. HPV-type distribution by age in prevalent infections, for example, suffer from variation in infection onset times because of differences in screening history, whereas HPV-type incidences do not. Type-specific incidences can therefore provide an estimate of the effect of vaccination on the occurrence of screen positives in HPV-based screening and about the necessity of continuing screening for hrHPV-negative women beyond a certain age.

Here, we report the age- and type-specific distribution of screen-detected incident hrHPV infections among participants of two large European population-based screening trials over the course of two screening rounds, including a total of 24,105 women.

## Materials and Methods

### Studies

Data collected in the context of two population-based randomized controlled clinical trials evaluating the efficacy of HPV-based screening compared with cytology-based screening were included in the current analyses: the Population Based Screening Study Amsterdam (POBASCAM) and the New Technologies for Cervical Cancer (NTCC) screening study. Both trials were

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conducted in the setting of regular (cytology-based) cervical cancer screening programs in the Netherlands and Italy, respectively. The designs of both trials have been previously described in detail and are only briefly outlined here (3–6).

### Study populations and design

Between 1999 and 2002, the POBASCAM trial invited women from a well-defined area southwest of Amsterdam, attending routine cervical cancer screening visits. In the Netherlands, women become screen-eligible in the year they turn 30 years of age and are subsequently screened at 5-year intervals until 60 years of age. In addition, to be eligible for POBASCAM, women should not have had a hysterectomy and not have a history of abnormal cytology or cervical intraepithelial neoplasia (CIN) in the last two years. Eligible, consenting women (44,102 women) were randomly assigned (1:1) to the intervention (hrHPV testing and cytology) or control group (cytology only). hrHPV testing was performed in the control group but only unblinded during data analysis.

Between 2002 and 2004, the NTCC trial invited women from nine areas in Italy, attending routine (3-year) screening visits to participate in the study. In Italy, women become screen-eligible in the year they turn 25 years of age and are subsequently screened at 3-year intervals until 60 years of age. In addition, to be eligible for NTCC, women should not be pregnant, not have had a hysterectomy, and not been treated for CIN in the last 5 years. Eligible, consenting women (94,370 women) were randomly assigned (1:1) to the intervention (phase I hrHPV testing and cytology; phase II hrHPV testing only) or control group (cytology only).

In both trials, women with borderline or mild dyskaryosis or worse ( $\geq$ ASCUS) (intervention and control arms) or who tested hrHPV positive (intervention arm only) at the baseline screening round were referred for intensified follow-up or colposcopy. All other women were advised to return for the next screening round during which cytology and hrHPV testing was performed in POBASCAM and cytology only in NTCC. At the next screen, all hrHPV-positive samples in the intervention and control group of POBASCAM were genotyped. In NTCC, hrHPV testing and genotyping at the next screen was performed in 5 of 9 study sites to a random sample of women in the intervention arm with baseline HPV negative results.

### Laboratory testing

Only hrHPV testing and genotyping are relevant in the context of the current analyses and the procedures are described below. Other laboratory test conducted in the context of the trials were described previously (3, 7–9).

In the POBASCAM study, endocervical brush material for hrHPV testing was stored in collection medium (5 mL PBS and 0.5% thiomersal) and tested by the Department of Pathology at the VU University Medical Center (Amsterdam, the Netherlands). Duplicate GP5<sup>+</sup>/6<sup>+</sup> PCR enzyme immunoassay (EIA) followed by reverse line blot analysis (RLB) on positive samples were carried out as described previously (10). A mixture of PCR probes was used for the detection of 14 hrHPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68).

In the NTCC study, hrHPV testing was performed on endocervical brush samples stored in PreservCyt transport medium (Cytoc Corporation) during enrollment phase I and stored in Specimen Transport Medium during enrollment phase II and retesting (STM, DNAPAP Cervical Sampler, Qiagen). Samples

were tested with the Hybrid Capture 2 (HC2) hybridization assay (HC2, Qiagen Incorporated) as previously described (7). HC2 targets 13 hrHPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Relative light units  $\geq$  1 pg/mL were considered positive, conforming to manufacturer's recommendations. Genotyping by GP5<sup>+</sup>/6<sup>+</sup> PCR and RLB was performed on HC2-positive samples including probes for the hrHPV types targeted by HC2 (10).

### Statistical analysis

We estimated age- and type-specific HPV incidence as the proportion positive at the first visit in second screening round for HPV type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, or 68 among women who tested hrHPV-negative at baseline. Enrollment age was categorized in 5-year age groups. In POBASCAM (5-year screening interval), the time window for the second round was defined as lasting from 48 to 108 months after enrollment, and in NTCC (3-year screening interval) as lasting from 24 to 60 months after enrollment.

