HPV16/18 vaccination in the Netherlands: Monitoring long-term effects on HPV infections and immunogenicity

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Chapter 1

Introduction

General

A genital infection with human papillomavirus (HPV) is the most common sexually transmitted infection, which passes transiently in most people. However, some infections with high-risk (hr) HPV types persist and are associated with an increased risk for the development of anogenital cancers, while low-risk (lr) HPV type infections may lead to anogenital warts. Cancer of the cervix is the most common HPV associated cancer, as well as the most common gynecological cancer worldwide. While HPV infections may be acquired early after initiation of sexual activity, cervical cancer can take many years to develop and has a peak prevalence between 35-55 years of age. Three prophylactic HPV vaccines have been developed, aiming to prevent HPV infection and subsequent disease. All vaccines protect against the two most common oncogenic HPV genotypes, HPV16 and HPV18. In the Netherlands, vaccination was introduced in the National Immunization Program (NIP) in 2009 for girls only, using the bivalent HPV vaccine. The program started with a catch-up campaign for 12- to 16-year-old girls born in 1993-1996, according to a three-dose schedule. From 2010 onwards, HPV vaccination was offered to girls in the year they turn 13 years old and since 2014, the program has used a two-dose schedule. Since 2022, HPV vaccination is offered to both girls and boys in the year they turn 10 years old.

The goal of this thesis is to monitor and evaluate the effect of the HPV vaccination program on HPV infections and immune responses, with a special focus on long term effects.

Papillomaviruses

Papillomaviruses (PVs) come in a large variety of types, but all consist of a small, circular genome of about 8000 base pairs. The base pairs code for the most important early genes (E1, E2, and E4-E7), and late genes one and two (L1 and L2), amongst others [1]. Only a subset of all PVs can infect human tissue (HPV), of which over 200 have been identified to date based on genetic variation. The types are subdivided in genera; about 40 commonly studied HPV types are able to infect the human mucosae and are from the alpha genus [2]. Within this genus different clades can be distinguished indicating variation between the types. HPV infections of the human mucosae may appear anogenital, but also oropharyngeal. Moreover, a distinction has been made based on the oncogenic potential, subdividing hr and lr HPV types.

HPV types

Currently, twelve HPV types are considered as group 1 carcinogens, and are therefore classified as hr HPV: HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 [3, 4]. A handful of other types is classified as potentially carcinogenic [3]. Although 80 to 90% of the infections are asymptomatic [5] and will be cleared spontaneously within

18 months on average [6], a persistent infection with a hr HPV type may lead to the development of (pre) malignant lesions on anogenital and oropharyngeal sites, including cervical, vaginal, vulvar, penile, anal, oral and larynx cancer [7]. Of these, cervical cancer is the most common cancer associated with HPV [8]. HPV16 and 18 are the dominant oncogenic types, associated with 70% of all cervical cancers and responsible for the majority of other HPV associated cancers [4]. LrHPV types are associated with benign lesions of the anogenital site known as anogenital warts or condyloma acuminata and low-grade lesions of the cervix. HPV6 and 11 are the most common lrHPV types with an estimated attributable fraction of 90% for anogenital warts [9].

Epidemiology and risk factors

HPV infections are very common and mostly present as sexually transmitted infections with an estimated lifetime risk to acquire an infection of 80% [10]. Among women, age related distributions of anogenital HPV infection vary across populations. For most populations, prevalence increases from the start of sexual debut until age 25-30. Thereafter, a plateau is reached followed by a declining prevalence of genital HPV infections, although inconsistent patterns are observed in middle-aged women [11, 12]. Other observed trends are flatter(mainly seen in Asian countries) or bimodal, especially in Latin-America or Africa [13]. Prevalence among men is more equally spread over age groups with no clear decline [14, 15]. HPV16 is the most common HPV type in cervical infections among women with normal cytology across the world [13, 16]. Prevalence of other hr HPV type infections shows heterogeneity between different populations, which can be due to host immunogenic factors or other geographical characteristics. Moreover, prevalence is less well studied at other anatomical sites but seems dependent on gender and sexual preference. For example, HPV prevalence at the anal site is much higher among men who have sex with men (MSM) compared to women or men who have sex with women. Anal HPV prevalence is also associated with the presence of an HIV infection [17]. For the oral cavity, HPV prevalence estimates are inconsistent and dependent on the collection method, with some studies showing a higher prevalence among men than among women [18].

As transmission of mucosal HPV infections is most likely to happen during (vaginal) intercourse, risk factors for contracting an infection are mostly related to sexual behavior and comparable to other sexually transmitted infections (STI). An increasing number of sexual partners and a younger age of sexual debut (high-risk sexual behavior), but also smoking, drinking alcohol, and oral contraceptive use have been associated with HPV infection [6]. Consistent condom use likely reduces transmission, although not as much as observed in other STIs [19].

HPV infections can be classified as incident or prevalent when observed once, or persistent when the same HPV type infection is observed in subsequent measurements. Whether HPV infections that are not longer detected have completely cleared is still under debate as latency and reactivation cannot be ruled out [20]. This indicates that the virus remains present in the body below the viral load detection limit, but reactivates when the immune system gets suppressed or circumstances change [21].

Cervical carcinogenesis

Cancerous lesions of the cervix were the first to be identified as related to a persistent hr HPV infection, and they are also the only HPV related cancer of which virtually all cases are attributable to HPV infections [8]. Other HPV related cancers may have a more heterogeneous origin, including a role for environmental factors [22]. Therefore, carcinogenesis has been most clearly documented for the cervix. Microtrauma in the epithelium of the cervical tissue (often the cervical transformation zone) can be induced during sexual intercourse, giving the virus the opportunity to infect the host basal cells. Viral proteins inhibit normal differentiation of the basal cells while initiating viral genome replication [23]. When viral parts are shed at the epithelial surface, a productive infection state is reached. If the infection is maintained and not cleared by the host's immune system, expression of the virus in dividing host cells can occur, leading to deregulation of the cell cycle and genetic instability also known as a transforming infection. This causes morphological changes of the cervical cells which may eventually lead to invasive cancer [24].

Cervical squamous cell carcinomas are the most common form of cervical cancer and are preceded by different stages of cervical intraepithelial neoplasia (CIN). These are categorized as mild dysplasia (CIN1) up to severe dysplasia and carcinoma in situ (CIN3), with a larger part of the epithelium affected in the later stages [25]. Progression and regression of lesion stages is possible, but the chance to regress decreases with increasing stage. The time between acquiring an HPV infection and CIN2/3 progression has been estimated to be 3-5 years, while it may take up to 15-30 years to develop cervical cancer [23, 26]. A less frequently observed form of cervical cancer is adenocarcinoma (preceded by adenocarcinoma in situ), although its precancerous stages are less well defined [26]. Also, pathogenesis at other anatomical sites has been less well described but is supposed to be comparable to the cervix.

Disease burden of HPV related cancer and anogenital warts

HPV associated cancers pose an important disease burden worldwide, although the total number of non-cervical cases is smaller and the HPV attributable fraction is reduced compared to that of cervical cancer [27]. Regional differences in HPV associated cancer incidence have been observed; cervical cancer disproportionally

affects women in the developing world (85% of cases reside in South-Eastern Asia, Latin America, and sub-Saharan Africa), while non-cervical cancer has the highest burden in Europe and North-America [27]. This inconsistency between cervical and non-cervical cancer occurrence is probably related to the lack of preventive measures like screening programs, which are available for cervical cancer (mainly in developed countries) but do not exist for non-cervical HPV-related cancer. Together with the age at which non-cervical cancer is often diagnosed, this leads to different regional patterns in disease burden [7]. In the Netherlands, about 800 women are diagnosed with cervical cancer each year, and about 200 women die from this disease [28].

Anogenital warts add a substantial part to the total number of HPV-related disease cases, albeit with lower disease severity. In the Netherlands, the annual rate of diagnosed AGW at general practices fluctuated between 3.7 and 4.2 per 1000 persons between 2017 and 2021 [29].

Vaccination

Since the first licensure in 2006 and 2007, different prophylactic HPV vaccines have been introduced to the market to prevent HPV associated disease. The working mechanism is based on virus-like ps (VLPs), which resemble the L1 gene of the virus but do not contain actual HPV DNA and can therefore not cause disease [32]. All vaccines are based on the same mechanism but are produced in different vectors and use different adjuvants. The bivalent vaccine (Cervarix, 2vHPV) induces protection against infection with hr HPV types 16/18 types [33], while the quadrivalent vaccine (Gardasil, 4vHPV) combines protection against HPV16/18 with lr types HPV6/11 [34]. A nonavalent vaccine (Gardasil9, 9vHPV) was introduced in 2015 and protects against HPV types HPV6/11/16/18/31/33/45/52/58 [35]. All vaccines were primarily targeting cervical cancers (and anogenital warts in case of HPV6/11 inclusion),

Table 1: Number of incident HPV associated cancer cases with HPV attributable fractions.

Anatomical site	Number of incident cases (2020) [27]	HPV attributable fraction (%) [24]	Number of HPV attributable cases*	Relative attribution of HPV16/18 (%) [28]	Relative attribution of HPV31/33/45/52/58 (%) [28]
Cervix	604 100	100	604 100	71	18
Anus	50 900	88	44 800	87	8
Oropharynx	98 400	31	30 500	90	5
Vagina	17 900	78	14 000	64	21
Vulva	45 200	25	11 300	79	14
Penis	36 100	50	18 000	77	11

** Based on attributable fraction from Martel [27] and Globocan 2020 [30]*

but over the last years the European Medicines Agency (EMA) registration has been extended to include protection against vulvar, vaginal, and anal lesions according. Vaccines were initially licensed according to three-dose schedules for people from 9 years of age onwards. Licensure was replaced by a two-dose recommendation for 9-13/14-year-olds based on immunobridging data in 2014 [36-38]. In 2022, the World Health Organization (WHO) changed their recommendation to a one or two-dose schedule for all children and adolescents until 20 years of age and to a two-dose schedule with a 6-month interval for adults ≥21 years. However, such schedules have not yet been approved by the European Medicines Agency (EMA) [39].

Vaccination in the Netherlands was implemented as a primary prevention strategy for HPV related cancers targeting girls-only, as the initial aim was to reduce cervical cancer. Following a catch-up campaign for 13-16-year-old girls in 2009, the bivalent HPV vaccine was added to the regular National Immunization Program (NIP) in 2010. Girls were eligible for a 3-dose vaccination schedule in the year they turned 13 [40]. In 2014, a switch towards a two-dose schedule was made based on an advice of the EMA. In 2019, an updated advice from the Dutch Health Council stated that it would be beneficial to offer HPV vaccination to boys in addition to girls and to lower the age of vaccination to nine years (in the year a child turns ten). This change in schedule has been implemented in 2022, combined with a catch-up campaign for all adolescents (boys and girls) up to the age of 18 years in 2022 and 2023. Additionally, the opportunity to vaccinate is provided for everyone up to the age of 26 years in 2023 [41].

Uptake of HPV vaccination in the Netherlands has been fluctuating; the catch-up campaign in 2009/2010 for birth cohorts 1993-1996 had an uptake around 50%. From 2010 onwards, HPV in the regular NIP has shown a varying uptake. In 2018 the uptake was at its lowest at 45.5% and since 2020 an increasing trend can be observed [42]. The uptake of HPV vaccination is lower than that of the infant vaccines offered within the NIP (all >90%). However, some young people do receive HPV vaccination after the targeted age; if these are included in the calculation, the uptake is slightly higher. Also the Covid-19 pandemic has caused some fluctuations in vaccine uptake over the past years, as invitations for HPV vaccination were postponed.

While some countries like England and Scotland achieved a high uptake since the start of the HPV vaccination program, others encountered similar difficulties reaching high HPV vaccine uptake; negative media attention and parental concerns about vaccine safety and effectiveness that were spread by lobby groups, caused drastic declines in the HPV vaccination uptake in Ireland and Denmark [43,44]. Similar factors likely affected the uptake in the Netherlands [45] but studies additionally showed that not

Table 2: Different licensed HPV vaccinations with their specifications as described by EMA.

	Cervarix® (bivalent)	**Gardasil® (quadrivalent)**	**Gardasil9® (nonavalent)**
Manufacturer	GlaxoSmithKline Biologicals, SA	Merck Sharp & Dohme	Merck Sharp & Dohme
VLP types included	HPV16 and 18	HPV6, 11, 16 and 18	HPV6, 11, 16, 18, 31, 33, 45, 52 and 58
Dose of L1 protein	20 µg (HPV16 and 18)	20 µg (HPV6 and 18) 40 µg (HPV11 and 16)	20 µg (HPV31, 33, 45, 52, 58) 30 µg (HPV6) 40 µg (HPV11 and 18) 60 µg (HPV16)
Producer cells	Trichoplusia ni (Hi 5) insect cell line infected with L1 recombinant	Saccharomyces cerevisiae expressing L1	Saccharomyces cerevisiae expressing L1
Registered for	Boys and girls ≥9 year	Boys and girls 9-26 year	Boys and girls 9-26 year
Adjuvant	500 µg aluminum hydroxide and 50 µg 3–0-deacylated-4′-monophosphoryl lipid A (AS04)	225 µg aluminum hydroxy phosphate sulphate (AAHS)	500 µg aluminum hydroxy phosphate sulphate (AAHS)
Schedule	9-14 years of age: two doses (0, 5-13 months) ≥15 years of age: three doses (0,1,6 months)	9-13 years of age: two doses (0, 6 months) ≥14 years of age: three doses (0,2,6 months)	9-14 years of age: two doses (0, 6 months) ≥15 years of age: three doses (0,2,6 months)
Indications	Protection against HPV-related: - Precancerous cervical, vulvar, vaginal, and anal lesions; - Cervical and anal cancers	Protection against HPV-related: - Precancerous cervical, vulvar, vaginal, and anal lesions; - Cervical and anal cancers; - Genital warts	Protection against HPV-related: - Precancerous cervical, vulvar, vaginal, and anal lesions; - Cervical, vulvar, vaginal, and anal cancers; - Genital warts

having received measles vaccination, having one or two parents born in Morocco or Turkey, living in an area with lower socioeconomic status and higher municipal voting proportions for Christian political parties or populist parties are associated with a lower vaccination uptake [46].

In the Netherlands, monitoring of vaccine-related adverse events is performed by The Netherlands Pharmacovigilance Centre Lareb [47]. Mild adverse events following HPV vaccination have been frequently reported, including local and systemic reactions. Both in- and outside the Netherlands, research has been conducted to evaluate possible severe HPV vaccine related adverse events, including migraine, chronic fatigue syndrome (CFS), and complex regional pain syndromes or postural orthostatic tachycardia syndrome (CRPS or POTS) [48-50]. No associations or indications for a biological relation between HPV vaccination and any of these health issues were established and the vaccine is considered safe [51].

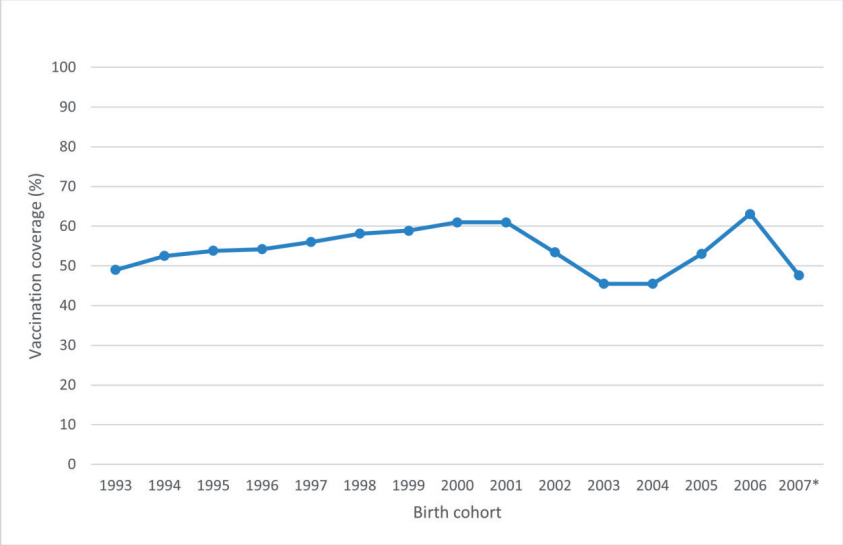

Figure 1: HPV vaccination uptake by birth cohort in the Netherlands since start of the HPV vaccination program
**: Since reporting year 2022 (birth cohort 2007), an informed consent procedure is in place for the registration of vaccination data with personal data, such as year of birth and postal codes. If a parent/adolescent does not permit use of the data, , the vaccinee is not included in the vaccine uptake calculations, leading to an underestimation of the total vaccine uptake.*

Immune responses

The exact working mechanism of HPV vaccination is not known, but neutralizing antibodies are the assumed mediators of protection following vaccination. The vaccine induces strong immunological responses and vaccinated individuals almost always seroconvert (nearly 100%), with antibody levels up to 100-fold higher compared to unvaccinated individuals [52,53]. This indicates the HPV vaccines are very immunogenic with antibody levels that remain high over time, as is also predicted by modeling studies [54,55]. On the other hand, observed seroconversion following a natural infection is limited. Not everyone seroconverts and it is unknown whether a current infection prevents a subsequent one with the same HPV type [56]. A correlate of protection is not determined for HPV so far. Nevertheless, serology measurements or seroprevalence studies can be indicative of cumulative past exposure among the population. As earlier discussed, different dosing schedules have been in place.. Up to now these reduced dosing schedules seem appropriate especially among children en adolescents, leading to comparable and durable immune responses at least against vaccine type infections [57-59].

Vaccine impact

Impact of vaccination can be measured in different ways. Regarding HPV vaccines, various endpoints can be included on the trajectory from incident infection to actual cancer. They come with their own (dis)advantages, such as the time it takes to observe a clinical endpoint and the number of people needed to measure an effect. Furthermore, the impact of vaccination can be expressed as efficacy when measured within randomized clinical trials under ideal, controlled circumstances, and as effectiveness when measured with observational, real-world data [60]. For HPV vaccines, evaluation in real-world settings is especially important, since registration and clinical trials were mostly based on an older study population as compared to preadolescents to which the vaccines are administered within vaccination programs [61]. Lastly, it is informative to not only include the observed effect among vaccinated individuals as compared to unvaccinated ones, but also to consider the indirect effect of vaccination on the population (population-level effects). This provides information about the herd effects; effects of vaccination outside the targeted group through reduced infection transmission in the total population, including unvaccinated individuals. For girls-only vaccination herd effects may occur among men (first order effects) or unvaccinated women (second order effects) [62]. It may also be interesting to evaluate the effect of HPV vaccination for specific types combined or subsets, such as vaccine types, cross-protective types, lr and hr HPV types. With regard to cross-protective types, protection against numerous HPV types has been shown following bivalent and quadrivalent vaccination and is important to consider in the evaluation of the total protective effect of these vaccines. Generally, cross protection is stronger following bivalent compared to quadrivalent vaccination and cross-protective effects have been observed for HPV types HPV31/33/45/52/58, with HPV31 and HPV45 most consistently reported [63, 64]. Randomized clinical trials (RCTs) that were conducted for the vaccine registration mainly included 16–26-year-old females and showed very high efficacy (>95%) against vaccine type CIN2(+) lesions among those without evidence of previous HPV exposure [65-67]. This study population was assumed to be the best approximation of the target population of vaccination, being preadolescents before sexual debut. Protection is generally lower in populations that might have already been exposed and is often lower against (persistent) infections as compared to lesions.

Secondary prevention

As opposed to primary HPV related cancer prevention through vaccination, the Netherlands also has a national cervical cancer screening program for secondary prevention of cervical cancer. The current organized program invites all women aged 30 years for their first visit, which consists of a cervical sample at the general practitioner's office or a self-collected vaginal sample on request. In 2021, the regular program reached 55% of the invited women; this uptake included some catch-up related to delays in 2020

Table 3: Vaccine effectiveness from the three licensed HPV vaccines against different endpoints.

Adapted from [68]	**Gardasil**	**Gardasil9**	**Cervarix**
Among women 15/16–26 years			
4–6 months HPV 16/18 infection	96% (83, 100)	na	94% (92, 96)
6-month HPV 31/33/45/52/58 infection	18% (5, 29)	96% (94, 98)	na
6-month HPV 31 infection	46% (15, 66)	96% (91, 98)	77% (69, 83)
6-month HPV 33 infection	NS	99% (95, 100)	45% (25, 60)
6-month HPV 45 infection	NS	97% (92, 99)	74% (58, 84)
6-month HPV 51 infection	na	na	17% (4, 28)
6-month HPV 52 infection	NS	97% (95, 99)	na
6-month HPV 58 infection	NS	95% (91, 97)	na
CIN 2 + related to HPV 16/18	98% (94, 100)	na	98% (88, 100)
CIN 2 + related to HPV 31	70% (32, 88)	100% (40, 100)	88% (68, 96)
CIN 2 + related to HPV 33	NS	100% (33, 100)	68% (40, 84)
CIN 2 + related to HPV 39	NS	na	75% (22, 94)
CIN 2 + related to HPV 45	NS	NS	82% (17, 98)
CIN 2 + related to HPV 51	NS	na	54% (22, 74)
CIN 2 + related to HPV 52	NS	100% (67, 100)	na
CIN 2 + related to HPV 58	NS	NS	na
CIN 2 + caused by any HPV type	22% (3, 38)	63% (35, 79)	62% (47, 73)
CIN 3 + caused by any HPV type	43% (24, 57)	na	93% (79, 99)
Among women older than 25 years			
6-month infection or disease related to HPV 16/18	85% (68, 94)	na	91% (79, 97)
6-month HPV 31 infection	na	na	66% (25, 86)
6-month HPV 45 infection	na	na	71% (34, 88)

Vaccine efficacies are presented with 95% confidence intervals.
NS means not significant; na means not applicable/available.

due to the Covid-19 pandemic. The 5-year uptake of the cervical cancer screening program with opportunistic screens and follow-up management is 72% [69]. Since 2017, hr HPV DNA testing has replaced cytology testing as primary screening tool: Only HPV positive samples are tested for cytology and HPV-positive women are managed according to cytology and HPV16/18 genotyping results [70]. This process is repeated every 5 years, but women who have a negative hr HPV test at age 40 or 50 years are re-invited after 10 years.

In 2023, women who were eligible for HPV vaccination through the catch-up campaign will enter the HPV-based screening program. This provides a new source for vaccine monitoring and evaluation: through comparing screening outcomes in

vaccinated and unvaccinated women the impact of vaccination on clinical outcomes will become measurable.

Monitoring NIP

Surveillance of the NIP is important and aims to evaluate the current immunization program. The evaluation is based on several monitoring areas divided in five pillars [71]. 1) Pathogen surveillance including virological changes and viral load, 2) surveillance of the disease or intermediate endpoints such as (persistent) infections, 3) surveillance of adverse events possibly related to vaccination, 4) surveillance of vaccination uptake, 5) immunosurveillance including serological responses among vaccinated and unvaccinated, duration of protection, and correlates of protection.

Along with the introduction of the HPV vaccine, monitoring was advised by the Health Council to assess effectiveness and safety as long-term follow-up data were not yet available [72]. The expected effects on cancer reduction can only be observed after many years, so early endpoints such as (persistent) infections and pre-stages of cervical lesions form an important alternative source of information (surveillance of disease). Together with the other pillars this provides extensive insight in the effects of HPV vaccination in the Netherlands.

Aim and content of thesis

The aim of this thesis is to monitor the long-term effects of HPV vaccination within the NIP of the Netherlands regarding virological and serological outcomes. More specifically, genital HPV infections, which are considered important early outcomes, and serological IgG responses are described and studied. Additionally, we describe effects of both a three-dose and a reduced dosing schedule on the long-term protection. This information is important for bridging the gap between the initial vaccination implementation and the upcoming expected results on clinical outcomes. Both the HAVANA cohort (three-dose schedule among girls invited through the catch-up campaign) and the HAVANA2 cohort (two-dose schedule) are important sources of information within this thesis.

In **part 1** the focus is on the serological responses following HPV infection and vaccination. **Chapter 2** describes the population-based changes in seroprevalence of unvaccinated individuals over a ten-year time period, during which HPV vaccination was implemented in the Netherlands. IgG antibody levels as induced by natural infection against 7 hr HPV types in the general population are described. In **chapter 3,** the focus is on vaccine derived immune responses following vaccination. Within the HAVANA cohort, antibody levels among both vaccinated and unvaccinated participants are described. Furthermore, the association between type-specific antibody

levels one-year before infection is studied among vaccinated individuals. **Chapter 4** reviews the currently available information on immunological responses following vaccination, with special focus on long-term effects. Chapter 4 identifies the current state of knowledge and aspects that could be considered for further research.

In **part 2** of this thesis, the focus is on the effects of HPV vaccination on genital infections. First, in **chapter 5**, we look at the trends of type-specific HPV prevalence over time since the introduction of HPV vaccination. Both the trends among vaccinated women, heterosexual men and unvaccinated women are described in order to gain insight into the population-level impact of a girls-only HPV vaccination program. **Chapter 6** focusses on genital infections among vaccinated and unvaccinated participants from the HAVANA2 cohort in order to estimate the effectiveness against vaccine type and cross protective type infections from the reduced dosing schedule at four-year post-vaccination. In **chapter 7**, methodological challenges regarding vaccine effectiveness are described, specifically the selection of a method for vaccine effectiveness (VE) estimation. Different methods as identified in the literature are described, compared, and applied to the HAVANA data in order to make a statement on the most suitable method for VE estimation from observational cohort data. Finally, **chapter 8** focusses on the long-term protection from the three-dose schedule up to 10 years after vaccination using HAVANA data. **Chapter 9** contains the general discussion of this thesis.

References

1. De Villiers E-M, Fauquet C, Broker TR, Bernard H-U, zur Hausen H. Classification of papillomaviruses. Virology 2004; 324:17-27.
2. de Villiers E-M. Cross-roads in the classification of papillomaviruses. Virology 2013; 445:2-10.
3. International Agency for Research on Cancer Lyon, France. Cancer fact sheets. Available at: http://gco.iarc.fr/today/fact-sheets-cancers.
4. De Sanjose S, Quint WG, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. The lancet oncology 2010; 11:1048-56.
5. Moscicki A-B, Schiffman M, Burchell A, et al. Updating the natural history of human papillomavirus and anogenital cancers. Vaccine 2012; 30:F24-F33.
6. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. Journal of clinical virology 2005; 32:16-24.
7. Chaturvedi AK. Beyond cervical cancer: burden of other HPV-related cancers among men and women. Journal of Adolescent Health 2010; 46:S20-S6.
8. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. The Journal of pathology 1999; 189:12-9.
9. Egawa N, Doorbar J. The low-risk papillomaviruses. Virus research 2017; 231:119-27.
10. Chesson HW, Dunne EF, Hariri S, Markowitz LE. The estimated lifetime probability of acquiring human papillomavirus in the United States. Sexually transmitted diseases 2014; 41:660.
11. Cuzick J, Clavel C, Petry KU, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. International journal of cancer 2006; 119:1095-101.
12. Smith JS, Melendy A, Rana RK, Pimenta JM. Age-specific prevalence of infection with human papillomavirus in females: a global review. Journal of Adolescent Health 2008; 43(4), S5-e1.
13. Bruni L, Diaz M, tellsagué M, Ferrer E, Bosch FX, de Sanjosé S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. Journal of Infectious Diseases 2010; 202:1789-99.
14. Anic GM, Giuliano AR. Genital HPV infection and related lesions in men. Preventive medicine 2011; 53:S36-S41.
15. Smith JS, Gilbert PA, Melendy A, Rana RK, Pimenta JM. Age-specific prevalence of human papillomavirus infection in males: a global review. Journal of Adolescent Health 2011; 48(6), 540-552.
16. Clifford G, Gallus S, Herrero R, et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. The Lancet 2005; 366:991-8.
17. Machalek DA, Poynten M, Jin F, et al. Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic review and meta-analysis. The lancet oncology 2012; 13:487-500.
18. Wierzbicka M, Klussmann JP, San Giorgi MR, Wuerdemann N, Dikkers, FG. Oral and laryngeal HPV infection: Incidence, prevalence and risk factors, with special regard to concurrent infection in head, neck and genitals. Vaccine 2021; 39(17), 2344-2350.
19. Manhart LE, Koutsky LA. Do condoms prevent genital HPV infection, external genital warts, or cervical neoplasia?: A meta-analysis. Sexually transmitted diseases 2002; 29:725-35.
20. Gravitt PE. The known unknowns of HPV natural history. The Journal of clinical investigation 2011; 121:4593-9.

21. Doorbar J, Quint W, Banks L, et al. The biology and life-cycle of human papillomaviruses. Vaccine 2012; 30:F55-F70.
22. Munoz N, tellsagué X, de González AB, Gissmann L. HPV in the etiology of human cancer. Vaccine 2006; 24:S1-S10.
23. Doorbar J. Molecular biology of human papillomavirus infection and cervical cancer. Clinical science 2006; 110:525-41.
24. Galloway DA, Laimins LA. Human papillomaviruses: shared and distinct pathways for pathogenesis. Current opinion in virology 2015; 14:87-92.
25. Snijders PJ, Steenbergen RD, Heideman DA, Meijer CJ. HPV-mediated cervical carcinogenesis: concepts and clinical implications. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland 2006; 208:152-64.
26. Schiffman M, tle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. The Lancet 2007; 370:890-907.
27. de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. International journal of cancer 2017; 141:664-70.
28. van 't Klooster TS, de Melker HE. The National Immunisation Programme in the Netherlands : Surveillance and developments in 2019-2020. Bilthoven: National Institute for Public Health and Environment, 2020.
29. National Institute for Public Health and Environment. Sexually transmitted infections in the Netherlands in 2022: National Institute for Public Health and the Environment (RIVM), 2023.
30. International Agency for Research on Cancer (IARC). Global Cancer Observatory (Globocan) 2020. Lyon, France., 2020.
31. de Sanjosé S, Serrano B, Tous S, et al. Burden of human papillomavirus (HPV)-related cancers attributable to HPVs 6/11/16/18/31/33/45/52 and 58. JNCI cancer spectrum 2018; 2:pky045.
32. Schiller JT, Lowy DR. Papillomavirus-like ps and HPV vaccine development. In: Seminars in cancer biology 1996. Elsevier:373-82.
33. Committee for Medicinal Products for Human Use (CHMP). Assessment report Cervarix: European Medicines Agency, 2013.
34. Committee for Medicinal Products for Human Use (CHMP). Gardasil - EPAR: Product information, 2009.
35. Committee for Medicinal Products for Human Use (CHMP). Gardasil9 - EPAR: Product information, 2015.
36. Romanowski B, Schwarz TF, Ferguson LM, et al. Immunogenicity and safety of the HPV-16/18 AS04-adjuvanted vaccine administered as a 2-dose schedule compared to the licensed 3-dose schedule: Results from a randomized study. Human vaccines 2011; 7:1374-86.
37. Dobson SR, McNeil S, Dionne M, et al. Immunogenicity of 2 doses of HPV vaccine in younger adolescents vs 3 doses in young women: a randomized clinical trial. Jama 2013; 309:1793-802.
38. Iversen OE, Miranda MJ, Ulied A, et al. Immunogenicity of the 9-Valent HPV Vaccine Using 2-Dose Regimens in Girls and Boys vs a 3-Dose Regimen in Women. Jama 2016; 316:2411-21.
39. World Health Organization. Weekly Epidemiological Record, vol. 97, 24 [full issue].2022 ; 97(24):261-76.
40. De Melker HE, Conyn-Van Spaendonck MA, Boot HJ, Coutinho RA. STAND VAN ZAKEN-Introductie van vaccinatie tegen baarmoederhalskanker. Nederlands tijdschrift voor geneeskunde 2009; 153:658.
41. Health Council of the Netherlands. Vaccination against HPV. The Hague, 2019.
42. National Institute for Public Health and Environment. Vaccinatiegraad en jaarverslag Rijksvaccinatieprogramma Nederland 2021. Bilthoven, the Netherlands, 2022.

43. Corcoran B, Clarke A, Barrett T. Rapid response to HPV vaccination crisis in Ireland. The Lancet 2018; 391:2103.
44. Hansen PR, Schmidtblaicher M, Brewer NT. Resilience of HPV vaccine uptake in Denmark: decline and recovery. Vaccine 2020; 38:1842-8.
45. Conyn-van Spaendonck M. Harde lessen uit introductie van HPV-vaccinatie. TSG 2010; 88:5-6.
46. de Munter AC, Klooster TM, van Lier A, Akkermans R, de Melker HE, Ruijs WLM. Determinants of HPV-vaccination uptake and subgroups with a lower uptake in the Netherlands. BMC public health 2021; 21:1-13.
47. Bijwerkingencentrum Lareb. Long-lasting adverse events following immunization with Cervarix®, 2015.
48. European Medicines Agency (EMA). HPV vaccines: EMA confirms evidence does not support that they cause CRPS or POTS, 2016.
49. Schurink-Van't Klooster TM, Kemmeren J, van der Maas N, et al. No evidence found for an increased risk of long-term fatigue following human papillomavirus vaccination of adolescent girls. 2018; 36:6796-802.
50. Feiring B, Laake I, Bakken IJ, et al. HPV vaccination and risk of chronic fatigue syndrome/myalgic encephalomyelitis: a nationwide register-based study from Norway. Vaccine 2017; 35:4203-12.
51. Schurink-van t Klooster TM, Kemmeren JM, Hilgersom WJA, Hoes J, de Melker HE. Surveillance van mogelijke bijwerkingen na HPV-vaccinatie: het vaccin is veilig Infectieziekten Bulletin 2019; 30.
52. Turner TB, Huh WK. HPV vaccines: Translating immunogenicity into efficacy. Human vaccines & immunotherapeutics 2016; 12:1403-5.
53. Harro CD, Pang Y-YS, Roden RB, et al. Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like p vaccine. Journal of the National Cancer Institute 2001; 93:284-92.
54. Schwarz TF, Huang LM, Valencia A, et al. A ten-year study of immunogenicity and safety of the AS04-HPV-16/18 vaccine in adolescent girls aged 10-14 years. Human vaccines & immunotherapeutics 2019; 15:1970-9.
55. Einstein MH, Takacs P, Chatterjee A, et al. Comparison of long-term immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine and HPV-6/11/16/18 vaccine in healthy women aged 18-45 years: end-of-study analysis of a Phase III randomized trial. Human vaccines & immunotherapeutics 2014; 10:3435-45.
56. Mollers M, Vossen JM, Scherpenisse M, van der Klis FR, Meijer CJ, de Melker HE. Current knowledge on the role of HPV antibodies after natural infection and vaccination: implications for monitoring an HPV vaccination programme. Journal of medical virology 2013; 85:1379-85.
57. Romanowski B, Schwarz TF, Ferguson L, et al. Sustained immunogenicity of the HPV-16/18 AS04-adjuvanted vaccine administered as a two-dose schedule in adolescent girls: five-year clinical data and modeling predictions from a randomized study. Human vaccines & immunotherapeutics 2016; 12:20-9.
58. Donken R, Dobson SRM, Marty KD, et al. Immunogenicity of 2 and 3 Doses of the Quadrivalent Human Papillomavirus Vaccine up to 120 Months Postvaccination: Follow-up of a Randomized Clinical Trial. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2019.
59. Olsson SE, Restrepo JA, Reina JC, et al. Long-term immunogenicity, effectiveness, and safety of nine-valent human papillomavirus vaccine in girls and boys 9 to 15 years of age: Interim analysis after 8 years of follow-up. Papillomavirus research (Amsterdam, Netherlands) 2020; 10:100203.

60. Weinberg GA, Szilagyi PG. Vaccine epidemiology: efficacy, effectiveness, and the translational research roadmap. The Journal of infectious diseases 2010; 201:1607-10.
61. Schiller JT, tellsagué X, Garland SM. A review of clinical trials of human papillomavirus prophylactic vaccines. Vaccine 2012; 30:F123-F38.
62. Garnett GP. Role of herd immunity in determining the effect of vaccines against sexually transmitted disease. The Journal of infectious diseases 2005; 191:S97-S106.
63. Malagón T, Drolet M, Boily M-C, et al. Cross-protective efficacy of two human papillomavirus vaccines: a systematic review and meta-analysis. The Lancet infectious diseases 2012; 12:781-9.
64. Brown DR, Joura EA, Yen GP, et al. Systematic literature review of cross-protective effect of HPV vaccines based on data from randomized clinical trials and real-world evidence. Vaccine 2021; 39(16), 2224-2236.
65. Joura EA, Giuliano AR, Iversen O-E, et al. A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. New England Journal of Medicine 2015; 372:711-23.
66. Future II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. New England Journal of Medicine 2007; 356:1915-27.
67. Lehtinen M, Paavonen J, Wheeler CM, et al. Overall efficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervical intraepithelial neoplasia: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. The lancet oncology 2012; 13:89-99.
68. Harper DM, DeMars LR. HPV vaccines - A review of the first decade. Gynecologic Oncology 2017; 146:196-204.
69. Integraal Kankercentrum Nederland. Monitor Bevolkingsonderzoek Baarmoederhalskanker 2021, 2022.
70. Rijkaart DC, Berkhof J, Rozendaal L, et al. Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial. The lancet oncology 2012; 13:78-88.
71. Schurink-van 't Klooster TM, de Melker HE. The National Immunisation Programme in the Netherlands : Surveillance and developments in 2017-2018: National Insitite for Public Health and the Environment, 2018.
72. Gezondheidsraad. Advies vaccinatie baarmoederhalskanker, 2008.

Part I

Vaccine and natural infection induced immune responses

Chapter 2

Changes in HPV seroprevalence from an unvaccinated toward a girls-only vaccinated population in the Netherlands.

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ABSTRACT

Background: In the Netherlands, bivalent human papillomavirus (HPV) vaccination was included in the National Immunization Program for 12-year-old girls in 2010 (vaccination coverage, 45%–60%). We examined possible changes in HPV seroprevalence in the HPV-unvaccinated Dutch population aged 0–89 years, comparing prevaccination data with data of approximately 6 years after implementation of national vaccination.

Methods: Serum samples of men and women were used from two cross-sectional population-based serosurveillance studies performed before (2006–07, n = 6,384) and after (2016–17, n = 5,645) implementation of HPV vaccination in the Netherlands. Seven high-risk HPV-specific antibodies (HPV16, 18, 31, 33, 45, 52, and 58) were tested in a virus-like particle-based multiplex immunoassay.

Results: Type-specific HPV seroprevalence increased in women between 2006–07 and 2016–17. Also, a higher seroprevalence for at least one type in women >15 years was found in 2016–17 (31.7%) compared with 2006–07 (25.2%). In men, overall HPV seroprevalence remained similar; however, a lower seroprevalence was found for HPV16 in 2016–17 (7.5%) compared with 2006–07 (10.6%).

Conclusions: Our results indicate an increase in high-risk HPV types in women and a rather stable exposure in men. No clear effects of the strategy of girls-only vaccination were observed in men, probably because of the short time after introduction combined with suboptimal coverage.

Impact: No herd immunity has been observed yet in a population with suboptimal HPV vaccination coverage.

Introduction

Human papillomavirus (HPV), a virus capable of infecting the epithelial cells of the mucosa, is the cause of anogenital warts and cervical cancer [1]. Besides cervical cancer, HPV is also linked to various other cancers in the anogenital tract and oral cavity [2]. By routine HPV vaccination and effective cervical cancer screening programs, countries can reduce the burden of HPV-related disease.

All current globally available vaccines provide protection against HPV types 16 and 18, for example, and are included in the current bivalent vaccine. HPV types 6 and 11 are added in the quadrivalent vaccine and the nonavalent vaccine included additional HPV types 31, 33, 45, 52, and 58. Vaccination against HPV has been implemented in many countries, with the primary aim to protect women against cervical cancer. In the Netherlands, the bivalent HPV vaccine was implemented in the Dutch National Immunization Program as a girls-only vaccine for 12-year olds in a three-dose schedule in 2010, and is currently still being used, protecting them against HPV types 16 and 18. In addition, a catch-up campaign was initiated for girls from the birth cohorts 1993–1996 (i.e., 13–16 year olds) in 2009. From 2014 onward, the Netherlands shifted to a two-dose schedule (starting from birth cohort 2001 onward). The HPV vaccination coverage in girls in the Netherlands varied from 2009–2017 from 45% to 62% [3].

To gain information about previous HPV exposure, HPV serology is established as an important tool for population-based studies [4]. This provides a view on type-specific cumulative lifetime exposure to HPV. Antibodies against HPV L1 virus-like particles (VLP) remain stable over time, and therefore reflect past infection and cumulative exposure. However, not everyone who contracted HPV will seroconvert, and the rate of seroconversion is known to be sex dependent [5]. After HPV vaccination, HPV-specific antibodies are 10–100 times higher than [natural] infection-induced antibodies in serum [6], and therefore could be used to monitor vaccine uptake.

We assessed the (natural) infection-induced HPV seroprevalence for seven high-risk (hr) HPV types in the Dutch population in 2006/2007 (i.e., 4 years before the introduction of HPV) and 2016/2017 (i.e., 6 years postvaccination implementation). In addition, we investigated the effects of the introduction of the HPV vaccination on the seroprevalence of HPV types in our population.

Materials and Methods

Study design

Serum samples from two cross-sectional population-based serosurveillance studies performed from February 2006 to June 2007 and from September 2016 to October 2017 in the Netherlands were used for this study. Participants were 0–79 years of age in the 2006–2007 survey (n = 6,384), and 0–89 years of age for the 2016–17 cohort (n = 5,645). Study designs have been previously described in detail [7, 8]. Briefly, the randomly invited participants were asked to fill in a questionnaire and to provide a blood sample. Questionnaires of both surveys included data on demographic characteristics, ethnicity (first- and second-generation migrants), vaccination history, and sexual behavior. Vaccination history was determined via the individuals' registration booklet and the Dutch vaccination registration Praeventis [9]. The questionnaire used in 2006–7 was extended in the 2016–17 survey with more questions regarding sexual behavior. Information related to sexual behavior was only available from participants older than 14 years of age in both the 2006–07 study and the 2016–17 study.