Only baseline hrHPV-negative women who had their second hrHPV test within the time window for the second screening round were included in these analyses. Women who were invited for a second screening round had an enrollment age between 29 and 56 years in POBASCAM (women aged older than 56 years at baseline are not eligible for a subsequent program screen) and 24 to 60 years of age in NTCC. In total, POBASCAM contributed 16,671 women ( $n = 8,254$  control,  $n = 8,417$  intervention) and NTCC 7,434 women ( $n = 2,081$  enrollment phase I,  $n = 5,353$  enrollment phase II). Women who received intensified follow-up and/or referral for colposcopy based upon their enrollment HPV and/or cytology results were excluded. Consequently, eligible women had either normal cytology results (POBASCAM and NTCC phase I) or unknown cervical cytology (NTCC phase II). The intervention and control arms of the POBASCAM did not differ with respect to hrHPV incidence, and were combined. The enrollment phase I and phase II groups of the NTCC did not differ with respect to hrHPV incidence, and were combined as well.

Using a public health perspective, HPV types were grouped according to a hierarchical definition based on the inclusion of HPV types in vaccines. A woman was considered HPV16/18 positive if the HPV test was positive for at least one of the two currently vaccine-preventable high-risk types 16 and 18 ("bivalent"), and HPV16/18/31/33/45/52/58 positive ("polyvalent") if positive for at least one of these high-risk types targeted by the investigational polyvalent vaccine (11). Consistently, nonbivalent vaccine HPV included types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and nonpolyvalent vaccine HPV included types 35, 39, 51, 56, 59, and 68. Because of infections with multiple hrHPV types, the bivalent and nonbivalent group are not mutually exclusive neither are the polyvalent and nonpolyvalent group. In the current article, the term bivalent HPV vaccine is related to all vaccines preventing HPV16/18 infections.

Age-stratified type-specific incidence proportions are reported with Clopper–Pearson 95% confidence intervals (CI). To further study the relation between vaccine and nonvaccine HPV type-specific incidence and age, a logistic regression analysis was performed, including age and age squared as continuous variables, to evaluate nonlinear (quadratic) associations. Overlapping age ranges were used for these analyses, including women ages 29 to 56 years in POBASCAM and NTCC. In sensitivity analyses, we included women of all screen-eligible ages in NTCC,

ages 24 to 60 years. We furthermore evaluated the age trend of the detection of vaccine and nonvaccine HPV types among women with incident infection(s) by fitting logistic regression models including vaccine or nonvaccine HPV types as dependent variable, and age, age squared, and the number of new infection types as independent variables. The latter was included to account for the age-dependent clustering of infections with multiple hrHPV types.

The main results are reported separately for POBASCAM and NTCC. However, in the absence of heterogeneity in age-specific hrHPV incidence, estimates between POBASCAM and NTCC-pooled analysis were performed as well.

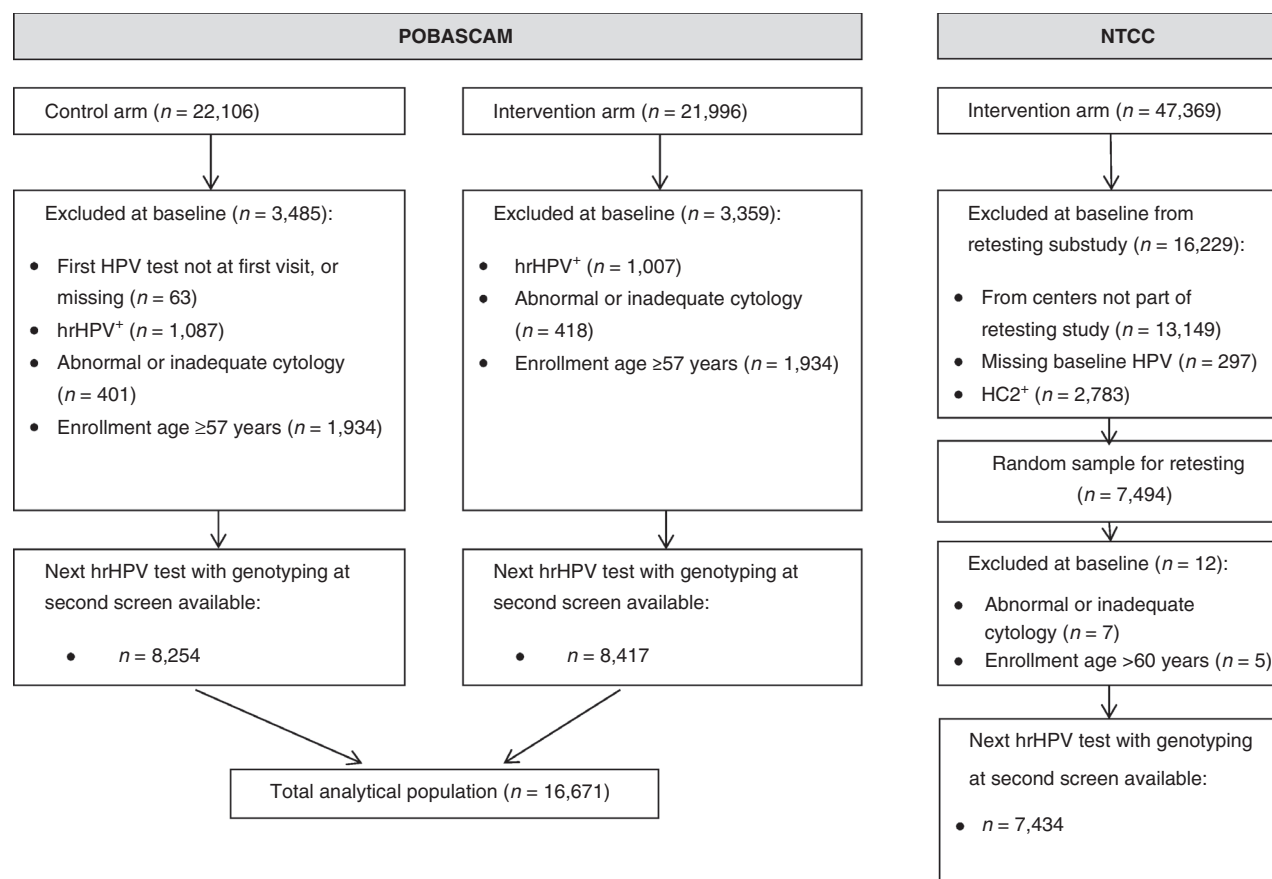
Statistical analyses were done using STATA (STATA/IC 11, Stata Corp).