We obtained written informed consent from all participants or their guardians before participation. The studies were conducted in accordance with recognized ethical guidelines (the Declaration of Helsinki) and were approved by an institutional review board "The Medical Ethics Committee Noord-Holland" in the Netherlands (METC number: ISRCTN 20164309 and M015–022).

Serologic measurement

Serum samples of both surveys were stored at –80°C until analysis, samples were measured at random for age and sex. For the measurement of HPV-specific IgG serum antibodies against L1 VLP of HPV16, 18, 31, 33, 45, 52, and 58, a VLP-based multiplex immunoassay was used as described previously [10]. GSK (2006–07 survey) and MSD (Merck Sharp & Dohme; 2016–17 survey) produced the HPV-VLPs in used these studies. Briefly, VLPs were conjugated to seven distinct fluorescent microspheres via amine coupling. Serum samples were 1/50, 1/100, or 1/10,000 diluted and incubated with the VLP-coupled microspheres. HPV-specific IgG serum antibodies were detected using a secondary goat anti-human phycoerythrin-labeled antibody. Four in-house control sera and an in-house standard were used on each plate. The in-house standard (IVIG, lot LE12H227AF, Baxter) was calibrated against reference serum of GSK for all the seven HPV types. HPV-specific IgG antibodies were analyzed using the Bioplex system 200 with Bioplex software (Bio-Rad Laboratories). Samples were assumed to be seropositive above cutoffs according to the 99% Frey method (with 99% one-sided t values, based on concentrations measured in children of 1–10 years old (n = 859; [11]) and found to be 9, 13, 27, 11, 19, 14, and 31 Luminex units/mL

(LU/mL; ref. 10) for HPV16, 18, 31, 33, 45, 52, and 58, respectively. As samples from 2016–17 were measured using a different batch of VLPs than those used in 2006–07, a correction formula was applied on the data of the 2016–17 survey. This correction formula was based on retesting of a random subset of 160 samples of the 2006–07 samples with the new VLPs. The correction formula was applied to the 2016–17 antibody measurements to align them with the 2006–07 measurements.

Statistical analysis

Data analyses were conducted using SAS version 9.4 and GraphPad Prism version 8.0.2. Women who were vaccinated against HPV according to the vaccination registry (n = 228) were excluded from analysis. In addition, women under 31 years of age and with arbitrary antibody concentration cutoff of >100 LU/mL for HPV16 and >50 LU/mL for HPV18 were considered to be "highly likely to have been vaccinated" and were excluded from the analyses (n = 18). Characteristics of the study population were compared among the 2006–2007 cohort and the 2016–2017 cohort using $\chi 2$ tests. Seroprevalence for "any" or "all" hr-HPV-type(s) refer to the seven hr-serotypes that have been measured in this study. The study design (i.e., a two-stage cluster sampling method including specific regions and municipalities from which participants were invited) was taken into account in the analyses, as well as weights determined proportional to the reference population (Dutch population, January 1, 2007 and January 1, 2017, respectively) taking into account sex, age, ethnic origin, and urbanization degree. Seroprevalences were calculated per age-cohort and as large differences already have been observed between men and women [10], analyses were stratified for men and women. Crude seroprevalences of the different cohorts, age groups, and/or sexes were compared using Monte Carlo simulations. Parameters of the beta distribution for both seroprevalences were estimated and used in the simulations to obtain P values. Geometric mean concentrations (GMC) were calculated among HPV16 and HPV18 seropositive individuals from both cohorts, taking the study design into account. P values of <0.05 were considered statistically significant.

The associations between HPV seropositivity (positive for at least one out of the seven HPV types) in sexually active individuals older than 14 years of age who were not vaccinated and demographic characteristics (age, ethnic origin, degree of urbanization, education level, and socioeconomic status), was examined for the 2016–2017 cohort for men and women separately. In addition, associations with (sexual) behavior characteristics were taken into account, including: body mass index (BMI), alcohol consumption, smoking, having a steady partner, age of sexual debut (being defined as the first time of vaginal and/or penile intercourse), condom use at last sex act, number of partners in the last 6 months, lifetime number of partners, and reported history of STI (note: participants with missing values for a specific variable were allocated

to a unknown category). We used generalized estimation equation (GEE) logistic regression models with a log link function and robust error variance. The incorporation of a GEE with exchangeable correlation structure accounted for dependency of multiple HPV types within an individual. First, univariate logistic regression analyses were conducted to study characteristics associated with HPV seropositivity. Variables that had $P < 0.1$ in univariate analyses were included in the multivariate analysis and backward selection (dropping variables one-by-one) was then applied. Hence, a multivariate model only including independently associated risk factors ($P < 0.05$) remained.

To study the differences in seroprevalence between the 2006–2007 and 2016–2017 cohort more closely, a pooled dataset was created including all HPV-unvaccinated participants from both cohorts. Again, the association between HPV seropositivity in sexually active individuals older than 14 years of age and demographics and sexual behavior characteristics was studied, in addition to the variable defining the cohort. Only characteristics available from both surveys were considered for inclusion in the model. Using a Poisson regression with robust error variance, we first calculated the crude prevalence ratio (PR). Next, we included the variables of interest to adjust for differences between the two surveys resulting in an adjusted PR (aPR). The analyses were performed for seroprevalence of any HPV type as well as type-specific. In addition, we stratified the analyses for men and women; we assumed that if herd effects on seroprevalence were to be observed this short after HPV vaccine introduction, this would be among men (first-order effect), in particular, younger males. Therefore, we looked also into the aPR for younger males (15–39 years of age).

Results

Study and participant characteristics

We tested 5,645 serum samples, with corresponding response rates of 13.2% for men and 18.4% for women from the 2016–17 survey, and 6,384 serum samples, with corresponding response rates 28.9% for men and 34.7% for women from the 2006–07, which were tested previously [10, 12]. Study characteristics were stratified for sex. In the 2016–17 survey, for both men and women, participants of 15 years and older were higher educated and had a higher net monthly income in comparison with both men and women in the 2006–07 survey. The mean age of sexual debut for people under 25 years of age was similar between the different surveys and sexes. However, age of sexual debut across all ages was lower in the 2016–17 survey compared with 2006–07 survey, for both men and women. In addition, the percentage of participants reporting to have a current steady partner was lower in 2016–2017, while “the

number of sex partners in the last 6 months" and "ever having been diagnosed with a sexually transmitted disease (STD)" were higher in the 2016–17 survey compared with 2006–07 (Table 1).

Table 1: Sociodemographic and sexual behavior characteristics of participants aged 15 years and older without vaccination, with a blood sample for HPV IgG antibody determination in the Netherlands, by sex and survey.

	Men	Men		Women	Women	
	2006–07	2016–17		2006–07	2016–17	
	% (*n*)	% (*n*)		% (*n*)	% (*n*)	
Sociodemographic characteristic	*N* = 1,937	*N* = 1,911	*P*	*N* = 2,535	*N* = 2,415	*P*
Age group, years						
15–19	6.87 (133)	5.67 (110)		6.51 (165)	1.98 (43)	
20–24	7.12 (138)	10.36 (198)		8.92 (226)	4.93 (107)	
25–29	6.50 (126)	9.00 (172)		8.76 (222)	9.36 (203)	
30–39	14.97 (290)	15.07 (288)		16.76 (425)	20.2 (438)	
40–49	14.51 (281)	13.55 (259)		14.20 (360)	18.44 (400)	
50–59	15.07 (292)	14.49 (277)		16.65 (422)	16.74 (363)	
60–69	18.79 (364)	16.27 (311)		17.16 (435)	16.04 (348)	
70–79	16.15 (313)	12.55 (240)		11.05 (280)	10.01 (217)	
80–89		2.93 (56)			2.31 (50)	
Educational level[a]			<0.0001			<0.0001
High	29.17 (565)	40.24 (769)		23.59 (598)	35.04 (760)	
Middle	29.94 (580)	28.78 (550)		31.52 (799)	29.74 (645)	
Low	39.34 (762)	25.64 (490)		43.35 (1,099)	28.82 (625)	
Unknown	1.55 (30)	5.34 (102)		1.54 (39)	6.41 (139)	
Net monthly income			<0.0001			<0.0001
<850/<970	5.94 (115)	502 (96)		8.72 (221)	5.3 (115)	
851–1,150/971–1,335	7.80 (151)	6.65 (127)		9.47 (240)	9.04 (196)	
1,151–1,750/1,356–1,969	19.00 (368)	11.93 (228)		17.87 (453)	14.66 (318)	
1,751–3,050/1,970–3,314	32.27 (625)	28.52 (545)		25.68 (651)	29.28 (635)	
3,051–3,500/3,315–3,500	7.02 (136)	8.16 (156)		6.11 (155)	6.69 (145)	
>3,501	11.31 (219)	26.95 (515)		8.36 (212)	20.89 (453)	
Unknown	16.68 (323)	12.77 (244)		23.79 (603)	14.15 (307)	
Ethnicity			0.0307			0.0018
Dutch	81.78 (1,584)	79.96 (1,528)		81.85 (2,075)	78.98 (1,713)	
First-generation migrant	10.84 (210)	13.50 (258)		11.76 (298)	15.26 (331)	
Second-generation migrant	7.38 (143)	6.54 (125)		6.39 (162)	5.76 (125)	

Table 1: Continued

	Men	Men		Women	Women	
	2006–07	2016–17		2006–07	2016–17	
	% (*n*)	% (*n*)		% (*n*)	% (*n*)	
Sociodemographic characteristic	*N* = 1,937	*N* = 1,911	*P*	*N* = 2,535	*N* = 2,415	*P*
Smoking						
Yes		48.77 (932)			43.11 (935)	
No		42.96 (821)			47.76 (1,036)	
Unknown		8.27 (158)			9.13 (198)	
Alcohol						
Yes		76.35 (1,459)			64.04 (1,389)	
No		14.70 (281)			26.09 (566)	
Unknown		8.95 (171)			9.87 (214)	
BMI						
<18.5		1.99 (38)			1.89 (41)	
18.5–25		43.22 (826)			43.52 (944)	
25–30		33.18 (634)			26.97 (585)	
≥30		10.52 (201)			14.98 (325)	
Unknown		11.09 (212)			12.63 (274)	
Current steady partner			0.0005			<0.0001
Yes	79.50 (1,540)	77.60 (1,483)		75.31 (1,909)	77.04 (1,671)	
No	19.05 (369)	19.05 (364)		22.72 (139)	18.86 (409)	
Unknown	1.45 (28)	3.35 (64)		1.97 (50)	4.1 (89)	
Ever had sexual intercourse			<0.0001			<0.0001
Yes	89.93 (1,742)	91.63 (1,751)		90.49 (2,294)	95.67 (2,075)	
No	6.87 (133)	8.37 (160)		5.48 (139)	4.33 (409)	
Unknown	3.20 (62)	0.00 (0)		4.02 (102)	0.00	
Median age at sexual debut (<26 years of age)	16.9	16.8		16.7	16.7	
Age at sexual debut			<0.0001			<0.0001
<17 years	12.80 (248)	18.32 (350)		17.32 (439)	24.57(533)	
17–19 years	24.37 (472)	31.08 (594)		30.73 (779)	34.76 (754)	
≥20 years	31.96 (619)	28.57 (546)		27.14 (688)	24.85 (539)	
Unknown	30.87 (598)	22.03 (421)		24.81 (629)	15.81 (343)	
Number of partners last 6 months			<0.0001			<0.0001
0	14.09 (273)	11.15 (213)		15.35 (389)	16.32 (354)	
1–2 partners	62.36 (1,208)	68.13 (1,302)		60.79 (1,541)	67.31 (1,460)	
>2 partners	0.98 (19)	2.30 (44)		0.79 (20)	0.83 (18)	
Unknown	22.56 (437)	18.42 (352)		23.08 (585)	15.54 (337)	

Table 1: Continued

	Men	Men		Women	Women	
	2006–07	2016–17		2006–07	2016–17	
	% (*n*)	% (*n*)		% (*n*)	% (*n*)	
Sociodemographic characteristic	*N* = 1,937	*N* = 1,911	*P*	*N* = 2,535	*N* = 2,415	*P*
Lifetime sexual partners						
1–2 partners		38.72 (740)			47.63 (1,033)	
3–5 partners		21.04 (402)			23.19 (503)	
6–9 partners		10.57 (202)			8.21 (178)	
≥10 partners		12.87 (246)			8.85 (192)	
Unknown		16.80 (321)			12.13 (263)	
Condom use last time sex			<0.0001			<0.0001
Yes	8.00 (155)	14.08 (269)		7.65 (991)	13.05 (283)	
No	56.01 (1,085)	69.65 (1,331)		53.25 (1,350)	73.95 (1,604)	
Unknown	35.98 (697)	16.27 (311)		39.09 (991)	13 (282)	
Ever had sexually transmitted disease			0.0016			<0.0001
Yes	3.82 (74)	6.17 (118)		5.44 (138)	8.16 (177)	
No	89.00 (1,724)	82.42 (1,575)		86.04 (2181)	79.07 (1,715)	
Unknown	7.18 (139)	11.41 (218)		8.52 (216)	12.77 (277)	

a: Educational level was used for participants 0–11 years, active education was used for participants 12–25 years, and highest accomplished educational level was used for participants >25 years. Low, no education, primary school, prevocational education (VMBO), lower vocational education (LBO/MBO-1), lower general secondary education (MAVO/VMBO); Middle, intermediate/secondary vocational education (MBO-2–4), higher/senior vocational education (HAVO), preuniversity education (VWO/Gymnasium); High, higher professional education (HBO), University BSc., University MSc., Doctorate; Missing, ethnicity n = 13.

HPV Seroprevalence

Age-specific seroprevalence and GMC in an unvaccinated population, by sex and survey

An increase in seroprevalence for any hr-HPV type was observed in women in the age cohort from 15–19 years old, which reflects the median age of sexual debut. In the 2016–17 survey, seroprevalence for any type increased from 3.0% (10–14 years old) to 30.5% (20–24 years old) and 33.7% (25–29 years old) and peaked at 37.0% in the 30–39 year old. The greatest rise was seen for HPV16 and HPV18. This increase in seroprevalence was much more gradual for men, and mainly in the 2006–07 survey was most pronounced for any hr-HPV type and HPV16 (Fig. 1). Samples sizes of age cohorts can be found in Table 1 and Supplementary Table S1. Low seroprevalences were observed in children 0–14 years of age in both sexes and surveys. In the 2016–17 survey, the highest seroprevalences in children (0–14) were detected for HPV16 and HPV18 (Fig. 1).

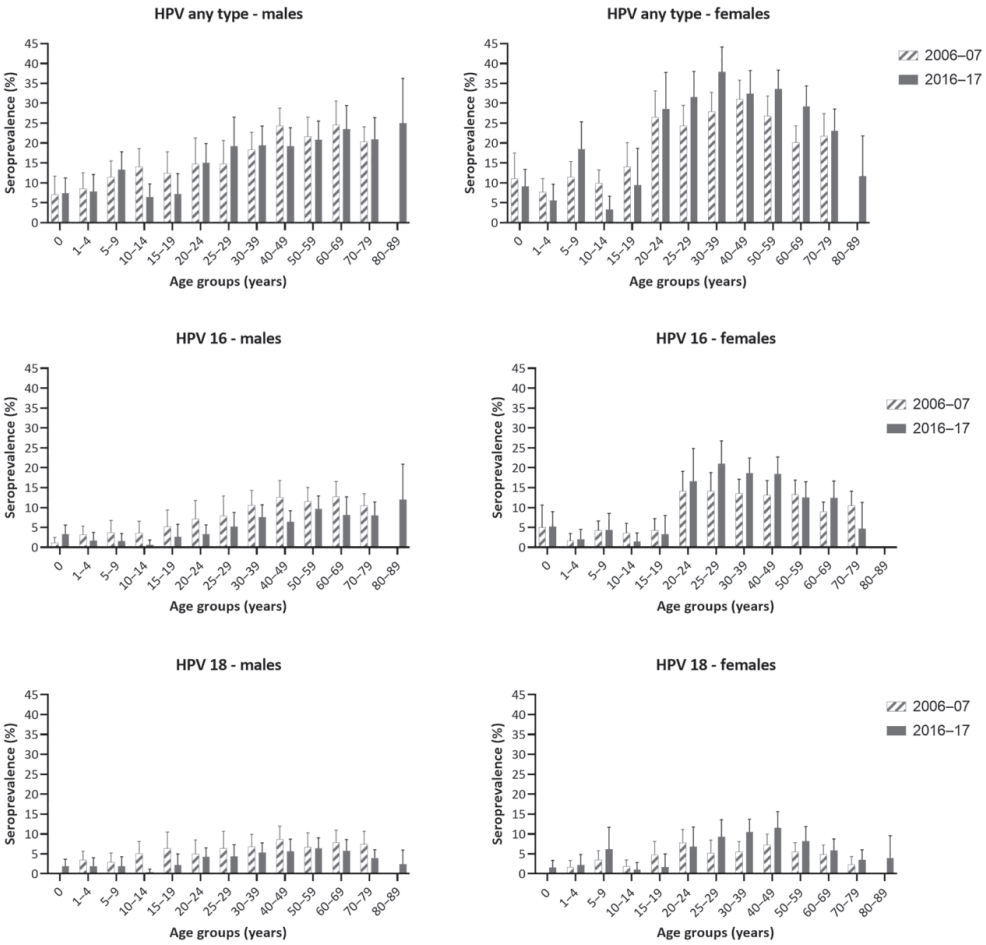


Figure 1: Age-specific seroprevalence (%; with 95% CIs) of any high-risk type HPV IgG antibodies for men (A) and women (B), HPV 16 for men (C) and women (D), and HPV18 for men (E) and women (F) in the unvaccinated general population of the Netherlands.

In the older female age groups, overall seroprevalence decreased from the age of 49 years onward in the 2006–07 survey and from 60–69 years onward in the 2016–17 survey. Age-specific higher seroprevalence for any hr-HPV type was observed in 2016–17 compared with 2006–07, being significant in age groups 30–39 (P = 0.0108), 50–59 (P = 0.0406), and 60–69 (P = 0.0056) years of age. A lower seroprevalence for any hr-HPV type was observed in the age group 10–14 (P = 0.0118) in 2016–17 compared with 2006–07. In men, a lower age-specific seroprevalence for any hr-HPV type was observed in 2016–17 compared with 2006–07, being only significant in the age groups 10–14 (P = 0.0056). No significant difference was found for the age-specific

seroprevalence for any hr-HPV type excluding 16 and 18 in the age groups 10–14 and 15–19 years of age between the two surveys, P = 0.091 and P = 0.1206, respectively.

Overall HPV seroprevalence from unvaccinated individuals 15 years and onward

Unvaccinated female participants older than 15 years of age showed significantly higher seroprevalence for any hr-HPV type in 2016–17 compared with 2006–07; 31.4% [95% confidence interval (CI), 29.1–33.7] and 25.2% (95% CI, 23.1–2.3), respectively. For men from 15 years of age and older, seroprevalence for any hr-HPV type was similar between the 2006–07 and 2016–17 surveys; 19.7% (95% CI, 17.9–21.6) and 20.3% (95% CI, 18.4–22.1), respectively. In women, also seropositivity for one up to all seven types was significantly higher in 2016–17 and hr-type specific. Type-specific HPV16, HPV18, HPV31, and HPV58 were higher in 2016–17 compared with 2006–07, which was also true for the combinations HPV16 and 18, HPV16 or 18, and HPV16 and/or 18 (Table 2).

For men, this was true for the combination HPV16 or 18, positivity for more than two hr-HPV types and type-specific HPV18, 31, 33, 45, 52, and 58. For HPV16 a lower seroprevalence was seen in 2016–17 (7.5%; 95% CI, 6.5–8.5) compared with 2006–07 (10.6%; 95% CI, 9.2–12.0). Just as for the combination HPV16 and 18, HPV16 and/or HPV18 and positivity for more than one hr-HPV type (Table 2). HPV16 was also most prevalent in both surveys, followed by HPV18, HPV45, and the rest of the types (Table 2). Only a very small percentage of the males were seropositive for all seven hr-HPV types, 0.6% and 0.3% for 2006–07 and 2016–17, respectively.

HPV type-specific antibody concentrations among seropositive individuals

The age-specific HPV16 GMCs of (natural) infection-induced seropositive women as well as of seropositive men were comparable in all age cohorts between both studies. No differences were found between the GMCs of the HPV16 and HPV18 seropositive individuals between 2006–07 and 2016–17 (Supplementary Fig. S3).

Risk factors for hr-HPV seropositivity

For women, the univariate analysis showed an association for HPV seropositivity for any hr-HPV type with middle educational level, being a first- or second-generation migrant, having a lower income, ever used alcohol, not having a steady partner, lower age of sexual debut, having more than two sexual partners last 6 months, history of reported STD, and having more than two sexual partners during lifetime. In the backward selection model, low and middle educational level, first-generation migrants, more than two sexual partners during lifetime, and history of self-reported STDs

Table 2: Weighted seroprevalence, and corresponding 95% CIs, for seven high-risk HPV types and combinations in the total population of the Netherlands from 15 years of age without vaccination, stratified by sex and survey.

Total population from 15 years of age, without vaccination	Men (2006–07)	Men (2016–17)		Women (2006–07)	Women (2016–17)	
	(*n* = 1,937)	(*n* = 1,916)	*P*	(*n* = 2,535)	(*n* = 2,177)	*P*
High-risk HPV types						
HPV16	10.6 (9.2–12.0)	7.2 (6.2–8.2)	0.0000	11.9 (10.3–13.6)	15.8 (13.9–17.7)	0.0006
HPV18	7.2 (6.1–8.2)	5.0 (3.8–6.2)	0.0078	5.6 (4.7–6.4)	7.9 (6.7–9.1)	0.0006
HPV31	2.5 (1.7–3.4)	1.4 (0.9–1.9)	0.0158	3.3 (2.4–4.2)	5.2 (4.1–6.3)	0.009
HPV33	6.0 (4.7–7.2)	5.4 (4.4–6.3)	0.4338	6.5 (5.5–7.6)	8.0 (6.3–9.7)	0.116
HPV45	6.8 (5.4–8.1)	9.6 (8.2–10.9)	0.0022	7.5 (6.2–8.9)	9.6 (8.2–10.9)	0.0304
HPV52	5.4 (4.4–6.3)	5.1 (4.1–6.0)	0.6576	6.9 (5.9–8.0)	8.7 (7.1–10.2)	0.0610
HPV58	3.7 (2.8–4.5)	3.0 (2.1–3.9)	0.2914	4.5 (3.6–5.3)	6.4 (5.2–7.5)	0.0052
HPV combinations						
HPV16 and 18	5.7 (4.9–6.6)	2.8 (2.0–3.6)	0.0000	3.6 (2.9–4.4)	4.8 (3.8–5.9)	0.0572
HPV16 or 18	6.3 (5.1–7.5)	6.6 (5.5–7.7)	0.7376	10.2 (8.8–11.6)	14.1 (12.4–15.7)	0.00040
HPV16 and/or 18	12.0 (10.6–13.5)	9.4 (8.2–10.5)	0.0026	13.8 (12.1–15.5)	18.9 (17.0–20.7)	0.0000
Positive for at least 1 hr-HPV type	20.3 (18.4–22.1)	19.3 (17.7–21.0)	0.4378	25.2 (23.1–27.3)	30.1 (27.7–32.4)	0.0052
Positive for at least 1 hr-HPV type, excluding HPV16 and 18	13.6 (11.7–15.5)	15.3 (13.7–16.8)	0.1826	17.9 (16.0–19.8)	21.0 (18.9–23.2)	0.0234
Positive for more than 1 hr-HPV type	9.8 (8.8–10.8)	7.3 (6.0–8.7)	0.0066	10.4 (9.1–11.7)	14.4 (12.3–16.4)	0.0004
Positive for more than 2 hr-HPV types	5.2 (4.0–6.3)	4.3 (3.4–5.2)	0.2078	4.9 (3.9–6.0)	7.8 (6.4–9.3)	0.001
Positive for 7 hr-HPV types	0.6 (0.2–0.9)	0.3 (0.0–0.5)	0.1686	0.3 (0.1–0.5)	0.7 (0.2–1.1)	0.1146

remained and were independently associated with seropositivity for any hr-HPV type (Table 3A and B). For men, the univariate analyses showed only an association of seropositivity for any hr-HPV type with history of self-reported STDs (Table 3A and B).

Pooled risk factor analysis associated with HPV seropositivity for the 2006–07 and 2016–17 surveys

HPV seropositivity for any hr-HPV type for women from 15 years onward was 25.2% in 2006–07 and 31.4% in 2016–17. After pooling both surveys, and adjusting for demographic characteristics (age, sex, urbanization, education, income, ethnicity) and sexual risk factors (age of sexual debut, number of partners during the last 6 months,

Table 3A: Risk factor analysis for any high-risk type HPV IgG seropositivity among sexually active and unvaccinated participants from 15 years of age in the Netherlands, by sex.

Males (n = 1,751)	Univariate model		Multivariate model	
Risk factor	OR	95% CI limits	OR	95% CI limits
Age				
15–19	Ref		Ref	
20–24	0.60	0.18–2.01		
25–29	0.68	0.21–2.26		
30–39	0.98	0.31–3.08		
40–49	0.94	0.30–2.99		
50–59	1.04	0.33–3.29		
60–69	1.01	0.32–3.16		
70–79	0.94	0.30–3.00		
80–89	0.93	0.25–3.46		
Education[a]				
High	Ref		Ref	
Middle	0.86	0.61–1.20		
Low	1.11	0.81–1.54		
Unknown	0.81	0.44–1.46		
Net monthly income[b]				
<850/<970	Ref		Ref	
851–1,150/971–1,335	0.68	0.31–1.48		
1,151–1,750/1,970–3,314	1.59	0.77–3.30		
1,751–3,050/1,970–3,314	1.18	0.59–2.35		
3,051–3,500/3,315–3,500	1.82	0.84–3.95		
>3,501	1.17	0.59–2.32		
Unknown	1.01	0.48–2.11		
Ethnicity[c]				
Dutch	Ref		Ref	
First-generation migrant	1.00	0.69–1.44		
Second-generation migrant	0.78	0.42–1.45		
Smoking ever				
No	Ref		Ref	
Yes	0.95	0.72–1.25		
Unknown	0.98	0.61–1.59		
Alcohol use				
No	Ref		Ref	
Yes	1.04	0.70–1.56		
Unknown	0.92	0.53–1.61		

Table 3A: Continued

Males (n = 1,751)	Univariate model		Multivariate model	
Risk factor	OR	95% CI limits	OR	95% CI limits
BMI				
<18.5	Ref		Ref	
18.5–25	1.06	0.34–3.34		
25–30	1.23	0.39–3.87		
>30	0.88	0.26–2.93		
Unknown	1.03	0.32–3.41		
Current steady partner				
No	Ref		Ref	
Yes	1.12	0.74–1.71		
Unknown	1.08	0.47–2.49		
Age of sexual debut				
<17 years	Ref		Ref	
17–19 years	1.21	0.84–1.73		
≥20 years	0.86	0.58–1.27		
Unknown	1.18	0.76–1.84		
History STD				
No	Ref		Ref	
Yes	**1.70**	**1.11–2.61**		
Unknown	0.88	0.58–1.34		
Condom use				
No	Ref		Ref	
Yes	0.77	0.50–1.17		
Unknown	0.76	0.46–1.26		
Partners last 6 months (sexual)				
0	Ref		Ref	
1–2	0.95	0.62–1.45		
>2	0.84	0.37–1.91		
Unknown	1.03	0.58–1.83		
Partners lifetime (sexual)				
1–2	Ref		Ref	
3–5	0.83	0.57- 1.21		
6–9	0.97	0.62–1.50		
>10	1.34	0.94–1.91		
Unknown	1.45	0.92–2.28		

Table 3B: Risk factor analysis for any high-risk type HPV IgG seropositivity among sexually active and unvaccinated participants from 15 years of age in the Netherlands, by sex.

Females (n = 2,075)	Univariate model		Multivariate model	
Risk factor	OR	95% CI limits	OR	95% CI limits
Age				
15–19	Ref		Ref	
20–24	1.90	0.50–7.23		
25–29	2.18	0.59–8.05		
30–39	2.16	0.60–7.78		
40–49	2.12	0.58–7.68		
50–59	2.19	0.60–7.91		
60–69	1.83	0.51–6.65		
70–79	1.19	0.32–4.36		
80–89	0.69	0.15–3.10		
Education[a]				
High	Ref		Ref	
Middle	**1.36**	**1.08–1.70**	**1.47**	**1.17–1.85**
Low	1.10	0.88–1.38	**1.40**	**1.09–1.78**
Unknown	1.17	0.78–1.75	1.22	0.83–1.80
Net monthly income[b]				
<850/<970	Ref		Ref	
851–1,150/971–1,335	1.14	0.75–1.73		
1,151–1,750/1,970–3,314	0.93	0.62–1.39		
1,751–3,050/1,970–3,314	**0.68**	**0.46–0.98**		
3,051–3,500/3,315–3,500	**0.45**	**0.28–0.73**		
>3,501	**0.61**	**0.41–0.90**		
Unknown	0.73	0.48–1.10		
Ethnicity[c]				
Dutch	Ref		Ref	
First-generation migrant	**2.27**	**1.83–2.81**	**2.47**	**1.97–3.10**
Second-generation migrant	**1.57**	**1.04–2.36**	1.27	0.85–1.91
Smoking ever				
No	Ref		Ref	
Yes	1.20	0.99–1.45		
Unknown	**1.49**	**1.07–2.06**		
Alcohol use				
No	Ref		Ref	
Yes	**1.29**	**1.03–1.62**		
Unknown	**1.61**	**1.15–2.27**		

Table 3B: Continued

Females (n = 2,075)	Univariate model		Multivariate model	
Risk factor	OR	95% CI limits	OR	95% CI limits
BMI				
<18.5	Ref		Ref	
18.5–25	1.07	0.52–2.20		
25–30	1.16	0.56–2.40		
>30	1.29	0.61–2.72		
Unknown	1.38	0.65–2.91		
Current steady partner				
No	Ref		Ref	
Yes	**0.71**	**0.59–0.90**		
Unknown	0.96	0.58–1.57		
Age of sexual debut				
<17 years	Ref		Ref	
17–19 years	**0.77**	**0.61–0.95**		
≥20 years	**0.52**	**0.0–0.67**		
Unknown	0.79	0.58–1.07		
History STD				
No	Ref		Ref	
Yes	**2.92**	**2.27–3.77**	**1.54**	**1.18–2.01**
Unknown	1.22	0.93–1.60	1.04	0.74–1.47
Condom use				
No	Ref		Ref	
Yes	1.07	0.82–1.40		
Unknown	0.88	0.62–1.26		
Partners last 6 months (sexual)				
0	Ref		Ref	
1–2	0.92	0.73–1.17		
>2	**3.03**	**1.58–5.78**		
Unknown	0.91	0.64–1.29		
Partners lifetime (sexual)				
1–2	Ref		Ref	
3–5	**2.22**	**1.75–2.81**	**2.03**	**1.60–2.59**
6–9	**3.71**	**2.80–4.90**	**3.47**	**2.58–4.67**
>10	**6.93**	**5.30–9.06**	**6.23**	**4.68–8.29**
Unknown	**2.72**	**1.93–3.83**	**2.55**	**1.66–3.93**

Note: Boldface text indicates that the OR is significant. a: According to definition of CBS in 2018. b: Left 2006–07 survey, right 2016–17 survey. c: Country of birth or country of birth of parents.

history of STDs), this resulted in a smaller, but still significant, difference between 2006–07 and 2016–17, (aPR 1.16; 95% CI, 1.02–1.32). Before adjustment of any variables, all seven hr-HPV types were significantly higher in 2016–17 compared with 2006–07. However, after adjustment for the demographic characteristics and sexual risk factors, the differences remained only significant for HPV16 (aPR 1.29; 95% CI, 1.07–1.55), HPV18 (aPR 1.31; 95% CI, 1.01–1.70), HPV31 (aPR 1.54; 95% CI, 1.111–2.14), and HPV52 (aPR 1.27; 95% CI, 1.00–1.62; Table 4).

Table 4: Pooled analysis of the 2006–7 and 2016–17 survey after adjustments for sociodemographic characteristics.

	Men		**Women**	
	***N* = 3,493**		***N* = 4,369**	
	HPV seropositive, *n* (%)	**aPR (95% CI)**	**HPV seropositive, *n* (%)**	**aPR (95% CI)**
Any HPV type				
2006–2007	366 (21.0)	Ref	596 (26.0)	**Ref**
2016–2017	361 (20.6)	0.99 (0.83–1.17)	989 (33.1)	**1.16 (1.02–1.32)**
HPV16				
2006–2007	192 (11.0)	**Ref**	279 (12.2)	**Ref**
2016–2017	137 (7.8)	**0.71 (0.55–0.91)**	363 (17.5)	**1.29 (1.07–1.55)**
HPV18				
2006–2007	130 (7.5)	Ref	128 (5.6)	**Ref**
2016–2017	98 (5.6)	0.77 (0.57–1.05)	187 (9.0)	**1.31 (1.01–1.70)**
HPV31				
2006–2007	49 (2.8)	Ref	80 (3.5)	**Ref**
2016–2017	29 (1.7)	0.66 (0.38–1.12)	128 (6.2)	**1.54 (1.11–2.14)**
HPV33				
2006–2007	111 (6.4)	Ref	151 (6.6)	Ref
2016–2017	96 (5.5)	0.89 (0.65–1.22)	186 (9.0)	1.08 (0.84–1.38)
HPV45				
2006–2007	123 (7.1)	**Ref**	184 (8.0)	Ref
2016–2017	180 (10.3)	**1.47 (1.13–1.92)**	222 (10.7)	1.16 (0.92–1.45)
HPV52				
2006–2007	103 (5.9)	Ref	167 (7.3)	**Ref**
2016–2017	92 (5.3)	0.95 (0.68–1.32)	212 (10.2)	**1.27 (1.00–1.62)**
HPV58				
2006–2007	70 (4.0)	Ref	113 (4.9)	**Ref**
2016–2017	54 (3.1)	0.71 (0.47–1.06)	152 (7.3)	1.28 (0.96–1.70)

For men from 15 years onward, HPV seropositivity for any hr-HPV type was significantly lower in 2016–17(18.2%) compared with 2006–07 (20.3%). After adjust-

ment for the demographic characteristics and sexual risk factors, this did not remain significant (aPR 0.99; 95% CI, 0.83–1.17). Before adjustment, HPV16, HPV18, and HPV31 were significantly lower in 2016–17 compared with 2006–07 and HPV45 was significantly higher in 2016–17 compared with 2006–07. This difference only remained significant for HPV16 after adjustment (aPR 0.71; 95% CI, 0.55–0.91; Table 4) and HPV45 (aPR 1.47; 95% CI, 1.13–1.92). Zooming in on men in the age cohort of 15–39, a nonsignificant decrease for HPV16 between 2006–07 and 2016–17 was observed (aPR 0.84; 95% CI, 0.52–1.37).

Discussion

In this study, we assessed the (natural) infection-induced seroprevalence of seven hr-HPV types in the Dutch population before and 6 to 7 years after the introduction of a girls-only bivalent HPV vaccination program, with an uptake varying over the years between 42% and 61%. Surprisingly, HPV seroprevalence in female age cohorts of 15 years and older has increased in a 10-year time period, mainly due to a significant increase in HPV16, 18, 31, and 58. In men, however, seroprevalence for any hr-HPV type remained similar with a decreasing trend found for HPV16 and increasing trend for HPV45.

We restricted the analyses to unvaccinated individuals thereby estimating naturally acquired and cumulative type-specific HPV exposure. The increase in seroprevalence with age for women was in line with the age of sexual debut. The peak in HPV seropositivity was highest in women ages 30–39, which has been reported in other publications [13–15]. This peak in seroprevalence around 10 to 20 years after sexual debut might reflect repeated exposures resulting in a subsequent increase of the seroconversion rate to induce a detectable antibody response [16].

In the 2006–07 survey, the seroprevalence in middle-aged and older women declined at an earlier age than in the 2016–17 survey, where levels started to decrease from 70 years and onward. In males, this decline in HPV seropositivity is not seen. The slight decrease of seropositivity observed in older women could be explained by waning of antibodies which was suggested by af Geijersstam and colleagues [4]. This would mean that seroprevalence is underestimating lifetime cumulative exposure. Alternatively, it could reflect a cohort effect, which is more likely as this effect is not seen in both sexes. Indeed, although age of sexual debut is similar, the sexual behavior pattern differs in the younger women having more lifetime sexual partners than the older women in this cohort [17].

Hr-HPV antibodies, albeit at very low concentrations, could be detected in children, which confirm other population studies [10, 16]. These antibodies might be derived from vertical or horizontal transmission [18].

In both surveys, a lower seroprevalence is observed in males compared with women, which conform other population studies [10, 13, 15, 16, 19]. It is unlikely that the overall lower seroprevalence seen in males is due to lower infection rates, because males reported a significantly higher number of lifetime sexual partners compared with females in the 2016–17 cohort. HPV DNA prevalence studies showed similar results among both sexes [20, 21]. The fact that women display a higher seroprevalence then men is likely to be explained by the anatomic site of the HPV infection, influencing its immune response. Infections at the epithelium of the cervix and anal tract induce higher immune response in comparison with infections that occur at the keratinized epithelia, such as genital skin [22–25].

Seroprevalence for HPV16 was highest of all HPV types in both surveys, which is in accordance with other population studies [15, 16, 26–29]. In a 10-year time period, HPV type-specific seroprevalence for HPV16, 18, 31, and 58 has increased in the female population of 15 years and older in the Netherlands. In addition, being seropositive for one up to all seven types increased over the years. This is possibly explained by the observed change toward a higher number of sexual partners in the last 6 months and history of self-reported STDs in the 2016–17 survey compared with the 2006–07 survey.

The risk factor analysis was restricted to the HPV unvaccinated, sexually active population from 15 years onward and stratified for sex. For women several behavioral factors, such as number of lifetime partners, history of STDs, and ethnicity, were independently associated with HPV seropositivity, which was also found in other studies [10, 15, 30, 31]. Alcohol use and smoking were only associated with HPV seropositivity in the univariate model. This is especially interesting with respect to the current increased risk in HPV-associated head and neck cancers [32, 33]. In these studies, alcohol use is often allied together with tobacco use; however, in our study, we could not find an association with smoking or alcohol use in the multivariate model. Other studies find varying results, showing a negative association [29], a positive association [14, 34], or no association at all [35]. Thereby leaving the relation between smoking and HPV seropositivity unclear.

In the male part of our study, we only found history of self-reported STDs to be significantly associated with HPV seropositivity in the univariate analyses. In the backward selection model, this factor did not remain independently associated with

HPV seropositivity anymore. Comparison with other studies is challenging as most population studies combine men and women in their risk factor analysis. Studies including separate male analysis reported a variety of associated factors. Most consistent findings were associations related to age [13, 31, 36–42], number of male anal sexual partners [30, 40, 41, 43], and [self-reported] circumcision [44]. Number of male anal sexual partners and [self-reported] circumcision were unfortunately not included in our questionnaire. In addition, some of these studies were performed among men who have sex with men, which is considered a high-risk population with specific behavior, complicating direct comparison.

Also after pooling both surveys and adjusting for demographic and sexual risk factors, the increase in HPV seroprevalence in women in the years after the introduction of the HPV vaccine remained significant.

An interesting finding is the decreased HPV16 seroprevalence between the two surveys for the Dutch male population. Although this might be due to herd immunity of the girls-only vaccination program, among 15–39 year old men, we observed a not statistically significant decline, while they seem likely to be the first age groups benefitting from the girls-only vaccination. In the even younger age group of 10–14 year old boys, we did find a difference between the two surveys for any hr-HPV type, but when excluding the vaccine types this difference was not significant anymore. This indicates that the. Albeit minor, difference in seroprevalence in the 10-year period was mainly attributable to the vaccine types. Please note that in this age group, the seroprevalences could not be adjusted as questionnaires including sexual behavior were only filled in by people above 15 years of age. In Australia, with high vaccine uptake percentages, herd protection impacts on seroprevalence in males (15–39 years of age) from a girls-only vaccination program were clearly shown 5 years after introduction of HPV vaccination [45]. Moreover, even a benefit for the nonvaccinated females was observed [46]. In contrast, no reduction was found in HPV seropositivity in males followed by a girls-only vaccination program in a study in the United States with comparable vaccination coverage in a girls-only program as in the Netherlands [47]. With a vaccination coverage of approximately 50% among vaccine-eligible girls in the Netherlands in 2016–17, herd effects on seroprevalence in the male part of the population might therefore be less pronounced. Nevertheless, in recent analysis among STI clinic visitors in the Netherlands, both first-order herd immunity effects among unvaccinated males as well as second-order herd immunity effects in unvaccinated women were found [48]. However, this was measured through infection rates where effects can be detected earlier than by seroprevalence.

A strength of this study is that we compared two surveys with a broad age range, one before and one 6 to 7 years after introduction of the HPV vaccination program, thereby enabling us to evaluate this program at a population level. An additional strength of this study is that an identical methodology and antibody assay is used between the two surveys. It must be kept in mind that the VLP sources have changed over time and could possibly cause some variance, for which we corrected by using QC and bridging. Furthermore, this study used two-stage cluster sampling strategy, including oversampling of minorities [7, 8], therefore being representative of the total Dutch population.

The use of different techniques and associated cutoff levels hinders the comparison with other (population) studies. International standardization for all hr-HPV types, in addition to HPV16 and 18 which are applied in this study, could help to overcome this difficulty in future studies. Besides this, it could be argued that HPV seropositivity is not a conclusive marker for cumulative exposure. HPV has the capability to evade the host immune system and as a consequence, detectable HPV-specific antibodies in serum are only developed in approximately 50%–70% of HPV-infected individuals [22]. Thus, seroprevalence will underestimate the actual lifetime HPV exposure and infection rate. Moreover, it should be noted that questions regarding sexual behavior were among the least well completed. Self-reporting of sexual behavior could lead to bias due to social desirability and this was also illustrated by our risk factor analysis for some variables [e.g., a high unknown category].