## Results

In total, results of 16,671 and 7,434 women were available for HPV incidence analyses in POBASCAM and NTCC, respectively (Fig. 1). Median age at baseline was 40 years in POBASCAM [interquartile range (IQR) 35–49 years] and 42 years in NTCC (IQR 34–51 years). Median time to the second HPV test was 5.02 years in POBASCAM (IQR 4.70–5.46) and 3.01 years in NTCC (IQR 2.98–3.11). Only 9 women in NTCC had an interval length of more than 4 years, indicating minimal overlap with the intervals in POBASCAM.

## POBASCAM

The overall 5-year incidence of hrHPV was 2.54% (95% CI, 2.30–2.78). The most common hrHPV types among women with an incident hrHPV infection were: HPV16 (0.70% of study women); HPV31 and HPV51 (both 0.29%); HPV52 (0.26%) and HPV56, HPV18 and HPV45 (all three 0.25%; Table 1). The highest hrHPV incidence was found among the youngest age group [29–33 years; 5.69% (4.69–6.83)] and the lowest one among the oldest age group [54–56 years; 1.03% (0.639–1.57; Table 2, Fig. 2)]. The age-associated decline of hrHPV was nonlinear and leveled off in older women ( $P$  value quadratic term 0.016). Nonlinear declines of type-specific incidence by age were also found for vaccine-HPV types ( $P$  value quadratic term: bivalent 0.024; polyvalent 0.002). Instead, a linear decline with increasing age was observed for nonvaccine HPV types (Table 2). The majority of incident infections were single infections (86.5%), but 11.6% included two hrHPV types and 1.90% more than two types. The proportion of women with multiple types infections declined with age [ $P$  value (1 df) 0.007]. Among women with at least one incident hrHPV infection, bivalent vaccine type infections were found in 36.4% (31.8–41.2); nonbivalent vaccine types in 71.1% (66.6–78.4); polyvalent vaccine types in 73.3% (68.8–77.4); and nonpolyvalent vaccine types in 34.8% (30.2–39.5; Table 3). The presence of polyvalent vaccine types in women with incident hrHPV infections showed a nonlinear trend with age (adjusted  $P$  value quadratic term 0.038; Table 3; Fig. 3). However,



**Figure 1.** Flow diagram of the analytical population.

**Table 1.** Screen-detected type-specific hrHPV incidence

	<b>POBASCAM (n = 16,671)</b>	<b>NTCC (n = 7,434)</b>
hrHPV (%)	426 (2.54)	233 (3.13)
HPV16 (%)	117 (0.7)	67 (0.9)
HPV18 (%)	41 (0.25)	28 (0.38)
HPV31 (%)	48 (0.29)	37 (0.5)
HPV33 (%)	27 (0.16)	8 (0.11)
HPV35 (%)	24 (0.14)	4 (0.05)
HPV39 (%)	22 (0.13)	16 (0.22)
HPV45 (%)	41 (0.25)	14 (0.19)
HPV51 (%)	48 (0.29)	31 (0.42)
HPV52 (%)	43 (0.26)	16 (0.22)
HPV56 (%)	42 (0.25)	20 (0.27)
HPV58 (%)	22 (0.13)	14 (0.19)
HPV59 (%)	17 (0.1)	14 (0.19)
HPV68 (%)	5 (0.03)	15 (0.2)

NOTE: Screenings interval POBASCAM 5 years; NTCC 3 years.

in all age groups, more than 20% of women with incident infections had bivalent vaccine types detected and more than 60% polyvalent vaccine types.

### NTCC

The overall 3-year incidence of hrHPV among women 24–60 years of age was 3.13% (2.74–3.56) and the most common types were HPV16 (1.00%), HPV31 (0.54%), HPV51 (0.46%), HPV18 (0.41%), and HPV 56 (0.29%; Table 1). hrHPV incidence decreased from 8.69% (6.68–10.9) in women 24 to 28 years of age to 0.731% (0.279–1.18) among women over 54 years of age (Table 2). Among women aged 29–56 years (similar to the age range in POBASCAM), the overall incidence of hrHPV was 2.77% (2.36–3.19). Using logistic regression models, restricted to women ages 29 to 56 years, similar age-related trends were found as in POBASCAM. The decline in hrHPV incidence with increasing age was nonlinear for both bivalent and polyvalent vaccine types (*P* value quadratic term 0.015 and 0.010, respectively), while a linear decline with increasing age was again observed for non-vaccine hrHPV types (Table 2). The majority of incident infections were single infections (78.9%) and no association of multiple infections with age was found [*P* value (1 df) 0.375]. When we included women ages 24 to 60 years, according to the screening ages in NTCC, more complicated nonlinear age trends were observed. The decline of hrHPV overall was linear with age, but the age trends of bivalent and polyvalent vaccine types were nonlinear. A polynomial to the fourth degree (quartic function) provided a better fit than a quadratic function (bivalent vaccine types *P* value quartic term 0.015; polyvalent vaccine types 0.013).

Bivalent vaccine type infections were found in 38.6% (32.3–45.2) of women with incident hrHPV infection(s); nonbivalent vaccine types in 73.0% (66.8–78.6); polyvalent vaccine type infections in 68.7% (62.3–74.6); and non-polyvalent vaccine types in 41.2% (34.8–47.8; Table 3). The presence of bivalent and polyvalent vaccine types in women with incident hrHPV infections showed a nonlinear trend with age (adjusted *P* value quadratic term 0.039 and 0.005, respectively; Table 3; Fig. 3). However, in all age groups, more than 20% of women with incident infections had bivalent vaccine types detected and over 50% polyvalent vaccine types.

### Pooled data

No statistically significant differences were found between POBASCAM and NTCC in age-stratified incidences (data not

shown). All the age-related trends described above, were more pronounced and clearly significant in the pooled dataset: restricting the population to women ages 29 to 56 years, the decline of incidence by age was nonlinear for hrHPV overall (*P* value quadratic term 0.012), for bivalent vaccine types (*P* value 0.001) and for polyvalent vaccine types (*P* value <0.001), but not for nonbivalent vaccine types (*P* value 0.192) or nonpolyvalent vaccine types (*P* value 0.683). The results were similar when excluding women with multiple incident infections (*P* value quadratic term hrHPV overall 0.042; bivalent vaccine types 0.006; polyvalent vaccine types 0.001; nonbivalent vaccine types 0.608; nonpolyvalent vaccine types 0.129).

The presence of vaccine types among women with (one or more) incident hrHPV infection varied with age [*P* value quadratic term (adjusted for the number of infections) bivalent vaccine types 0.022; polyvalent vaccine types 0.015]. Excluding women with multiple incident infections, the presence of especially polyvalent vaccine types varied with age (*P* value quadratic term 0.003). The nonlinear age effect was borderline significant for the presence of bivalent vaccine types (*P* value quadratic term 0.056; Fig. 3).

## Discussion

Type-specific hrHPV incidence was estimated using data collected from two large population-based screening trials, by computing the proportion of hrHPV-negative women who tested hrHPV positive at the second screening round (i.e., the point prevalence among initially hrHPV negative women). This measure corresponds to the incidence proportion during the screening interval of infections persisting until the second screen. Although the screening intervals were long (3–5 years), these incidence estimates are relevant from a public health perspective with respect to screening and vaccination, especially because the entire screen-eligible age range is included.

Screen-detected age-specific hrHPV incidence in POBASCAM and NTCC followed a decreasing trend as has been previously described for hrHPV prevalence and incidence (1, 12, 13). The observed decrease in hrHPV incidence by age is furthermore in line with a general decrease in the incidence of sexually transmitted diseases with increasing age, which has been explained by a decreasing number of new sexual partners per year. Natural immunity may furthermore play a role in the decline of hrHPV incidence with increasing age, although there are still many uncertainties about the level of protection naturally acquired antibodies provide and whether this effectiveness wanes over time. Remarkably, despite the different screening intervals, no substantial differences were found in the age-stratified incidence estimates from POBASCAM and NTCC. This could either be explained by a more sexually active population (more new sexual partners per year) in NTCC compared with POBASCAM, or by a fast clearance of the majority of infections early-on, after which the net result of incidence and clearance stabilizes. When screening intervals become very long the type distribution of incident infections becomes comparable with the type distribution of prevalent infections, including more persistent types. However, we did not find a different relative proportion of vaccine- and nonvaccine HPV-type infections in different durations of screening intervals in either study. Moreover, the proportion of bivalent and polyvalent vaccine types were similar between the two studies despite different re-testing interval, suggesting that the interval