HPV prophylactic vaccination programs are most effective when offered to nonsexually active preadolescents. In the Netherlands, the age of receiving the HPV vaccine is approximately 12 years old and recently the Health Council has advised to lower the age to 9 years. Our data support this change as HPV seropositivity begins to increase markedly after 10 years of age. In addition, the Health Council also advised to implement a sex-neutral HPV program which will be effective from 2021. On top of that, a catch-up vaccination will be offered to all young adults up to 26 years of age [49].

To conclude, our data showed that HPV infection-specific seroprevalence in women has increased in the Netherlands in a 10-year period. In men, however, seroprevalence for any hr-HPV type remained similar, a decrease was found for HPV16 and an increase for HPV45. Whether the decline in HPV16 is a first sign of a herd effect remains uncertain because a less pronounced effect was observed in men ages 15–39 years of age, where we would have expected that a herd effect would be visible first. Future seroprevalence studies will be interesting to capture the effect of a longer follow-up period after introduction of the girls-only program and possibly effects of the sex-neutral vaccination.

Supplementary data

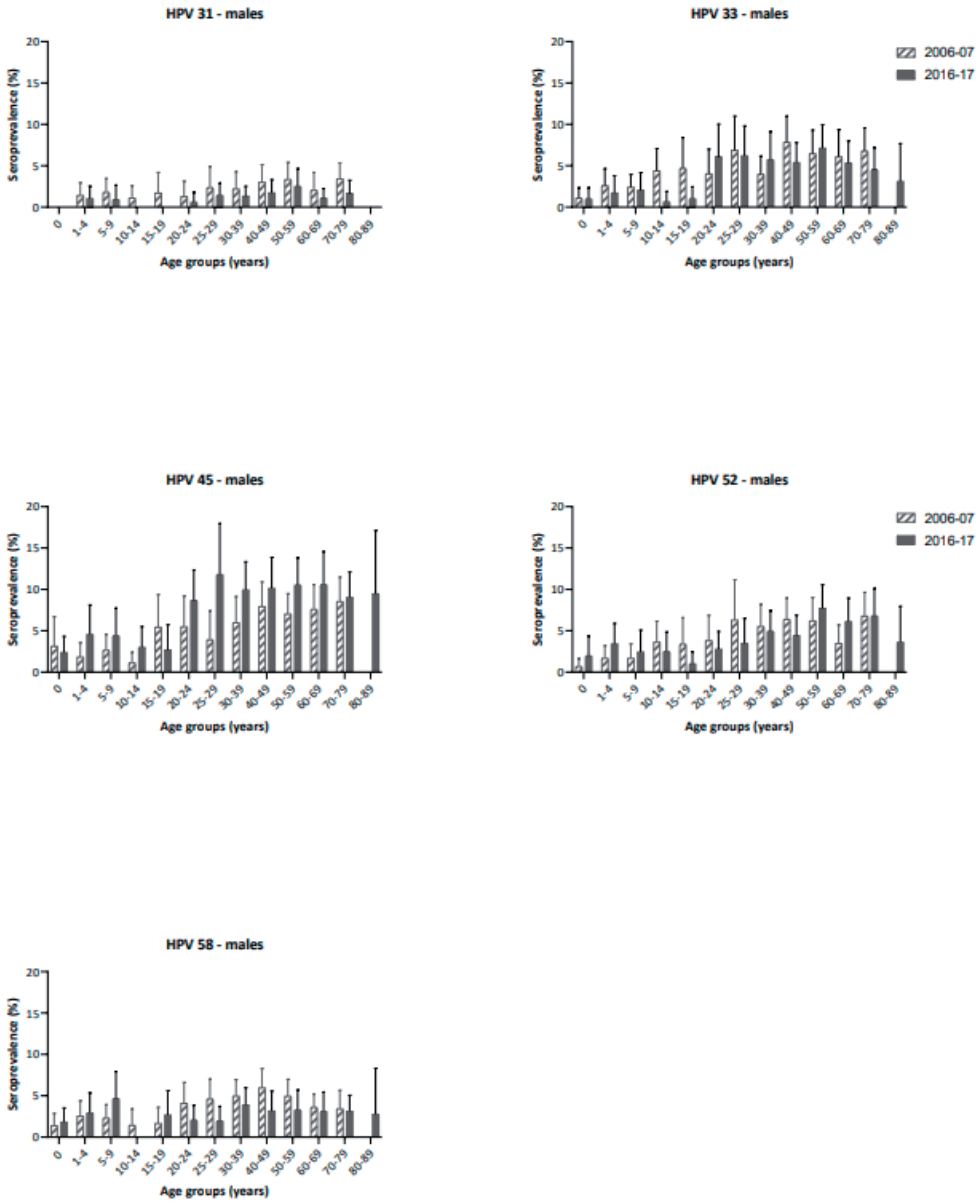


Figure 1: Age-specific seroprevalence (%) (with 95% confidence intervals) of HPV31(a), 33(b), 45(c), 52(d) and 58(e) in the unvaccinated male population of the Netherlands.

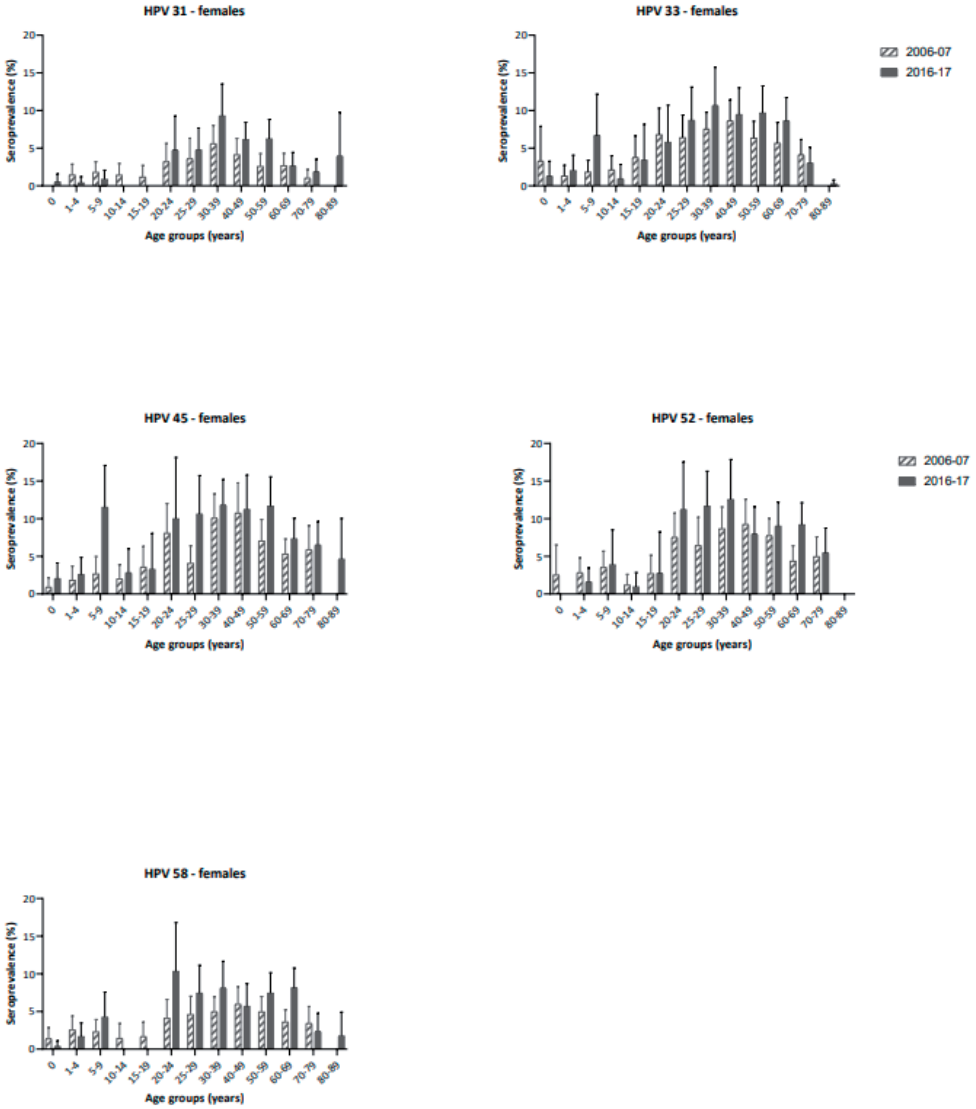


Figure 2: Age-specific seroprevalence (%) (with 95% confidence intervals) of HPV31(a), 33(b), 45(c), 52(d) and 58(e) in the unvaccinated female population of the Netherlands.

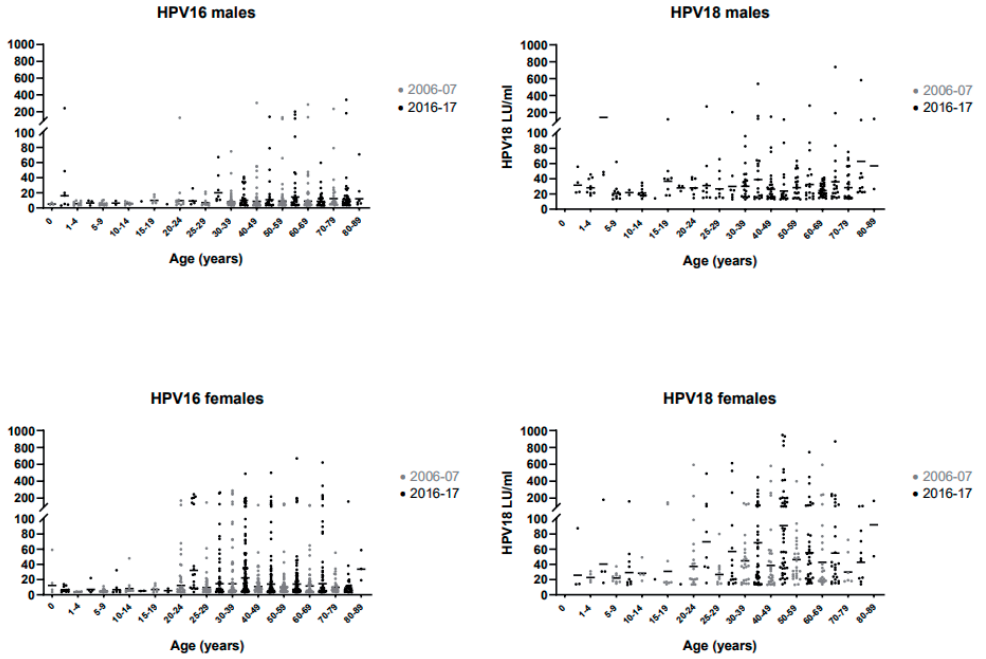


Figure 3: Age-specific geometric mean concentration (GMC) (with 95% confidence intervals (CI)) of HPV16 antibodies in men (a) and women (b) and HPV18 in men (c) and women (d) in the Netherlands.

Supplementary Table 1: Sample sizes of the total population under 15 years of age without vaccination, by sex and survey

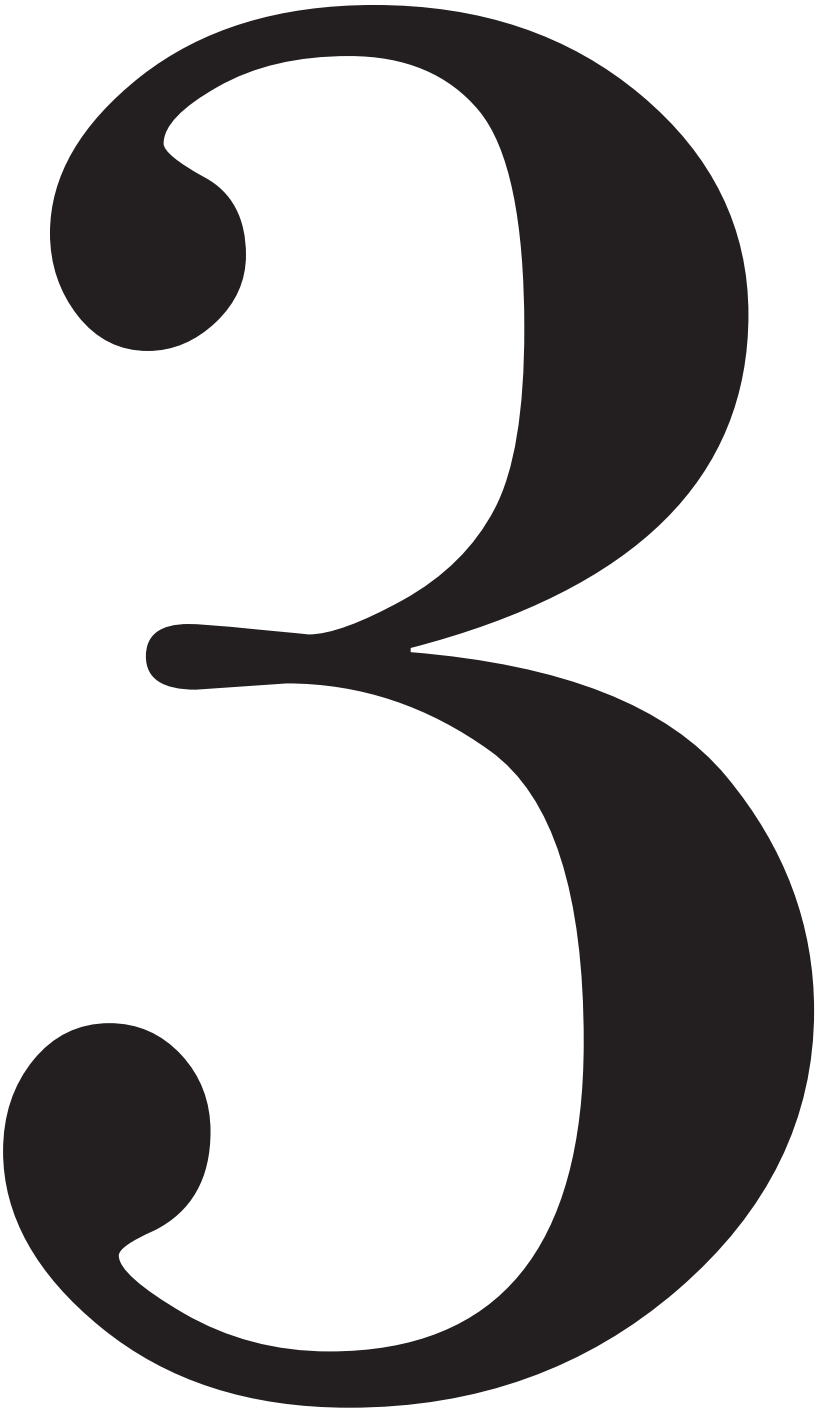
Total population under 15 years of age, without vaccination	Men (2006-07) N	Men (2016-17) N	Women (2006-07) N	Women (2016-17) N
0 year	187	202	159	195
1-4 years	267	171	247	167
5-9 years	314	170	306	157
10-14 years	206	167	226	118

References

1. Einstein MH, Schiller JT, Viscidi RP, Strickler HD, Coursaget P, Tan T, et al Clinician's guide to human papillomavirus immunology: knowns and unknowns. Lancet Infect Dis 2009;9:347–56.
2. Muñoz N, Bosch FX, Castellsagué X, Díaz M, de Sanjose S, Hammouda D, et al Against which human papillomavirus types shall we vaccinate and screen? The international perspective. Int J Cancer 2004;111:278–85.
3. van Lier EA, Oomen PJ, Giesbers H, van Vliet JA, Drijfhout IH, Zonnenberg-Hoff IF, et al Vaccination Coverage and Annual Report National Immunisation Programme Netherlands 2018 [in Dutch: Vaccinatiegraad en Jaarverslag Rijksvaccinatieprogramma Nederland 2018]. Bilthoven (the Netherlands): RIVM; 2019.
4. af Geijersstam V, Kibur M, Wang Z, Koskela P, la Eero P, Schiller J, et al Stability over time of serum antibody levels to human papillomavirus type 16. J Infect Dis 1998;177:1710–4.
5. Mollers M, Vossen JM, Scherpenisse M, van der Klis FRM, Meijer CJLM, de Melker HE. Review: current knowledge on the role of HPV antibodies after natural infection and vaccination: implications for monitoring an HPV vaccination programme. J Med Virol 2013;85:1379–85.
6. Hoes J, Pasmans H, Knol MJ, Donken R, van Marm-Wattimena N, Schepp RM, et al Persisting antibody response nine years after bivalent HPV vaccination in a cohort of Dutch women: immune response and the relation with genital HPV infections. J Infect Dis 2020;221:1884–94.
7. van der Klis FR, Mollema L, Berbers GAM, de Melker HE, Coutinho RA. Second national serum bank for population-based seroprevalence studies in the Netherlands. Neth J Med 2009;67:301–8.
8. Verberk JDM, Vos Regnerus A, Mollema L, van Vliet J, van Weert JWM, de Melker HE, et al Third national biobank for population-based seroprevalence studies in the Netherlands, including the Caribbean Netherlands. BMC Infect Dis 2019;19:470.
9. van Lier A, Oomen P, de Hoogh P, Drijfhout I, Elsinghorst B, Kemmeren J, et al Praeventis, the immunisation register of the Netherlands: a tool to evaluate the National Immunisation Programme. Euro Surveill 2012;17:20153.
10. Scherpenisse M, Mollers M, Schepp RM, Boot HJ, de Melker HE, Meijer CJLM, et al Seroprevalence of seven high-risk HPV types in the Netherlands. Vaccine 2012;30:6686–93.
11. Frey A, Di Canzio J, Zurakowski D. A statistically defined endpoint titer determination method for immunoassays. J Immunol Methods 1998;221:35–41.
12. Scherpenisse M, Mollers M, Schepp RM, Boot HJ, Meijer Chris JLM, Berbers GAM, et al Changes in antibody seroprevalence of seven high-risk HPV types between nationwide surveillance studies from 1995–96 and 2006–07 in The Netherlands. PLoS One 2012;7:e48807.
13. Newall AT, Brotherton JML, Quinn HE, McIntyre PB, Backhouse J, Gilbert L, et al Population seroprevalence of human papillomavirus types 6, 11, 16, and 18 in men, women, and children in Australia. Clin Infect Dis 2008;46:1647–55.
14. Wang SS, Schiffman M, Shields TS, Herrero R, Hildesheim A, Bratti MC, et al Seroprevalence of human papillomavirus-16, -18, -31, and -45 in a population-based cohort of 10000 women in Costa Rica. Br J Cancer 2003;89:1248–54.
15. Loenenbach AD, Poethko-Müller C, Pawlita M, Thamm M, Harder T, Waterboer T, et al Mucosal and cutaneous human papillomavirus seroprevalence among adults in the prevaccine era in Germany - results from a nationwide population-based survey. Int J Infect Dis 2019;83:3–11.
16. Michael KM, Waterboer T, Sehr P, Rother A, Reidel U, Boeing H, et al Seroprevalence of 34 human papillomavirus types in the German general population. PLoS Pathog 2008;4:e1000091.
17. Lindau ST, Schumm LP, Laumann EO, Levinson W, O'Muircheartaigh CA, Waite LJ. A study of sexuality and health among older adults in the United States. N Engl J Med 2007;357:762–74.

18. Syrjanen S. Current concepts on human papillomavirus infections in children. APMIS 2010;118:494–509.
19. Markowitz LE, Liu G, Hariri S, Steinau M, Dunne EF, Unger ER. Prevalence of HPV after introduction of the vaccination program in the United States. Pediatrics 2016;137:e20151968.
20. Giuliano AR, Lu B, Nielson CM, Flores R, Papenfuss MR, Lee Ji-Hyun, et al Age-specific prevalence, incidence, and duration of human papillomavirus infections in a cohort of 290 US men. J Infect Dis 2008;198:827–35.
21. Kjaer SK, Munk C, Winther JF, Jørgensen HO, Meijer CJLM, van den Brule AJC. Acquisition and persistence of human papillomavirus infection in younger men: a prospective follow-up study among Danish soldiers. Cancer Epidemiol Biomarkers Prev 2005;14:1528–33.
22. Carter JJ, Koutsky LA, Hughes JP, Lee SK, Kuypers J, Kiviat N, et al Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. J Infect Dis 2000;181:1911–9.
23. Heiligenberg M, Alberts CJ, Waterboer T, Speksnijder AGCL, De Vries Henry JC, Pawlita M, et al Route of sexual exposure is independently associated with seropositivity to HPV-16 and HPV-18 among clients of an STI clinic in the Netherlands. J Infect Dis 2013;208:1081–5.
24. Lu B, Viscidi RP, Wu Y, Nyitray AG, Villa LL, Lazcano-Ponce E, et al Seroprevalence of human papillomavirus (HPV) type 6 and 16 vary by anatomic site of HPV infection in men. Cancer Epidemiol Biomarkers Prev 2012;21:1542–6.
25. Vriend HJ, Bogaards JA, van der Klis FRM, Scherpenisse M, Boot HJ, King AJ, et al Patterns of human papillomavirus DNA and antibody positivity in young males and females, suggesting a site-specific natural course of infection. PLoS One 2013;8:e60696.
26. Castro FA, Dominguez A, Puschel K, Van De Wyngard V, Snijders PJF, Franceschi S, et al Serological prevalence and persistence of high-risk human papillomavirus infection among women in Santiago, Chile. BMC Infect Dis 2014;14:361.
27. Desai S, Chapman R, Jit M, Nichols T, Borrow R, Wilding M, et al Prevalence of human papillomavirus antibodies in males and females in England. Sex Transm Dis 2011;38:622–9.
28. Sudenga SL, Torres BN, Botha MH, Zeier M, Abrahamsen ME, Glashoff RH, et al HPV serostatus pre- and post-vaccination in a randomized phase II preparedness trial among Young Western Cape, South African women: the Evri Trial. Papillomavirus Res 2017;3:50–6.
29. Wilson LE, Pawlita M, Castle PE, Waterboer T, Sahasrabuddhe V, Gravitt PE, et al Natural immune responses against eight oncogenic human papillomaviruses in the ASCUS-LSIL Triage study. Int J Cancer 2013;133:2172–81.
30. Stone KM, Karem KL, Sternberg MR, McQuillan GM, Poon AD, Unger ER, et al Seroprevalence of human papillomavirus type 16 infection in the United States. J Infect Dis 2002;186:1396–402.
31. Thompson DL, Douglas JM Jr, Foster M, Hagensee ME, DiGuiseppi C, Barón AE, et al Seroepidemiology of infection with human papillomavirus 16, in men and women attending sexually transmitted disease clinics in the United States. J Infect Dis 2004;190:1563–74.
32. Guha N, Boffetta P, Filho VW, Neto JE, Shangina O, Zaridze D, et al Oral health and risk of squamous cell carcinoma of the head and neck and esophagus: results of two multicentric case-control studies. Am J Epidemiol 2007;166:1159–73.
33. Hashibe M, Brennan P, Chuang S-C, Boccia S, Castellsague X, Chen C, et al Interaction between tobacco and alcohol use and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. Cancer Epidemiol Biomarkers Prev 2009;18:541–50.

34. Coseo S, Porras C, Hildesheim A, Rodriguez AC, Schiffman M, Herrero R, et al Seroprevalence and correlates of human papillomavirus 16/18 seropositivity among young women in Costa Rica. Sex Transm Dis 2010;37:706–14.
35. Bedoya AM, Gaviria AM, Baena A, Borrero M, Duarte DF, Combita AL, et al Age-specific seroprevalence of human papillomavirus 16, 18, 31, and 58 in women of a rural town of Colombia. Int J Gynecol Cancer 2012;22:303–10.
36. Hagensee ME, Kiviat N, Critchlow CW, Hawes SE, Kuypers J, Holte S, et al Seroprevalence of human papillomavirus types 6 and 16 capsid antibodies in homosexual men. J Infect Dis 1997;176:625–31.
37. Slavinsky J 3rd, Kissinger P, Burger L, Boley A, DiCarlo RP, Hagensee ME. Seroepidemiology of low and high oncogenic risk types of human papillomavirus in a predominantly male cohort of STD clinic patients. Int J STD AIDS 2001;12:516–23.
38. Strickler HD, Kirk GD, Figueroa JP, Ward E, Braithwaite AR, Escoffery C, et al HPV 16 antibody prevalence in Jamaica and the United States reflects differences in cervical cancer rates. Int J Cancer 1999;80:339–44.
39. Dunne EF, Nielson CM, Hagensee ME, Papenfuss MR, Harris RB, Herrel N, et al HPV 6/11, 16, 18 seroprevalence in men in two US cities. Sex Transm Dis 2009;36:671–4.
40. Goldstone S, Palefsky JM, Giuliano AR, Moreira ED, Aranda C, Jessen H, et al Prevalence of and risk factors for human papillomavirus (HPV) infection among HIV-seronegative men who have sex with men. J Infect Dis 2011;203:66–74.
41. Lu B, Viscidi RP, Lee J-H, Wu Y, Villa LL, Lazcano-Ponce E, et al Human papillomavirus (HPV) 6, 11, 16, and 18 seroprevalence is associated with sexual practice and age: results from the multinational HPV Infection in Men Study (HIM Study). Cancer Epidemiol Biomarkers Prev 2011;20:990–1002.
42. Zhang C, Liu F, Deng Q, He Z, Xi L, Liu Y, et al Seroprevalence of 10 human papillomavirus types in the general rural population of Anyang, China: a cross-sectional study. Sex Transm Infect 2015;91:506–9.
43. Rahman S, Pierce Campbell CM, Rollison DE, Wang W, Waterboer T, Michel A, et al Seroprevalence and associated factors of 9-valent human papillomavirus (HPV) types among men in the multinational HIM Study. PLoS One 2016;11:e0167173.
44. Castellsagué X, Bosch FX, Muñoz N, Meijer CJLM, Shah KV, de Sanjosé S, et al Male circumcision, penile human papillomavirus infection, and cervical cancer in female partners. N Engl J Med 2002;346:1105–12.
45. Pillsbury AJ, Quinn HE, Evans TD, McIntyre PB, Brotherton JML. Population-level herd protection of males from a female human papillomavirus vaccination program: evidence from Australian serosurveillance. Clin Infect Dis 2017;65:827–32.
46. Tabrizi SN, Brotherton JML, Kaldor JM, Skinner SR, Liu B, Bateson D, et al Assessment of herd immunity and cross-protection after a human papillomavirus vaccination programme in Australia: a repeat cross-sectional study. Lancet Infect Dis 2014;14:958–66.
47. Brouwer AF, Eisenberg MC, Carey TE, Meza R. Trends in HPV cervical and seroprevalence and associations between oral and genital infection and serum antibodies in NHANES 2003–2012. BMC Infect Dis 2015;15:575.
48. Woestenberg PJ, Bogaards JA, King AJ, Leussink S, Ab van der Sande M, Jpa Hoebe C, et al Assessment of herd effects among women and heterosexual men after girls-only HPV16/18 vaccination in the Netherlands: a repeated cross-sectional study. Int J Cancer 2019;144:2718–27.
49. Health Council of the Netherlands. Vaccination against HPV [in Dutch: Vaccinatie tegen HPV]. The Hague (the Netherlands): Health Council of the Netherlands; 2019.



Chapter 3

Persisting antibody response 9 years after bivalent human papillomavirus (HPV) vaccination in a cohort of Dutch women: immune response and the relation to genital HPV infections.

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ABSTRACT

The bivalent human papillomavirus (HPV) vaccine is highly effective and induces robust serological responses. Using a Dutch prospective cohort initiated in 2009, including 744 vaccinated and 294 unvaccinated girls (1993–1994) who provide a vaginal self-swab sample, serum sample, and questionnaire yearly, we report a high, persisting antibody response up to 9 years after vaccination for vaccine types HPV-16 or HPV-18. Antibodies against nonvaccine HPV types 31, 33, 45, 52, and 58 were lower but still significantly higher than in unvaccinated individuals. This was also reflected in the seroprevalence. We compared participant characteristics and antibody levels between vaccinated women with and those without HPV infections 1 year before infection (204 incident and 64 persistent infections), but we observed no consistent difference in type-specific antibody levels. Having a high-risk HPV infection was associated with sexual risk behavior and smoking 1 year before infection. Although high antibody levels are necessary for protection, our study suggests that on the individual level other factors such as HPV exposure or antibody avidity could be important.

Introduction

Human papillomavirus (HPV) is a common, sexually transmitted virus, of which some types can cause anogenital and/or oropharyngeal infections. A persistent infection with a high-risk HPV type can lead to the progression of malignancies at specific anatomic sites [1]. The most frequently observed cancer type associated with HPV in women is cervical cancer. In total, 99% of all cervical cancer cases are caused by HPV infections, with HPV-16 or HPV-18 (HPV-16/18) responsible for about 70% of cases [2]. To prevent persistent HPV infections and subsequent lesions, prophylactic HPV vaccination was registered in 2006 with the ultimate goal of prevention of HPV-related cancers [3]. In 2010, the Netherlands implemented the bivalent vaccine into the National Immunization Program as a girls-only vaccine in a 3-dose schedule (0, 1, and 6 months), vaccinating girls in the year they reach age 13 years. Moreover, a catch-up campaign was initiated for birth cohorts 1993–1996 in 2009 (ie, 13–16-year-olds) [4]. From 2014, the Netherlands shifted to a 2-dose schedule (starting with birth cohort 2001).

Vaccine effectiveness (VE) of the bivalent vaccine against HPV-16/18 infections is high, with VE estimates >90% [5, 6]. Moreover, in the Dutch cohort described in this article, high VE estimates have been reported, with very few infections among vaccinated individuals [7]. Furthermore, for multiple nonvaccine types, varying rates of cross-protection against infections have been found [5, 8–10], and clinical trials and, more recently, population-based studies have demonstrated the effect of HPV-16/18 vaccination on cervical intraepithelial neoplasia and prestages of invasive cancer [11–14]. In addition, HPV vaccination induces robust serological responses [12, 15, 16], which are generally high and can be 100-fold higher compared with naturally elicited antibodies. Among vaccinated individuals the seroconversion rate is high for vaccine types (95%–100%), whereas a measurable immune response only occurs in 40%–60% of naturally infected individuals [17].

Even though high antibody levels are considered to be important for protection, a correlate of protection for HPV is lacking [18]. The observed high VE against vaccine types (HPV-16/18) is impeding this search, although at the infection level some breakthrough cases occur. The current study aimed to explore the longitudinal relationship between antibody response against HPV types 16, 18, 31, 33, 45, 52, and 58 and HPV DNA infections. We first describe antibody levels against these 7 high-risk HPV types in vaccinated and unvaccinated young women up to 9 years after vaccination with the bivalent vaccine in a 3-dose schedule. We then compare participant characteristics and antibody levels between vaccinated women with or without HPV DNA infections in the next year (infections with either vaccine [HPV-16/18], cross-

protective [HPV-31/45], or nonvaccine [HPV-33/52/58] types) to assess whether higher antibody levels protect against infection.

Methods

Study Design

In 2009, the HPV Among Vaccinated And Non-vaccinated Adolescents (HAVANA) study was initiated as a prospective cohort study, as described elsewhere [4]. In short, 9500 girls who were eligible for the catch-up campaign were randomly invited to participate in the study in 2009. One month and each consecutive year after vaccination, a vaginal self-swab sample, a blood sample, a cervical secretion sample obtained using a tampon (optionally), and a questionnaire were collected. A voucher of 25 euros was provided after each year of participation. The HAVANA study was approved by the Medical Ethics Committee of the VU University Medical Centre (no. 2009/022) and was conducted according to the Declaration of Helsinki. Informed consent was required before participants could be included.

Laboratory Procedures - Serology

Blood was collected using a serum tube (Vacuette; Greiner Bio-One) and participants who were not able to visit a blood collection session were offered a self-sample set to obtain finger-prick blood at home resulting, in dried blood spot samples (Whatman 903 Protein Saver Card) [19]. A viruslike particle (VLP)–based multiplex immunoassay was used to quantify type-specific HPV antibodies to types 16, 18, 31, 33, 45, 52, and 58 for both serum and dried blood spot samples. To analyze antibodies in the first 7 years of follow-up, we used HPV VLPs produced by GSK (GlaxoSmithKline Biologicals), and for the subsequent years we used VLPs donated by MSD (Merck Sharp & Dohme). VLPs were linked to 7 distinct color-coded fluorescent microspheres, and the multiplex immunoassay was performed as described elsewhere [19–21]. The HPV-specific antibodies were analyzed using a Bioplex system 200 with Bioplex software (Bio-Rad Laboratories). For each analyte, median fluorescent intensity was converted to Luminex units (LU) per milliliter. We assumed samples to be seropositive at type-specific cutoff levels determined previously [21]: 9, 13, 27, 11, 19, 14, and 31 LU/mL for HPV types 16, 18, 31, 33, 45, 52, and 58, respectively.

Laboratory Procedures - HPV DNA Detection and Genotyping

Vaginal self-samples were collected using a vaginal Viba-Brush (Rovers). After collection, samples were stored in 1 mL of phosphate-buffered saline at –20°C; 200 µL of the sample was used for DNA extraction with the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche). The DNA was then eluted in 100 µL of elution buffer.

A 10-μL sample of DNA extract was used for HPV amplification, making use of the sensitive SPF10 primer sets [22]. To detect the amplified HPV DNA, a DNA enzyme-linked immunoassay (HPV DEIA; DDL Diagnostic Laboratory) was applied. HPV DEIA–positive amplicons were then analyzed with a reverse line blot assay (HPV LiPA25; DDL Diagnostic Laboratory) to determine the genotype. Twenty-five HPV genotypes could be detected, including the following high-risk HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. Other HPV types that could be detected were 6, 11, 34, 40, 42, 43, 44, 53, 54, 66, 68, 70, 73, and 74 (of which types 53, 66, 68, 70, and 73 are classified as possibly oncogenic) [23]. All vaginal self-swab samples collected in 2009 (n = 1152) were subjected to a quality check by testing for β-actin as a marker for the presence of human DNA. Because 99.3% were positive for β-actin this control was not routinely performed for the remaining years of follow-up.

Statistical Analysis

To be included for analyses, participants had to be either nonvaccinated or fully vaccinated according to the 3-dose schedule and could not been vaccinated before the baseline measurement was performed or after the first follow-up moment (ie, participants vaccinated after year 1 of follow-up). Participants were allowed to miss follow-up moments (not censored). Differences between vaccinated and unvaccinated participants in type-specific seroprevalence based on immunoglobulin G (IgG) were explored per year using a $\times 2$ test. We also calculated geometric mean concentrations (GMCs) of serum antibodies. Differences between vaccination status groups were assessed per year by means of a t test on the log-transformed data, and trends over time within vaccination status group were studied using a linear mixed model.

We examined the association between demographic or sexual behavior characteristics 1 year before infection and HPV infection (irrespective of persistence) among vaccinated participants. To be included, participants needed to be HPV DNA negative at the baseline measurement (before vaccination) for the 7 included high-risk HPV types. Using univariate generalized estimation equation logistics regression models with exchangeable correlation structure, we combined data of participants over time. The uninfected comprised individuals who were negative for all high-risk types, as determined per study year, and they were compared with participants infected with HPV 1 year before infection, either with vaccine or cross-protective types (types 16, 18, 31, and 45, as defined by a significant type-specific VE in the current cohort [7]) or with nonvaccine types (types 33, 52, and 58). The year of follow-up was added to the model to adjust for the fluctuation related to time.

Baseline measurements were used to determine HPV DNA status but were not included in these analyses, because participants were not vaccinated yet at baseline.

The association between log-transformed type-specific serum IgG and infection status in the next year was assessed as well, for incident and persistent infections in multilevel linear models with unstructured covariance matrix. Participants had to be baseline HPV DNA negative for the respective type. An incident HPV infection was defined as being HPV DNA negative in the previous year and being HPV DNA positive in the current year. A persistent infection was defined as being HPV DNA positive in ≥2 consecutive years. Random intercept at the participant level was added to the model. Again, year of follow-up was added to the model. Participants added to the uninfected group in years they were HPV negative for the respective type. The outcome was expressed as the GMC ratio with 95% confidence intervals for antibody levels before infection, comparing vaccinated participants without infection to those with infection. All analyses were conducted using SAS software (version 9.4).

Results

Study Population

Characteristics of the participants are described in Table 1. In total, 1038 participants with baseline measurement (of whom 71.7% were vaccinated) were included in the current analyses. Owing to loss to follow-up, the number of participants decreased to 514 in the ninth year after vaccination (of whom 76.7% were vaccinated). Among vaccinated participants we observed a total of 204 incident and 64 persistent infections for HPV types 16, 18, 31, 33, 45, 52, and 58, which were included in the type-specific analyses.

Immunogenicity

In all years after vaccination, a significant difference in seroprevalence was observed between vaccinated and unvaccinated participants for all HPV types ($P < .001$) (Table 2). Seropositivity was 100% among vaccinated girls for vaccine types HPV-16 and HPV-18 directly after vaccination and remained at 99-100% up to 9 years after vaccination. Among the unvaccinated, these rates were only 9.7% and 4.8% for HPV-16 and HPV-18, respectively, in the first year of follow-up, increasing to 20.8% and 9.3% in the last year. Moreover, for other HPV types (31, 33, 45, 52, and 58), remarkably higher seroprevalence was observed among vaccinated girls (up to 92.9% at 2 years after vaccination for HPV-45) compared with unvaccinated participants in the same time frame (11.8% for HPV-45).

Prevaccination GMCs were comparable between vaccinated and unvaccinated participants ($P > .05$) (Figure 1). Thereafter, significant differences ($P < .05$) were observed between vaccinated and unvaccinated participants for all types at all time points.

Table 1: Characteristics of Study Participants Over Time

Sociodemographic Characteristic	Participants, No. (%)[a]									
	Baseline (N = 1038)	Round 1 (n = 797)	Round 2 (n = 765)	Round 3 (n = 722)	Round 4 (n = 624)	Round 5 (n = 641)	Round 6 (n = 569)	Round 7 (n = 589)	Round 8 (n = 579)	Round 9 (n = 514)
Fully vaccinated	744 (71.7)	590 (74.0)	562 (73.5)	533 (73.8)	475 (76.1)	467 (72.9)	407 (71.5)	426 (72.3)	427 (73.7)	394 (76.7)
Age, mean (range), y	15 (14–16)	16 (15–17)	17 (16–18)	18 (17–19)	19 (18–20)	20 (19–21)	21 (20–22)	22 (21–23)	23 (22–24)	24 (23–25)
High urbanization	933 (89.9)	724 (90.8)	686 (89.7)	590 (81.7)	553 (88.6)	575 (89.7)	518 (91.0)	498 (84.6)	485 (83.8)	436 (84.8)
Dutch ethnicity	898 (86.5)	699 (87.7)	670 (87.6)	644 (89.2)	557 (89.3)	573 (89.4)	518 (91.0)	535 (90.8)	526 (90.8)	467 (90.9)
High educational level	572 (55.1)	498 (62.5)	491 (64.2)	476 (65.9)	417 (66.8)	460 (71.8)	422 (74.2)	430 (73.0)	439 (75.8)	380 (73.9)
History of ever smoking	345 (33.2)	330 (41.4)	300 (39.2)	368 (51.0)	344 (55.1)	367 (57.3)	335 (58.9)	339 (57.6)	345 (59.6)	317 (61.7)
Status as current smoker	137 (13.2)	245 (30.7)	252 (32.9)	272 (37.7)	238 (38.1)	158 (24.6)	205 (36.0)	215 (36.5)	192 (33.2)	166 (32.3)
Any history of using contraception	408 (39.3)	489 (61.4)	580 (75.8)	623 (86.3)	577 (92.5)	602 (93.9)	537 (94.4)	570 (96.8)	559 (96.5)	503 (97.9)
Any history of sexual intercourse	239 (23.0)	341 (42.8)	445 (58.2)	506 (70.1)	509 (81.6)	552 (86.1)	503 (88.4)	521 (88.5)	531 (91.7)	478 (93.0)
Sexual behavior among sexually active participants										
Age <15 y at sexual debut	119 (49.8)	84 (24.6)	80 (18.0)	84 (16.6)	78 (15.3)	76 (13.8)	82 (16.3)	75 (14.4)	75 (14.1)	73 (15.3)
Lifetime sexual partners, mean (range), no.	1.7 (1–16)	2.0 (1–15)	2.6 (1–20)	2.8 (1–11)	3.4 (1–20)	4.3 (1–50)	5.0 (1–50)	5.4 (1–45)	6.5 (1–50)	6.8 (1–40)
New sexual partners in past 12 mo, mean (range), no.	-	1.2 (0–7)	1.1 (0–12)	1.0 (0–7)	1.0 (0–11)	1.2 (0–31)	0.9 (0–14)	1.7 (0–10)	1.7 (0–12)	1.8 (0–8)
Current steady partner	156 (65.3)	212 (62.2)	299 (67.2)	356 (70.4)	374 (73.5)	396 (71.7)	402 (79.9)	421 (80.8)	430 (81.0)	393 (82.2)
Age of current partner, mean (range), y	17 (13–23)	18 (14–24)	19 (16–30)	20 (16–43)	22 (17–44)	23 (17–45)	24 (19–46)	25 (19–47)	26 (18–51)	26 (20–36)
STI diagnosed in past 12 mo	2 (0.8)	5 (1.5)	9 (2.0)	20 (4.0)	17 (3.3)	26 (4.7)	28 (5.6)	33 (6.3)	28 (5.3)	16 (3.3)

Round means: study year. After the baseline measurement, participants are followed over study years/rounds/years of follow up (post vaccination, in case of vaccinated individuals). High urbanization was defined as: very to moderately urban (as opposed to low urban and country side). High educational level was defined as: higher general secondary education, pre-university education, university of applied sciences and university (as compared to Low/middle educational level which included all other levels of education). A: Data represent no. (%) of participants unless otherwise specified.

Table 2: Seroprevalence by Vaccination Status Over Time

HPV Type by Vaccination Status	Seropositivity, No. (%)[a]									
	Baseline	Round 1	Round 2	Round 3	Round 4	Round 5	Round 6	Round 7	Round 8	Round 9
Unvaccinated										
HPV-16	10 (3.4)	20 (9.7)[b]	19 (9.4)[b]	24 (12.7)[b]	29 (19.5)[b]	27 (15.5)[b]	27 (16.7)[b]	30 (18.40)[b]	27 (20.0)[b]	25 (20.8)[b]
HPV-18	5 (1.7)	10 (4.8)[b]	8 (3.9)[b]	9 (4.8)[b]	15 (10.4)[b]	6 (3.5)[b]	10 (6.2)[b]	17 (10.4)[b]	16 (11.9)[b]	11 (9.3)[b]
HPV-31	2 (0.7)	0 (0.0)[b]	3 (1.5)[b]	2 (1.1)[b]	4 (2.9)[b]	3 (1.7)[b]	5 (3.1)[b]	6 (3.7)[b]	9 (6.7)[b]	9 (7.5)[b]
HPV-33	8 (2.7)	10 (4.8)[b]	16 (7.9)[b]	6 (3.2)[b]	7 (5.1)[b]	14 (8.1)[b]	11 (6.8)[b]	22 (13.5)[b]	14 (10.5)[b]	11 (9.2)[b]
HPV-45	7 (2.4)	11 (5.3)[b]	24 (11.8)[b]	7 (3.7)[b]	14 (9.7)[b]	12 (6.9)[b]	19 (11.7)[b]	20 (12.3)[b]	22 (16.4)[b]	14 (11.7)[b]
HPV-52	15 (5.1)	11 (5.3)[b]	9 (4.4)[b]	6 (3.2)[b]	7 (5.0)[b]	11 (6.3)[b]	13 (8.0)[b]	17 (10.4)[b]	12 (8.9)[b]	12 (10.0)[b]
HPV-58	5 (1.7)	3 (1.5)[b]	5 (2.5)[b]	5 (2.7)[b]	3 (2.2)[b]	7 (4.0)[b]	6 (3.7)[b]	10 (6.1)[b]	13 (9.6)[b]	13 (10.8)[b]
Vaccinated										
HPV-16	18 (2.4)	590 (100.0)[b]	561 (99.8)[b]	531 (99.6)[b]	475 (100.0)[b]	466 (99.8)[b]	406 (99.8)[b]	426 (100.0)[b]	427 (100)[b]	393 (99.8)[b]
HPV-18	20 (2.7)	590 (100.0)[b]	560 (99.6)[b]	528 (99.1)[b]	471 (99.2)[b]	466 (99.8)[b]	404 (99.2)[b]	423 (99.3)[b]	426 (99.8)[b]	392 (99.5)[b]
HPV-31	8 (1.1)	383 (64.9)[b]	336 (59.8)[b]	252 (47.3)[b]	196 (42.9)[b]	225 (48.2)[b]	206 (50.6)[b]	154 (36.2)[b]	195 (45.7)[b]	189 (48.1)[b]
HPV-33	21 (2.8)	314 (53.2)[b]	384 (68.3)[b]	312 (58.5)[b]	227 (52.4)[b]	279 (59.7)[b]	232 (57.0)[b]	227 (53.3)[b]	209 (49.0)[b]	203 (51.7)[b]
HPV-45	16 (2.2)	529 (89.7)[b]	522 (92.9)[b]	460 (86.3)[b]	377 (80.2)[b]	386 (82.7)[b]	291 (71.5)[b]	329 (77.2)[b]	320 (75.0)[b]	286 (72.8)[b]
HPV-52	38 (5.1)	368 (62.4)[b]	325 (57.8)[b]	236 (44.3)[b]	219 (49.9)[b]	246 (52.7)[b]	218 (53.6)[b]	266 (62.4)[b]	215 (50.4)[b]	212 (53.9)[b]
HPV-58	14 (1.9)	237 (40.2)[b]	251 (44.7)[b]	248 (46.5)[b]	141 (31.5)[b]	228 (48.8)[b]	214 (52.6)[b]	219 (51.4)[b]	154 (36.1)[b]	146 (37.2)[b]

Round means: study year. After the baseline measurement, participants are followed over study years/rounds/years of follow up (post vaccination, in case of vaccinated individuals).

a: Cutoffs for seropositivity were defined as 9, 13, 27, 11, 19, 14, and 31 Luminex units/mL, respectively, for HPV types 16, 18, 31, 33, 45, 52, and 58, respectively.

b :Significant at P < .001.

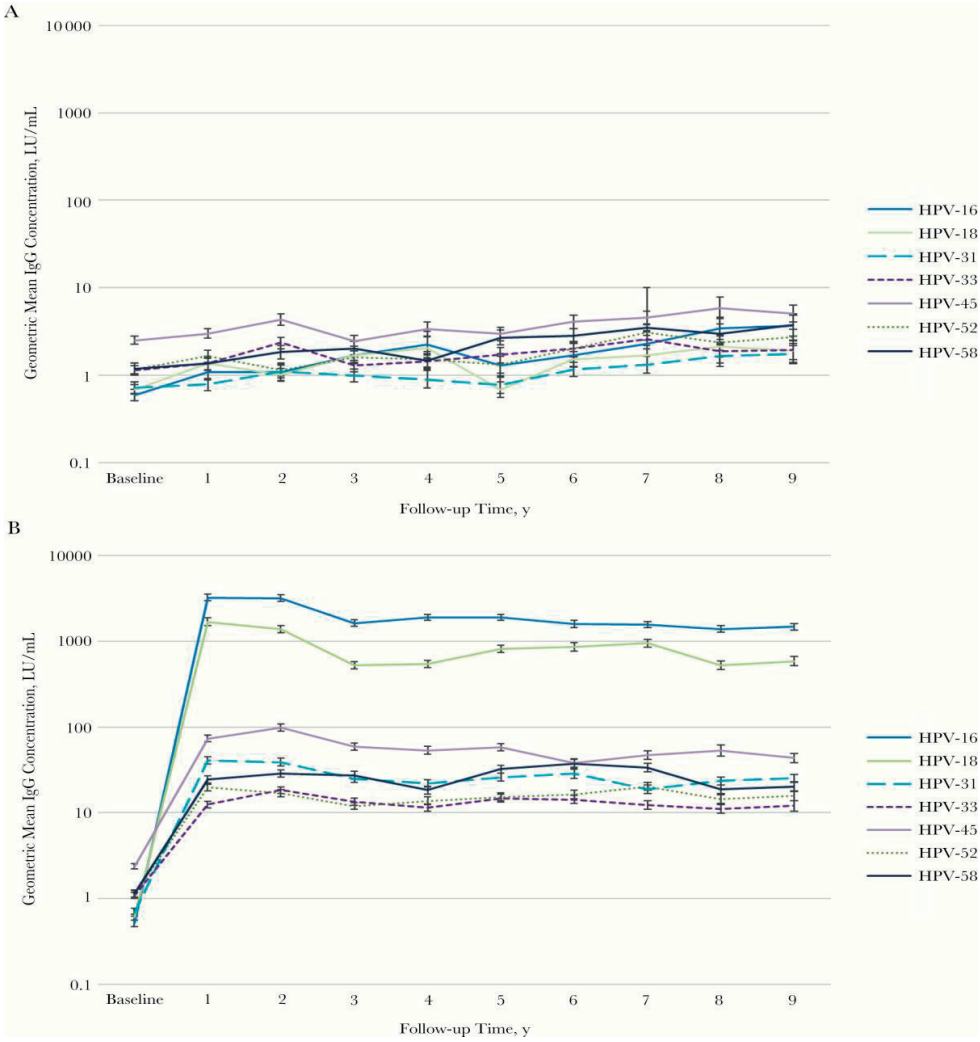

Figure 1: Geometric mean antibody concentrations of immunoglobulin G (IgG) against 7 human papillomavirus (HPV) types among unvaccinated (A) and fully vaccinated (B) participants.

Among vaccinated participants, antibodies against vaccine types HPV-16/18 showed a peak after vaccination (GMC, 3215 and 1680 LU/mL for HPV-16 and HPV-18, respectively) followed by a significant decline 3 years after vaccination (GMC, 1617 and 520 LU/mL). GMCs remained high and more or less stable up to 9 years after vaccination (GMC at 9 years, 1462 and 582 LU/mL). IgG antibody levels against other HPV types among vaccinated participants were considerably lower compared with vaccine types (range, 11–97 LU/mL), but still significantly higher than in unvaccinated participants. Antibody levels against cross-protective type HPV-45 displayed

the highest overall concentration. In addition, after a peak following vaccination, the GMCs of other HPV types remained stable in the postvaccination follow-up period. Among unvaccinated girls, antibody concentrations increased over time from 0.6 to 5.1 LU/mL but remained far beneath the levels observed among vaccinated participants.

Characteristics and Antibody Levels 1 Year Before Infection

Risk factors for contracting a vaccine/cross-protective or nonvaccine HPV type infection 1 year before infection are depicted in Table 3 and include smoking (both current smoking and any history of smoking) and characteristics related to sexual behavior. No substantial differences in risk factors were observed between infections with HPV types 16, 18, 31, or 45, and those with types 33, 52, or 58, although the association between smoking behavior and infection was stronger for vaccine and cross-protective type infections than for infections with other HPV types. However, because multivariable analyses could not be performed due to small numbers, we could not exclude a possible confounding effect of sexual behavior in this association.

There were no consistent significant differences in IgG antibody levels 1 year before infection between vaccinated individuals with or without an infection in the next year (Figure 2) for vaccine or cross-protective types. However, we did find significant differences for nonvaccine type HPV-52 (Figure 3). GMC ratios of 1.57 (95% confidence interval, 1.33–1.87) and 2.09 (1.63–2.70) were observed for incident and persistent infections, respectively, showing higher antibody levels in uninfected than in infected individuals in the year before infection. GMC ratios did not show a consistent pattern across HPV types or across incident and persistent infections. In a sensitivity analysis on incidence infections, we excluded the infections that turned out to be persistent. However, this did not change the results (data not shown).

Table 3: Risk Factors for Contracting Human Papillomavirus (HPV) Infection in the Next Year Among Vaccinated Women, Stratified by Vaccine or Cross-Protective Versus Nonvaccine HPV Types

Risk Factor	OR (95% CI) 1 y Before Infection	
	Vaccine or Cross-Protective HPV Types (16, 18, 31, and 45)	Nonvaccine HPV Types (33, 52, and 58)
Urbanization		
High	Reference	Reference
Low	1.8 (.4–7.9)	0.8 (.4–1.5)
Ethnicity		
Dutch	Reference	Reference
Other	0.7 (.3–1.8)	1.4 (.8–2.3)
Education		
High	Reference	Reference
Low	1.2 (.6–2.2)	1.0 (.7–1.6)
Any history of smoking		
No	Reference	Reference
Yes	3.9 (2.0–7.5)	2.1 (1.4–3.0)
Current smoker		
No	Reference	Reference
Yes	2.6 (1.4–4.7)	1.6 (1.1–2.4)
Any history of using contraception		
No	Reference	Reference
Yes	2.6 (.9–7.3)	4.8 (1.8–13.2)
Any history of sexual activity		
No	Reference	Reference
Yes	6.4 (2.1–19.3)	4.6 (2.5–8.6)
Age at sexual debut		
≥15 y	Reference	Reference
<15 y	0.8 (.3–2.0)	1.8 (1.1–2.9)
Lifetime sexual partners, no.		
0	Reference	Reference
1	3.6 (1.0–12.7)	2.4 (1.2–5.0)
≥2	8.0 (2.6–24.7)	5.5 (2.9–10.7)
Sexual partners in past 12 mo, no.		
0	Reference	Reference
1	3.7 (1.9–7.5)	2.3 (1.5–3.7)
≥2	3.4 (1.5–8.1)	3.0 (1.8–5.1)
Current steady partner		
No	Reference	Reference
Yes	0.5 (.3–.9)	0.4 (.3–.6)

Table 3: Continued

Risk Factor	OR (95% CI) 1 y Before Infection	
	Vaccine or Cross-Protective HPV Types (16, 18, 31, and 45)	Nonvaccine HPV Types (33, 52, and 58)
STI diagnosed in past 12 mo		
No	Reference	Reference
Yes	5.8 (2.5–13.2)	1.8 (.8–4.4)

Round means: study year. After the baseline measurement, participants are followed over study years/rounds/years of follow up (post vaccination, in case of vaccinated individuals). High urbanization was defined as: very to moderately urban (as opposed to low urban and country side). High educational level was defined as: higher general secondary education, pre-university education, university of applied sciences and university (as compared to Low/middle educational level which included all other levels of education).

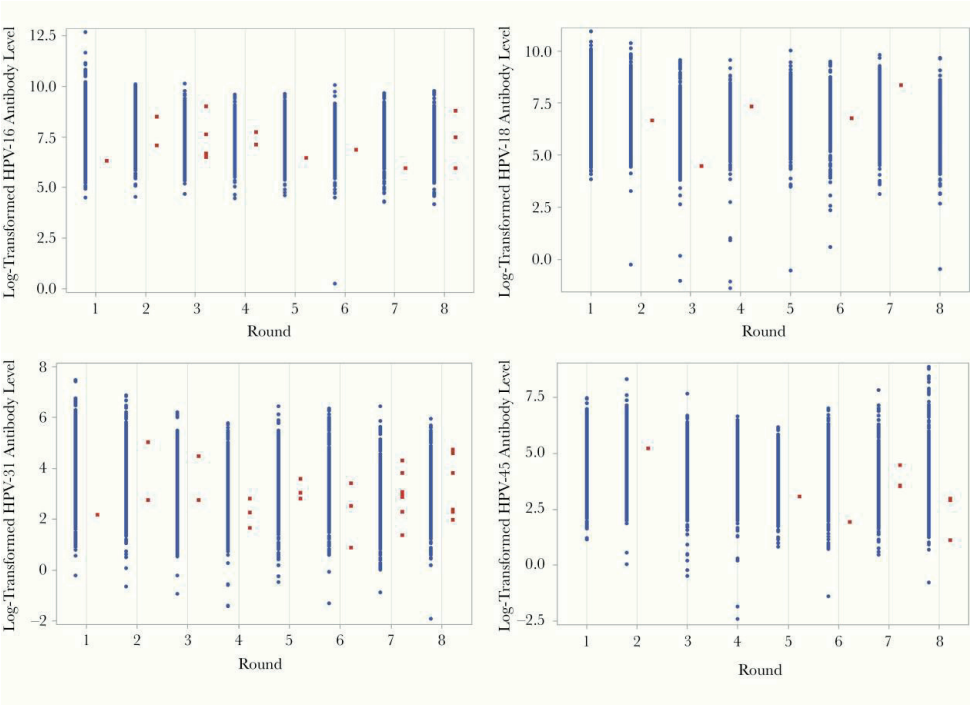

Figure 2: Antibody levels against HPV-16 (A), HPV-18 (B), HPV-31 (C), and HPV-45 (D) among vaccinated individuals with infections (squares) and without infections (dots) in the subsequent year. Round means: study year. After the baseline measurement, participants are followed over study years/rounds/years of follow up (post vaccination, in case of vaccinated individuals).

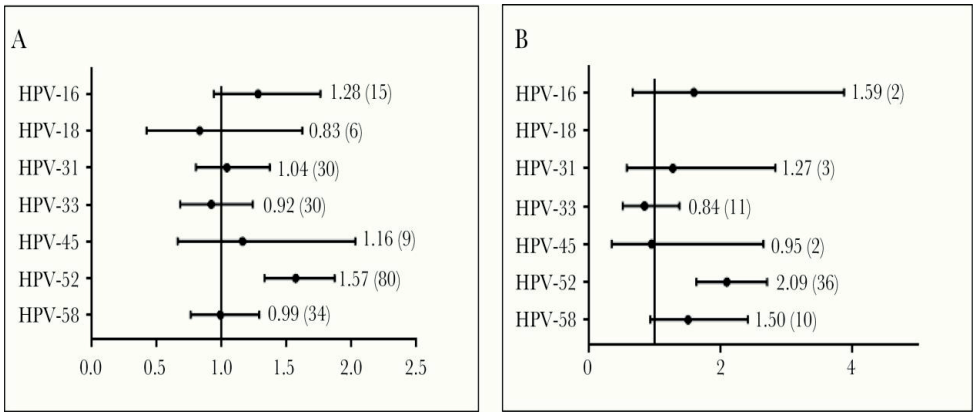

Figure 3: Geometric mean concentration ratios with 95% confidence intervals 1 year before infection, by human papillomavirus (HPV) type, comparing noninfected vaccinated with infected vaccinated participants for incident (A) and persistent (B) infections. The absolute numbers of type-specific infections are given in parentheses.

Discussion

We provided an overview of the effect of the bivalent HPV vaccine on serological response against vaccine types (HPV-16/18), cross-protective types (HPV-31/45) and nonvaccine types (HPV-33/52/58) up to 9 years after vaccination in a population-based setting. We observed high geometric mean antibody concentrations up to 9 years after vaccination against vaccine types and cross-protective types. In addition, we explored the longitudinal relationship between antibody response and HPV infections and showed that antibody levels among vaccinated individuals 1 year before infection were similar for those with or without type-specific HPV infections (with the exception of HPV-52 infection). As expected, only a few infections occurred among vaccinated individuals. We found indications that contracting an infection in the next year despite being vaccinated, was associated with smoking and sexual risk behavior.

As expected, HPV seroprevalence was high among vaccinated participants and amounted to 100% for vaccine types 1 month after vaccination. Our data confirm clinical trial results reporting seropositivity rates up to 100% 9–10 years after vaccination [11, 24]. Among unvaccinated participants, seropositivity was considerably lower. However, rates among unvaccinated increased to 20% in the ninth year of follow-up. This is probably the result of increased exposure over time and is supported by increased self-reported sexual behavior as well as HPV DNA prevalence (as reported by Donken et al [7]).

Serum IgG antibody concentrations against vaccine types remained high up to 9 years after vaccination in a population-based setting. Both clinical trials [11, 12] and data from the Finnish maternity cohort showed sustained antibody levels against vaccine types up to 12 years after vaccination with the bivalent vaccine [25]. Our study adds an overview of 5 other HPV types over time. For vaccine types HPV-16 and HPV-18, we observed a peak in antibody level 1 year after vaccination and stable antibody levels thereafter with no sign for a significant decline in the near future. The same pattern was observed for HPV types 31, 33, 45, 52, and 58, although at a lower level, with cross-protective type HPV-45 presenting the highest concentration. This is in line with the cross-protection that was observed earlier in this cohort for HPV types 31, 33, and 45 [7]. GMCs of vaccinated participants against all HPV types remained significantly above those from unvaccinated participants.

Because a correlate of protection is lacking, it remains difficult to interpret antibody concentrations with regard to protection or effectiveness [18]. This was also shown by our further analyses, in which we studied whether vaccinated, infected individuals already have lower antibody levels before the infection is established, making them more prone to infection. An association between preinfection GMC and infection status for vaccine types or cross-protective types was not found although the number of infections was possibly too low to expect this. For HPV-52 we did find an association, but because this is not a vaccine type or a cross-protective type, this does not explain the supposed relationship between vaccine-derived antibody levels and protection. Perhaps an association for HPV-52 could be more easily detected owing to more infections and a relative low antibody response compared with the other types.

Overall, high antibody levels and, especially, neutralizing antibodies are considered indicative of protection [26]. Our assay quantifies antibodies directed against the L1 VLP in a type-specific way but is not restricted to neutralizing antibodies [26, 27]. This suggests that quality of antibodies, instead of quantity, could be relevant in determining the level of protectiveness; as was earlier proposed by Scherpenisse and colleagues [28], accumulated binding strength/affinity of antibodies (avidity) could be used as a marker for this. Moreover, earlier studies also suggested that local immune responses (antibodies at the site of entry, ie, the cervix) could be important to consider [29]. Previous research showed that correlations between serum and cervical secretion sample antibody levels exist [30, 31], suggesting that comparable patterns could be expected. More recently, van der Weele et al [32] showed that HPV-16/18 breakthrough infections among vaccinated participants in the HAVANA cohort had significantly lower viral load values than HPV-16/18 infections in unvaccinated young women.

These findings could indicate that the vaccine-induced antibody response results in a reduction in viral load in breakthrough vaccine-type infections. This might lead to limited capacity of the virus to cause a persistent infection, possibly via the action of neutralizing antibodies. Finally, we hypothesize that antibody concentrations rising above certain levels or physiological maxima could have no further increasing value with regard to protection or immunity [18]; if this is the case, other discriminating factors, such cell-mediated immunity or genetic host or pathogen factors, might play a role in who acquires an infection despite vaccination. To study this more closely, in-depth immune cell analyses could be performed on peripheral blood mononuclear cells from infected vaccinated participants, or HPV DNA from infections could be analyzed in more detail (eg, by sequencing).

The associations between sexual risk behavior and HPV infection among vaccinated participants 1 year before infection might suggest that higher exposure to HPV results in a higher chance of high-risk HPV infection, including HPV-16/18 and HPV-31/45, despite vaccination. On the other hand, among visitors to Dutch sexually transmitted infection clinics, who represent a high-risk population, high VE has been reported as well [9]. Still, the proportion of risky sexual behavior could be more equally distributed across vaccinated and unvaccinated individuals in sexually transmitted infection clinics, resulting in high VE estimates. Furthermore, the observed association with smoking might be a proxy for more overall risky behavior resulting in higher exposure or could be related to an impaired immune response. Comparable risk factors were found among individuals infected with HPV types 16, 18, 31, or 45 and those infected with types 33, 52, or 58, although odds ratios for smoking were slightly higher among individuals infected with vaccine or cross-protective HPV types. An earlier pilot study found that smoking did not affect geometric mean titers after bivalent HPV vaccination but increased the risk of having low-avidity antibodies after vaccination [33]. Moreover, among unvaccinated young women, an impaired immune response after natural HPV infection due to smoking was suggested [34].

Strengths of the current study include the long follow-up time in a large population-based cohort; we did lose participants over time, but our cohort still has enough power to provide insight into the effects of the bivalent vaccine on the Dutch female population. Despite the yearly provided incentive, which could possibly lead to an included population with lower socioeconomic status, girls in this cohort were less likely to be second-generation migrants and were more highly educated than the general population. Therefore, we think that the effect of this possible bias on our estimates of immunogenicity of the vaccine is limited [4, 35]. We do acknowledge some limitations of the current study. The first is the limited number of (type-specific) HPV infections with regard to analyses. The high VE estimates are very reassuring [7]

but decreased the power to detect differences 1 year before infection in our analyses. Another challenge remains in the detection of infections; it could not be determined whether detected infections represent active infection of the cervix or the transient presence of HPV DNA in the lower genital tract. Furthermore, we did not have information on the exact timing of infection acquisition.

In conclusion, we observed high serum IgG antibody responses against vaccine types up to 9 years after vaccination, in a population-based setting among thrice-vaccinated girls from a catch-up campaign. Although antibody concentrations remain an important monitoring tool at population level, the question remains how insightful they are at an individual level, as long as a cutoff for protection is lacking and infections still occur despite high antibody levels. For future studies, it remains important to monitor vaccine responses but also failures, to see how infections occur and whether they can still induce lesions. In this respect, other factors such as antibody avidity and local antibodies at the site of infection, degree of HPV exposure, and possibly immune-related factors could also be interesting to take into account when evaluating HPV vaccines.

References

1. Burchell AN, Winer RL, de Sanjosé S, Franco EL. Chapter 6: epidemiology and transmission dynamics of genital HPV infection. Vaccine 2006; 24:S52–S61.
2. Smith JS, Lindsay L, Hoots B, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 2007; 121:621–32.
3. Harper DM, DeMars LR. HPV vaccines—a review of the first decade. Gynecol Oncol 2017; 146:196–204.
4. Mollers M, Scherpenisse M, van der Klis FR, et al. Prevalence of genital HPV infections and HPV serology in adolescent girls, prior to vaccination. Cancer Epidemiol 2012; 36:519–24.
5. Kavanagh K, Pollock KG, Cuschieri K, et al. Changes in the prevalence of human papillomavirus following a national bivalent human papillomavirus vaccination programme in Scotland: a 7-year cross-sectional study. Lancet Infect Dis 2017; 17:1293–302.
6. Lehtinen M, Dillner J. Clinical trials of human papillomavirus vaccines and beyond. Nat Rev Clin Oncol 2015; 12:2.
7. Donken R, King AJ, Bogaards JA, Woestenberg PJ, Meijer CJLM, de Melker HE. High effectiveness of the bivalent human papillomavirus (HPV) vaccine against incident and persistent HPV infections up to 6 years after vaccination in young Dutch women. J Infect Dis 2018; 217:1579–89.
8. Tota JE, Struyf F, Merikukka M, et al. Evaluation of type replacement following HPV16/18 vaccination: pooled analysis of two randomized trials. J Natl Cancer Inst 2017; 109.
9. Woestenberg PJ, King AJ, van Benthem BHB, et al. Bivalent vaccine effectiveness against type-specific HPV positivity: evidence for cross-protection against oncogenic types among Dutch STI clinic visitors. J Infect Dis 2018; 217:213–22.
10. Wheeler CM, Castellsagué X, Garland SM, et al. ; HPV PATRICIA Study Group. Cross-protective efficacy of HPV-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by non-vaccine oncogenic HPV types: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. Lancet Oncol 2012; 13:100–10.
11. Naud PS, Roteli-Martins CM, De Carvalho NS, et al. Sustained efficacy, immunogenicity, and safety of the HPV-16/18 AS04-adjuvanted vaccine: final analysis of a long-term follow-up study up to 9.4 years post-vaccination. Hum Vaccin Immunother 2014; 10:2147–62.
12. Harper DM, Franco EL, Wheeler CM, et al. HPV Vaccine Study group. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. Lancet 2006; 367:1247–55.
13. Palmer T, Wallace L, Pollock KG, et al. Prevalence of cervical disease at age 20 after immunisation with bivalent HPV vaccine at age 12-13 in Scotland: retrospective population study. BMJ 2019; 365:l1161.
14. Luostarinen T, Apter D, Dillner J, et al. Vaccination protects against invasive HPV-associated cancers. Int J Cancer 2018; 142:2186–7.
15. Muñoz N, Manalastas R Jr, Pitisuttithum P, et al. Safety, immunogenicity, and efficacy of quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine in women aged 24-45 years: a randomised, double-blind trial. Lancet 2009; 373:1949–57.
16. Joura EA, Giuliano AR, Iversen O-E, et al. Broad Spectrum HPV Vaccine Study. A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. N Engl J Med 2015; 372:711–23.
17. Mollers M, Vossen JM, Scherpenisse M, van der Klis FR, Meijer CJ, de Melker HE. Review: current knowledge on the role of HPV antibodies after natural infection and vaccination: implications for monitoring an HPV vaccination programme. J Med Virol 2013; 85:1379–85.

18. Turner TB, Huh WK. HPV vaccines: translating immunogenicity into efficacy. Hum Vaccin Immunother 2016; 12:1403–5.
19. Donken R, Schurink-Van't Klooster TM, Schepp RM, et al. Immune responses after 2 Versus 3 doses of HPV vaccination up to 4½ years after vaccination: an observational study among Dutch routinely vaccinated girls. J Infect Dis 2017; 215:359–67.
20. Opalka D, Matys K, Bojczuk P, et al. Multiplexed serologic assay for nine anogenital human papillomavirus types. Clin Vaccine Immunol 2010; 17:818–27.
21. Scherpenisse M, Mollers M, Schepp RM, et al. Seroprevalence of seven high-risk HPV types in the Netherlands. Vaccine 2012; 30:6686–93.
22. Kleter B, van Doorn LJ, ter Schegget J, et al. Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses. Am J Pathol 1998; 153:1731–9.
23. International Agency for Research on Cancer. Biological agents: a review of human carcinogens. Lyon, France: World Health Organization, 2012.
24. Schwarz TF, Galaj A, Spaczynski M, et al. Ten-year immune persistence and safety of the HPV-16/18 AS04-adjuvanted vaccine in females vaccinated at 15-55 years of age. Cancer Med 2017; 6:2723–31.
25. Artemchuk H, Eriksson T, Poljak M, et al. Long-term antibody response to human papillomavirus vaccines: up to 12 years of follow-up in the Finnish maternity cohort. J Infect Dis 2018; 219:582–9.
26. Pinto LA, Dillner J, Beddows S, Unger ER. Immunogenicity of HPV prophylactic vaccines: serology assays and their use in HPV vaccine evaluation and development. Vaccine 2018; 36:4792–9.
27. Scherpenisse M, Schepp RM, Mollers M, et al. A comparison of different assays to assess HPV16 and 18-specific antibodies after HPV infection and vaccination. Clin Vaccine Immunol 2013; 20:1329–32.
28. Scherpenisse M, Schepp RM, Mollers M, Meijer CJ, Berbers GA, van der Klis FR. Characteristics of HPV-specific antibody responses induced by infection and vaccination: cross-reactivity, neutralizing activity, avidity and IgG subclasses. PLoS One 2013; 8:e74797.
29. Bierl C, Karem K, Poon AC, et al. Correlates of cervical mucosal antibodies to human papillomavirus 16: results from a case control study. Gynecol Oncol 2005; 99:S262–8.
30. Scherpenisse M, Mollers M, Schepp RM, et al. Detection of systemic and mucosal HPV-specific IgG and IgA antibodies in adolescent girls one and two years after HPV vaccination. Hum Vaccin Immunother 2013; 9:314–21.
31. Petäjä T, Pedersen C, Poder A, et al. Long-term persistence of systemic and mucosal immune response to HPV-16/18 AS04-adjuvanted vaccine in preteen/adolescent girls and young women. Int J Cancer 2011; 129:2147–57.
32. van der Weele P, Breeuwsma M, Donken R, et al. Effect of the bivalent HPV vaccine on viral load of vaccine and non-vaccine HPV types in incident clearing and persistent infections in young Dutch females. PLoS One 2019; 14:e0212927.
33. Namujju PB, Pajunen E, Simen-Kapeu A, et al. Impact of smoking on the quantity and quality of antibodies induced by human papillomavirus type 16 and 18 AS04-adjuvanted virus-like-particle vaccine—a pilot study. BMC Res Notes 2014; 7:445.
34. Simen-Kapeu A, Kataja V, Yliskoski M, et al. Smoking impairs human papillomavirus (HPV) type 16 and 18 capsids antibody response following natural HPV infection. Scand J Infect Dis 2008; 40:745–51.
35. Giuliano AR, Lazcano-Ponce E, Villa L, et al. Impact of baseline covariates on the immunogenicity of a quadrivalent (types 6, 11, 16, and 18) human papillomavirus virus-like-particle vaccine. J Infect Dis 2007; 196:1153–62.

Chapter 4

Review of long-term immunogenicity following HPV vaccination: Gaps in current knowledge.

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ABSTRACT

The licensed HPV vaccines are highly efficacious and induce high levels of neutralizing antibody levels, the assumed mediators of protection. However, a correlate of protection against HPV is lacking, and the evidence is still limited as to long-term persistence of antibodies, especially following reduced dosing schedules. The World Health Organization (WHO) urges immunization of young girls as part of the strategy to eliminate cervical cancer, thus long-lasting protection is required. The current review describes long-term follow-up regarding vaccine-induced seropositivity and antibody level development following the different vaccines and dosing schedules. Implications and opportunities of long-term vaccine-induced immune responses are discussed, such as the gaps in monitoring of long-term immunogenicity, the possibilities of reduced dosing schedules, and the importance of evidence for durable immunity.

Introduction

The human and non-human papillomaviruses can be subdivided into genera. This review focusses on human papillomavirus (HPV) types from the alpha-genus, which are able to infect the human genital tract where they may cause disease [1]. Within this genus, high-risk (hr) and low-risk (lr) types can be distinguished; when hrHPV type infections persist, they have the potential to cause the development of cervical (pre-) cancer, whereas lrHPV types are associated with anogenital warts [2,3]. However, the vast majority of the infections are asymptomatic and clear spontaneously.

HPV is a very common sexually transmitted infection with an estimated cumulative lifetime risk of 80% in Western countries [4,5]. To prevent HPV infection and ultimately preclude the development of cervical cancer, prophylactic HPV vaccines have been developed. The first two were licensed in 2006 and 2007, respectively. The first is the bivalent vaccine (2vHPV), Cervarix®) [6], which targets the most important hrHPV types 16 and 18.The second is the quadrivalent vaccine (4vHPV), Gardasil® [7], which targets HPV types 6,11,16, and 18. In 2014, a vaccine targeting these four types plus five additional hrHPV types was licensed: the nonavalent vaccine (9vHPV), Gardasil9® [8]. In recent years, vaccine registration has been expanded to protection against non-cervical HPV-associated disease (including other anogenital cancers) and to males as well as females.

High efficacy of the HPV vaccines against cervical infections and lesions was shown by randomized controlled trials (RCTs), indicating protection up to 98% against virological and clinical endpoints caused by the targeted vaccine types [9]. These findings were reiterated in observational research following implementation of HPV vaccines in national immunization programs. For example, a large meta-analysis in high-income countries showed an 83% reduction of HPV16/18 prevalence among girls aged 13–19 y comparing pre- and post-vaccination implementation periods up to 8 y following implementation [10]. This reduction was likewise observed in field efficacy studies; in Australia, the prevalence of HPV16/18 was flat across age groups following vaccination [11], whereas in Sweden, a substantial risk reduction for cervical cancer was observed among vaccinated women [12]. Furthermore, HPV vaccines are known to be highly immunogenic able to provoke a solid systemic immune response, especially through the formation of antibodies [13]. Virtually all HPV-vaccinated individuals seroconvert [14] and RCTs found peak antibody levels in vaccinated individuals up to 100-fold higher compared to naturally infected individuals [15].

HPV vaccines can be provided according to a three-dose schedule (0,1–2, and 6 months), currently recommended for those 15 y and above or two-dose schedule (0,

5–13 months), recommended for those 9–14 y of age. After the initial registration of the HPV vaccines according to three-dose schedules, new evidence implied that two doses induce protection equally well. The underlying concept is known as immunobridging: Efficacy against virological and clinical endpoints was first observed among 15–26-y-old women who were vaccinated three times. Among 9-to-14-y-old girls who were vaccinated twice, non-inferior serum antibody responses were observed as compared to the three-dose-vaccinated women. Immunobridging assumes the same efficacy can be expected in groups where non-inferior antibody responses are found. Hence, from 2014 onwards, two–dose schedules were approved and advised for young vaccine recipients by the Food and Drug Administration (FDA), European Medicines Agency (EMA), and World Health Organization (WHO) [6–8].

Registered HPV vaccines are prophylactic and thus provide optimal protection among HPV-naïve individuals. Since the prevalence of HPV infection starts to rise from the beginning of sexual activity, vaccination in young adolescence is preferable, feasible, and pursued in most countries where HPV vaccination has been implemented. For optimal benefit, the induced protection should ideally cover the entire life period of sexual activity. As of 2018, the WHO has committed to elimination of cervical cancer as a global health problem. To reach this goal, one of the targets proposed for countries is to have 90% of girls fully vaccinated against HPV by age 15 [16]. While neutralizing antibodies are the supposed primary mediators of the protection, the minimum level required for protection has not yet been established, nor has the duration of protection [17]. In this review, the current knowledge on long-term immunogenicity following HPV vaccines is described and the implications for global HPV reduction are discussed.

Measurement units and assays

We can roughly distinguish three types of assays that are commonly used in RCTs to evaluate HPV antibodies following vaccination: the pseudovirus-based neutralization assay (PBNA), competitive (epitope-specific) immunoassays (cLIA), and VLP-IgG binding assay (ELISA or MIA). The first is considered relevant for measuring the biological activity, whereas the cLIA reflects neutralizing activity with high affinity. ELISA detects all antibodies regardless of neutralization [18]. Respectively, the methods require pseudovirions, type-specific monoclonal antibodies, and/or intact VLPs, and their quality affects the individual assay quality. WHO suggests PBNA was as the reference standard for assessing HPV-specific neutralizing antibodies. This method is very time-consuming and costly, but a recently developed high-throughput PBNA assay allows more assays to be processed sequentially and also has increased

sensitivity [19]. On the other hand, cLIA and ELISA/MIA are fast and suitable for high-throughput but, measure, respectively, a subset of total neutralizing antibodies and the total amount of HPV-specific antibodies.

Variations on these three assays are used in studies because reagents and assay standards are not always available and there are no official guidelines on methods for determining cutoffs. This use of varied techniques gives rise to variation in findings. For example, a comparison study showed that the PBNA is more sensitive than IgG-cLIA for the detection of HPV16- and HPV18-neutralizing antibodies [20]. Another study indicated that assays showed reasonable correlation, but that improvement in correlation could be achieved by small alterations [21]. To overcome such problems, the International Unit (IU) measure has been established for HPV16 and 18. It should be used to express findings in order to facilitate comparability,18 but not all studies use standardized measurements, and the IU for hrHPV types other than 16 and 18 has yet to be established.

Outcomes used to describe the immunogenicity of the HPV vaccines include the geometric mean concentration or titer (GMC/T), or the percentage of seropositives (i.e., number of study participants with an antibody level above a certain cutoff level). Both provide information on the long-term performance of the vaccine regarding stimulation of antibody production. Arbitrary study-specific cutoffs have been applied to determine seropositivity [13]. Additionally, antibody avidity can be used as a marker to express affinity maturation, i.e., how well an antibody binds to an antigen. This can for instance be measured with the chaotropic thiocyanate ion method in the ELISA/MIA assays [22,23]. Nevertheless, avidity is considered a crude marker for affinity maturation and should therefore be interpreted with caution.

Biological mechanisms underlying HPV vaccination

All three prophylactic HPV vaccines now on the market consist of virus-like particles (VLPs), although these are produced in various expression systems. Also, the vaccines differ in their adjuvant systems (Table 1). The VLPs resemble the L1 protein of HPV, the major capsid protein, which is morphologically indistinguishable from real HPV particles [24]. All the vaccines contain aluminum salts as an adjuvant to ensure a slow release of the antigen and activation of the innate immune system. However, the 2vHPV vaccine uses the AS04 adjuvants system, which contains both aluminum salt and monophosphoryl lipid A (MPL), which is believed to activate the innate immune response [25].

Table 1: Characteristics of the three available HPV VLP vaccines

	Cervarix® (bivalent)	Gardasil® (quadrivalent)	Gardasil9® (nonavalent)
Manufacturer	GlaxoSmithKline Biologicals, SA	Merck Sharp & Dohme	Merck Sharp & Dohme
VLP types included	HPV16 and 18	HPV6, 11, 16 and 18	HPV6, 11, 16, 18, 31, 33, 45, 52 and 58
Dose of L1 protein	20 µg (HPV16 and 18)	20 µg (HPV6 and 18), 40 µg (HPV11 and 16)	20 µg (HPV31, 33, 45, 52, 58), 30 µg (HPV6), 40 µg (HPV11 and 18), 60 µg (HPV16)
Registered for	Boys and girls ≥9 yr.	Boys and girls 9-26 yr.	Boys and girls 9-26 yr.
Adjuvant	500 µg aluminium hydroxide and 50 µg 3–0-deacylated-4′-monophosphoryl lipid A (AS04)	225 µg aluminium hydroxyphosphate sulphate (AAHS)	500 µg aluminium hydroxyphosphate sulphate (AAHS)
Schedule	9-14 years of age: two doses (0, 5-13 months) ≥15 years of age: three doses (0,1,6 months)	9-13 years of age: two doses (0, 6 months) ≥14 years of age: three doses (0,2,6 months)	9-14 years of age: two doses (0, 6 months) ≥15 years of age: three doses (0,2,6 months)

In animal models, vaccination with L1 VLPs has been shown to induce neutralizing antibody levels and protection against an HPV infection [26]. After passive transfer of immune sera, naïve animals were protected against infection [17]. These findings were supportive of the general assumption that protection following HPV vaccination is primarily antibody-mediated. Even more supportive were trials in human participants, which showed high and durable, type-restricted titers of VLP antibodies after vaccination [22].

Naïve B- and T-cell activation is important in HPV antibody production. Upon entering the body, the VLP antigen part of the vaccine is bound to antigen-presenting cells (APCs). The antigen is then presented to T-cells, which have various functions and can differentiate into one of several T-cell lineages, including cytotoxic, T-helper, or memory T-cells. The T-helper cells in turn stimulate naive B-cells to become either plasma cells or memory B-cells [27]. Long-lived plasma cells (LLPC) are generated upon vaccination and secrete antigen-specific antibodies, thereby enabling the persistence of circulating antibodies. However, circulating memory B-cells can be still detected after vaccination and could therefore also assist in a rapid recall when the HPV antigen is encountered again [28]. Thus, LLPCs, memory B- and T-cells are essential for establishing long-term protection, i.e., by inducing and maintaining high levels of neutralizing antibodies.

Neutralizing antibodies are the assumed mediators of protection following HPV vaccination. They can bind to a virion and prevent it from binding to a cell, thereby neu-

tralizing the toxin. There are various isotype forms of antibodies, IgG and IgA, which can be further subdivided. After HPV vaccination the subclasses IgG1 and IgG3 are most frequently detected [17]. Serum antibodies are thought to arrive at the side of infection via exudate (antibody leak from a damaged blood vessel or membrane) and/or transudate (antibody transfer from the intravascular compartment due to an imbalance of hydrostatic or oncotic pressure or though antibody-transporting receptors) to block HPV binding to the basement membrane [29]. To date, no correlate of protection has been established for HPV, as vaccine efficacy after HPV vaccination is high, with few breakthrough infections and hence few vaccinated individuals who are infected with vaccine types. This limits the opportunities to study which antibody levels are needed to give adequate protection. Also, studies might be biased by the difficulties in distinguishing rare breakthrough infections from emergence of prevalent infection at the time of vaccination or reactivation of latent infection [30].