**Table 2.** Age-specific incidence of hrHPV, (non-)bivalent vaccine types and (non-)polyvalent vaccine types

Age	At risk N	hrHPV		Bivalent vaccine types <sup>a</sup>		Nonbivalent vaccine types <sup>b</sup>		Polyvalent vaccine types <sup>c</sup>		Nonpolyvalent vaccine types <sup>d</sup>	
		n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
POBASCAM											
29-33	1,899	108	5.69 (4.69-6.83)	44	2.32 (1.67-3.10)	77	4.05 (3.22-5.04)	88	4.63 (3.73-5.68)	34	1.79 (1.24-2.49)
34-38	3,671	125	3.41 (2.84-4.04)	48	1.31 (0.97-1.73)	89	2.42 (1.95-2.98)	94	2.56 (2.07-3.12)	45	1.23 (0.90-1.64)
39-43	3,251	68	2.09 (1.63-2.64)	25	0.77 (0.50-1.13)	47	1.46 (1.06-1.92)	47	1.45 (1.06-1.92)	24	0.74 (0.48-1.10)
44-48	3,174	56	1.76 (1.34-2.29)	13	0.41 (0.22-0.70)	45	1.42 (1.04-1.89)	34	1.07 (0.74-1.49)	24	0.76 (0.49-1.12)
49-53	2,639	45	1.71 (1.25-2.28)	16	0.61 (0.35-0.93)	30	1.14 (0.77-1.62)	31	1.17 (0.80-1.66)	15	0.57 (0.32-0.94)
54-56	2,037	21	1.03 (0.64-1.57)	8	0.39 (0.17-0.77)	13	0.64 (0.34-1.09)	16	0.79 (0.45-1.27)	5	0.25 (0.08-0.57)
Total	16,671	423	2.54 (2.30-2.78)	154	0.92 (0.78-1.08)	301	1.81 (1.61-2.02)	310	1.86 (1.66-2.08)	147	0.88 (0.75-1.04)
Linear effect:			-0.066 (0.007) <sup>h</sup>		-0.079 (0.012) <sup>h</sup>		-0.068 (0.008) <sup>h</sup>		-0.076 (0.008) <sup>h</sup>		-0.063 (0.012) <sup>h</sup>
regression											
coefficient											
(SE) <sup>e</sup>											
Nonlinear effect:			0.002 (<0.001) <sup>i</sup>		0.003 (0.001) <sup>i</sup>		0.001 (0.001)		0.003 (0.001) <sup>i</sup>		<0.001 (0.002)
regression											
coefficient											
(SE) <sup>f</sup>											
NTCC											
24-28	748	65	8.69 (6.77-10.94)	26	3.48 (2.28-5.05)	47	6.28 (4.65-8.27)	46	6.15 (4.54-8.12)	28	3.74 (2.5-5.36)
29-33	961	53	5.52 (4.16-7.15)	28	2.91 (1.94-4.18)	32	3.33 (2.29-4.67)	44	4.58 (3.35-6.1; 44)	15	1.56 (0.88-2.56)
34-38	1222	40	3.27 (2.35-4.43)	10	0.82 (0.39-1.5)	23	2.62 (1.8-3.68)	27	2.21 (1.46-3.2; 27)	18	1.47 (0.88-2.32)
39-43	1123	29	2.58 (1.74-3.69)	10	0.89 (0.43-1.63)	23	2.05 (1.3-3.06)	15	1.34 (0.75-2.19; 15)	15	1.34 (0.75-2.19)
44-48	1112	24	2.16 (1.39-3.19)	8	0.72 (0.31-1.41)	18	1.62 (0.96-2.55)	12	1.08 (0.56-1.88; 12)	12	1.08 (0.56-1.88)
49-53	901	12	1.33 (0.69-2.31)	4	0.44 (0.12-1.13)	10	1.11 (0.53-2.03)	8	0.89 (0.38-1.74; 8)	4	0.44 (0.12-1.13)
54-56	667	8	1.2 (0.52-2.35)	4	0.6 (0.16-1.53)	6	0.9 (0.33-1.95)	7	1.05 (0.42-2.15; 7)	3	0.45 (0.09-1.31)
57-60	700	2	0.29 (0.03-1.03)	0	0 (0-0.53)	2	0.29 (0.03-1.03)	1	0.14 (0-0.79; 1)	1	0.14 (0-0.79)
Total	7434	233	3.13 (2.75-3.56)	90	1.21 (0.97-1.49)	170	2.29 (1.96-2.65)	160	2.15 (1.83-2.51; 160)	96	1.29 (1.05-1.57)
Linear effect:			-0.068 (0.011) <sup>h</sup>		-0.083 (0.018) <sup>h</sup>		-0.057 (0.012) <sup>h</sup>		-0.084 (0.014) <sup>h</sup>		-0.049 (0.016) <sup>i</sup>
regression											
coefficient											
(SE) <sup>e,g</sup>											
Nonlinear effect:			0.001 (0.001)		0.005 (0.002) <sup>i</sup>		<0.001 (0.002)		0.004 (0.002) <sup>i</sup>		-0.002 (0.002)
regression											
coefficient											
(SE) <sup>f,g</sup>											

<sup>a</sup>Bivalent vaccine types includes HPV types 16 and 18.

<sup>b</sup>Nonbivalent vaccine types includes HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.

<sup>c</sup>Polyvalent vaccine types includes HPV types 16, 18, 31, 33, 45, 52, and 58.

<sup>d</sup>Nonpolyvalent vaccine types includes HPV types 35, 39, 51, 56, 59, and 68. The classification in HPV types is not mutually exclusive and totals of bivalent + nonbivalent and polyvalent + nonpolyvalent types exceed the 100% as a result of multiple type infections.