Unvaccinated individuals acquiring an HPV infection can likewise develop an immune response. However, detectable antibody levels are not always present after a natural infection (not everyone seroconverts), and it is not known whether a previous infection protects against subsequent exposure to the same HPV type [31]. Nonetheless, GMC/Ts reached among naturally infected individuals can provide benchmarks for the evaluation of antibody levels after vaccination [32].

Long-term immune responses following three doses of HPV vaccination

Seropositivity rates

Given the initial registration for HPV vaccination, the longest follow-up has been reported in studies adhering to this schedule. In Table 2, an overview is given of RCTs conducted for the three different vaccines with follow-up of seropositivity rates. Diverse study populations have been included, both younger and older age groups and women and men. For all three vaccines, follow-up was at least 7.5 y, and the longest was 14 y for the 4vHPV vaccine. ELISA and cLIA were the assays commonly used to assess antibody levels. Although high seroprevalence rates were maintained, a slight decline was observed with increased follow-up, notably for HPV18 after 4vHPV (47.9% seropositive after 4 y) [44]. Previous research indicated a decline in seropositivity for HPV18 after 4vHPV vaccination, but no breakthrough infections/lesions were reported [48]. However, follow-up may have been too short and statistical power too limited to fully examine this.

Table 2: Long-term seropositivity and geometric mean concentration (GMC) following three doses of HPV vaccination (in RCTs) [33–47].

Vaccine	Study and population (age at vaccination)	Immunogenicity endpoint: percentage seropositive	Immunogenicity endpoint: GMC/T	Techniques	Follow-up
2vHPV	NCT00309166 ♂ 10-14y	HPV16 100% HPV18 100%	27891.6 EU/mL 10593.7 EU/mL	ELISA	7m
	VIVIANE ♀ >25y to at least 46y	26-35y: HPV16 100% HPV18 98.0% 36-45y: HPV16 100% HPV18 97.1% >45y: HPV16 95.7% HPV18 93.3%	n.a.	ELISA	7y
	CVT ♀ 18-25y	HPV16 100% HPV18 100%	716 EU/mL 322 EU/mL	ELISA	7y
	HPV001/007/023 ♀ 15-25y	HPV16 100% HPV18 100%	n.a.	ELISA	9.4y
	NCT00196924 ♀ 10-14y	HPV16 100% HPV18 100% HPV-31 87.7% HPV-45 85.1%	1589.9 EU/mL 597.2 EU/mL 242.9 EU/mL 204.7 EU/mL	ELISA	10y
	NCT00196937 ♀ 15-55y	15-25y: HPV16 100% HPV18 99.2% 26-45y: HPV16 99.2% HPV18 93.7% 46-55y: HPV16 96.3% HPV18 83.3%	965.4 EU/mL 321.1 EU/mL 334.4 EU/mL 115.4 EU/mL 157.4 EU/mL 69.7 EU/mL	ELISA	10y
4vHPV	MAM study ♂ 27-45y	HPV06 100% HPV11 100% HPV16 100% HPV18 100%	419.5 mMu/mL 516.6 mMu/mL 2228.6 mMu/mL 300.0 mMu/mL	cLIA	7m
	NCT00090285 ♂ 16-26y	HPV06 88.9% HPV11 94.0% HPV16 97.9% HPV18 57.0%	71.5 mMu/mL 82.6 mMu/mL 293.3 mMu/mL 33.1 mMu/mL	cLIA	3y
	NCT00090220 ♀ 24-45y	HPV06 91.5% HPV11 92.0% HPV16 97.4% HPV18 47.9%	61.0 mMu/mL 66.0 mMu/mL 202.0 mMu/mL 23.0 mMu/mL	cLIA	4y
	V501-018 ♀ ♂ 9-16y	9-12y: HPV06 90.1% HPV11 89.7% HPV16 97.0% HPV18 90.1% 13-16y: HPV06 86.8% HPV11 86.8% HPV16 94.0% HPV18 86.8%	91.4 mMu/mL 78.7 mMu/mL 336.4 mMu/mL 41.0 mMu/mL 76.9 mMu/mL 66.9 mMu/mL 289.4 mMu/mL 28.9 mMu/mL	cLIA	10.5y
	FUTURE(I/II) ♀ 16-23y	HPV06 90.6% HPV11 91.1% HPV16 98.3% HPV18 52.4%	78.4 mMu/mL 66.8 mMu/mL 291.2 mMu/mL 26.1 mMu/mL	cLIA	14y

Table 2: Continued

Vaccine	Study and population (age at vaccination)	Immunogenicity endpoint: percentage seropositive	Immunogenicity endpoint: GMC/T	Techniques	Follow-up
9vHPV	V503-003, NCT01651949	HPV06 99.6%	782.0 mMu/mL	cLIA	7m
		HPV11 100%	616.7 mMu/mL		
		HPV16 100%	3346.0 mMu/mL		
	♂ 16-26y	HPV18 99.9%	808.2 mMu/mL		
		HPV31 100%	708.5 mMu/mL		
		HPV33 100%	384.8 mMu/mL		
		HPV45 99.8%	235.6 mMu/mL		
		HPV52 100%	386.8 mMu/mL		
		HPV58 100%	509.8 mMu/mL		
	V503-002	HPV06 98.5%	252.8 mMu/mL	cLIA	3y
		HPV11 99.3%	145.8 mMu/mL		
	♀ 9-15y	HPV16 99.8%	857.4 mMu/mL		
		HPV18 94.5%	167.8 mMu/mL		
		HPV31 99.3%	216.6 mMu/mL		
		HPV33 98.5%	94.1 mMu/mL		
		HPV45 93.8%	64.7 mMu/mL		
		HPV52 99.0%	109.6 mMu/mL		
		HPV58 99.0%	147.4 mMu/mL		
	V503-002	HPV06 98.7%	262.7 mMu/mL	cLIA	3y
		HPV11 98.3%	156.6 mMu/mL		
	♂ 9-15y	HPV16 99.6%	944.1 mMu/mL		
		HPV18 96.6%	244.2 mMu/mL		
		HPV31 98.5%	246.3 mMu/mL		
		HPV33 98.7%	120.8 mMu/mL		
		HPV45 93.0%	76.7 mMu/mL		
		HPV52 97.9%	104.9 mMu/mL		
		HPV58 99.1%	170.9 mMu/mL		
	NCT00543543	HPV06 95.0%	143.1 mMu/mL	cLIA	5y
		HPV11 95.5%	82.9 mMu/mL		
	♀ 16-26y	HPV16 100%	324.4 mMu/mL		
		HPV18 77.5%	62.5 mMu/mL		
		HPV31 96.3%	69.2 mMu/mL		
		HPV33 96.5%	44.7 mMu/mL		
		HPV45 81.1%	20.8 mMu/mL		
		HPV52 91.0%	33.7 mMu/mL		
		HPV58 92.4%	50.9 mMu/mL		
	V503-002; NCT00943722	HPV06 94.0%	135.4 mMu/mL	cLIA	7.5y
		HPV11 91.1%	87.8 mMu/mL		
		HPV16 99.5%	490.4 mMu/mL		
	♀ 9-15y	HPV18 96.8%	150.0 mMu/mL		
		HPV31 95.9%	125.8 mMu/mL		
		HPV33 95.0%	65.3 mMu/mL		
		HPV45 92.4%	48.9 mMu/mL		
		HPV52 96.8%	69.7 mMu/mL		
		HPV58 98.6%	85.6 mMu/mL		
	V503-002; NCT00943722	HPV06 88.2%	139.0 mMu/mL	cLIA	7.5y
		HPV11 90.4%	94.6 mMu/mL		
		HPV16 99.5%	497.9 mMu/mL		
	♂ 9-15y	HPV18 96.1%	161.4 mMu/mL		
		HPV31 96.1%	138.8 mMu/mL		
		HPV33 92.8%	76.7 mMu/mL		
		HPV45 96.0%	58.1 mMu/mL		
		HPV52 92.8%	63.8 mMu/mL		
		HPV58 98.5%	103.5 mMu/mL		

n.a. = not available, geometric mean concentration or titer was not specified; EU = ELISA units; mMu = milli-Merck units

Long-term seropositivity appears to be higher when the vaccine is administered at a younger age as compared to older populations, especially those 25 y or older [43]. In general, males and females were comparable regarding achieved seropositivity rates, but small differences have been observed [46]. This might be due to different populations across studies or minor differences in immune response following vaccination [49]. The 2vHPV and 4vHPV vaccine also induce some cross-protection against non-targeted hrHPV types [50]. For example, after 2vHPV vaccination, seropositivity rates for HPV31 and HPV45 were slightly lower compared to the 9vHPV vaccine and were maintained until at least 10 y [42]. For the 4vHPV vaccine also, cross-protection for these types was observed, although seropositivity rates were slightly lower than for the 2vHPV vaccine [51,52].

Similar to RCTs, observational studies have been conducted to monitor the long-term immunogenicity following the implementation of HPV vaccination into national immunization programs. High seroprevalence for HPV16 and HPV18 up to 12 y after 2vHPV vaccination was observed [30,53]. Also for cross-protective types HPV31, 33 and 45, persisting seropositivity rates up to 12 y were reported [53]. For the 4vHPV vaccine, comparable vaccine-type immunogenicity following three doses was shown in observational studies [54] Regarding cross-reactivity, seropositivity rates were generally lower for non-vaccine types following 4vHPV compared to 2vHPV vaccination [55]. A 5-y observational follow-up study of the 9vHPV vaccine indicated long-term seropositivity for all types included in the vaccine, with patterns comparable to those in RCTs [56].

Antibody levels over time

Antibody levels against HPV16/18 and cross–protective types over time are reported from an observational cohort study in which 15–16-y-old girls received three doses of 2vHPV vaccination (Figure 1) [30]. An initial peak is observed, followed by a rapid, significant decline for the vaccine types between y 2 and 3 post-vaccination. Thereafter a more gradual antibody decline is observed as time progresses. Antibody levels against cross-protective types show a comparable pattern at a lower level. GMCs against HPV16 are higher than for other types from the start and remain so over time [30].

In Table 2, GMC/Ts from the RCTs are included if reported. Although various arbitrary measurement units were used, the pattern of antibody level development for all the vaccines and HPV types is comparable to the one described above [42,45–47,57,58]. However, the initial decline in HPV18 following both the 4vHPV and 9vHPV vaccination seems more pronounced as measured by cLIA assay (less so in the total binding assay), which could also explain the observed faster decline in

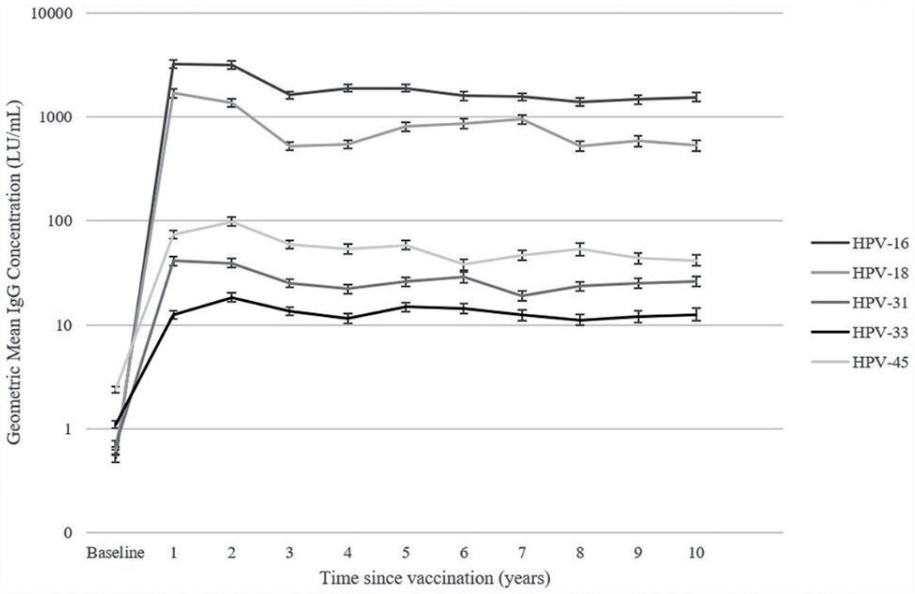

Figure 1: Adapted from Hoes et al. [30] with 1 additional y of follow-up. Geometric mean concentration (GMC) of HPV16, 18, 31, 33, and 45 IgG before and every year after 3 doses of 2vHPV vaccination (declining number of women due to loss-to-follow–up).

seropositivity rates. Furthermore, there were small deviations between the 4vHPV and 9vHPV vaccine that could be related to changes in the VLP concentration. Regarding cross-protective types, the observed responses are higher following 2vHPV compared to 4vHPV vaccination.59 In the first 7 months post-vaccination, the 2vHPV vaccine induced neutralizing HPV31/33/45/52/58 antibodies significantly more often and to higher levels than did the 4vHPV vaccine [52]. Neutralizing antibodies remained detectable up to at least 7–12 y post-vaccination, but with expected three- to fourfold higher titers after 2vHPV vaccination than 4vHPV vaccination [55,60]. Evidenced by clinical trial data, cross protection against HPV31 and HPV45 and subsequent lesions indeed seems to be higher following 2vHPV compared to 4vHPV vaccination [50], which could be due to the observed higher antibody levels.

Statistical modeling studies indicated that among young girls (10–14 y) receiving timely 2vHPV vaccination, durability of antibody levels above natural infection level was predicted to be 70.1 y for anti-HPV-16 and 78.8 y for anti-HPV–18, or even lifelong, depending on the model used.42 Another modeling study among older women receiving HPV vaccination (15–55 y) indicated that antibody levels after 2vHPV vaccination for vaccine types HPV16 and 18 would remain higher than after natural infection for up to 30 y. However, the age at which participants received vaccination was important, as with older age the predicted prolonged immune response decreased,

probably due to lower initial antibody responses [43]. For the 4vHPV vaccine, the predicted GMTs up to 20 y after vaccination were also above the level induced by natural infection for anti-HPV-16 antibodies but below the natural infection level for anti-HPV–18 antibodies (among 18–45-y-olds). In general, longer durability of antibodies was predicted following 2vHPV than 4vHPV vaccination [61]. This is likely due to the high initial antibody levels after 2vHPV vaccination.

Some RCTs compared the different vaccines directly. In general, they showed that the 2vHPV vaccine induces higher antibody responses for both HPV16 and 18 compared to the 4vHPV vaccine (up to threefold higher as measured by PBNA) [62,63], while 9vHPV vaccination induced anti-HPV16/18 responses similar to the 4vHPV vaccine, as measured by cLIA [9,64]. Especially, the response against HPV18 differs between the 2vHPV and 4vHPV vaccine, as the 4vHPV vaccine was less immunogenic for HVP18 (shown in lower GMTs in the first year following vaccination) [62]. This difference persisted with increased follow-up [65]. A comparison trial between the 2vHPV and 9vHPV vaccine is ongoing (NCT02834637).

Other immune parameters

Besides seroprevalence and antibody levels, other immune parameters may provide an indication of long-term protection following HPV vaccination. Research showed that avidity levels of antibodies against vaccine types increased with every dose of 2vHPV vaccination, peaked after the third, and remained relatively constant up to 3 y post-vaccination [66]. Nevertheless, a correlation between avidity and neutralizing antibody levels could not be established, suggesting that neutralizing activity of antibodies is relatively independent of their avidity (once a threshold level is reached following primary vaccination) [66]. Furthermore, a study comparing two and three doses of 2vHPV vaccination indicated no differences in avidity up to four and a half years post-vaccination [67], leaving the correlation between avidity and number of doses inconclusive.

Another parameter that is less well studied is cellular immunity following HPV vaccination. Research showed that both the 2vHPV and 4vHPV vaccines give an HPV-specific memory B- and T-cell response [68,69] up to at least 4 y post-vaccination [63]. Age at vaccination was found to impact memory B-cell formation, whereas T-cell memory formation influenced by dose number but not by age of vaccination [70]. Recipients of the 2vHPV vaccine showed higher numbers of memory B-cells after vaccination compared to those receiving 4vHPV vaccine [63,71]. Likewise, for HPV31 and HPV45 numbers of both memory T- and memory B-cells were detected up to 36 months post-vaccination for the 2vHPV vaccine. Again, this level of cross-protection was higher for the 2vHPV compared to the 4vHPV vaccine [72]. Generally speaking,

although cell-mediated immune effectors provide information on the responsiveness to the vaccine, they do not directly indicate how well the vaccine protects or how long antibodies are maintained (which is due to the production by LLPCs) [22]. Nevertheless, further research on the long-term persistence of memory B- and T-cells could provide an indication for the sustainability of protection from disease, as is the case in hepatitis B studies [73].

Immune responses following fewer than three doses of HPV vaccination

Non-inferiority of the two-dose schedule

To compare immune responses for various dosing schedules, GMT/C ratios are often used (in combination with non-inferiority margins). For all three vaccines, RCTs comparing two (at the required 0-, >5-month interval)- and three-dose schedules have been conducted indicating non-inferiority of the two-dose schedule regarding the vaccine types (among girls aged 9–14 y) according to study-dependent cutoffs, when compared to a three-dose schedule (in women aged 15–26 y) [74–76]. The longest follow-up with direct comparison of doses was measured for the 4vHPV vaccine (up to 10 y post-vaccination), showing sustained immunogenicity for HPV6/11/16/18 and steady seropositivity rates, following two doses [77]. However, a meta–analysis showed that, compared to three doses, two doses of the 4vHPV vaccine could produce an inferior antibody response for HPV18 within 18 months and, likewise, two doses of 2vHPV could produce an inferior responses for HPV16 within 2 y (again, women receiving three doses were older, at 15–26 y, than those receiving two doses, at 9–13 y) [78]. Moreover, a study by Leung and colleagues compared the 2vHPV and 4vHPV vaccine in a two-dose schedule and found that, as with three doses, the 2vHPV vaccine elicits antibody responses that are up to sixfold higher for the vaccine types [65] compared to 4vHPV.

Antibody levels and the development of GMC/T over time are comparable following two doses and three doses of the same vaccine, at least for the first period (up to 36 months) post–vaccination [75,76,79,80]. Among young girls (9–14 y) receiving two doses of 2vHPV vaccination, modeling studies predicted lifelong durability of antibody levels above natural infection level, comparable to the three-dose schedule [75]. No differences in avidity following a two-dose 2vHPV schedule were observed at months 7, 24 or 48 post-vaccination, suggesting that the quality of the antibody response in terms of avidity was similar in the two-dose recipients compared to three-dose recipients [81]. Also following two doses, cross-protection against non-vaccine types can be observed. For the 2vHPV vaccine, similar antibody concentrations against

HPV31 and HPV45 were measured up to 5 y after both two and three doses of vaccination [72]. For the 4vHPV vaccine, higher antibody concentrations against HPV31 were observed up to 6 y after one-, two-, or three-dose-vaccination as compared to no vaccination, but this was not observed for other cross-protective serotypes [82]. This difference in antibody response against cross-protective types between vaccines might be due to the AS04 adjuvant system used in the 2vHPV vaccine, which is claimed to induce a broader immune response and may hence lead to higher antibody levels [51].

One-dose HPV vaccination

Besides RCTs, other prospective studies investigated optimal dosing schedules, including one-dose vaccination. This most reduced number of doses makes HPV vaccination cheaper and logistically more accessible, especially for low- and middle-income countries. Nevertheless, one-dose delivery would require sufficiently high efficacy and long-term immunogenicity. Several studies have shown seropositivity for HPV16 and HPV18 following one-dose 2vHPV vaccination (up to 11 y of follow-up), although antibody levels were lower compared to two or three doses [41,83]. With one-dose delivery of 4vHPV, avidity was non-inferior, and detectable concentrations of neutralizing antibodies to all four vaccine-targeted HPV types were present, but at much lower concentration after one dose than after two/three doses (up to 6 y of follow-up) and seropositivity rates decreased rapidly; however, protection from infection seemed comparable between dosing schedules [54,84,85]. Another study indicated that besides lower antibody levels following one-dose 2vHPV, also lower levels of memory B- and T-cells were measured. Altogether, these findings suggest that one-dose vaccine recipients are at higher risk of waning immunity [86]. Despite the potential of the one dose schedule, the trade-off between costs and accessibility on one side and the effectiveness and immunogenicity on the other should be considered carefully, especially regarding long-term protection.

Implications of long-term HPV vaccine-induced immunogenicity

Long–term protection following HPV vaccination is important to prevent people from acquiring an HPV infection throughout their lives. Therefore, protection during sexually active life is desirable, as this reduces lifetime risk of HPV infection and subsequent disease, and supports the WHO goal to eliminate cervical cancer [16]. If protection following vaccination persists for a limited amount of time, the peak prevalence of HPV infections may shift toward older age, influencing the outcomes of and the need for secondary prevention (i.e., screening) of cervical cancer. The pattern in which HPV prevalence peaks and thus protection is needed might differ geographi-

cally, causing the HPV burden to be unequally distributed across countries. In higher income countries, the HPV infection prevalence peaks between 20 and 25 y of age and is followed by a decline and finally a plateau in prevalence [87]. However, a large meta–analysis including 1 million women with normal cytology from all over the world indicated that HPV prevalence in less developed countries remains at a higher level following the initial peak. In some cases, a second peak can be observed around age 45-65, although the actual risk of developing cancer from these infections might be limited [88].

While a correlate of protection is still lacking for HPV, protection is assumed to be antibody-mediated. The three vaccines are very immunogenic and induce solid protection against HPV infections and cervical lesions [9], which could indicate that protection will last if there is a high and detectable immune response. In general, long-term follow-up studies align with regard to immunogenicity. Following all three vaccines, seropositivity rates for targeted vaccine types remain high up to at least 7.5 y after vaccination, with few indications of waning. This finding is underscored by modeling studies and the development of GMC/Ts over time. Nevertheless, some decline of 4vHPV-induced antibodies against HPV18 is observed with all dosing schedules [44,78]. This could indicate an increased risk of waning immunity, although no supporting evidence in the form of breakthrough cases has been reported [48]. The observation does emphasize the importance of continued immunosurveillance and proper comparisons between vaccines, e.g., between 2vHPV, which induces the highest levels of cross protection, and 9vHPV, which provides broad-spectrum protection.

In Figure 2, the vaccines are summarized as to their dosing schedules and longest reported follow-up of immunogenicity. It shows that the current knowledge gaps mainly concern long-term follow-up of reduced dosing schedules and 9vHPV vaccine, all requiring further research. Despite the benefits of immunosurveillance over clinical surveillance (e.g., less invasive, easier to collect samples, especially from males), vaccine efficacy is often seen as most important outcome, with immunogenicity outcomes considered separately [9]. Accordingly, research is needed to study the linkage between protection and observed immune responses over time, which can aid in our understanding of long-term efficacy following HPV vaccination. However, such research remains challenging due to the low number of breakthrough infections, the large confidence intervals around effectiveness estimates, and the variety in antibody levels among individuals [30]. In RCTs, prolonged or incidental follow-up of vaccine recipients with relatively low antibody response reveal the individual level of antibodies that must be achieved for protection.

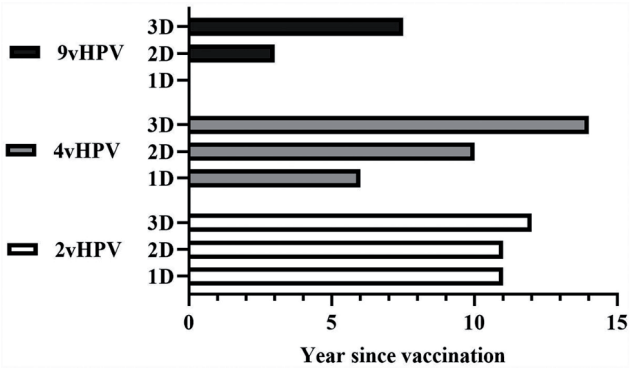

Figure 2: Longest reported follow-up time concerning antibody levels and/or seropositivity rate. Studies can be both RCT or observational. Stratified for HPV vaccine and dosing schedule [38,47,53,76,77,83,89].

Vaccine uptake, age at vaccination, and the optimal dosing schedule remain critical research areas. Since HPV vaccination was introduced, uptake has remained behind in low- and middle-income countries, although they have the highest burden and minimal screening opportunities [90]. Even in high-income countries uptake has been suboptimal, possibly due to negative media attention or parental concerns [91]. Thus, increased effort is required to offer timely vaccination to young girls around the globe. Age of vaccination remains important, since vaccination at higher ages increases pre-vaccination HPV exposure risk. Moreover, the number of doses might affect GMT/Cs; more evidence is needed on the non-inferiority and long-term follow-up of a one-dose schedule regarding both immunogenicity and effectiveness [92]. Besides RCTs in which some participants received one dose and virological endpoints were evaluated [41,93], there are ongoing comparison studies between two- and one-dose recipients as to both non-inferiority of immune response and protection against clinical outcomes (NCT03180034 and NCT03675256, both focus on 2vHPV and 9vHPV vaccination). Additionally, some early initiated studies will continue their follow-up and frequently report their findings [92] Evidence for or against one-dose vaccination, based on efficacy against persistent infection and immunogenicity as to targeted HPV types, will be available from the RCTs and other studies in the coming years, aiding in the evaluation and formal implementation of a one-dose schedule.

To conclude, long-term immunogenicity following HPV vaccination looks promising, with little indication for a decline. Future studies should focus on establishing a correlate of protection in order to optimize dosing schedules and to realize sustained protection through sexually active life. This will aid in reducing HPV infections and subsequent disease, with the ultimate goal of worldwide elimination of cervical cancer.

References

1. De Villiers E-M, Fauquet C, Broker TR, Bernard H-U, zur Hausen H. Classification of papillomaviruses. Virology. 2004;324(1):17–11. doi:10.1016/j.virol.2004.03.033.
2. Chaturvedi AK. Beyond cervical cancer: burden of other HPV-related cancers among men and women. J Adolesc Health. 2010;46(4):S20–S6. doi:10.1016/j.jadohealth.2010.01.016.
3. Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, Stanley MA. The biology and lifecycle of human papillomaviruses. Vaccine. 2012;30:F55–F70. doi:10.1016/j.vaccine.2012.06.083.
4. Chesson HW, Dunne EF, Hariri S, Markowitz LE. The estimated lifetime probability of acquiring human papillomavirus in the United States. Sex Transm Dis. 2014;41(11):660. doi:10.1097/OLQ.0000000000000193.
5. Syrjänen K, Hakama M, Saarikoski S, Väyrynen M, Yliskoski M, Syrjänen S, Kataja V, Castren O. Prevalence, incidence, and estimated life-time risk of cervical human papillomavirus infections in a nonselected Finnish female population. Sex Transm Dis. 1990;17(1):15–19. doi:10.1097/00007435-199017010-00004.
6. Committee for Medicinal Products for Human Use (CHMP). European public assessment report (EPAR) for Cervarix. UK: European Medicines Agency. 2009.
7. Committee for Medicinal Products for Human Use (CHMP). European public assessment report (EPAR) for Gardasil. UK: European Medicines Agency, 2009.
8. Committee for Medicinal Products for Human Use (CHMP). European public assessment report (EPAR) for Gardasil9. UK: European Medicines Agency, 2015.
9. Harper DM, DeMars LR. HPV vaccines - a review of the first decade. Gynecol Oncol. 2017;146(1):196–204. doi:10.1016/j.ygyno.2017.04.004.
10. Drolet M, Bénard É, Pérez N, Brisson M, Ali H, Boily M-C, Baldo V, Brassard P, Brotherton JML, Callander D, et al. Population-level impact and herd effects following the introduction of human papillomavirus vaccination programmes: updated systematic review and meta-analysis. Lancet. 2019;394(10197):497–509. doi:10.1016/S0140-6736(19)30298-3.
11. Brotherton JM, Hawkes D, Sultana F, Malloy MJ, Machalek DA, Smith MA, Garland SM, Saville M. Age-specific HPV prevalence among 116,052 women in Australia's renewed cervical screening program: a new tool for monitoring vaccine impact. Vaccine. 2019;37(3):412–16. doi:10.1016/j.vaccine.2018.11.075.
12. Lei J, Ploner A, Elfström KM, Wang J, Roth A, Fang F, Sundström K, Dillner J, Sparén P. HPV vaccination and the risk of invasive cervical cancer. N Engl J Med. 2020;383(14):1340–48. doi:10.1056/NEJMoa1917338.
13. Turner TB, Huh WK. HPV vaccines: translating immunogenicity into efficacy. Hum Vaccine Immunother. 2016;12(6):1403–05. doi:10.1080/21645515.2015.1103936.
14. Harro CD, Pang -Y-YS, Roden RB, Hildesheim A, Wang Z, Reynolds MJ, Mast TC, Robinson R, Murphy BR, Karron RA, et al. Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like particle vaccine. J Natl Cancer Inst. 2001;93(4):284–92. doi:10.1093/jnci/93.4.284.
15. Petaja T, Pedersen C, Poder A, Strauss G, Catteau G, Thomas F, Lehtinen M, Descamps D. Long-term persistence of systemic and mucosal immune response to HPV-16/18 AS04-adjuvanted vaccine in preteen/adolescent girls and young women. Int J Cancer. 2011;129(9):2147–57. doi:10.1002/ijc.25887.
16. World Health Organization. Draft: global strategy towards eliminating cervical cancer as a public health problem. World Health Organization: Geneva, 2020.

17. Schiller JT, Lowy DR. Understanding and learning from the success of prophylactic human papillomavirus vaccines. Nat Rev Microbiol. 2012;10(10):681–92. doi:10.1038/nrmicro2872.
18. Pinto LA, Dillner J, Beddows S, Unger ER. Immunogenicity of HPV prophylactic vaccines: serology assays and their use in HPV vaccine evaluation and development. Vaccine. 2018;36(32 Pt A):4792–99. doi:10.1016/j.vaccine.2017.11.089.
19. Sehr P, Rubio I, Seitz H, Putzker K, Ribeiro-Müller L, Pawlita M, Müller M. High-throughput pseudovirion-based neutralization assay for analysis of natural and vaccine-induced antibodies against human papillomaviruses. PloS One. 2013;8(10):e75677. doi:10.1371/journal.pone.0075677.
20. Brown D, Müller M, Sehr P, Pawlita M, Seitz H, Rubio I, Antonello J, Radley D, Roberts C, Saah A, et al. Concordance assessment between a multiplexed competitive Luminex immunoassay, a multiplexed IgG Luminex immunoassay, and a pseudovirion-based neutralization assay for detection of human papillomaviruse types 16 and 18. Vaccine. 2014;32(44):5880–87. doi:10.1016/j.vaccine.2014.08.004.
21. Scherpenisse M, Schepp RM, Mollers M, Mooij SH, Meijer CJ, Berbers GA, van der Klis FR. A comparison of different assays to assess HPV16 and 18-specific antibodies after HPV infection and vaccination. Clin Vaccine Immunol. 2013;CVI:00153–13.
22. Schiller J, Lowy D. Explanations for the high potency of HPV prophylactic vaccines. Vaccine. 2018;36(32):4768–73. doi:10.1016/j.vaccine.2017.12.079.
23. Scherpenisse M, Schepp RM, Mollers M, Meijer CJLM, Berbers GAM, van der Klis FRM. Characteristics of HPV-specific antibody responses induced by infection and vaccination: cross-reactivity, neutralizing activity, avidity and IgG subclasses. PloS One. 2013;8(9):e74797. doi:10.1371/journal.pone.0074797.
24. Schiller JT, Lowy DR Papillomavirus-like particles and HPV vaccine development. Seminars in cancer biology; 1996; Elsevier. p. 373–82. ,
25. Didierlaurent AM, Morel S, Lockman L, Giannini SL, Bisteau M, Carlsen H, Kielland A, Vosters O, Vanderheyde N, Schiavetti F, et al. AS04, an aluminum salt-and TLR4 agonist-based adjuvant system, induces a transient localized innate immune response leading to enhanced adaptive immunity. J Immunol. 2009;183(10):6186–97. doi:10.4049/jimmunol.0901474.
26. Day PM, Kines RC, Thompson CD, Jagu S, Roden RB, Lowy DR, Schiller JT. In vivo mechanisms of vaccine-induced protection against HPV infection. Cell Host Microbe. 2010;8(3):260–70. doi:10.1016/j.chom.2010.08.003.
27. Stanley M. Potential mechanisms for HPV vaccine-induced long-term protection. Gynecol Oncol. 2010;118(1):S2–S7. doi:10.1016/j.ygyno.2010.04.002.
28. Siegrist C-A. Vaccine immunology. Vaccines. 2008;5:17–36.
29. Stanley M. Immunobiology of HPV and HPV vaccines. Gynecol Oncol. 2008;109(2):S15–S21. doi:10.1016/j.ygyno.2008.02.003.
30. Hoes J, Pasmans H, Knol MJ, Donken R, Van Marm-wattimena N, Schepp RM, King AJ, van der Klis FRM, De Melker HE. Persisting antibody response 9 years after bivalent Human Papillomavirus (HPV) vaccination in a Cohort of Dutch women: immune response and the relation to genital HPV infections. J Infect Dis. 2020;221(11):1884–94. doi:10.1093/infdis/jiaa007.
31. Mollers M, Vossen JM, Scherpenisse M, van der Klis FR, Meijer CJ, De Melker HE. Current knowledge on the role of HPV antibodies after natural infection and vaccination: implications for monitoring an HPV vaccination programme. J Med Virol. 2013;85(8):1379–85. doi:10.1002/jmv.23616.
32. Schwarz TF, Spaczynski M, Schneider A, Wysocki J, Galaj A, Perona P, Poncelet S, Zahaf T, Hardt K, Descamps D, et al. Immunogenicity and tolerability of an HPV-16/18 AS04-adjuvanted

prophylactic cervical cancer vaccine in women aged 15–55 years. Vaccine. 2009;27(4):581–87. doi:10.1016/j.vaccine.2008.10.088.

33. Petäjä T, Keränen H, Karppa T, Kawa A, Lantela S, Siitari-Mattila M, Levänen H, Tocklin T, Godeaux O, Lehtinen M, et al. Immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine in healthy boys aged 10–18 years. J Adolesc Health. 2009;44(1):33–40. doi:10.1016/j.jadohealth.2008.10.002.
34. Wheeler CM, Skinner SR, Del Rosario-Raymundo MR, Garland SM, Chatterjee A, Lazcano-Ponce E, Salmerón J, McNeil S, Stapleton JT, Bouchard C, et al. Efficacy, safety, and immunogenicity of the human papillomavirus 16/18 AS04-adjuvanted vaccine in women older than 25 years: 7-year follow-up of the phase 3, double-blind, randomised controlled VIVIANE study. Lancet Infect Dis. 2016;16(10):1154–68. doi:10.1016/S1473-3099(16)30120-7.
35. Naud PS, Roteli-Martins CM, De Carvalho NS, Teixeira JC, de Borba PC, Sanchez N, Zahaf T, Catteau G, Geeraerts B, Descamps D. Sustained efficacy, immunogenicity, and safety of the HPV-16/18 AS04-adjuvanted vaccine: final analysis of a long-term follow-up study up to 9.4 years post-vaccination. Hum Vaccine Immunother. 2014;10(8):2147–62. doi:10.4161/hv.29532.
36. Giuliano AR, Isaacs-Soriano K, Torres BN, Abrahamsen M, Ingles DJ, Sirak BA, Quiterio M, Lazcano-Ponce E. Immunogenicity and safety of Gardasil among mid-adult aged men (27–45 years)—the MAM study. Vaccine. 2015;33(42):5640–46. ,
37. Hillman RJ, Giuliano AR, Palefsky JM, Goldstone S, Moreira ED, Vardas E, Aranda C, Jessen H, Ferris DG, Coutlee F, et al. Immunogenicity of the quadrivalent human papillomavirus (type 6/11/16/18) vaccine in males 16 to 26 years old. Clin Vaccine Immunol. 2012;19(2):261–67. doi:10.1128/CVI.05208-11.
38. Kjaer SK, Nygård M, Sundström K, Dillner J, Tryggvadottir L, Munk C, Berger S, Enerly E, Hortlund M, Ágústsson ÁI, et al. Final analysis of a 14-year long-term follow-up study of the effectiveness and immunogenicity of the quadrivalent human papillomavirus vaccine in women from four Nordic countries. EClinicalMedicine. 2020;23:100401. doi:10.1016/j.eclinm.2020.100401.
39. Castellsagué X, Giuliano A, Goldstone S, Guevara A, Mogensen O, Palefsky JM, Group T, Shields C, Liu K, Maansson R, et al. Immunogenicity and safety of the 9-valent HPV vaccine in men. Vaccine. 2015;33(48):6892–901. doi:10.1016/j.vaccine.2015.06.088.
40. Huh WK, Joura EA, Giuliano AR, Iversen OE, de Andrade RP, Ault KA, Bartholomew D, Cestero RM, Fedrizzi EN, Hirschberg AL, et al. Final efficacy, immunogenicity, and safety analyses of a nine-valent human papillomavirus vaccine in women aged 16–26 years: a randomised, double-blind trial. Lancet. 2017;390(10108):2143–59. doi:10.1016/S0140-6736(17)31821-4.
41. Safaeian M, Sampson JN, Pan Y, Porras C, Kemp TJ, Herrero R, Quint W, Van Doorn LJ, Schussler J, Lowy DR, et al. Durability of protection afforded by fewer doses of the HPV16/18 vaccine: the CVT trial. J Natl Cancer Inst. 2018;110(2):205–12. doi:10.1093/jnci/djx158. ,
42. Schwarz TF, Huang LM, Valencia A, Panzer F, Chiu C-H, Decreux A, Poncelet S, Karkada N, Folschweiller N, Lin L, et al. A ten-year study of immunogenicity and safety of the AS04-HPV-16/18 vaccine in adolescent girls aged 10–14 years. Hum Vaccine Immunother. 2019;15(7–8):1970–79. doi:10.1080/21645515.2019.1625644.
43. Schwarz TF, Galaj A, Spaczynski M, Wysocki J, Kaufmann AM, Poncelet S, Suryakiran PV, Folschweiller N, Thomas F, Lin L, et al. Ten-year immune persistence and safety of the HPV-16/18 AS04-adjuvanted vaccine in females vaccinated at 15–55 years of age. Cancer Med. 2017;6(11):2723–31. doi:10.1002/cam4.1155.

44. Castellsague X, Munoz N, Pitisuttithum P, Ferris D, Monsonego J, Ault K, Luna J, Myers E, Mallary S, Bautista OM, et al. End-of-study safety, immunogenicity, and efficacy of quadrivalent HPV (types 6, 11, 16, 18) recombinant vaccine in adult women 24–45 years of age. Br J Cancer. 2011;105(1):28–37. doi:10.1038/bjc.2011.185.
45. Ferris DG, Samakoses R, Block SL, Lazcano-Ponce E, Restrepo JA, Mehlsen J, Chatterjee A, Iversen O-E, Joshi A, Chu J-L, et al. 4-valent human papillomavirus (4vHPV) vaccine in preadolescents and adolescents after 10 years. Pediatrics. 2017;140(6):e20163947. doi:10.1542/peds.2016-3947.
46. Van Damme P, Olsson SE, Block S, Castellsague X, Gray GE, Herrera T, Huang L-M, Kim DS, Pitisuttithum P, Chen J, et al. Immunogenicity and safety of a 9-valent HPV vaccine. Pediatrics. 2015;136(1):e28–39. doi:10.1542/peds.2014-3745.
47. Olsson S-E, Restrepo JA, Reina JC, Pitisuttithum P, Ulied A, Varman M, Van Damme P, Moreira ED, Ferris D, Block S, et al. Long-term immunogenicity, effectiveness, and safety of nine-valent human papillomavirus vaccine in girls and boys 9 to 15 years of age: interim analysis after 8 years of follow-up. Papillomavirus Res. 2020;10:100203. doi:10.1016/j.pvr.2020.100203. ,
48. Villa L, Costa R, Petta C, Andrade RP, Paavonen J, Iversen O-E, Olsson S-E, Høye J, Steinwall M, Riis-Johannessen G, et al. High sustained efficacy of a prophylactic quadrivalent human papillomavirus types 6/11/16/18 L1 virus-like particle vaccine through 5 years of follow-up. Br J Cancer. 2006;95(11):1459–66. doi:10.1038/sj.bjc.6603469.
49. Klein SL, Jedlicka A, Pekosz A. The Xs and Y of immune responses to viral vaccines. Lancet Infect Dis. 2010;10(5):338–49. doi:10.1016/S1473-3099(10)70049-9.
50. Malagón T, Drolet M, Boily M-C, Franco EL, Jit M, Brisson J, Brisson M. Cross-protective efficacy of two human papillomavirus vaccines: a systematic review and meta-analysis. Lancet Infect Dis. 2012;12(10):781–89. doi:10.1016/S1473-3099(12)70187-1.
51. Einstein MH, Baron M, Levin MJ, Chatterjee A, Fox B, Scholar S, Rosen J, Chakhtoura N, Lebacq M, van der Most R, et al. Comparison of the immunogenicity of the human papillomavirus (HPV)-16/18 vaccine and the HPV-6/11/16/18 vaccine for oncogenic non-vaccine types HPV-31 and HPV-45 in healthy women aged 18–45 years. Hum Vaccine. 2011;7(12):1359–73. doi:10.4161/hv.7.12.18282.
52. Mariz FC, Bender N, Anantharaman D, Basu P, Bhatla N, Pillai MR, Prabhu PR, Sankaranarayanan R, Eriksson T, Pawlita M, et al. Peak neutralizing and cross-neutralizing antibody levels to human papillomavirus types 6/16/18/31/33/45/52/58 induced by bivalent and quadrivalent HPV vaccines. NPJ Vaccines. 2020;5:14. doi:10.1038/s41541-020-0165-x.
53. Artemchuk H, Eriksson T, Poljak M, Surcel HM, Dillner J, Lehtinen M, Faust H. Long-term antibody response to human papillomavirus vaccines: up to 12 years of follow-up in the Finnish maternity cohort. J Infect Dis. 2018;219(4):582–89.
54. Sankaranarayanan R, Prabhu PR, Pawlita M, Gheit T, Bhatla N, Muwonge R, Nene BM, Esmy PO, Joshi S, Poli URR, et al. Immunogenicity and HPV infection after one, two, and three doses of quadrivalent HPV vaccine in girls in India: a multicentre prospective cohort study. Lancet Oncol. 2016;17(1):67–77. doi:10.1016/S1470-2045(15)00414-3.
55. Kann H, Lehtinen M, Eriksson T, Surcel H-M, Dillner J, Faust H. Sustained cross-reactive antibody responses after human papillomavirus vaccinations: up to 12 years follow-up in the Finnish maternity cohort. J Infect Dis. 2020. doi:10.1093/infdis/jiaa617.
56. Guevara A, Cabello R, Woelber L, Moreira ED, Joura E, Reich O, Shields C, Ellison MC, Joshi A, Luxembourg A, et al. Antibody persistence and evidence of immune memory

at 5 years following administration of the 9-valent HPV vaccine. Vaccine. 2017;35(37):5050–57. doi:10.1016/j.vaccine.2017.07.017.

57. Schwarz TF, Huang L, Medina DMR, Valencia A, Lin TY, Behre U, Catteau G, Thomas F, Descamps D. Four-year follow-up of the immunogenicity and safety of the HPV-16/18 AS04-adjuvanted vaccine when administered to adolescent girls aged 10–14 years. J Adolesc Health. 2012;50(2):187–94. doi:10.1016/j.jadohealth.2011.11.004.
58. Reisinger KS, Block SL, Lazcano-Ponce E, Samakoses R, Esser MT, Erick J, Puchalski D, Giacoletti KE, Sings HL, Lukac S, et al. Safety and persistent immunogenicity of a quadrivalent human papillomavirus types 6, 11, 16, 18 L1 virus-like particle vaccine in preadolescents and adolescents: a randomized controlled trial. Pediatr Infect Dis J. 2007;26(3):201–09. doi:10.1097/01.inf.0000253970.29190.5a. ,
59. Barzon L, Squarzon L, Masiero S, Pacenti M, Marcati G, Mantelli B, Gabrielli L, Pascucci MG, Lazzarotto T, Caputo A, et al. Neutralizing and cross-neutralizing antibody titres induced by bivalent and quadrivalent human papillomavirus vaccines in the target population of organized vaccination programmes. Vaccine. 2014;32(41):5357–62. doi:10.1016/j.vaccine.2014.07.014.
60. Godi A, Panwar K, Haque M, Cocuzza CE, Andrews N, Southern J, Turner P, Miller E, Beddows S. Durability of the neutralizing antibody response to vaccine and non-vaccine HPV types 7 years following immunization with either Cervarix® or Gardasil® vaccine. Vaccine. 2019;37(18):2455–62. doi:10.1016/j.vaccine.2019.03.052.
61. Einstein MH, Takacs P, Chatterjee A, Sperling RS, Chakhtoura N, Blatter MM, Lalezari J, David M-P, Lin L, Struyf F, et al. Comparison of long-term immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine and HPV-6/11/16/18 vaccine in healthy women aged 18–45 years: end-of-study analysis of a phase III randomized trial. Hum Vaccine Immunother. 2014;10(12):3435–45. doi:10.4161/hv.36121.
62. Leung TF, Liu AP, Lim FS, Thollot F, Oh HM, Lee BW, Rombo L, Tan NC, Rouzier R, Friel D, et al. Comparative immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine and HPV-6/11/16/18 vaccine administered according to 2- and 3-dose schedules in girls aged 9–14 years: results to month 12 from a randomized trial. Hum Vaccine Immunother. 2015;11(7):1689–702. doi:10.1080/21645515.2015.1050570.
63. Einstein MH, Levin MJ, Chatterjee A, Chakhtoura N, Takacs P, Catteau G, Dessy FJ, Moris P, Lin L, Struyf F, et al. Comparative humoral and cellular immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine and HPV-6/11/16/18 vaccine in healthy women aged 18–45 years: follow-up through month 48 in a phase III randomized study. Hum Vaccine Immunother. 2014;10(12):3455–65. doi:10.4161/hv.36117.
64. Vesikari T, Brodszki N, Van Damme P, Diez-Domingo J, Icardi G, Petersen LK, Tran C, Thomas S, Luxembourg A, Baudin M, et al. A randomized, double-blind, phase III study of the immunogenicity and safety of a 9-valent human papillomavirus L1 virus-like particle vaccine (V503) versus Gardasil® in 9–15-year-old girls. Pediatr Infect Dis J. 2015;34(9):992–98. doi:10.1097/INF.0000000000000773.
65. Leung TF, Liu AP, Lim FS, Thollot F, Oh HML, Lee BW, Rombo L, Tan NC, Rouzier R, De Simoni S, et al. Comparative immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine and 4vHPV vaccine administered according to two- or three-dose schedules in girls aged 9–14 years: results to month 36 from a randomized trial. Vaccine. 2018;36(1):98–106. doi:10.1016/j.vaccine.2017.11.034.
66. Kemp TJ, Safaeian M, Hildesheim A, Pan Y, Penrose KJ, Porras C, Schiller JT, Lowy DR, Herrero R, Pinto LA, et al. Kinetic and HPV infection effects on cross-type neutralizing antibody

and avidity responses induced by Cervarix®. Vaccine. 2012;31(1):165–70. doi:10.1016/j.vaccine.2012.10.067.

67. Donken R, Schurink-Van't Klooster TM, Schepp RM, van der Klis FRM, Knol MJ, Meijer CJLM, de Melker HE Immune responses after 2 versus 3 doses of HPV vaccination up to 4½ years after vaccination: an observational study among Dutch routinely vaccinated girls. J Infect Dis. 2017;215(3):359–67. doi:10.1093/infdis/jiw588
68. Matsui K, Adelsberger JW, Kemp TJ, Baseler MW, Ledgerwood JE, Pinto LA. Circulating CXCR5(+)CD4(+) T follicular-like helper cell and memory B cell responses to human Papillomavirus vaccines. PLoS One. 2015;10(9):e0137195. doi:10.1371/journal.pone.0137195.
69. Dauner JG, Pan Y, Hildesheim A, Harro C, Pinto LA. Characterization of the HPV-specific memory B cell and systemic antibody responses in women receiving an unadjuvanted HPV16 L1 VLP vaccine. Vaccine. 2010;28(33):5407–13. doi:10.1016/j.vaccine.2010.06.018.
70. Smolen KK, Gelinas L, Franzen L, Dobson S, Dawar M, Ogilvie G, Krajden M, Fortuno ES, Kollmann TR. Age of recipient and number of doses differentially impact human B and T cell immune memory responses to HPV vaccination. Vaccine. 2012;30(24):3572–79. doi:10.1016/j.vaccine.2012.03.051.
71. Giannini S, Hanon E, Moris P, Vanmechelen M, Morel S, Dessy F, Fourneau M, Colau B, Suzich J, Losonksy G, et al. Enhanced humoral and memory B cellular immunity using HPV16/18 L1 VLP vaccine formulated with the MPL/aluminium salt combination (AS04) compared to aluminium salt only. Vaccine. 2006;24(33–34):5937–49. doi:10.1016/j.vaccine.2006.06.005.
72. Folschweiller N, Behre U, Dionne M, Durando P, Esposito S, Ferguson L, Ferguson M, Hillemanns P, McNeil SA, Peters K, et al. Long-term cross-reactivity against nonvaccine human Papillomavirus types 31 and 45 after 2- or 3-dose schedules of the AS04-adjuvanted human HPV-16/18 vaccine. J Infect Dis. 2019;219(11):1799–803. doi:10.1093/infdis/jiy743.
73. Stanley MA, Sudenga SL, Giuliano AR. Alternative dosage schedules with HPV virus-like particle vaccines. Expert Rev Vaccines. 2014;13(8):1027–38. doi:10.1586/14760584.2014.935767.
74. Romanowski B, Schwarz TF, Ferguson LM, Ferguson M, Peters K, Dionne M, Schulze K, Ramjattan B, Hillemanns P, Behre U, et al. Immune response to the HPV-16/18 AS04-adjuvanted vaccine administered as a 2-dose or 3-dose schedule up to 4 years after vaccination: results from a randomized study. Hum Vaccine Immunother. 2014;10(5):1155–65. doi:10.4161/hv.28022.
75. Romanowski B, Schwarz TF, Ferguson L, Peters K, Dionne M, Behre U, Schulze K, Hillemanns P, Suryakiran P, Thomas F, et al. Sustained immunogenicity of the HPV-16/18 AS04-adjuvanted vaccine administered as a two-dose schedule in adolescent girls: five-year clinical data and modeling predictions from a randomized study. Hum Vaccine Immunother. 2016;12(1):20–29. doi:10.1080/21645515.2015.1065363.
76. Iversen OE, Miranda MJ, Ulied A, Soerdal T, Lazarus E, Chokephaibulkit K, Block SL, Skrivanek A, Nur Azurah AG, Fong SM, et al. Immunogenicity of the 9-valent HPV vaccine using 2-dose regimens in girls and boys vs a 3-dose regimen in women. Jama. 2016;316(22):2411–21. doi:10.1001/jama.2016.17615.
77. Donken R, Dobson SRM, Marty KD, Cook D, Sauvageau C, Gilca V, Dionne M, McNeil S, Krajden M, Money D, et al. Immunogenicity of 2 and 3 doses of the quadrivalent human Papillomavirus vaccine up to 120 months postvaccination: follow-up of a randomized clinical trial. Clin Infect Dis. 2019;71:1022–1029.
78. Donken R, Knol MJ, Bogaards JA, van der Klis FR, Meijer CJ, de Melker HE. Inconclusive evidence for non-inferior immunogenicity of two-compared with three-dose HPV immunization schedules in preadolescent girls: a systematic review and meta-analysis. J Infect. 2015;71(1):61–73. doi:10.1016/j.jinf.2015.02.005.

79. Dobson SR, McNeil S, Dionne M, Dawar M, Ogilvie G, Krajden M, Sauvageau C, Scheifele DW, Kollmann TR, Halperin SA, et al. Immunogenicity of 2 doses of HPV vaccine in younger adolescents vs 3 doses in young women: a randomized clinical trial. Jama. 2013;309(17):1793–802. doi:10.1001/jama.2013.1625.
80. Schurink-van't Klooster TM, Donken R, Schepp RM, van der Klis FR, de Melker HE. Persistence of immune response following bivalent HPV vaccination: a follow-up study among girls routinely vaccinated with a two-dose schedule. Vaccine. 2018;36(49):7580–87. doi:10.1016/j.vaccine.2018.10.018.
81. Boxus M, Lockman L, Fochesato M, Lorin C, Thomas F, Giannini SL. Antibody avidity measurements in recipients of Cervarix® vaccine following a two-dose schedule or a three-dose schedule. Vaccine. 2014;32(26):3232–36. doi:10.1016/j.vaccine.2014.04.005.
82. Toh ZQ, Kosasih J, Russell FM, Reyburn R, Fong J, Tuivaga E, Ratu FT, Nguyen CD, Matanitobua S, Do LAH, et al. Selective persistence of HPV cross-neutralising antibodies following reduced-dose HPV vaccine schedules. Vaccines. 2019;7(4):200. doi:10.3390/vaccines7040200.
83. Kreimer AR, Sampson JN, Porras C, Schiller JT, Kemp T, Herrero R, Wagner S, Boland J, Schussler J, Lowy DR, et al. Evaluation of durability of a single dose of the bivalent HPV vaccine: the CVT trial. J Natl Cancer Inst. 2020;112(10):1038–46. doi:10.1093/jnci/djaa011.
84. Toh ZQ, Cheow KWB, Russell FM, Hoe E, Reyburn R, Fong J, Tuivaga E, Ratu FT, Nguyen CD, Matanitobua S, et al. Cellular immune responses 6 years following 1, 2, or 3 doses of quadrivalent HPV vaccine in Fijian girls and subsequent responses to a dose of bivalent HPV vaccine. Open Forum Infect Dis. 2018;5(7):ofy147. doi:10.1093/ofid/ofy147.
85. Sankaranarayanan R, Joshi S, Muwonge R, Esmy PO, Basu P, Prabhu P, Bhatla N, Nene BM, Shaw J, Poli URR, et al. Can a single dose of human papillomavirus (HPV) vaccine prevent cervical cancer? Early findings from an Indian study. Vaccine. 2018;36(32 Pt A):4783–91. doi:10.1016/j.vaccine.2018.02.087.
86. Pasmans H, Schurink-Van't Klooster TM, Bogaard MJM, Van Rooijen DM, de Melker HE, Welters MJP, van der Burg SH, van der Klis FRM, Buisman A-M. Long-term HPV-specific immune response after one versus two and three doses of bivalent HPV vaccination in Dutch girls. Vaccine. 2019;37(49):7280–88. doi:10.1016/j.vaccine.2019.09.066.
87. Cuzick J, Clavel C, Petry KU, Meijer CJLM, Hoyer H, Ratnam S, Szarewski A, Birembaut P, Kulasingam S, Sasieni P, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. Int J Cancer. 2006;119(5):1095–101. doi:10.1002/ijc.21955.
88. Bruni L, Diaz M, Castellsagué M, Ferrer E, Bosch FX, De Sanjosé S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis. 2010;202(12):1789–99. doi:10.1086/657321.
89. Toh ZQ, Russell FM, Reyburn R, Fong J, Tuivaga E, Ratu T, Nguyen CD, Devi R, Kama M, Matanitobua S, et al. Sustained antibody responses 6 years following 1, 2, or 3 doses of quadrivalent human papillomavirus (HPV) vaccine in adolescent Fijian girls, and subsequent responses to a single dose of bivalent HPV vaccine: a prospective cohort study. Clin Infect Dis. 2017;64(7):852–59. doi:10.1093/cid/ciw865. ,
90. Bruni L, Diaz M, Barrionuevo-Rosas L, Herrero R, Bray F, Bosch FX, De Sanjosé S, Castellsagué X. Global estimates of human papillomavirus vaccination coverage by region and income level: a pooled analysis. Lancet Global Health. 2016;4(7):e453–e63. doi:10.1016/S2214-109X(16)30099-7.
91. Corcoran B, Clarke A, Barrett T. Rapid response to HPV vaccination crisis in Ireland. Lancet. 2018;391(10135):2103. doi:10.1016/S0140-6736(18)30854-7.

92. Basu P, Brisson M, Campos N, Clarke E, Drolet M, Gallagher K, Henão R, Ana M, Howard N, Hutubessy R, et al. Review of the current published evidence on single-dose HPV vaccination. 2nd ed. Single-Dose Seattle, USA: HPV Vaccine Evaluation Consortium; 2019.
93. Kreimer AR, Sampson JN, Porras C, Schiller JT, Kemp T, Herrero R, Wagner S, Boland J, Schussler J, Lowy DR, et al. Evaluation of durability of a single-dose of the bivalent HPV vaccine: the CVT trial. J Natl Cancer Inst. 2020;112:1038–46.

Part II

Effects of HPV vaccination on genital HPV infection

Chapter 5

Population impact of girls-only human papillomavirus 16/18 vaccination in the Netherlands: Cross-protective and second-order herd effects.

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ABSTRACT

Background: Human papillomavirus (HPV) vaccination programs achieve substantial population-level impact, with effects extending beyond protection of vaccinated individuals. We assessed trends in HPV prevalence up to 8 years postvaccination among men and women in the Netherlands, where bivalent HPV vaccination, targeting HPV types 16/18, has been offered to (pre)adolescent girls since 2009 with moderate vaccination coverage.

Methods: We used data from the PASSYON study, a survey initiated in 2009 (prevaccination) and repeated biennially among 16- to 24-year-old visitors of sexual health centers. We studied genital HPV positivity from 2009 to 2017 among women, heterosexual men, and unvaccinated women using Poisson generalized estimating equation models, adjusted for individual- and population-level confounders. Trends were studied for 25 HPV types detected by the SPF10-LiPA25 platform.

Results: A total of 6354 women (64.7% self-reported unvaccinated) and 2414 heterosexual men were included. Percentual declines in vaccine types HPV-16/18 were observed for all women (12.6% per year [95% confidence interval {CI}, 10.6–14.5]), heterosexual men (13.0% per year [95% CI, 8.3–17.5]), and unvaccinated women (5.4% per year [95% CI, 2.9–7.8]). We observed significant declines in HPV-31 (all women and heterosexual men), HPV-45 (all women), and in all high-risk HPV types pooled (all women and heterosexual men). Significant increases were observed for HPV-56 (all women) and HPV-52 (unvaccinated women).

Conclusions: Our results provide evidence for first-order herd effects among heterosexual men against HPV-16/18 and cross-protective types. Additionally, we show second-order herd effects against vaccine types among unvaccinated women. These results are promising regarding population-level and clinical impact of girls-only bivalent HPV vaccination in a country with moderate vaccine uptake.

Introduction

Infections with human papillomavirus (HPV) are usually transient; however, persistent infections may induce illness in the anogenital or oropharyngeal regions among men and women. Most common are warts, but persistent infections with a high-risk (hr) HPV type can also lead to various cancers [1]. From 2006 onward, 3 prophylactic vaccines have been registered for prevention of HPV-related (pre)cancers, all targeting the most oncogenic hrHPV types 16 and 18. The Netherlands has included the bivalent HPV (2vHPV) vaccine, targeting HPV-16/18, in its national immunization program. HPV-16/18 vaccination is offered to 12-year-old girls in the routine program since 2010, after a one-off catch-up campaign in 2009 for 12- to 16-year-old girls (birth cohorts 1993–1996) [2]. So far, vaccine uptake among vaccine-eligible girls in the general population has fluctuated between 46% and 61% per year between 2009 and 2017 [3].

Vaccine effectiveness of HPV-16/18 vaccination against (persistent) infection with vaccine and cross-protective HPV types has been shown in vaccine recipients relative to controls [4, 5]. However, the population impact of HPV vaccination extends beyond direct protection of vaccinated individuals, as infection dynamics change through vaccination implementation. In countries achieving high coverage in girls-only quadrivalent HPV vaccination programs, also targeting low-risk (lr) HPV types 6 and 11, indirect benefits were evident early on through reduced prevalence of genital warts in unvaccinated young men [6]. Additionally, declining hrHPV prevalence in young vaccinated women was sufficient to provide herd protection to unvaccinated women within 6–7 years after initiation of girls-only HPV vaccination in settings with >80% coverage [7, 8]. Herd effects among unvaccinated women are mainly derived from unvaccinated, but indirectly protected, heterosexual men. However, men have been underrepresented in studies assessing population trends in HPV prevalence over time since vaccine introduction [9].

Previously, we demonstrated herd effects for vaccine types HPV-16/18 among heterosexual men 6 years after introduction of girls-only HPV-16/18 vaccination in the Netherlands [10]. Herd effects among unvaccinated women were not yet observed within 6 years postvaccination, presumably due to the moderate vaccine uptake in the Netherlands. In a girls-only vaccination program, herd protection of unvaccinated women constitutes a second-order effect and is strongly determined by vaccination coverage [11]. As we observed herd effects among heterosexual men within 6 years postvaccination, we hypothesized that herd effects among unvaccinated women in the Netherlands should also become measurable with prolonged monitoring. Continued monitoring of trends in type-specific HPV prevalence over time is also relevant for

detection of type replacement, a still unresolved possibility in the wake of HPV vaccination [12].

To further assess the population impact of the girls-only HPV-16/18 vaccination program in the Netherlands on postvaccination trends in vaccine-targeted and nonvaccine HPV types, we updated and expanded our previous analyses. Here, we present trends in HPV positivity of 25 HPV types from prevaccination up to 8 years postvaccination among 16- to 24-year-old women and heterosexual men visiting sexual health centers (SHCs) throughout the Netherlands.

Methods

Study Design

For this updated analysis, we used data from the PASSYON (Papillomavirus Surveillance Among STI Clinic Youngsters in the Netherlands) study: a biennial cross-sectional survey to assess HPV prevalence among young visitors to SHCs [10]. In the Netherlands, SHCs offer sexually transmitted infection (STI) testing to those with increased risk, including individuals ≤24 years of age. The study design has been described previously [13]. In brief, the PASSYON study was initiated in 2009 (prevaccination) and repeated in 2011, 2013, 2015, and 2017 in SHCs throughout the country (Supplementary Figure 1). Male and female SHC visitors 16–24 years of age were approached for participation and asked to collect a genital self-swab (Copan Diagnostics, Italy). Women were instructed to take a vaginal sample, while men took a penile sample. A questionnaire on sexual risk behavior, demographics, and vaccination status was collected, which was supplemented with information from regular SHC consults. The Medical Ethical Committee (University of Utrecht, the Netherlands), provided a waiver for full medical ethical review (protocol number 08/397). Data were obtained using a unique code per person and all participants gave informed consent.

Laboratory Analyses

HPV laboratory testing was conducted similarly across all study years [13]. In brief, DNA was extracted using the MagnaPure platform (Total Nucleic Acid Isolation Kit, Roche, the Netherlands) and HPV DNA was amplified using SPF10 primer sets and detected using DNA enzyme-linked immunoassays (HPV-DEIA, DDL Diagnostics Laboratory, the Netherlands). Positive samples were genotyped with line-probe assay (HPV-LiPA25, DDL Diagnostics Laboratory, the Netherlands), which is able to detect hrHPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and lrHPV types 6, 11, 34, 40, 42, 43, 44, 53, 54, 66, 70, and 74. Also HPV-68, -73, and -97 can be

detected, but these types cannot be distinguished from each other and are therefore classified as HPV-68.

Statistical Analysis

Trends in HPV positivity were studied for all women (irrespective of vaccination status) and self-reported heterosexual men. To study second-order herd effects, we additionally analyzed trends in self-reported unvaccinated women (only women reporting to be unvaccinated).

The crude prevalence of hrHPV and lrHPV types was calculated for each study year, stratified by sex and vaccination status. Trends in type-specific HPV prevalence over time were assessed using generalized estimating equation (GEE) Poisson models with a log link function and robust error variance. Incorporation of an exchangeable correlation structure accounted for dependency of HPV types within individuals and ensured efficient estimation of regression coefficients. PASSYON year was added as a continuous variable to the model, resulting in a linear trend of HPV prevalence over time on a log scale. Percentual changes in HPV prevalence per year were estimated by exponentiating the (adjusted) regression coefficient for each HPV type.

To select possible confounding variables in the estimation of HPV prevalence trends, we first examined the study population over time regarding participant characteristics. Using $\chi 2$ tests, we checked whether characteristics were comparable between different study years. Next, we studied the association between HPV positivity (any type) and participant characteristics, again using $\chi 2$ tests. Characteristics associated with study year and with HPV positivity were included as explanatory variables in a logistic regression stepwise selection model (with $P < .05$ as entry and stay criteria), using HPV positivity as outcome. Variables included in the final model were used for adjustment in the Poisson GEE models. This process was performed separately for all women, men, and unvaccinated women.

Next to individual-level confounders indicated by the selection models, trends in HPV prevalence were adjusted for age group (16–20 vs 21–24 years) and for changes in SHC access policy (population-level confounder). Due to funding restrictions from 2015 onward, SHCs became stricter in prioritizing individuals at high risk for STIs, which could have resulted in a study population at systematically increased risk for HPV infection starting from 2015 [14]. As we assumed we were unable to fully adjust for this by only including changes over time in the known variables [10], we adjusted for policy change by including a categorical variable indicating the policy change from 2015 onward.

Additionally, we estimated pooled (adjusted) trends in HPV positivity over time. Pooled estimates were obtained as a weighted average of type-specific trends in the GEE Poisson models. Pooled trends were estimated for vaccine types (16/18), hrHPV types of the nonavalent vaccine (16/18/31/33/45/52/58), all hrHPV types (16/18/31/33/35/39/45/51/52/56/58/59), and all types measured in the SPF10-DEIA-LiPA25 assay. As the impact of vaccination among 16- to 24-year-olds on overall prevalence was likely to be still very low in 2011 [15], we performed sensitivity analyses pooling data from PASSYON years 2009 and 2011 to create more stable baseline measurements.

All analyses were performed using SAS version 9.4 (SAS Institute, Cary, North Carolina). We performed complete case analyses, as none of the variables had >5% missing.

Results

We included a total of 6354 women (1574 vaccinated [≥1 dose], 4111 unvaccinated, and 669 unknown, all self-reported) and 2414 heterosexual men who all provided a genital swab; the study population was 1524 in 2009, 1775 in 2011, 1816 in 2013, 1782 in 2015, and 1871 in 2017. The percentage of women reporting to be vaccinated increased over the years; 2.3% in 2009, 6.4% in 2011, 19.2% in 2013, 36.7% in 2015, and 54.1% in 2017. In total, 13.2% of vaccinated women were vaccinated within the regular program. Characteristics of the total study population are presented in Table 1, with the association between characteristics and study year and the association between characteristics and HPV positivity given separately for all women, heterosexual men, and unvaccinated women in Supplementary Tables 1–3. In general, sexual risk behavior increased over time in all groups.

Figure 1 displays the crude HPV prevalence over time for all women, heterosexual men, and unvaccinated women. HPV prevalence was positively affected by the SHC policy change from 2015 onward. After adjustment for age and changes in individual-level characteristics over time, policy change was predicted to have elevated the HPV type-specific positivity by 9% among women and up to 30% among men in trend analyses. Overall, after adjustment for age and selected participant characteristics, and more so after adjustment for policy change, declining trends in HPV prevalence over time became stronger, while increasing trends became weaker (Supplementary Table 4). In the final adjusted GEE models, decreasing trends in both vaccine types HPV-16 and HPV-18 were estimated for women, heterosexual men, and unvaccinated women separately (Figure 2). The pooled percentual decline in HPV-16/18 prevalence per year was 12.6% (95% confidence interval [CI], 10.6%–14.5%) among

Table 1: Characteristics of the Study Population for All Women, Heterosexual Men, and Unvaccinated Women

Characteristic	All Women (n = 6354)		Heterosexual Men (n = 2414)		Unvaccinated Women (n = 4111)	
Age						
16–20 y	2478	(39.0)	691	(28.6)	1336	(32.5)
21–24 y	3876	(61.0)	1723	(71.4)	2775	(67.5)
Self-defined ethnicity						
Dutch	5514	(86.8)	1933	(80.1)	3579	(87.1)
Not Dutch	837	(13.2)	479	(19.9)	530	(12.9)
Educational levela						
Low/middle	1550	(24.5)	708	(29.4)	964	(23.5)
High	4773	(75.5)	1699	(70.6)	3133	(76.5)
Sexual preference						
Heterosexual	6070	(95.5)	2414	(100.0)	3940	(95.8)
Gay or bisexual	284	(4.5)	0	(0.0)	171	(4.2)
Age of sexual debut						
≤14 y	813	(13.0)	405	(16.9)	504	(12.4)
15–16 y	3036	(48.3)	961	(40.2)	1934	(47.5)
≥17 y	2428	(38.7)	1024	(42.9)	1630	(40.1)
No. of sex partners in past 6 mo						
0–1	1960	(30.9)	499	(20.7)	1342	(32.7)
2–3	3047	(48.0)	876	(36.3)	1956	(47.6)
4–5	947	(14.9)	510	(21.1)	591	(14.4)
≥6	394	(6.2)	529	(21.9)	219	(5.3)
No. of lifetime sex partners						
0–2	682	(10.9)	130	(5.6)	438	(10.8)
3–4	1176	(18.9)	245	(10.6)	770	(19.0)
5–6	1220	(19.6)	284	(12.3)	789	(19.5)
7–14	2196	(35.2)	749	(32.4)	1417	(35.0)
≥15	962	(15.4)	903	(39.1)	635	(15.7)
Anal sex past 6 mo						
No	5527	(87.4)	2021	(84.8)	3568	(87.2)
Yes, insertive only	0	(0.0)	351	(14.7)	0	(0.0)
Yes, receptive only	796	(12.6)	3	(0.2)	526	(12.8)
Yes, both insertive and receptive	0	(0.0)	7	(0.3)	0	(0.0)
Notified for STIb						
No	5630	(89.1)	1992	(82.9)	3684	(90.0)
Yes	688	(10.9)	410	(17.1)	408	(10.0)

Table 1: Continued

Characteristic	All Women (n = 6354)		Heterosexual Men (n = 2414)		Unvaccinated Women (n = 4111)	
STI-related symptomsb						
No	4818	(76.4)	1742	(72.7)	3100	(75.9)
Yes	1491	(23.6)	655	(27.3)	984	(24.1)
Self-reported history of any STI						
No	3549	(56.2)	1298	(54.0)	2361	(57.6)
Yes	1683	(26.6)	508	(21.1)	1087	(26.6)
Never tested	1089	(17.2)	598	(24.9)	648	(15.8)
Genital chlamydia infectionb						
No	5403	(85.5)	2010	(83.8)	3534	(86.4)
Yes	914	(14.5)	388	(16.2)	557	(13.6)
Steady partner						
No	3883	(62.6)	1359	(58.3)	2470	(61.5)
Yes, for 0–5 mo	1366	(22.0)	584	(25.1)	908	(22.6)
Yes, ≥6 mo	959	(15.4)	386	(16.6)	641	(15.9)
Condom use past 6 mo, casual partnerc						
Inconsistent	2624	(41.5)	1139	(47.6)	1601	(39.1)
Consistent	2243	(35.5)	810	(33.8)	1525	(37.2)
No casual partners past 6 mo	1455	(23.0)	445	(18.6)	972	(23.7)

Data are presented as No. (%). Numbers vary because of missing values.
a: High educational level included school of higher general secondary education, pre–university education, university of applied sciences, and university. Low/middle educational level included all other levels of education.
b: Based on information of the sexual health center visit.
c: Inconsistent included reporting never, rarely, and "sometimes I do, sometimes I do not" condom use. Consistent included reporting often or always condom use.

all women, 13.0% (95% CI, 8.3%–17.5%) among heterosexual men, and 5.4% (95% CI, 2.9%–7.8%) among unvaccinated women (Table 2). Declining trends were also observed for cross-protective types. We estimated significantly declining trends in the prevalence of HPV-31, with a 6.8% annual decline (P < .001) among all women and a 9.7% annual decline (P = .005) among men, and in the prevalence of HPV-45, with a 4.9% annual decline (P = .036) among all women. The decline in HPV-45 prevalence of 10.4% annually among heterosexual men was borderline nonsignificant (P = .065). Other significantly declining trends in adjusted analyses were seen for HPV-59 among unvaccinated women and for low-risk types HPV-34 (all women and heterosexual men) and HPV-44 (heterosexual men).

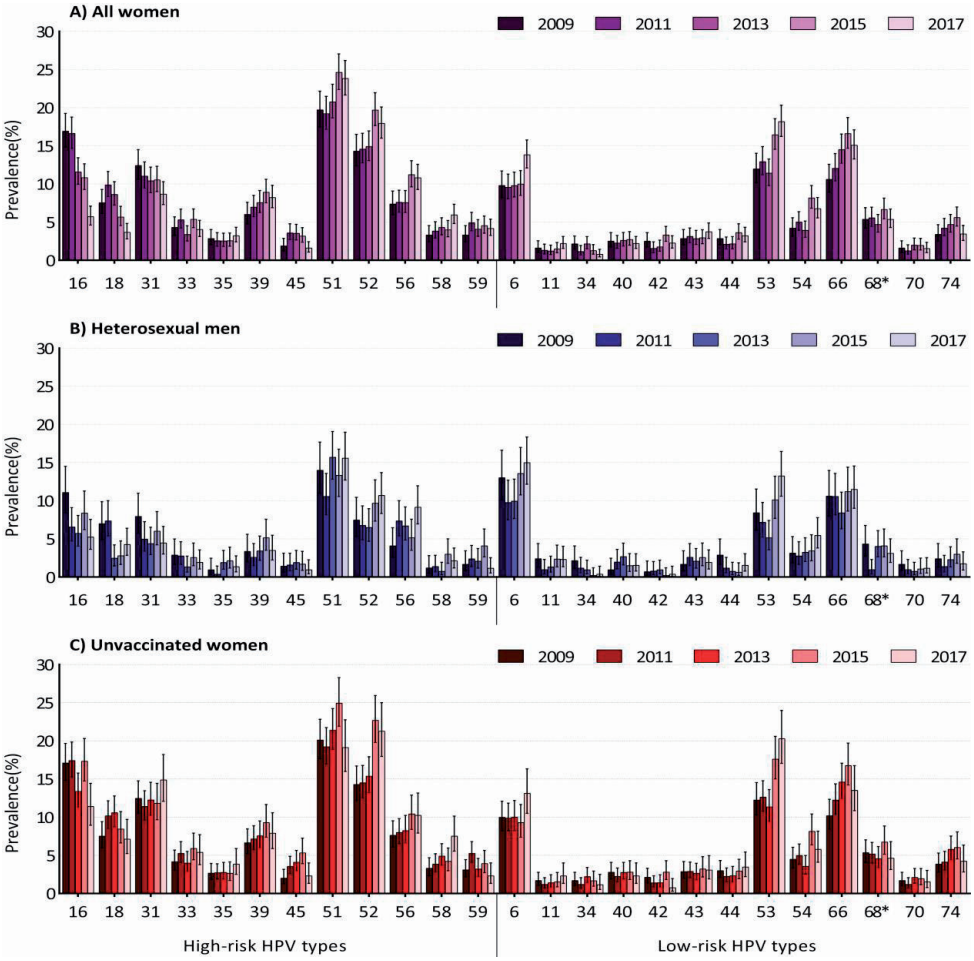


Figure 1: Prevalence of human papillomavirus (HPV) for the different years of the Papillomavirus Surveillance Among STI Clinic Youngsters in the Netherlands (PASSYON) study among all women (A), heterosexual men (B), and unvaccinated women (C). From 2015 onward, the access policy at the sexual health centers had changed, leading to prioritizing of individuals at high risk for sexually transmitted infections. *HPV-68 also includes HPV-73 and HPV-97.

Pooling trends of hrHPV types of the nonavalent vaccine and all hrHPV types (as a weighted average) resulted in a 5.7% and 3.0% annual decline among women and in a 7.8% and 5.3% annual decline among heterosexual men (Table 2). The pooled trend of all measured HPV types including hrHPV and lrHPV types was declining among women and heterosexual men (1.6% and 4.1% annual decline, respectively). Among unvaccinated women, none of the pooled trends were statistically significant.

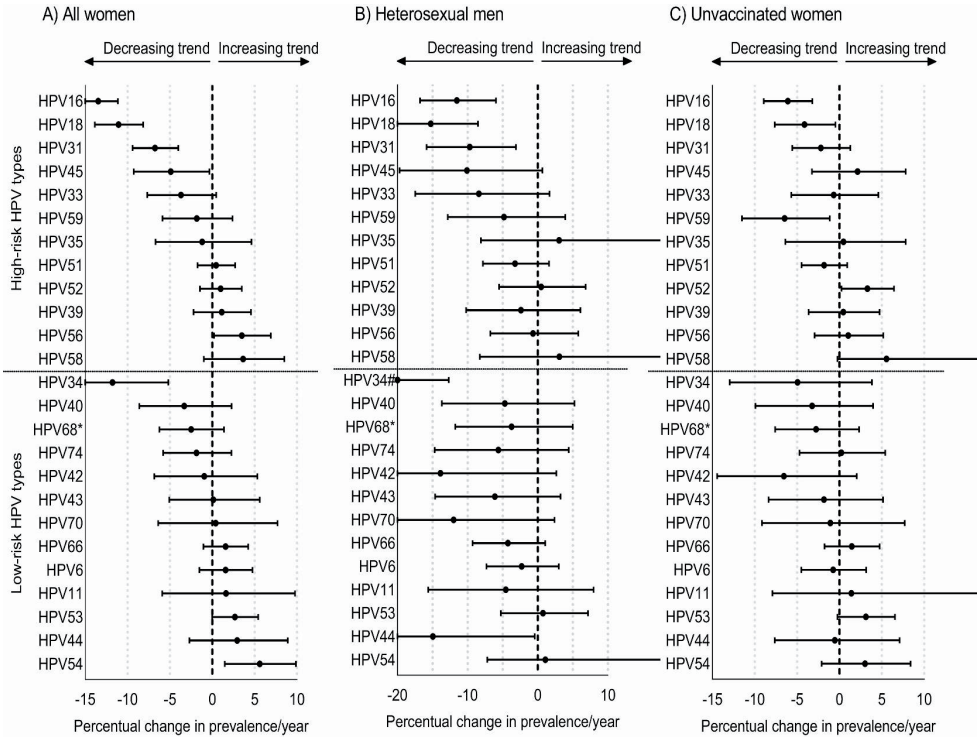


Figure 2: Percentual change in prevalence of high-risk and low-risk human papillomavirus (HPV) types per year, among all women (A), heterosexual men (B), and unvaccinated women (C). Percentual change in prevalence per year was calculated by exponentiating the adjusted regression coefficients of study year, which was added as a continuous variable in generalized estimating equation analyses. For the exact Percentual changes per year, see Supplementary Table 4. *HPV-68 also includes HPV-73 and HPV-97. #Point estimate for HPV-34 among heterosexual men was –26%. The x-axes differ between all women, heterosexual men, and unvaccinated women. Regression coefficients for all women were adjusted for age, policy change at the sexual health center, lifetime sex partners, history of any sexually transmitted infection (STI), steady partner, notified for STI, sex partners past 6 months, and condom use with casual partner. Regression coefficients for heterosexual men were adjusted for age, policy change at the sexual health center, lifetime sex partners, and history of any sexually transmitted infection. Regression coefficients for the unvaccinated women were adjusted for age, policy change at the sexual health center, lifetime sex partners, history of any sexually transmitted infection, notified for STI, sex partners past 6 months, and condom use with casual partner.

Sensitivity analyses in which the first two PASSYON years were pooled and considered as one baseline measurement yielded comparable results regarding adjusted trends for HPV vaccine types. Some type specific estimates became more pronounced, for example, the decline of HPV-45/33 among all women, whereas others were attenuated, such as the decrease in HPV-31 among heterosexual men. Increasing trends in HPV-56 among all women and in HPV-52 among unvaccinated women were no longer statistically significant (Supplementary Table 5).

Table 2: Pooled Trends in Percentual Change of Human Papillomavirus Prevalence per Year Among All Women, Heterosexual Men, and Unvaccinated Women

Vaccine Type	All Women, % (95% CI)	Heterosexual Men, % (95% CI)	Unvaccinated Women, % (95% CI)
Bivalent vaccine types[a]	–12.58 (–14.53 to –10.59)	–13.04 (–17.54 to –8.30)	–5.38 (–7.84 to –2.87)
Nonavalent vaccine hrHPV types[b]	–5.73 (–7.42 to –4.02)	–7.82 (–11.81 to –3.64)	–1.28 (–3.40 to .87)
All hrHPV types[c]	–3.02 (–4.61 to –1.42)	–5.29 (–8.96 to –1.48)	–1.29 (–3.22 to .67)
All hrHPV and lrHPV types[d]	–1.59 (–3.13 to –.03)	–4.10 (–7.69 to –.38)	–0.67 (–2.53 to 1.22)

Pooled trends were obtained as a weighted average of the type-specific trends in the generalized estimating equation Poisson models. Percentual change in prevalence per year was calculated by exponentiating the adjusted regression coefficients of study year.
a: Including HPV types 16 and 18.
b: Including HPV types 16, 18, 31, 33, 45, 52, and 58.
c: Including HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59.
d: Including HPV types 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 70, 74, and 68/73/97.

Also increasing trends in HPV prevalence were observed. In adjusted GEE models, the prevalence of HPV-56/54 increased among all women, and the prevalence of HPV-52 increased among unvaccinated women. A complete overview of the trends for all 25 HPV types is provided in Figure 2.

Discussion

We assessed trends in type-specific HPV prevalence for 25 HPV types up to 8 years after HPV-16/18 vaccination implementation in the Netherlands. We demonstrated significant population impact of girls-only vaccination on vaccine-type HPV infection, with HPV-16/18 prevalence declining each year by 13% among women and heterosexual men, and by 5.4% among unvaccinated women. We also demonstrated significant declines in HPV-31 and HPV-45 among women and heterosexual men, providing strong evidence that cross-protection of the 2vHPV vaccine extends to unvaccinated individuals. Our results show that HPV-16/18 vaccination induces herd effects against vaccine and cross-protective HPV types in a setting with moderate vaccination uptake.

Decreasing trends in HPV-16/18 prevalence were observed among all groups. For women, this is partly explained by an increased proportion of vaccinated women over time who benefit from direct protection of HPV-16/18 vaccination. In a previous analysis with data up to 6 years postvaccination, we reported that heterosexual men already benefited indirectly from this through herd protection [10]. This finding is reiterated in the current analyses with data up to 8 years postvaccination. Addi-

tionally, we were now able to measure reductions in HPV-16/18 prevalence among unvaccinated women, which constitutes a second-order effect that takes more time to develop. Meanwhile, no effects of vaccination implementation were observed among men who have sex with men in the same period [16]. Our results are in line with observations from the United States, where vaccine coverage has been suboptimal as well (around 50%) and herd effects among unvaccinated women were not yet present 3–6 years after vaccination, but became measurable 5–8 years after vaccination [17].

Cross-protection of HPV-16/18 vaccination has been most clearly established for HPV types 31/33/45 [18]. In line with this cross-protection, the current analyses showed significantly decreasing trends in HPV-31/45 among all women, and in HPV-31 among heterosexual men. Declining trends in HPV-33 among all women and in HPV-45 among heterosexual men were also pronounced, albeit nonsignificant. Declines in cross-protective HPV types were not yet observed in previous analyses [10]. Although natural fluctuation could occur over time, consistency of these results suggests that cross-protection of the 2vHPV vaccine also leads to herd effects for these types, although second-order herd effects against cross-protective types remain to be demonstrated. Second-order effects against the pooled outcome HPV-31/33/45 were seen in Scotland 7 years after 2vHPV vaccine introduction, but this was in a setting with a much higher vaccination uptake of around 90% [7]. Likewise, in a community-randomized trial with moderate vaccination uptake, significant second-order herd effects could only be demonstrated in the sex-neutral vaccination arm, and not in the girls-only vaccination arm [19]. Presumably, the speed at which herd effects become apparent is a composite of vaccine effectiveness, which is lower against HPV-31/33/45 as compared to HPV-16/18, and vaccination coverage. Therefore, we suspect herd protection against cross-protective HPV types will also become apparent in unvaccinated women in the Netherlands with prolonged follow-up.