<sup>e</sup>Regression coefficient and standard error of the linear term in logistic regression models, including age as continuous variable.

<sup>f</sup>Regression coefficient and standard error of the nonlinear (quadratic) term in logistic regression models, including age and age squared as continuous variable.

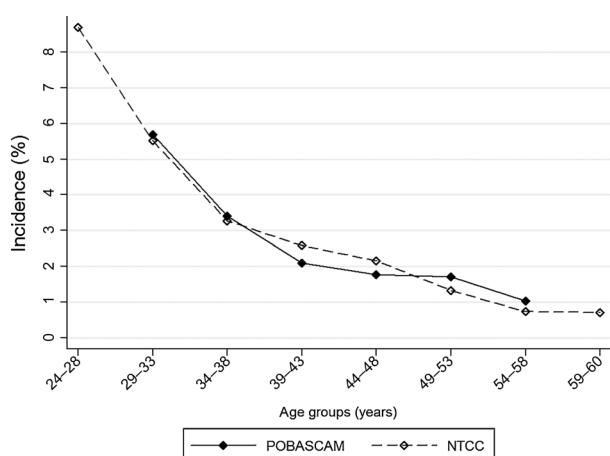
<sup>g</sup>Restricted to the same age range as POBASCAM (29-56 years).

<sup>h</sup>P < 0.001.

<sup>i</sup>P < 0.05.

length we used was not long enough to lead to any difference. An interesting finding was that different age trends were observed for the incidence of vaccine and nonvaccine HPV type. For vaccine HPV types, the decline of incidence was nonlinear (quadratic) and leveled off in older ages, whereas this nonlinearity was not found for nonvaccine HPV types. We found this in the pooled study but also in the two studies separately when we used overlapping ages, from 29 to 56 years of age. Moreover, the presence of vaccine HPV types among all women with incident infections, significantly changed by age, following a nonlinear (quadratic) curve, also after adjusting for the number of types detected. To further explore confounding by multiple HPV type infections, we assessed the age- and type-specific distribution among women with an HPV incident infection with only one HPV type in the pooled dataset. The same age-related trends were observed, thereby excluding confounding by multiple infections. In contrast to the age-associated decrease in hrHPV incidence overall, these differences in the age-related HPV type-

specific distribution cannot easily be explained by sexual behavior or by screening efforts. The latter may lead to a change in the HPV type distribution of prevalent infections but by restricting our analysis population to women with a documented negative previous HPV screening test result, we are able to focus on infections with a known time of onset and screening history is less likely to influence these results. A possible explanation might be a biologic difference between HPV types. For example, protective natural immunity may be more widespread to the most common and most persisting types. Several studies, including mostly young women, have observed protective effects from naturally derived antibodies, especially for HPV16 (14-17), whereas a recent publication suggests differences between types (18). Another analysis among women in the placebo arm of a quadrivalent HPV vaccine study, showed direct evidence of seroprotection in younger, but not in older women (19). These type- and age-specific differences in natural immunity could explain the age-associated differences in type-



**Figure 2.**

The incidence of screen-detected hrHPV. Incidence is defined as the proportion of women HPV-negative at baseline who were positive at the second screening round [POBASCAM median interval 5.0 years (IQR, 4.7–5.5); NTCC median interval 3.0 years (IQR, 3.0–3.1)]. In POBASCAM, women with an enrollment age between 29 and 56 years were eligible; in NTCC, women with an enrollment age between 24 and 60 years were eligible. hrHPV types include HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

specific HPV incidence. Differential reactivation of latent infections after menopause could also be an explanation.

Notwithstanding these interesting age-related distributional differences, the main findings of our study are that (1) hrHPV infections continue to be detected after a documented negative result even among older women, and (2) a substantial proportion of the newly detected hrHPV infections among older women contain vaccine hrHPV types (bivalent and polyvalent vaccine types). We cannot determine whether these infections are new infections or reactivation of previously cleared infections. These findings can be used to explore issues related to the impact of HPV vaccination of adult women (e.g., up to 40 or 45 years of age). Clinical trials have shown that vaccine efficacy against (persistent) HPV16/18 infections among adult sexually experienced HPV DNA-negative women is well more than 80%, without distinguishing between (re)infection or reactivation (20). Initial vaccine efficacy estimates of a polyvalent HPV vaccine showed non-inferiority compared with the quadrivalent vaccine against persistent HPV16/18 and 96.0% vaccine efficacy against persistent HPV 31/35/45/52/58 types (21). It can thus be expected that adult vaccination will considerably reduce the burden of new hrHPV infections, especially when a polyvalent vaccine is used. Adult vaccination may then provide an opportunity to reduce the