Another declining trend is observed for HPV-34, showing a decrease in both women and heterosexual men. Of the lrHPV types, HPV-34 is phylogenetically most closely related to HPV-16 [20]. Hence, the observed decrease could be related to vaccine introduction, although cross-protection against HPV-34 has not been noticed before. Furthermore, we also observed increasing trends for a few HPV types; for HPV-54/56 among all women, and for HPV-52 among unvaccinated women. Increasing trends in HPV-58/53 were borderline nonsignificant among (unvaccinated) women. Interestingly, HPV-53/54/56 are phylogenetically very distant from the vaccine types and are located on different clades (α6 and α13, respectively), and are therefore the least likely to benefit from cross-reactivity of vaccine-induced immune responses [20]. However, HPV-52 is relatively closely related to HPV-16 (both on clade α9) while also showing an increase [21]. Together, these findings could signify early effects of type replace-

ment, but an increasing HPV prevalence over time could also be due to unmasking and secular trends irrespective of vaccination, for example, due to behavioral changes over time [22]. No significantly increasing trends in HPV prevalence were observed among men, and all increases disappeared in sensitivity analyses. However, detection of type replacement in our study was complicated by the SHC policy change during our study period, and adjusting for this policy change could have resulted in an overcorrection. Other studies assessing trends in type-specific HPV prevalence also showed increases in HPV types following vaccination implementation. In meta-analyses, increases were observed in HPV-39/52/53/73 [23], and in a community-randomized trial a tendency for increasing prevalence of HPV-39/51 was observed among unvaccinated participants [24]. In both studies, results were inconsistent when analyzed by age or birth cohort, and other studies reported no increases in HPV types [25, 26]. Because type replacement following HPV vaccination probably takes many years to develop if present [12], continued surveillance is needed on a type-specific level. Additionally, eventual replacement in disease burden also depends on the oncogenic potential of HPV types becoming more common, emphasizing the need for type-specific surveillance in (pre)cancer screening following vaccination implementation.

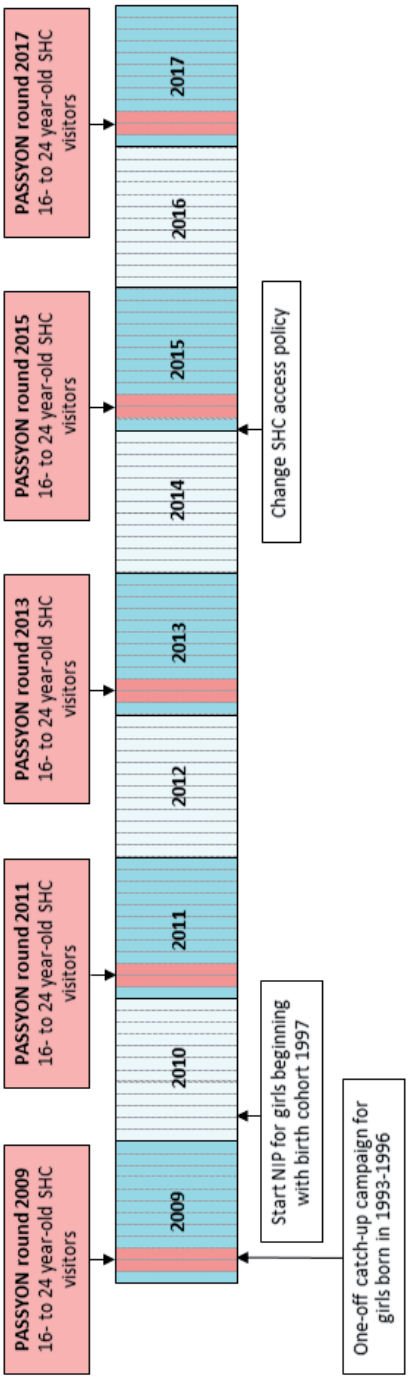
The current study has some limitations. First, the prioritization of high-risk individuals eligible for testing at SHCs has changed due to policy changes over the last years. While we corrected for this by including both individual- and population-level confounders in our model, we cannot rule out residual bias. Still, declines in prevalence were already observed without adjustment, both for vaccine-targeted and cross-protective types. Second, vaccination status was self-reported and could be subject to recall bias. If part of the unvaccinated women were truly vaccinated, this would result in an overestimation of the decreasing trends for vaccine types among unvaccinated women. However, previous confirmation analyses based on serology showed good correlations between self-reported vaccination status and observed antibody levels [4]. Therefore, the possible bias by using self-reported vaccination status in this setting can be considered minimal. Third, only 1 year of official prevaccination data was available, affecting our ability to consider possible background trends or natural fluctuations in HPV prevalence. In sensitivity analyses, we repeated all trend analyses in which study years 2009 and 2011 were pooled and considered to represent the prevaccination situation, and these yielded comparable results.

Our current findings indicate that transmission of vaccine-targeted and cross-protective HPV types is decreasing throughout the population. Our study included a high-risk population with a higher HPV prevalence compared to the general Dutch population, hampering generalizability [27]. As the population-level impact of HPV vaccination is generally attenuated in a high-prevalence setting, the estimates of herd

protection as provided in this study are probably conservative, and population effects of girls-only HPV-16/18 vaccination in the Netherlands are likely to be stronger in the general population [28]. Our findings also emphasize the importance of monitoring nonvaccine HPV types. Prevalence changes of hrHPV types other than HPV-16/18 are relevant to assess the residual risk of (pre-)cancerous lesions and screening need in vaccinated cohorts. Our results showed declining pooled trends in all hrHPV types and all hrHPV and lrHPV types together, among both women and heterosexual men. This is reassuring for the overall benefit of HPV-16/18 vaccination and demonstrates that the 2vHPV vaccine generates broad-spectrum protection against HPV infections.

In conclusion, the current study showed substantial population-level impact of girls-only HPV-16/18 vaccination in a high-risk study population in the Netherlands, a country with moderate vaccination coverage. Apart from significant declines in vaccine-type HPV infections, we also demonstrated that cross-protection of HPV-16/18 vaccination extended to unvaccinated individuals. Our study provides unique documentation of the unfolding of first- and second-order herd effects, and suggests a significant eventual clinical impact of a girls-only HPV vaccination program.

Supplementary data



Supplementary Figure 1: Human papillomavirus (HPV) vaccination in the Netherlands and the PASSYON study design.

Supplementary Table 1: Characteristics over time and relation with human papillomavirus (HPV) positivity among all women.

	2009 (n=1110)	2011 (n=1274)	2013 (n=1294)	2015 (n=1318)	2017 (n=1358)		Any HPV[1]	
	N (%)	N (%)	N (%)	N (%)	N (%)	X^2 p-value	n positive (%)	X^2 p-value
Vaccinated								
Unvaccinated	929 (83.7)	1060 (83.2)	896 (69.2)	709 (53.8)	517 (38.1)			
1 or 2 doses	0 (0.0)	11 (0.9)	24 (1.9)	56 (4.3)	100 (7.4)			
3 doses	0 (0.0)	62 (4.9)	199 (15.4)	310 (23.5)	428 (31.5)			
No. doses unknown	26 (2.3)	8 (0.6)	25 (1.9)	118 (8.9)	207 (15.2)			
Unknown	155 (14.0)	133 (10.4)	150 (11.6)	125 (9.5)	106 (7.8)			
Age						<0.01		<0.01
16-20 years	480 (43.2)	465 (36.5)	539 (41.7)	528 (40.1)	466 (34.3)		1726 (69.7)	
21-24 years	630 (56.8)	809 (63.5)	755 (58.3)	790 (59.9)	892 (65.7)		3020 (77.9)	
Self-defined ethnicity						0.42		0.85
Dutch	964 (87.0)	1093 (85.8)	1113 (86.0)	1149 (87.2)	1195 (88.1)		4121 (74.7)	
Not Dutch	144 (13.0)	181 (14.2)	181 (14.0)	169 (12.8)	162 (11.9)		623 (74.4)	
Educational level[2]						<0.01		0.63
Low/middle	232 (21.2)	335 (26.4)	349 (27.2)	330 (25.1)	304 (22.4)		1164 (75.1)	
High	863 (78.8)	933 (73.6)	936 (72.8)	987 (74.9)	1054 (77.6)		3555 (74.5)	
Sexual preference						<0.01		0.99
Heterosexual	1079 (97.2)	1235 (96.9)	1241 (95.9)	1249 (94.8)	1266 (93.2)		4534 (74.7)	
Gay or bisexual	31 (2.8)	39 (3.1)	53 (4.1)	69 (5.2)	92 (6.8)		212 (74.7)	
Age sexual debut						0.12		0.23
≤14 years	118 (10.7)	158 (12.6)	193 (15.0)	178 (13.7)	166 (12.4)		619 (76.1)	
15-16 years	539 (48.8)	614 (48.9)	606 (47.2)	637 (49.1)	640 (47.9)		2285 (75.3)	
≥17 years	447 (40.5)	484 (38.5)	485 (37.8)	482 (37.2)	530 (39.7)		1787 (73.6)	

Supplementary Table 1: Continued

	2009 (n=1110)	2011 (n=1274)	2013 (n=1294)	2015 (n=1318)	2017 (n=1358)	Any HPV[1]		
	N (%)	N (%)	N (%)	N (%)	N (%)	X^2 p-value	n positive (%)	X^2 p-value
Sex partners past 6 months						<0.01		<0.01
0-1 partner	391 (35.3)	476 (37.4)	421 (32.6)	339 (25.7)	333 (24.5)		1260 (64.3)	
2-3 partners	528 (47.7)	599 (47.1)	649 (50.2)	636 (48.3)	635 (46.8)		2323 (76.2)	
4-5 partners	141 (12.7)	152 (12.0)	156 (12.1)	238 (18.1)	260 (19.2)		801 (84.6)	
≥6 partners	48 (4.3)	45 (3.5)	67 (5.2)	105 (8.0)	129 (9.5)		358 (90.9)	
Lifetime sex partners						<0.01		<0.01
≤2 partners	138 (12.6)	183 (14.6)	128 (10.1)	121 (9.4)	112 (8.4)		320 (46.9)	
3-4 partners	235 (21.5)	257 (20.5)	265 (20.9)	216 (16.7)	203 (15.2)		761 (64.7)	
5-6 partners	224 (20.5)	239 (19.1)	249 (19.6)	254 (19.7)	254 (19.1)		900 (73.8)	
7-14 partners	360 (32.9)	414 (33.1)	458 (36.1)	470 (36.4)	494 (37.1)		1822 (83.0)	
≥15 partners	136 (12.4)	159 (12.7)	169 (13.3)	229 (17.8)	269 (20.2)		852 (88.6)	
Anal sex past 6 months						0.12		0.06
No	964 (87.5)	1138 (89.5)	1119 (87.1)	1130 (86.4)	1176 (86.6)		4104 (74.3)	
Yes	138 (12.5)	133 (10.5)	165 (12.9)	178 (13.6)	182 (13.4)		616 (77.4)	
Notified for STI[3]						<0.01		<0.01
No	1027 (92.8)	1167 (91.9)	1169 (90.5)	1148 (87.6)	1119 (83.5)		4152 (73.8)	
Yes	80 (7.2)	103 (8.1)	122 (9.5)	162 (12.4)	221 (16.5)		566 (82.3)	
STI related symptoms[3]						0.30		0.20
No	847 (76.9)	993 (78.3)	962 (74.7)	997 (76.0)	1019 (76.0)		3578 (74.3)	
Yes	254 (23.1)	276 (21.7)	326 (25.3)	314 (24.0)	321 (24.0)		1132 (75.9)	

Supplementary Table 1: Continued

	2009 (n=1110)	2011 (n=1274)	2013 (n=1294)	2015 (n=1318)	2017 (n=1358)		Any HPV[1]	
	N (%)	N (%)	N (%)	N (%)	N (%)	X^2 p-value	n positive (%)	X^2 p-value
Self-reported history of any STI						<0.01		<0.01
No	715 (65.1)	741 (58.4)	715 (55.5)	681 (51.9)	697 (51.6)		2575 (72.6)	
Yes	238 (21.7)	313 (24.7)	335 (26.0)	380 (29.0)	417 (30.8)		1446 (85.9)	
Never tested	146 (13.3)	215 (16.9)	239 (18.5)	251 (19.1)	238 (17.6)		707 (64.9)	
Genital chlamydia infection[3]						0.10		<0.01
No	959 (86.7)	1090 (85.9)	1095 (84.9)	1139 (86.8)	1120 (83.6)		3963 (73.4)	
Yes	147 (13.3)	179 (14.1)	195 (15.1)	173 (13.2)	220 (16.4)		754 (82.5)	
Steady partner						<0.01		<0.01
No	634 (58.1)	728 (58.7)	744 (58.9)	855 (66.7)	922 (69.2)		2976 (76.6)	
Yes, for 0-6 months	249 (22.8)	288 (23.2)	298 (23.6)	267 (20.8)	264 (19.8)		1047 (76.7)	
Yes, for ≥6 months	208 (19.1)	225 (18.1)	221 (17.5)	159 (12.4)	146 (11.0)		608 (63.4)	
Condom use past 6 months, casual partner[4]						<0.01		<0.01
Inconsistent	382 (34.5)	408 (32.1)	538 (41.7)	622 (47.4)	674 (50.2)		2083 (79.4)	
Consistent	421 (38.0)	492 (38.7)	446 (34.5)	447 (34.1)	437 (32.6)		1721 (76.7)	
No casual partners	304 (27.5)	371 (29.2)	307 (23.8)	242 (18.5)	231 (17.2)		920 (63.2)	

Numbers vary because of missing values. Abbreviations: STI: sexually transmitted infection.
Variables that were associated with PASSYON study year (p<0.05) and HPV positivity (p<0.05) were considered in a computerized stepwise selection model, to select confounders to adjust for. The variables that were selected in the stepwise model (p<0.05 entry and stay criteria) were: lifetime sex partners, history of any STI, steady partner, notified for STI, sex partners past 6 months, condom use with casual partners.
1. Being positive for HPV6/11/16/18/31/33/34/35/39/40/42/43/44/45/51/52/53/54/56/58/59/66/68/70 and/or 74.*
2. High educational level included school of higher general secondary education, pre-university education, university of applied sciences, and university. Low/middle educational level included all other levels of education.
3. Based on information of the sexual health center visit.
4. Inconsistent included reporting never, rarely and "sometimes I do, sometimes I do not" condom use. Consistent included reporting often or always condom use.

Supplementary Table 2: Characteristics over time and relation with human papillomavirus (HPV) positivity among heterosexual men.

	2009 (n=414)	2011 (n=501)	2013 (n=522)	2015 (n=464)	2017 (n=513)		Any HPV[1]	
	N (%)	N (%)	N (%)	N (%)	N (%)	X^2 p-value	n positive (%)	X^2 p-value
Age						0.25		<0.01
16-20 years	118 (28.5)	139 (27.7)	169 (32.4)	131 (28.2)	134 (26.1)		274 (39.7)	
21-24 years	296 (71.5)	362 (72.3)	353 (67.6)	333 (71.8)	379 (73.9)		945 (54.9)	
Self-defined ethnicity						0.87		0.47
Dutch	332 (80.6)	397 (79.2)	414 (79.3)	379 (81.7)	411 (80.1)		984 (50.9)	
Not Dutch	80 (19.4)	104 (20.8)	108 (20.7)	85 (18.3)	102 (19.9)		235 (49.1)	
Educational level[2]						<0.01		0.53
Low/middle	129 (31.2)	161 (32.3)	174 (33.5)	127 (27.4)	117 (22.8)		351 (49.6)	
High	284 (68.8)	337 (67.7)	345 (66.5)	337 (72.6)	396 (77.2)		866 (51.0)	
Age sexual debut						0.10		<0.01
≤14 years	65 (15.9)	95 (19.0)	92 (17.8)	70 (15.3)	83 (16.4)		209 (51.6)	
15-16 years	147 (36.0)	192 (38.5)	230 (44.4)	193 (42.1)	199 (39.3)		525 (54.6)	
≥17 years	196 (48.0)	212 (42.5)	196 (37.8)	195 (42.6)	225 (44.4)		475 (46.4)	
Sex partners past 6 months						<0.01		<0.01
0-1 partner	121 (29.2)	113 (22.6)	101 (19.3)	83 (17.9)	81 (15.8)		198 (39.7)	
2-3 partners	168 (40.6)	198 (39.5)	196 (37.5)	153 (33.0)	161 (31.4)		393 (44.9)	
4-5 partners	66 (15.9)	111 (22.2)	108 (20.7)	105 (22.6)	120 (23.4)		291 (57.1)	
≥6 partners	59 (14.3)	79 (15.8)	117 (22.4)	123 (26.5)	151 (29.4)		337 (63.7)	
Lifetime sex partners						<0.01		<0.01
≤2 partners	34 (8.5)	31 (6.5)	25 (5.0)	15 (3.4)	25 (5.1)		130 (19.2)	
3-4 partners	50 (12.5)	58 (12.2)	63 (12.6)	31 (7.0)	43 (8.7)		68 (27.8)	
5-6 partners	70 (17.5)	67 (14.1)	62 (12.4)	41 (9.3)	44 (8.9)		103 (36.3)	
7-14 partners	121 (30.2)	151 (31.8)	154 (30.8)	159 (35.9)	164 (33.3)		375 (50.1)	
≥15 partners	126 (31.4)	168 (35.4)	196 (39.2)	197 (44.5)	216 (43.9)		593 (65.7)	

Supplementary Table 2: Continued

	2009 (n=414)	2011 (n=501)	2013 (n=522)	2015 (n=464)	2017 (n=513)		Any HPV[1]	
	N (%)	N (%)	N (%)	N (%)	N (%)	X^2 p-value	n positive (%)	X^2 p-value
Anal sex past 6 months						0.38		<0.01
No	351 (85.4)	427 (85.9)	441 (86.0)	371 (81.9)	431 (84.2)		990 (49.0)	
Yes	60 (14.6)	70 (14.1)	72 (14.0)	82 (18.1)	81 (15.8)		212 (58.1)	
Notified for STI[3]						<0.01		0.02
No	366 (89.1)	427 (85.7)	442 (84.7)	373 (80.9)	384 (75.3)		986 (49.5)	
Yes	45 (10.9)	71 (14.3)	80 (15.3)	88 (19.1)	126 (24.7)		228 (55.6)	
STI related symptoms[3]						0.95		<0.01
No	295 (72.3)	363 (72.9)	373 (71.5)	336 (73.0)	375 (73.7)		850 (48.8)	
Yes	113 (27.7)	135 (27.1)	149 (28.5)	124 (27.0)	134 (26.3)		362 (55.3)	
Self-reported history of any STI						<0.01		<0.01
No	286 (69.8)	265 (53.1)	263 (50.5)	241 (51.9)	234 (47.6)		639 (49.2)	
Yes	64 (15.6)	99 (19.8)	103 (19.8)	111 (23.9)	131 (25.7)		332 (65.4)	
Never tested	60 (14.6)	135 (27.1)	155 (29.8)	112 (24.1)	136 (26.7)		243 (40.6)	
Genital chlamydia infection[3]						<0.01		<0.01
No	365 (89.2)	421 (84.7)	435 (83.5)	373 (80.9)	416 (81.6)		986 (49.1)	
Yes	44 (10.8)	76 (15.3)	86 (16.5)	88 (19.1)	94 (18.4)		226 (58.3)	
Steady partner						<0.01		0.06
No	206 (51.1)	253 (53.0)	284 (55.8)	294 (65.8)	322 (65.3)		689 (50.7)	
Yes, for 0-6 months	108 (26.8)	134 (28.1)	142 (27.9)	91 (20.4)	109 (22.1)		309 (52.9)	
Yes, for ≥6 months	89 (22.1)	90 (18.9)	83 (16.3)	62 (13.9)	62 (12.6)		175 (45.3)	

Supplementary Table 2: Continued

	2009 (n=414)	2011 (n=501)	2013 (n=522)	2015 (n=464)	2017 (n=513)		Any HPV[1]	
	N (%)	N (%)	N (%)	N (%)	N (%)	X^2 p-value	n positive (%)	X^2 p-value
Condom use past 6 months, casual partner[4]						<0.01		<0.01
Inconsistent	146 (35.4)	211 (42.4)	240 (46.1)	254 (55.0)	288 (57.6)		629 (55.2)	
Consistent	152 (36.8)	179 (35.9)	181 (34.7)	146 (31.6)	152 (30.4)		405 (50.0)	
No casual partners	115 (27.8)	108 (21.7)	100 (19.2)	62 (13.4)	60 (12.0)		178 (40.0)	

Abbreviations: STI: sexually transmitted infection.
Variables that were associated with PASSYON study year ($p<0.05$) and HPV positivity ($p<0.05$) were considered in a computerized stepwise selection model, to select confounders to adjust for. The variables that were selected in the stepwise model ($p<0.05$ entry and stay criteria) were: lifetime sex partners, history of any STI.
1. Being positive for HPV6/11/16/18/31/33/34/35/39/40/42/43/44/45/51/52/53/54/56/58/59/66/68/70 and/or 74.*
2. High educational level included school of higher general secondary education, pre-university education, university of applied sciences, and university. Low/middle educational level included all other levels of education.
3. Based on information of the sexual health center visit.
4. Inconsistent included reporting never, rarely and "sometimes I do, sometimes I do not" condom use. Consistent included reporting often or always condom use.
Numbers vary because of missing values.

Supplementary Table 3: Characteristics over time and relation with human papillomavirus (HPV) positivity among unvaccinated women.

	2009 (n=929)	2011 (n=1060)	2013 (n=896)	2015 (n=709)	2017 (n=517)		Any HPV[1]	
	N (%)	N (%)	N (%)	N (%)	N (%)	X^2 p-value	n positive (%)	X^2 p-value
Age						<0.01		<0.01
16-20 years	373 (40.2)	345 (32.5)	283 (31.6)	185 (26.1)	150 (29.0)		920 (68.9)	
21-24 years	556 (59.8)	715 (67.5)	613 (68.4)	524 (73.9)	367 (71.0)		2164 (78.0)	
Self-defined ethnicity						0.75		0.77
Dutch	816 (88.0)	919 (86.7)	771 (86.0)	621 (87.6)	452 (87.4)		2688 (75.1)	
Not Dutch	111 (12.0)	141 (13.3)	125 (14.0)	88 (12.4)	65 (12.6)		395 (74.5)	
Educational level[2]						<0.01		0.67
Low/middle	172 (18.8)	261 (24.6)	226 (25.2)	176 (24.8)	129 (25.0)		728 (75.5)	
High	743 (81.2)	799 (75.4)	670 (74.8)	533 (75.2)	388 (75.0)		2345 (74.85)	
Sexual preference						<0.01		0.96
Heterosexual	901 (97.0)	1027 (96.9)	860 (96.0)	669 (94.4)	483 (93.4)		2956 (75.0)	
Gay or bisexual	28 (3.0)	33 (3.1)	36 (4.0)	40 (5.6)	34 (6.6)		128 (74.9)	
Age sexual debut						0.36		0.04
≤14 years	95 (10.3)	122 (11.7)	125 (14.0)	97 (13.9)	65 (12.8)		393 (78.0)	
15-16 years	447 (48.3)	508 (48.6)	411 (46.2)	331 (47.4)	237 (46.6)		1467 (75.9)	
≥17 years	383 (41.4)	416 (39.8)	354 (39.8)	270 (38.7)	207 (40.6)		1191 (73.1)	
Sex partners past 6 months						<0.01		<0.01
0-1 partner	330 (35.6)	395 (37.3)	288 (32.1)	190 (26.8)	139 (26.9)		875 (65.2)	
2-3 partners	440 (47.4)	496 (46.8)	446 (49.8)	339 (47.8)	235 (45.5)		1497 (76.5)	
4-5 partners	122 (13.1)	131 (12.4)	116 (12.9)	130 (18.3)	92 (17.8)		507 (85.8)	
≥6 partners	36 (3.9)	37 (3.5)	46 (5.1)	50 (7.1)	50 (9.7)		204 (93.2)	

Supplementary Table 3: Continued

	2009 (n=929)	2011 (n=1060)	2013 (n=896)	2015 (n=709)	2017 (n=517)		Any HPV[1]	
	N (%)	N (%)	N (%)	N (%)	N (%)	X^2 p-value	n positive (%)	X^2 p-value
Lifetime sex partners						<0.01		<0.01
≤2 partners	104 (11.4)	150 (14.3)	82 (9.3)	60 (8.6)	42 (8.2)		211 (48.2)	
3-4 partners	200 (21.8)	205 (19.6)	163 (18.5)	120 (17.2)	82 (16.1)		496 (64.4)	
5-6 partners	195 (21.3)	198 (18.9)	163 (18.5)	138 (19.8)	95 (18.6)		579 (73.4)	
7-14 partners	303 (33.1)	360 (34.4)	339 (38.5)	243 (34.9)	172 (33.7)		1176 (83.0)	
≥15 partners	114 (12.4)	134 (12.8)	133 (15.1)	135 (19.4)	119 (23.3)		573 (90.2)	
Anal sex past 6 months						0.02		0.01
No	813 (87.8)	945 (89.2)	770 (86.7)	594 (84.4)	446 (86.3)		2650 (74.3)	
Yes	113 (12.2)	114 (10.8)	118 (13.3)	110 (15.6)	71 (13.7)		418 (79.5)	
Notified for STI[3]						<0.01		<0.01
No	861 (92.9)	975 (92.3)	810 (90.6)	617 (87.5)	421 (82.6)		2737 (74.3)	
Yes	66 (7.1)	81 (7.7)	84 (9.4)	88 (12.5)	89 (17.5)		333 (81.6)	
STI related symptoms[3]						0.26		0.09
No	710 (77.0)	821 (77.8)	668 (74.9)	522 (74.0)	379 (74.3)		2305 (74.4)	
Yes	212 (23.0)	234 (22.2)	224 (25.1)	183 (26.0)	131 (25.7)		758 (77.0)	
Self-reported history of any STI						<0.01		<0.01
No	613 (66.5)	620 (58.6)	505 (56.6)	363 (51.3)	260 (50.3)		1716 (72.7)	
Yes	199 (21.6)	258 (24.4)	232 (26.0)	231 (32.7)	167 (32.3)		941 (86.6)	
Never tested	110 (11.9)	180 (17.0)	155 (17.4)	113 (16.0)	90 (17.4)		418 (64.5)	
Genital chlamydia infection[3]						0.22		<0.01
No	798 (86.1)	913 (86.5)	760 (85.1)	627 (88.9)	436 (85.3)		2605 (73.7)	
Yes	129 (13.9)	142 (13.5)	133 (14.9)	78 (11.1)	75 (14.7)		464 (83.3)	

Supplementary Table 3: Continued

	2009 (n=929)	2011 (n=1060)	2013 (n=896)	2015 (n=709)	2017 (n=517)		Any HPV[1]	
	N (%)	N (%)	N (%)	N (%)	N (%)	X^2 p-value	n positive (%)	X^2 p-value
Steady partner						<0.01		<0.01
No	537 (58.8)	606 (58.5)	521 (59.4)	463 (67.6)	343 (67.5)		1897 (76.8)	
Yes, for 0-6 months	206 (22.6)	246 (23.7)	214 (24.4)	135 (19.7)	107 (21.1)		694 (76.4)	
Yes, for ≥6 months	170 (18.6)	184 (17.8)	142 (16.2)	87 (12.7)	58 (11.4)		418 (65.2)	
Condom use past 6 months, casual partner[4]						<0.01		<0.01
Inconsistent	306 (33.0)	343 (32.4)	374 (41.8)	321 (45.4)	257 (50.3)		1601 (80.8)	
Consistent	371 (40.0)	420 (39.7)	316 (35.3)	254 (35.9)	164 (32.1)		1170 (76.7)	
No casual partners	250 (27.0)	296 (28.0)	204 (22.8)	132 (18.7)	90 (17.6)		612 (62.9)	

Abbreviations: STI: sexually transmitted infection.
Variables that were associated with PASSYON study year (p<0.05) and HPV positivity (p<0.05) were considered in a computerized stepwise selection model, to select confounders to adjust for. The variables that were selected in the stepwise model (p<0.05 entry and stay criteria) were: lifetime sex partners, history of any STI, condom use with casual partners, notified for STI, sex partners past 6 months.
1. Being positive for HPV6/11/16/18/31/33/34/35/39/40/42/43/44/45/51/52/53/54/56/58/59/66/68/70 and/or 74.*
2. High educational level included school of higher general secondary education, pre-university education, university of applied sciences, and university. Low/middle educational level included all other levels of education.
3. Based on information of the sexual health center visit.
4. Inconsistent included reporting never, rarely and "sometimes I do, sometimes I do not" condom use. Consistent included reporting often or always condom use.
Numbers vary because of missing values.

Supplementary Table 4: Percentual change in HPV prevalence per year and the effect of adjustment, separately for all women, heterosexual men, and unvaccinated women.

		All women % (95% CI)			Heterosexual men % (95% CI)			Unvaccinated women % (95% CI)		
		Crude	Adjusted for confounders[1]	Adjusted for policy and confounders[1]	Crude	Adjusted for confounders[2]	Adjusted for policy and confounders[2]	Crude	Adjusted for confounders[3]	Adjusted for policy and confounders[3]
High-risk HPV types	**16**	-11.0 (-13.1 – -8.9)	-12.4 (-14.4 – -10.3)	-13.5 (-15.7 – -11.1)	-6.2 (-11.2 – -0.8)	-8.0 (-12.9 – -2.8)	-11.5 (-16.8 – -6.0)	-3.1 (-5.7 – -0.4)	-4.5 (-7.1 – -1.9)	-6.1 (-8.9 – -3.2)
	18	-8.5 (-11.2 – -5.7)	-9.9 (-12.6 – -7.2)	-11.1 (-13.9 – -8.2)	-10.2 (-16.3 – -3.6)	-12.0 (-18.0 – -5.5)	-15.2 (-21.5 – -8.5)	-1.0 (-4.4 – 2.5)	-2.5 (-5.8 – 0.9)	-4.1 (-7.6 – -0.5)
	31	-4.0 (-6.5 – -1.4)	-5.6 (-8.0 – -3.1)	-6.8 (-9.4 – -4.0)	-4.2 (-10.2 – 2.2)	-6.0 (-11.9 – 0.2)	-9.7 (-15.8 – -3.1)	1.1 (-2.1 – 4.3)	-0.5 (-3.6 – 2.7)	-2.2 (-5.6 – 1.3)
	33	-0.8 (-4.8 – 3.4)	-2.4 (-6.3 – 1.6)	-3.7 (-7.7 – 0.5)	-2.8 (-12.2 – 7.7)	-4.6 (-13.9 – 5.6)	-8.4 (-17.5 – 1.7)	2.7 (-2.4 – 8.0)	1.1 (-3.9 – 6.3)	-0.7 (-5.7 – 4.6)
	35	1.8 (-3.7 – 7.8)	0.1 (-5.3 – 5.9)	-1.2 (-6.7 – 4.6)	9.5 (-2.2 – 22.6)	7.4 (-4.1 – 20.4)	3.0 (-8.1 – 15.6)	3.9 (-3.2 – 11.4)	2.2 (-4.7 – 9.6)	0.5 (-6.4 – 7.8)
	39	4.3 (1.1 – 7.6)	2.5 (-0.6 –5.7)	1.1 (-2.2 – 4.6)	3.7 (-4.1 – 12.1)	1.7 (-6.0 – 10.0)	-2.4 (-10.2 – 6.1)	3.9 (-0.2 – 8.0)	2.2 (-1.7 – 6.3)	0.5 (-3.6 – 4.7)
	45	-2.0 (-6.4 – 2.6)	-3.7 (-8.0 – 0.8)	-4.9 (-9.3 – -0.3)	-4.6 (-14.5 – 6.5)	-6.4 (-16.2 – 4.5)	-10.1 (-19.7 – 0.7)	5.6 (0.2 – 11.4)	4.0 (-1.4 – 9.6)	2.1 (-3.2 – 7.8)
	51	3.6 (1.8 – 5.3)	1.8 (0.1 – 3.6)	0.5 (-1.7 – 2.7)	2.8 (-0.9 – 6.7)	0.8 (-2.8 – 4.6)	-3.2 (-7.8 – 1.6)	1.5 (-0.8 – 3.7)	-0.1 (-2.3 – 2.1)	-1.8 (-4.5 – 0.9)
	52	4.1 (2.0 – 6.3)	2.3 (0.3 – 4.4)	1.0 (-1.4 – 3.5)	6.8 (1.4 – 12.5)	4.8 (-0.6 – 10.4)	0.5 (-5.5 – 6.8)	6.8 (4.1 – 9.6)	5.1 (2.5 – 7.8)	3.3 (0.2 – 6.4)
	56	6.7 (3.6 – 9.9)	4.9 (1.9 – 8.0)	3.5 (0.2 – 6.9)	5.6 (-0.1 – 11.5)	3.5 (-2.0 – 9.4)	-0.7 (-6.7 – 5.8)	4.5 (0.6 – 8.5)	2.8 (-0.9 – 6.7)	1.0 (-2.9 – 5.2)
	58	6.9 (2.3 – 11.7)	5.1 (0.6 – 9.8)	3.6 (-1.0 – 8.5)	9.6 (-2.1 – 22.7)	7.5 (-4.1 – 20.4)	3.1 (-8.2 – 15.8)	9.2 (3.4 – 15.3)	7.5 (1.8 – 13.4)	5.6 (-0.2 – 11.7)
	59	1.2 (-2.9 – 5.4)	-0.5 (-4.5 – 3.6)	-1.8 (-5.9 – 2.4)	1.1 (-7.2 – 10.1)	-0.9 (-9.0 – 8.0)	-4.8 (-12.8 – 3.9)	-3.4 (-8.6 – 2.0)	-4.9 (-10.0 – 0.5)	-6.5 (-11.5 – -1.2)

Supplementary Table 4: Continued

		All women % (95% CI)			Heterosexual men % (95% CI)			Unvaccinated women % (95% CI)		
		Crude	Adjusted for confounders[1]	Adjusted for policy and confounders[1]	Crude	Adjusted for confounders[2]	Adjusted for policy and confounders[2]	Crude	Adjusted for confounders[3]	Adjusted for policy and confounders[3]
Low-risk HPV types	**6**	4.7 (1.9 – 7.6)	2.9 (0.2 – 5.8)	1.6 (-1.5 – 4.7)	3.8 (-0.4 – 8.2)	1.8 (-2.3 – 6.1)	-2.3 (-7.3 – 3.0)	2.6 (-1.0 – 6.3)	1.0 (-2.5 – 4.6)	-0.7 (-4.5 – 3.2)
	11	4.8 (-3.0 – 13.2)	3.0 (-4.6 – 11.1)	1.6 (-5.9 – 9.8)	1.4 (-10.1 – 14.4)	-0.6 (-11.9 – 12.2)	-4.5 (-15.6 – 8.0)	4.8 (-4.8 – 15.5)	3.2 (-6.3 – 13.6)	1.4 (-7.9 – 11.7)
	34	-9.2 (-15.5 – -2.4)	-10.7 (-16.8 – -4.1)	-11.8 (-17.9 – -5.2)	-21.7 (-33.7 – -7.4)	-23.2 (-35.1 – -9.2)	-26.0 (-37.2 – -12.7)	-1.8 (-10.1 – 7.3)	-3.3 (-11.4 – 5.6)	-4.9 (-13.0 – 3.8)
	40	-0.4 (-5.8 – 5.3)	-2.0 (-7.3 – 3.5)	-3.3 (-8.6 – 2.3)	1.2 (-8.1 – 11.4)	-0.7 (-9.9 – 9.3)	-4.7 (-13.7 – 5.2)	-0.0 (-6.9 – 7.3)	-1.6 (-8.3 – 5.7)	-3.2 (-9.9 – 4.0)
	42	2.1 (-3.9 – 8.5)	0.4 (-5.5 – 6.6)	-0.9 (-6.8 – 5.3)	-8.6 (-23.5 – 9.2)	-10.4 (-25.0 – 7.1)	-13.9 (-27.7 – 2.7)	-3.5 (-11.7 – 5.4)	-5.0 (-13.0 – 3.7)	-6.6 (-14.4 – 2.1)
	43	3.2 (-2.0 – 8.8)	1.5 (-3.7 – 6.8)	0.1 (-5.1 – 5.6)	-0.3 (-9.1 – 9.4)	-2.2 (-10.9 – 7.3)	-6.1 (-14.6 – 3.3)	1.4 (-5.2 – 8.6)	-0.1 (-6.7 – 6.9)	-1.8 (-8.4 – 5.1)
	44	6.2 (0.4 – 12.2)	4.3 (-1.3 – 10.2)	2.9 (-2.7 – 8.9)	-9.8 (-23.1 – 5.7)	-11.6 (-24.6 – 3.6)	-14.9 (-27.3 – -0.4)	2.8 (-4.5 – 10.6)	1.2 (-5.9 – 8.9)	-0.5 (-7.6 – 7.1)
	53	5.9 (3.5 – 8.3)	4.1 (1.8 – 6.4)	2.7 (0.0 – 5.4)	7.1 (1.7 – 12.8)	5.1 (-0.3 – 10.6)	0.8 (-5.2 – 7.1)	6.6 (3.6 – 9.8)	5.0 (2.0 – 8.0)	3.1 (-0.2 – 6.6)
	54	8.9 (4.9 – 13.0)	7.0 (3.1 – 11.1)	5.6 (1.5 – 9.9)	7.4 (-0.8 – 16.4)	5.4 (-2.7 – 14.1)	1.1 (-7.2 – 10.1)	6.5 (1.4 – 11.9)	4.9 (-0.2 – 10.1)	3.0 (-2.1 – 8.4)
	66	4.7 (2.4 – 7.1)	2.9 (0.7 – 5.2)	1.6 (-1.0 – 4.2)	1.7 (-2.7 – 6.3)	-0.3 (-4.5 – 4.2)	-4.2 (-9.3 – 1.1)	4.9 (1.9 – 7.9)	3.2 (0.4 – 6.2)	1.5 (-1.8 – 4.8)
	68*	0.5 (-3.2 – 4.3)	-1.2 (-4.8 – 2.5)	-2.5 (-6.2 – 1.4)	2.3 (-5.8 – 10.9)	0.3 (-7.6 – 8.8)	-3.7 (-11.7 – 5.0)	0.5 (-4.4 – 5.6)	-1.1 (-5.8 – 3.9)	-2.8 (-7.6 – 2.3)
	70	3.5 (-3.5 – 11.0)	1.8 (-5.1 – 9.1)	0.4 (-6.4 – 7.7)	-6.6 (-19.9 – 8.8)	-8.5 (-21.5 – 6.7)	-12.0 (-24.4 – 2.4)	2.2 (-6.2 – 11.4)	0.6 (-7.6 – 9.6)	-1.1 (-9.1 – 7.7)
	74	1.16 (-2.8 – 5.3)	-0.5 (-4.4 – 3.4)	-1.9 (-5.8 – 2.3)	0.2 (-9.1 – 10.6)	-1.7 (-10.9 – 8.4)	-5..6 (-14..7 – 4..4)	3..6 (-1..3 – 8..8)	2..0 (-2..8 – 7..0)	0..2 (-4..7 – 5..4)

Percentual change in prevalence per year, was calculated by exponentiating the regression coefficients of study year

1. Confounders are: age, lifetime sex partners, history of any sexually transmitted infection (STI), steady partner, notified for STI, sex partners past 6 months, condom use with casual partner

2. Confounders are: age, lifetime sex partners, history of any STI

3. Confounders are: age, lifetime sex partners, history of any STI, notified for STI, sex partners past 6 months, condom use with casual partner

HPV68 also includes HPV73 and HPV97*

Supplementary Table 5: Percentual change in HPV prevalence per year of sensitivity analyses pooling data of PASSYON study rounds 2009 and 2011.