**Table 3.** HPV (non-) vaccine-type positivity among women with incident hrHPV infections

Age group	hrHPV positive <i>N</i>	Bivalent vaccine types <sup>a</sup>		Nonbivalent vaccine types <sup>b</sup>		Polyvalent vaccine types <sup>c</sup>		Nonpolyvalent vaccine types <sup>d</sup>	
		<i>n</i>	% (95% CI)	<i>n</i>	% (95% CI)	<i>n</i>	% (95% CI)	<i>n</i>	% (95% CI)
<b>POBASCAM</b>									
29-33	108	44	40.7 (31.4–50.6)	77	71.3 (61.8–79.6)	88	81.5 (72.9–88.3)	34	31.4 (22.9–41.1)
34-38	125	48	38.4 (29.8–47.5)	89	71.2 (62.4–78.9)	94	75.2 (66.7–82.5)	45	36.0 (27.6–45.1)
39-43	68	25	36.8 (25.4–49.3)	47	69.1 (56.7–79.8)	47	69.1 (56.7–79.8)	24	35.3 (24.1–47.8)
44-48	56	13	23.2 (13.0–36.4)	45	80.4 (67.6–89.8)	34	60.7 (46.8–73.5)	24	42.3 (29.7–56.8)
49-53	45	16	35.6 (21.9–51.2)	30	66.7 (51.0–80.0)	31	68.9 (53.4–81.8)	15	33.3 (20.0–49.0)
54-56	21	8	38.1 (18.1–61.6)	13	61.9 (38.4–81.9)	16	76.2 (52.8–91.8)	5	23.8 (8.2–47.2)
Total	423	154	36.4 (31.8–41.2)	301	71.1 (66.6–78.4)	310	73.3 (68.8–77.4)	147	34.8 (30.2–39.3)
Linear effect: regression coefficient (SE) <sup>e</sup>			–0.011 (0.014)		0.004 (0.015)		–0.025 (0.147)		0.014 (0.014)
Nonlinear effect: regression coefficient (SE) <sup>f</sup>			0.002 (0.002)		–0.002 (0.002)		0.004 (0.002) <sup>g</sup>		–0.003 (0.002)
<b>NTCC</b>									
24-28	65	26	40.0 (28.0–52.9)	47	72.3 (59.8–82.7)	46	70.8 (58.2–81.4)	28	43.1 (30.8–56.0)
29-33	53	28	52.8 (38.6–66.7)	32	60.4 (46.0–73.5)	44	83.0 (70.2–91.9)	15	28.3 (16.8–42.3)
34-38	40	10	25.0 (12.7–41.2)	23	80.0 (64.4–90.9)	27	67.5 (50.9–81.4)	18	45.0 (29.3–61.5)
39-43	29	10	34.5 (17.9–54.3)	23	79.3 (60.3–92.0)	15	51.7 (32.5–70.6)	15	51.7 (32.5–70.6)
44-48	24	8	33.3 (15.6–55.3)	18	75.0 (53.3–90.2)	12	50 (29.1–70.9)	12	50.0 (29.1–70.9)
49-53	12	4	33.3 (9.9–65.1)	10	83.3 (51.6–97.9)	8	66.7 (34.9–90.1)	4	33.3 (9.9–65.1)
54-56	8	4	50.0 (15.7–84.3)	6	75.0 (34.9–96.8)	7	87.5 (47.3–99.7)	3	37.5 (8.5–75.5)
57-60	2	0	0	2	100.0 (0.16–100.0)	1	50.0 (1.26–98.7)	1	50.0 (1.3–98.7)
Total	233	90	38.6 (32.3–45.2)	170	73.0 (66.8–78.6)	160	68.7 (62.3–74.6)	96	41.2 (34.8–47.8)
Linear effect: regression coefficient (SE) <sup>e,g</sup>			–0.022 (0.229)		0.043 (0.025)		–0.046 (0.024)		0.030 (0.021)
Nonlinear effect: regression coefficient (SE) <sup>f,g</sup>			0.006 (0.030) <sup>h</sup>		–0.004 (0.003)		0.010 (0.004) <sup>h</sup>		–0.005 (0.003)

<sup>a</sup>Bivalent vaccine types includes HPV types 16 and 18.

<sup>b</sup>Nonbivalent vaccine types includes HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.

<sup>c</sup>Polyvalent vaccine types includes HPV types 16, 18, 31, 33, 45, 52, and 58.

<sup>d</sup>Nonpolyvalent vaccine types includes HPV types 35, 39, 51, 56, 59, and 68.

<sup>e</sup>Regression coefficient and standard error of the linear term in logistic regression models, including age as continuous variable and adjusted for the number of infections.

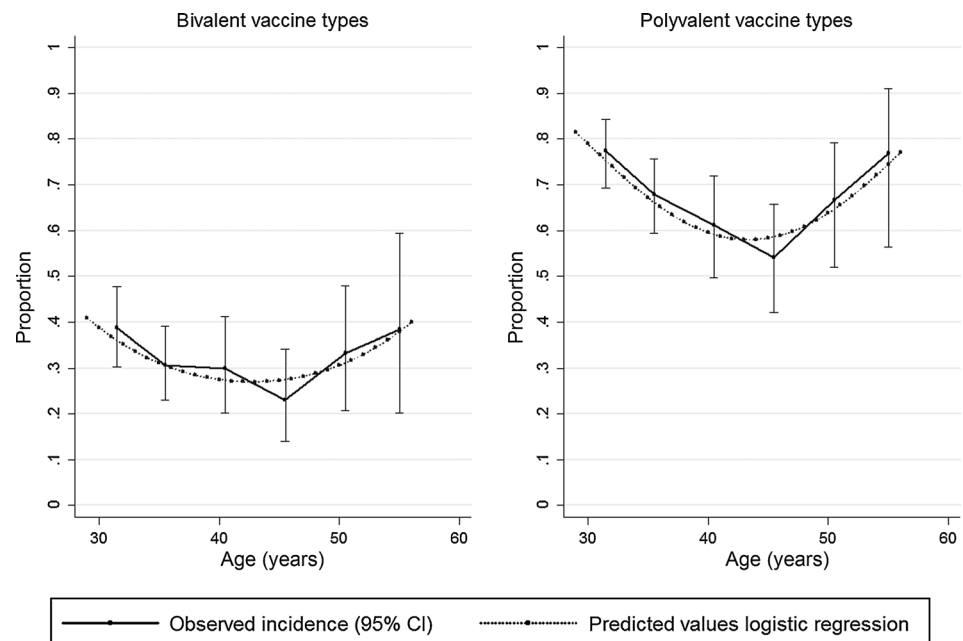
<sup>f</sup>Regression coefficient and standard error of the nonlinear (quadratic) term in logistic regression models, including age and age squared as continuous variable and adjusted for the number of infections.

<sup>g</sup>Restricted to the same age range as POBASCAM (29–56 years). The classification in HPV types is not mutually exclusive and totals of bivalent + nonbivalent and polyvalent + nonpolyvalent types exceed the 100% as a result of multiple type infections.

<sup>h</sup> $P < 0.05$ .

**Figure 3.**

HPV vaccine-type positivity among women with single hrHPV incident infection. Pooled data from POBASCAM and NTCC. Bivalent vaccine types include HPV types 16 and 18. Polyvalent vaccine types include HPV types 16, 18, 31, 33, 45, 52, and 58. Solid lines: the observed incidence (95% CIs); dashed lines: predicted values from logistic regression. *P* value of the quadratic term (adjusted for the number of infections) was 0.056 for bivalent types and 0.003 for polyvalent types.



number of screening visits in previously unvaccinated women. It should be noted that given the long time needed for progression from HPV infection to cancer (22) and given the fact that clearance of incident infections does not substantially decrease with increasing age, the risk of CIN3 and cancer in women with newly acquired hrHPV infections at older age may be low. A careful evaluation of the costs and benefits should not only take into account the hrHPV infection reduction and projected limited reductions in CIN and cancer but also cost savings from fewer screening visits.

Our analyses have some limitations. First of all, we have restricted our population to women who are screen-negative at baseline, and excluded hrHPV-positive women who could have acquired a different hrHPV type during follow up. However, this allowed us to reflect upon the hrHPV incidence from a public health (screening) perspective. Screen-positive women are referred to intensified follow-up and new hrHPV types detected during follow-up will be detected after a considerable shorter time frame than our screen-detected infections. Second, when we included the youngest women (aged 24–28 years) and women more than 56 years of age in NTCC, the quadratic age-related trends were no longer statistically significant, and a more complicated age-trend (polynomial to the fourth degree) provided a better explanation of the observed data. This was mainly driven by the different HPV type-specific distribution among the youngest women compared with the age-related trends found in women over 29 years of age, which could have a biologic or behavioral explanation. For example, although the incidence of hrHPV overall was significantly lower in women aged 29 to 33 years, compared with women ages 24 to 28 years, the incidence of HPV16/18 did not decline. The relative contribution of HPV16/18 also had a tendency to increase from women aged 24 to 28 years to women aged 29 to 33 years. The different transmission dynamics in this youngest age group significantly influences the observed trend in other ages. A final limitation is that it must be kept in mind that these are data collected over two screening rounds

among women at different ages, and not a cohort of women followed prospectively for more than two screening rounds. Therefore, we cannot exclude cohort effects. In fact, the decline in hrHPV with age could plausibly reflect a cohort effect, as a consequence of changes in sexual behavior. However, it is less obvious to explain the age-related differences in vaccine and non-vaccine hrHPV types by sexual behavior or other cohort effects.

In conclusion, using data collected from two large population-based screening trials we observed decreasing trends of HPV type-specific incidences by age. The observed differences across age in the contribution of specific types in new HPV infections cannot easily be explained by sexual behavior, cohort effects, or by previous screening history. Notwithstanding the reduced incidence by age, the contribution of vaccine types in new infections remained substantial. This suggests a considerable reduction of new hrHPV infections in vaccinated cohorts which demands for a revision of screening guidelines.

#### Disclosure of Potential Conflicts of Interest

J. Berkhof is a consultant/advisory board member for Roche Diagnostics. P.J.F. Snijders received speakers' bureau honoraria from Roche, Gen-Probe, Qiagen, and Abbott. No potential conflicts of interest were disclosed by the other authors.

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**Other (performed molecular analyses for HPV detection and genotyping and interpretation of the results):** A. Gillio-Tos

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# BLOOD CANCER DISCOVERY

## The Age Distribution of Type-Specific High-Risk Human Papillomavirus Incidence in Two Population-Based Screening Trials

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