		All women[1]	Heterosexual men[2]	Unvaccinated women[3]
		% (95%CI)	% (95%CI)	% (95%CI)
High-risk HPV types	HPV16	-18..75 (-21..74 – -15..67)	-10..68 (-17..47 – -3..34)	-9..29 (-13..11 – -5..30)
	HPV18	-17..05 (-20..71 – -13..21)	-16..60 (-25..04 – -7..21)	-8..51 (-13..16 – 3..60)
	HPV31	-9..67 (-13..01 – -6..19)	-7..86 (-15..58 – 0..55)	-3..78 (-8..16 – 0..81)
	HPV33	-6..64 (-11..47 – -1..54)	-7..36 (-18..66 – 5..49)	-3..14 (-9..40 – 3..54)
	HPV35	-2..66 (-9..17 – 4..32)	5..73 (-7..03 – 20..25)	-0..79 (-9..20 – 8..39)
	HPV39	-1..00 (-5..02 – 3..20)	0..03 (-9..48 – 10..54)	-1..38 (-6..52 – 4..04)
	HPV45	-10..26 (-15..73 – -4..44)	-11..50 (-23..65 – 2..58)	-1..51 (-8..23 – 5..70)
	HPV51	-1..34 (-4..15 – 1..55)	-1..40 (-7..43 – 5..03)	-3..82 (-7..42 – -0..09)
	HPV52	-0..82 (-3..89 – 2..36)	3..37 (-4..10 – 11..43)	2..48 (-1..49 – 6..61)
	HPV56	2..11 (-1..84 – 6..23)	0..10 (-7..76 – 8..62)	-0..41 (-5..42 – 4..86)
	HPV58	2..27 (-3..18 – 8..03)	5..92 (-7..52 – 21..32)	4..93 (-2..04 – 12..38)
	HPV59	-5..19 (-10..17 – 0..07)	-4..98 (-14..93 – 6..13)	-12..23 (-18..97 – -4..94)
Low-risk HPV types	HPV6	0..20 (-3..59 – 4..13)	0..62 (-5..79 – 7..48)	-2..15 (-6..97 – 2..93)
	HPV11	0..95 (-7..80 – 10..53)	-0..46 (-13..49 – 14..55)	1..53 (-9..48 – 13..87)
	HPV34	-15..37 (-22..55 – -7..53)	-30..00 (-45..01 – -10..89)	-7..58 (-17..14 – 3..08)
	HPV40	-5..73 (-12..01 – 0..99)	-6..07 (-17..59 – 7..07)	-4..86 (-12..98 – 4..02)
	HPV42	-1..89 (-8..67 – 5..39)	-16..01 (-33..48 – 6..05)	-8..24 (-17..58 – 2..16)
	HPV43	-2..01 (-8..21 – 4..60)	-6..69 (-17..53 – 5..57)	-3..63 (-11..64 – 5..11)
	HPV44	2..54 (-3..88 – 9..40)	-11..17 (-26..30 – 7..04)	-0..67 (-9..09 – 8..53)
	HPV53	1..19 (-2..17 – 4..66)	4..44 (-3..21 – 12..67)	2..37 (-1..95 – 6..87)
	HPV54	4..47 (-0..39 – 9..56)	4..11 (-6..00 – 15..33)	2..04 (-4..25 – 8..75)
	HPV66	-0..63 (-3..87 – 2..73)	-2..72 (-9..30 – 4..33)	-0..83 (-4..99 – 3..53)
	HPV68/73/97	-4..97 (-9..53 – -0..18)	-0..18 (-9..60 – 10..21)	-4..88 (-10..93 – 1..59)
	HPV70	-0..86 (-8..60 – 7..54)	-10..72 (-25..98 - 7..69)	-2..33 (-11..84 – 8..20)
	HPV74	-5..25 (-10..03 – -0..21)	-3..78 (-14..63 – 8..45)	-2..30 (-8..41 – 4..21)
Pooled outcome measures	2v types[4]	-18..13 (-20..76 – -15..41)	-13..03 (-19..10 – -6..51)	-9..01 (-12..42 – -5..46)
	9v hr types[5]	-8..92 (-11..23 – -6..56)	-6..27 (-11..66 – -0..55)	-3..41 (-6..44 – -0..28)
	All hr types[6]	-5..46 (-7..65 – -3..21)	-3..56 (-8..63 – 1..80)	-3..37 (-6..21 – -0..44)
	All types[7]	-3..61 (-5..79 – -1..39)	-1..79 (-6..77 – 3..46)	-2..52 (-5..31 – 0..35)

Percentual change in prevalence per year, was calculated by exponentiating the adjusted regression coefficients of study year. Pooled estimates were obtained as a weighted average of the type-specific trends in the GEE Poisson models
1. Adjusted for age, policy change at the sexual health center, lifetime sex partners, history of any sexually transmitted (STI) infection, steady partner, notified for an STI, sex partners past 6 months, condom use with casual partner
2. Adjusted for age, policy change at the sexual health center, lifetime sex partners, history of any STI
3. Adjusted for age, policy change at the sexual health center, lifetime sex partners, history of any STI, notified for STI, sex partners past 6 months, condom use with casual partner
4. Including HPV types 16, 18
5. Including HPV types 16, 18, 31, 33, 45, 52, 58
6. Including HPV types 16, 18, 31, 33, 45, 52, 58, 35, 39, 51, 56, 59
7. Including HPV types 16, 18, 31, 33, 45, 52, 58, 35, 39, 51, 56, 59, 6, 11, 34, 40, 42, 43, 44, 53, 54, 66, 70, 74, 68/73/97

References

1. de Martel C , Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. Int J Cancer2017; 141:664–70.
2. Qendri V , Schurink-Van 't Klooster TM, Bogaards JA, Berkhof J. Ten years of HPV vaccination in the Netherlands: current evidence and future challenges in HPV-related disease prevention. Expert Rev Vaccines2018; 17:1093–104.
3. van Lier E , Oomen P, Giesbers H, et al. Vaccinatiegraad en jaarverslag Rijksvaccinatieprogramma Nederland 2018. Report No.: 2019-0015. Bilthoven, the Netherlands: National Institute for Public Health and the Environment, 2019.
4. Woestenberg PJ , King AJ, van Benthem BHB, et al. ; Medical Microbiological Laboratories and the Public Health Services. Bivalent vaccine effectiveness against type-specific HPV positivity: evidence for cross-protection against oncogenic types among Dutch STI clinic visitors. J Infect Dis2018; 217:213–22.
5. Donken R , King AJ, Bogaards JA, Woestenberg PJ, Meijer CJLM, de Melker HE. High effectiveness of the bivalent human papillomavirus (HPV) vaccine against incident and persistent HPV infections up to 6 years after vaccination in young Dutch women. J Infect Dis2018; 217:1579–89.
6. Korostil IA , Ali H, Guy RJ, Donovan B, Law MG, Regan DG. Near elimination of genital warts in Australia predicted with extension of human papillomavirus vaccination to males. Sex Transm Dis2013; 40:833–5.
7. Kavanagh K , Pollock KG, Cuschieri K, et al. Changes in the prevalence of human papillomavirus following a national bivalent human papillomavirus vaccination programme in Scotland: a 7-year cross-sectional study. Lancet Infect Dis2017; 17:1293–302.
8. Machalek DA , Garland SM, Brotherton JML, et al. Very low prevalence of vaccine human papillomavirus types among 18- to 35-year old Australian women 9 years following implementation of vaccination. J Infect Dis2018; 217:1590–600.
9. Drolet M , Bénard É, Pérez N, Brisson M; HPV Vaccination Impact Study Group. Population-level impact and herd effects following the introduction of human papillomavirus vaccination programmes: updated systematic review and meta-analysis. Lancet2019; 394:497–509.
10. Woestenberg PJ , Bogaards JA, King AJ, et al. ; Medical Microbiological Laboratories and the Public Health Services. Assessment of herd effects among women and heterosexual men after girls-only HPV16/18 vaccination in the Netherlands: a repeated cross-sectional study. Int J Cancer2019; 144:2718–27.
11. Malagón T , Laurie C, Franco EL. Human papillomavirus vaccination and the role of herd effects in future cancer control planning: a review. Expert Rev Vaccines2018; 17:395–409.
12. Man I , Vänskä S, Lehtinen M, Bogaards JA. Human papillomavirus genotype replacement: still too early to tell? [manuscript published online ahead of print 27 January 2020]. J Infect Dis2020; doi:1093/infdis/jiaa032.
13. Vriend HJ , Boot HJ, van der Sande MA; Medical Microbiological Laboratories; Municipal Health Services. Type-specific human papillomavirus infections among young heterosexual male and female STI clinic attendees. Sex Transm Dis2012; 39:72–8.
14. Visser M , van Aar F, van Oeffelen A, et al. Sexually transmitted infections in the Netherlands in 2016. Bilthoven, the Netherlands: National Institute for Public Health and the Environment, 2017.
15. Vriend HJ , Bogaards JA, van der Klis FR, et al. ; Medical Microbiological Laboratories, Municipal Health Services. Patterns of human papillomavirus DNA and antibody positivity in young males and females, suggesting a site-specific natural course of infection. PLoS One2013; 8:e60696.

16. Woestenberg PJ , van Benthem BH, Bogaards JA, et al. HPV infections among young MSM visiting sexual health centers in the Netherlands: opportunities for targeted HPV vaccination. Vaccine2020; 38:3321–9.
17. Oliver SE , Unger ER, Lewis R, et al. Prevalence of human papillomavirus among females after vaccine introduction—National Health and Nutrition Examination Survey, United States, 2003–2014. J Infect Dis2017; 216:594–603.
18. Malagón T , Drolet M, Boily MC, et al. Cross-protective efficacy of two human papillomavirus vaccines: a systematic review and meta-analysis. Lancet Infect Dis2012; 12:781–9.
19. Lehtinen M , Luostarinen T, Vänskä S, et al. Gender-neutral vaccination provides improved control of human papillomavirus types 18/31/33/35 through herd immunity: results of a community randomized trial (III). Int J Cancer2018; 143:2299–310.
20. Bogaards JA , van der Weele P, Woestenberg PJ, van Benthem BHB, King AJ. Bivalent human papillomavirus (HPV) vaccine effectiveness correlates with phylogenetic distance from HPV vaccine types 16 and 18. J Infect Dis2019; 220:1141–6.
21. de Villiers EM . Cross-roads in the classification of papillomaviruses. Virology2013; 445:2–10.
22. Tota JE , Ramanakumar AV, Jiang M, et al. Epidemiologic approaches to evaluating the potential for human papillomavirus type replacement postvaccination. Am J Epidemiol2013; 178:625–34.
23. Mesher D , Soldan K, Lehtinen M, et al. Population-level effects of human papillomavirus vaccination programs on infections with nonvaccine genotypes. Emerg Infect Dis2016; 22:1732–40.
24. Gray P , Palmroth J, Luostarinen T, et al. Evaluation of HPV type-replacement in unvaccinated and vaccinated adolescent females-post-hoc analysis of a community-randomized clinical trial (II). Int J Cancer2018; 142:2491–500.
25. Mesher D , Panwar K, Thomas SL, et al. The impact of the national HPV vaccination program in England using the bivalent HPV vaccine: surveillance of type-specific HPV in young females, 2010–2016. J Infect Dis2018; 218:911–21.
26. Feiring B , Laake I, Christiansen IK, et al. Substantial decline in prevalence of vaccine-type and nonvaccine-type human papillomavirus (HPV) in vaccinated and unvaccinated girls 5 years after implementing HPV vaccine in Norway. J Infect Dis2018; 218:1900–10.
27. Mollers M , Vriend HJ, van der Sande MA, et al. Population- and type-specific clustering of multiple HPV types across diverse risk populations in the Netherlands. Am J Epidemiol2014; 179:1236–46.
28. Baussano I , Lazzarato F, Ronco G, Franceschi S. Impacts of human papillomavirus vaccination for different populations: a modeling study. Int J Cancer2018; 143:1086–92.

6

Chapter 6

Vaccine effectiveness following routine immunization with bivalent human papillomavirus (HPV) vaccine: Protection against incident genital HPV infections from a reduced-dosing schedule.

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ABSTRACT

Background: In the Netherlands, the bivalent human papillomavirus (HPV) vaccine has been offered to preadolescent girls via the National Immunization Program in a 2-dose schedule since 2014. The current study estimates vaccine effectiveness (VE) against HPV infections up to 4 years postvaccination among girls eligible for routine 2-dose immunization.

Methods: A cohort study (HAVANA2) was used in which participants annually filled out an online questionnaire and provided a vaginal self-sample for determination of HPV by the SPF10-LiPA25 assay, able to detect 25 HPV types. VE against incident type-specific infections and pooled outcomes was estimated by a Cox proportional hazards model with shared frailty between the HPV types.

Results :In total, 2027 girls were included in the study, 1098 (54.2%) of whom were vaccinated with 2 doses. Highest incidence rate was 5.0/1000 person-years (HPV-51) among vaccinated participants and 9.1/1000 person-years (HPV-74) among unvaccinated participants. Adjusted pooled VE was 84.0% (95% confidence interval [CI], 27.0%–96.5%) against incident HPV-16/18 infections and 86.5% (95% CI, 39.5%–97.08%) against cross-protective types HPV-31/33/45.

Conclusions: Four years postvaccination, 2 doses of bivalent HPV vaccine were effective in the prevention of incident HPV-16/18 infections and provided cross-protection to HPV-31/33/45. Our VE estimates rival those from 3-dose schedules, indicating comparable protection by 2-dose schedules.

Introduction

Persistent infections with human papillomavirus (HPV) are associated with development of clinical disease, including anogenital or oropharyngeal cancers (in case of high-risk HPV type infections) and anogenital or laryngeal warts (in case of low-risk HPV type infections) [1, 2]. From 2006 onward, 3 vaccines targeting different combinations of HPV types have been registered, which were initially licensed and offered according to a 3-dose (3D) schedule (recommended schedule: 0, 1, and 6 months). The European Medicines Agency licensed a 2-dose (2D) schedule (0 and 6 months) for all available HPV vaccines in 2014 for vaccine recipients aged 9–14 years [3]. Immunobridging studies demonstrated comparable immunogenicity between 9- to 14-year-old 2D-vaccinated and 15- to 26-year-old 3D-vaccinated individuals. As efficacy of vaccination was already shown among 3D vaccine recipients, comparable efficacy was expected after 2 doses in case of noninferior immunogenicity [4, 5]. When comparing girls vaccinated at similarly young age, antibody levels against HPV vaccine types following 2 doses are within acceptable ranges compared to 3 doses [6–8], although noninferiority of HPV-18 antibodies is still inconclusive [9, 10].

Ultimately, noninferiority of reduced-dosing schedules needs to be assessed on vaccine efficacy and effectiveness outcomes. However, assessment of protection against virological and clinical outcomes following a 2D schedule requires long follow-up and results are still limited, especially for the bivalent vaccine (2vHPV). Originally, HPV vaccine trials in young women used cervical intraepithelial neoplasia grade 2 (CIN2+) or higher as outcome. Since the introduction of HPV vaccination, HPV infections are endorsed as intermediate endpoint for monitoring vaccine effectiveness (VE) [11]. Only a few studies have shown protection against HPV infections or CIN2+ after 2 doses, including randomized controlled trials (RCTs), a cross-sectional study, and a linkage study [12–14]. Other observational data also indicated protection from reduced dosing schedules (including both 1- and 2-dose vaccination) [15–17]. However, the vast majority of these studies were not conducted in the context of a recommended 2D schedule, so information was retrieved from incompletely vaccinated individuals (ie, those who did not complete 3D vaccination series) [13, 18]. Therefore, numbers may be small, age at vaccination may be higher, and the interval between first and second vaccine can be shorter than recommended, possibly leading to lower antibody levels or waning of antibodies [19]. Together, this might affect VE estimates. As preadolescents who were vaccinated according to prescribed 2D regimens do not yet attend cervical cancer screening programs, monitoring of HPV infections is necessary to assess VE of 2D vaccination in a population-based setting.

The current study aims to estimate VE of 2vHPV vaccination against incident genital HPV infections after a 2-dose recommended schedule from a Dutch longitudinal cohort study. In the Netherlands, 2vHPV vaccination targeting high-risk types HPV-16 and HPV-18 was included in the National Immunization Program (NIP) beginning in 2010, initially as a girls-only vaccine in a 3D schedule [20]. The 3D schedule was replaced by a 2D schedule in 2014, starting with girls born in 2001 (eligible for vaccination in the year they turn 13). Vaccine uptake has been suboptimal in the Netherlands, ranging between 46% and 61% [21], which facilitates comparisons between vaccinated and unvaccinated individuals from the same birth cohorts. We report data up to 4 years postvaccination among routinely vaccinated Dutch girls from the first birth cohort eligible for the 2D schedule.

Methods

Study Design

In 2016, letters of invitation were sent to 11 770 vaccinated and 27 491 unvaccinated girls from birth cohort 2001. A longitudinal cohort study was initiated: HAVANA2 (HPV Amongst Vaccinated and Nonvaccinated Adolescents After 2 Doses). Girls and their parents signed an informed consent before inclusion in the study (n = 2476 correct informed consents, response rate 6.3%). Vaccination status of participants was acquired through the national vaccination registry, Praeventis [22]. Every year, participants filled out a web-based questionnaire and collected a vaginal self-sample (Viba-Brush, Rovers Medical Devices, Oss, the Netherlands). We report data from 2016, 2017, and 2018 (Figure 1), that is, up to 4 years postvaccination. This study adhered to the tenets of the Declaration of Helsinki and was approved by the Medical Ethics Committee of the VU University Medical Center (2009/022).

Laboratory Analyses

Self-collected vaginal samples were stored in 1 mL buffered saline at –20°C. DNA was isolated from 200 µL of suspension using the MagNA Pure DNA and viral NA small volume Kit (Roche, Mannheim, Germany). DNA was eluted in 100 µL of elution buffer, of which 10 µL was used for amplification of HPV DNA. Amplification was performed using the broad-spectrum SPF10 primer cocktail. Amplified HPV DNA was detected with a DNA enzyme-linked immunoassay (HPV-DEIA, Labo Biomedical Products, Rijswijk, the Netherlands). HPV-DEIA–positive amplicons were subsequently analyzed in a reverse line blot assay (HPV-LiPA25, Labo Biomedical Products, Rijswijk, the Netherlands). The reverse line blot assay is able to detect 25 HPV genotypes, including high-risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. Additionally, it can detect 13 low-risk types: 6, 11, 34, 40, 42, 43, 44, 53, 54,

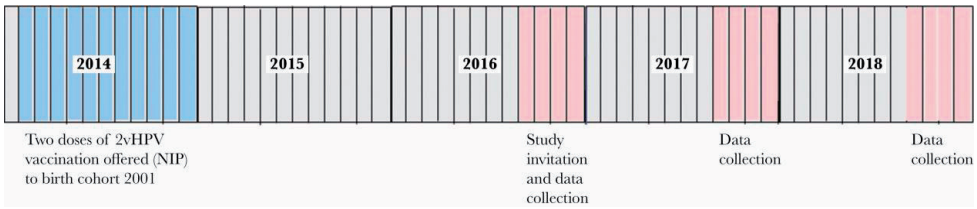

Figure 1: Study design and first years of follow-up.

66, 70, and 74. HPV types 68, 73, and 97 are also detected, but since no distinction between these types can be made, they are all classified as HPV-68.

Statistical Analyses

For inclusion in the analyses, participants needed to hand in a vaginal self-sample in the first study round and be either unvaccinated or vaccinated according to 2D schedule before study start. Unvaccinated participants who decided to initiate HPV vaccination during the study were included in the unvaccinated group until the year they started vaccination and were censored thereafter. For participants with missing follow-up data in the questionnaires, data from previous years were used for analyses if possible (last observation carried forward). Participants were censored for the remainder of the follow-up period if they did not contribute a self-sample to a year.

To explore possible associations with HPV vaccine uptake, sociodemographic characteristics among vaccinated and unvaccinated girls were described per study year. Differences in characteristics between vaccinated and unvaccinated girls over time were analyzed in a generalized estimating equation (GEE) binomial model with logit link and an exchangeable correlation structure. Each characteristic was modeled as a function of vaccination status, study round, and their interaction, to assess potential trend differences over time. Additionally, we examined which characteristics were associated with HPV infection (irrespective type or persistence) using a time-dependent GEE with a Poisson distribution and a log-link. Characteristics significantly associated with HPV in univariable models ($P < .05$) were included in a multivariable model to identify characteristics independently associated with HPV. These were considered as covariates to adjust for in VE analyses. An additional category was included for missing observations per characteristic. For the final models we included age, ethnicity, ever had sexual intercourse, and ever used contraception to adjust the estimates. The other characteristics that were associated with HPV were all related to sexual behavior and were not included in the model as this resulted in nonconvergence (to many variables in model, data not shown).

Type-specific HPV prevalence was determined per year. Incidence was defined as being positive for a specific HPV type, preceded by a negative sample in the previous year (except for infections in the first year). Persistence was defined as being HPV positive for the same HPV type in (at least) 2 consecutive years. Type-specific incidence and persistence rates were calculated as the number of infections divided by the person-years at risk (Poisson approach). Infections (events) were counted at the year in which they were detected. Person-years were counted as the time between vaccination or vaccine eligibility for unvaccinated participants (set at 30 June 2014, halfway during the year girls were eligible) and the end of follow-up or the time of an event, whichever came first. This reflects the time girls were at risk for developing an incident or persistent infection. The maximum number of person-years per participant was 4 (2014–2018).

VE against incident HPV infections was estimated for all HPV types available in the HPV-LiPA25 using a Cox proportional hazards model with shared frailty between HPV types. VE was calculated as 1 – hazard ratio × 100%. For HPV types with zero infection events among vaccinated girls (ie, VE = 100%), approximate lower bounds of the 95% confidence intervals (CIs) were obtained by the Peto estimator for the hazard ratio, based on the log-rank statistic [23]. For other types, event-specific hazards were adjusted using time-dependent sociodemographic characteristics as previously identified. The frailty term in the Cox model denotes a random effect on the individual level, representing residual heterogeneity in HPV infection risk irrespective of type. VE against all HPV types was estimated by 1 multivariate model, with covariate effects estimated simultaneously for all types. As pooled outcomes, we considered vaccine types (HPV-16/18), cross-protective types (HPV-31/33/45), high-risk types (HPV-16/18/31/33/45/52/58), low-risk types associated with genital warts (HPV-6/11), and a combination of vaccine and cross-protective types (HPV-16/18/31/33/45). Pooled outcomes were estimated as weighted averages of types included in a particular combination to obtain more precise estimates compared to type-specific VE or a priori specification of combined outcomes. All statistical analyses were performed using SAS software version 9.4 (SAS Institute, Cary, North Carolina).

Results

A total of 2027 girls handed in a vaginal self-sample in the first study year, 1098 (54.2%) of whom were vaccinated against HPV according to a 2D schedule at age 12 (in the year they turned 13, according to the NIP). The number of girls participating amounted to 1666 in the third year due to loss to follow-up (Table 1). At study start, the mean age was 15 years. In the first part of Table 1, the entire study population is

Table 1: Sociodemographic Characteristics by Study Year Among Vaccinated and Unvaccinated Participants` a: P values for main effect of vaccination status are reported (no time interaction).

Characteristic	Study Year 1 (2016)				Study Year 2 (2017)				Study Year 3 (2018)				Associated With Vaccination Status	Associated With HPV
	Unvaccinated (n = 929)		Vaccinated (n = 1098)		Unvaccinated (n = 802)		Vaccinated (n = 944)		Unvaccinated (n = 767)		Vaccinated (n = 899)			
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	P-value	P-value
Age, y, mean (range)	14.97 (14–15)		14.96 (14–15)		15.98 (15–16)		15.97 (15–16)		16.97 (16–17)		16.96 (16–17)			<.0001
Dutch ethnicity													.7328	.0468
Yes	769	(82.8)	926	(84.3)	671	(83.7)	772	(81.8)	651	(84.9)	748	(83.2)		
No	102	(11. 0)	120	(10.9)	75	(9.4)	97	(10.3)	79	(10.3)	85	(9.5)		
Degree of urbanization													.4166	.1892
Low	422	(45.4)	476	(43.4)	367	(45.8)	405	(42.9)	359	(46.8)	400	(44.5)		
High	443	(47.7)	558	(50.8)	379	(47.3)	462	(48.9)	371	(48.4)	433	(48.2)		
Highest educational level													.4803	.1249
Low	365	(39.3)	437	(39.8)	283	(35.3)	328	(34.7)	275	(35.9)	316	(35.2)		
High	506	(54.5)	609	(55.5)	463	(57.7)	541	(57.3)	450	(58.7)	510	(56.7)		
Ever used contraception													.03	.0261
No	625	(67.3)	734	(66.8)	416	(51.9)	420	(44.5)	282	(36.8)	275	(30.6)		
Yes	246	(26.5)	312	(28.4)	330	(41.1)	449	(47.6)	448	(58.4)	558	(62.1)		
Smoking past year													.3544	.4011
No	745	(80.2)	910	(82.9)	585	(72.9)	696	(73.7)	523	(68.2)	603	(67.1)		
Yes	126	(13.6)	136	(12.4)	161	(20.1)	173	(18.3)	207	(27.0)	230	(25.6)		
Sexual orientation													.6632	.5703
Heterosexual	822	(88.5)	984	(89.6)	693	(86.4)	808	(85.6)	672	(87.6)	764	(85.0)		
Other	47	(5.1)	58	(5.3)	50	(6.2)	61	(6.5)	58	(7.6)	67	(7.5)		
Ever had sexual intercourse													.6947	.0195
No	745	(80.2)	921	(83.9)	545	(68.0)	614	(65.0)	400	(52.2)	451	(50.2)		
Yes	126	(13.6)	123	(11.2)	201	(25.1)	255	(27.0)	330	(43.0)	382	(42.5)		

Table 1: Continued

Characteristic	Study Year 1 (2016)				Study Year 2 (2017)				Study Year 3 (2018)				Associated With Vaccination Status	Associated With HPV
	Unvaccinated (n = 929)		Vaccinated (n = 1098)		Unvaccinated (n = 802)		Vaccinated (n = 944)		Unvaccinated (n = 767)		Vaccinated (n = 899)			
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	P-value	P-value
Among sexually active participants only														
Ever used condoms													.6577	.9772
No	14	(11.1)	14	(11.4)	20	(10.0)	18	(7.1)	35	(10.6)	40	(10.5)		
Yes	112	(88.9)	109	(88.6)	181	(90.0)	237	(92.9)	295	(89.4)	342	(89.5)		
Age at first sexual intercourse													.5910	.0270
<15 y	68	(54.0)	70	(56.9)	55	(27.4)	62	(24.3)	50	(15.2)	57	(14.9)		
≥15 y	56	(44.4)	51	(41.5)	142	(70.6)	193	(75.7)	277	(83.9)	325	(85.1)		
No. of sex partners in past year													.6992	.3574
1–2	113	(89.7)	115	(93.5)	174	(86.6)	221	(86.7)	268	(81.2)	316	(82.7)		
≥3	7	(5.6)	6	(4.9)	15	(7.5)	21	(8.2)	35	(10.6)	45	(11.8)		
No. of sex partners in lifetime													.7329	<.0001
1–2	111	(88.1)	108	(87.8)	159	(79.1)	209	(82.0)	250	(75.8)	284	(74.3)		
≥3	15	(11.9)	14	(11.4)	38	(18.9)	45	(17.6)	77	(23.3)	96	(25.1)		
Has current sex partner													.9926	.0238
No	44	(34.9)	48	(39.0)	75	(37.3)	80	(31.4)	109	(33.0)	130	(34.0)		
Yes	81	(64.3)	75	(61.0)	126	(62.7)	175	(68.6)	220	(66.7)	251	(65.7)		
Condom use with partner													.6802	.0011
Never	12	(9.5)	12	(9.8)	23	(11.4)	30	(11.8)	59	(17.9)	67	(17.5)		
Always or sometimes	64	(50.8)	60	(48.8)	93	(46.3)	135	(52.9)	143	(43.3)	161	(42.1)		
Diagnosed with STI past year													.3394	.0092
No	125	(99.2)	121	(98.4)	196	(97.5)	253	(99.2)	321	(97.3)	373	(97.6)		
Yes	1	(0.8)	2	(1.6)	5	(2.5)	2	(0.8)	9	(2.7)	9	(2.4)		

described regarding sociodemographic characteristics. Sexual activity increased from 12% to 43% over the first 3 years. In general, vaccinated and unvaccinated girls were comparable regarding sociodemographic characteristics, except for contraception use: Vaccinated participants were more likely to ever have used contraception (odds ratio [OR], 1.18 [95% CI, 1.01–1.37]). Table 1 also shows sexual behavior characteristics among the sexually active participants only. No differences between vaccinated and unvaccinated participants were seen in sociodemographic or sexual characteristics over time.

The prevalence of type-specific HPV infections was low in the first study year among both vaccinated and unvaccinated girls (Figure 2). Prevalence of any HPV type infection (both low risk and high risk) increased from 1.7% in year 1 to 11.0% in year 3 for unvaccinated participants and from 1.1% to 8.0% for vaccinated participants, respectively. HPV-51 was the most prevalent high-risk type, while HPV-74 was the most prevalent low-risk type, irrespective of vaccination status. Type-specific incidence rates ranged from 0.0 to 9.1 per 1000 person-years (HPV-74) among unvaccinated and from 0.0 to 5.0 per 1000 person-years (HPV-51) for vaccinated girls. Due to the low number of persistent infections, persistence rates could only be calculated for a limited number of HPV types. Highest persistence rates were 1.2 per 1000 person-years (HPV-16) among unvaccinated participants and 0.8 per 1000 person-years (HPV-51) among vaccinated participants (Table 2).

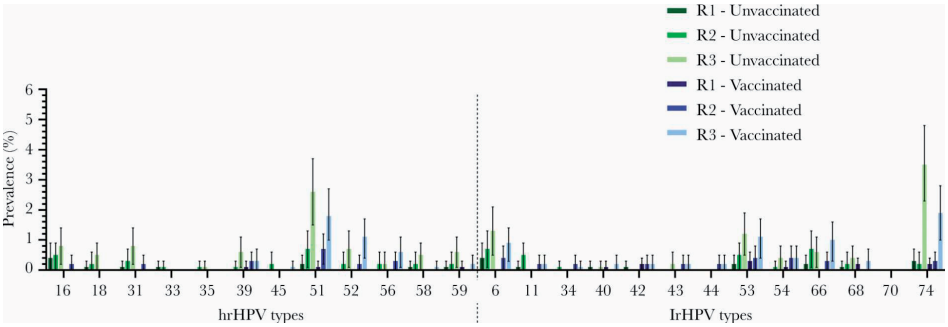

Figure 2: Type-specific prevalence with 95% confidence intervals of high-risk and low-risk human papillomavirus (hrHPV and lrHPV, respectively) types among vaccinated and unvaccinated participants per study year

VE against incident infections was calculated for all high-risk types (Figure 3A), all low-risk types (Figure 3B) and for pooled outcomes (Figure 4). Type-specific VE estimates against HPV-18, -33, -35, and -45 were all 100%, as no infections among vaccinated participants were detected, but only VE against HPV-18 was statistically

Table 2: Type-Specific Incidence and Persistence Rates per 1000 Person-Years Among Vaccinated and Unvaccinated Participants.

HPV Type	Incidence Rates per 1000 PY (95% CI)		Persistence Rates per 1000 PY (95% CI)	
	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
High-risk types				
16	1.8 (1.5–2.1)	0.5 (.3–.7)	1.2 (.5–3.2)	0.0
18	1.5 (1.3–1.8)	0.0 (.0–.3)	0.0	0.0
31	2.7 (2.4–3.1)	0.5 (.3–.7)	0.3 (.0–2.2)	0.0
33	0.6 (.4–.8)	0.0 (.0–.3)	0.0	0.0
35	0.6 (.4–.8)	0.0 (.0–.3)	0.0	0.0
39	1.8 (1.5–2.1)	1.8 (1.5–2.0)	0.0	0.0
45	0.6 (.4–.8)	0.0 (.0–.3)	0.0	0.0
51	6.7 (6.1–7.3)	5.0 (4.6–5.5)	0.6 (.2–2.4)	0.8 (.2–2.3)
52	2.1 (1.8–2.5)	2.3 (2.0–2.6)	0.0	0.3 (.0–1.8)
56	0.9 (.7–1.1)	2.0 (1.7–2.3)	0.3 (.0–2.2)	0.3 (.0–1.8)
58	1.2 (.9–1.5)	0.3 (.1–.4)	0.0	0.0
59	2.1 (1.8–2.5)	0.8 (.6–1.0)	0.3 (.0–2.2)	0.0
Low-risk types				
6	4.9 (4.4–5.4)	2.3 (2.0–2.6)	0.9 (.3–2.8)	0.0
11	1.2 (.9–1.5)	0.5 (.4–.7)	0.3 (.0–2.2)	0.3 (.0–1.8)
34	0.0 (.0–.3)	0.8 (.6–1.0)	0.0	0.0
40	0.3 (.2–.5)	0.8 (.6–1.0)	0.0	0.0
42	0.3 (.2–.5)	1.0 (.8–1.2)	0.0	0.5 (.1–2.0)
43	0.6 (.4–.8)	0.8 (.6–1.0)	0.0	0.0
44	0.0 (.0–.3)	1.0 (.8–1.2)	0.0	0.0
53	3.3 (2.9–3.8)	3.5 (3.2–3.9)	0.6 (.2–2.4)	0.5 (.1–2.0)
54	1.2 (.9–1.5)	1.8 (1.5–2.0)	0.0	0.5 (.1–2.0)
66	3.0 (2.7–3.4)	2.0 (1.8–2.3)	0.0	0.3 (.0–1.8)
68	1.2 (.9–1.5)	1.0 (.8–1.2)	0.0	0.0
70	0.0 (.0–.3)	0.0 (.0–.3)	0.0	0.0
74	9.1 (8.5–9.8)	4.8 (4.4–5.2)	0.0	0.0

The 95% confidence intervals for zero observations were calculated based on the rule of 3.

significant in unadjusted analyses (P = .0148, log-rank test). For other types, estimates were adjusted for age, ethnicity, ever had sexual intercourse, and ever used contraception. In the pooled outcomes analyses, adjusted VE against vaccine types HPV-16/18 was 84.0% (95% CI, 27.0%–96.5%). The VE against cross-protective types HPV-31/33/45 was 86.5% (95% CI, 39.5%–97.08%). Moreover, the VE against incident infections with high-risk types HPV-16/18/31/33/45/52/58 was 64.9% (95% CI,

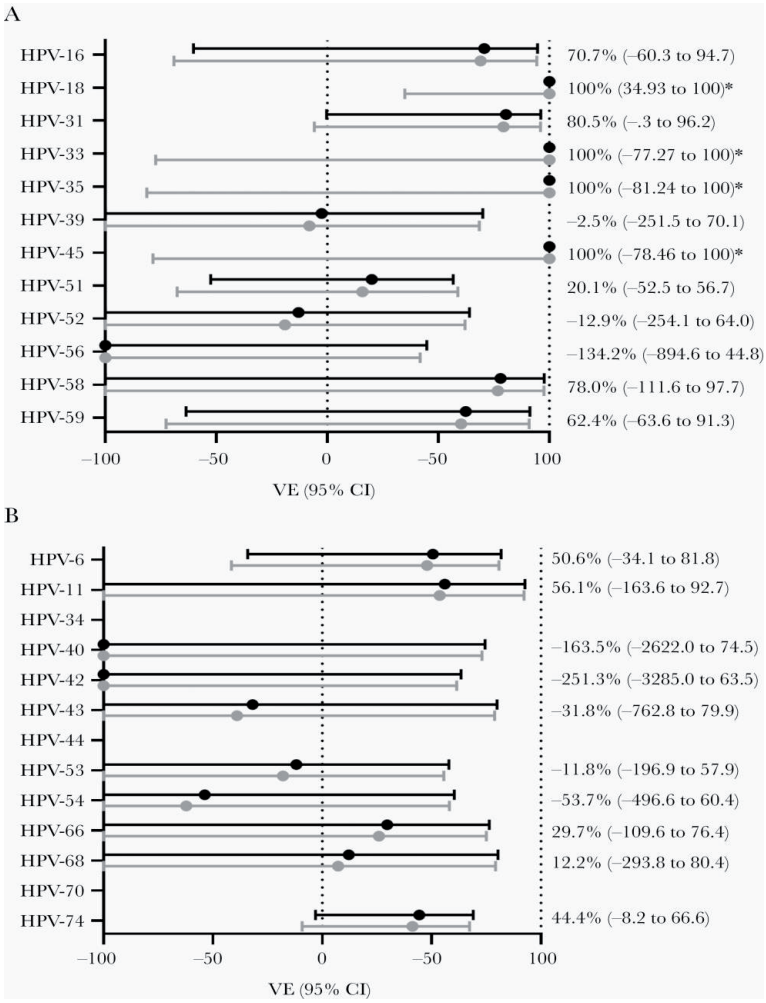

Figure 3: Type-specific vaccine effectiveness (VE) estimates against incident human papillomavirus (HPV) infections with 95% confidence intervals (CIs). Crude (gray dots) and adjusted estimates (black dots) are shown for high-risk (A) and low-risk (B) HPV types. VE was adjusted for age, ethnicity, ever had sexual intercourse, and ever used contraception. *For HPV types with no infections among vaccinated participants, confidence estimates could only be included for the crude estimates (using the Peto estimator for the hazard ratio, based on the log-rank statistic).

20.2%–81.0%), while VE against low-risk types 6 and 11 was 51.7% (95% CI, –3.1% to 77.4%). The complete models including estimates for covariates are included in Supplementary Data 1.

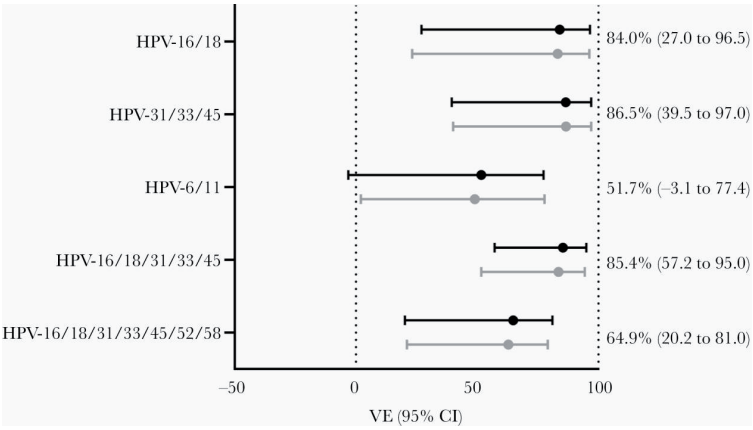

Figure 4: Vaccine effectiveness (VE) estimates for pooled outcomes against incident human papillomavirus (HPV) infections with 95% confidence intervals (CIs) for crude (gray dots) and adjusted estimates (black dots). VE was adjusted for age, ethnicity, ever had sexual intercourse, and ever used contraception.

Discussion

We studied VE of 2 doses of the HPV-16/18 vaccine in the first birth cohort eligible for reduced-dosing schedule vaccination in the routine vaccination program of the Netherlands. With a 4-year postvaccination follow-up, we demonstrate protection against incident HPV-16/18 infections as well as cross-protection against HPV-31/33/45 infections. To our knowledge, this is the first observational study reporting VE of 2vHPV vaccination against type-specific HPV positivity among routinely 2D vaccinated young women. Our VE estimates compare well to those derived from birth cohorts eligible for the 3D schedule, indicating similar protection of the 2D schedule [24–26].

An important aspect of this study is that the 2 doses of HPV vaccination were routinely offered in the NIP and replaced the initial 3D schedule based on immunological data. In this context, evidence for effectiveness based on virological and clinical outcomes is imperative and should be compared to the effectiveness following 3 doses. Previous research on the 3D schedule can provide various benchmarks, since studies may report effectiveness against incident, prevalent, or persistent infections, with increasing expectation for effectiveness, respectively. Data from Dutch surveillance studies among 3D vaccine-eligible girls from the catch-up campaign (slightly older at vaccination compared to our participants) indicated that VE against incident HPV-16/18 infections was 70% (95% CI, 52%–82%) 4 years postvaccination [24]. For HPV-16/18/31/45, VE was 72% (95% CI, 58%–82%). For persistent HPV-16/18

infections up to 6 years postvaccination from the same study, VE was 97.7% (95% CI, 83.5%–99.7%) [25]. Another Dutch surveillance study among sexual health clinic visitors eligible for 3D vaccination reported a VE of 89.9% (95% CI, 81.7%–94.4%) against prevalent HPV-16/18 infections [26]. Together, these findings align well with the observations from the current 2D study in which VE of 84% against incident HPV-16/18 infections is found. The primary and intermediate endpoints for HPV vaccination as indicated by the World Health Organization include persistent infections [11]. Due to low numbers, VE against persistent infections was not yet included in our current analyses. With prolonged follow-up of the current cohort, these estimates can be reported in the future.

Routinely offered 2vHPV vaccination according to a 3D schedule has been in place outside the Netherlands as well. A Scottish study indicated a VE of 89.1% (95% CI, 85.1%–92.3%) against vaccine-type infections among girls offered vaccination at age 12–13 [13]. For HPV-31/33/45 the VE was 85.1% (95% CI, 77.3%–90.9%). VE declined with increasing age of vaccination. In general, VE observed in the current study following a 2D schedule seems comparable to 3D schedule findings, which is in line with the immunological data and immunobridging studies on the basis of which the 2D schedule was licensed.

Two-dose VE estimates can also be evaluated based on clinical trial data. Even though the trials were not designed or powered to study the 2-dose schedule specifically, they often report their findings from minority groups receiving <3 doses. Our point estimate of 84.0% for HPV-16/18 is in line with those of the 2D-vaccinated, HPV-naive cohorts in the HPV PApilloma TRIal against Cancer In young Adults (PATRICIA) and the Costa Rica Vaccine Trial (CRVT) (81.2%) [18]. As indicated before, women receiving their first and second vaccination without proper time interval might have lower antibody levels following vaccination [5, 19]. Subanalyses in the CRVT data indeed showed higher efficacy among those receiving vaccinations 6 months apart as compared to a shorter interval, also affecting the possibility of cross-protection against HPV-31/33/45 [18]. In the current analyses, all 2D-vaccinated girls received their second dose at >5 months apart from their first dose, likely contributing to our high VE estimates in general. Our findings also agree well with observations from a Dutch serosurveillance study conducted among routinely 2D-vaccinated girls [27]. It was found that seroprevalence was 100% up to 2 years postvaccination for vaccine types with corresponding high avidity levels, likely resulting in solid protection against vaccine types and cross-protective types alike.

Other (high-risk) HPV types against which (cross-protective) effects were observed included HPV-31/33/45 (VE, 86.5%), which is in line with observations from pre-

vious research [28], although our estimates were higher. A linkage study between vaccination status and cervical screening from Scotland indicated cross-protection against HPV-31/33/45 of 40.3% among 2D-vaccinated women, but this was among those who did not complete routinely offered 3D vaccination. Regarding type-specific significant VE estimates in general, this was only observed for HPV-18 in unadjusted analyses. This could be due to rather low numbers of type-specific infections and could become measurable with prolonged follow-up time, as observed in 3D schedules and other 2D studies [25, 29].

We found a borderline nonsignificant VE against HPV-6/11 infections (51.7% [95% CI, –3.1% to 77.4%]). These low-risk types are not targeted by HPV-16/18 vaccination. Although 1 of the first RCTs on 2vHPV vaccination [30] also indicated partial protection against HPV-6/11 infections among the HPV-naive cohort, only very few other surveillance studies could replicate such an effect [31]. Some studies have shown a partial protective effect of 2vHPV vaccination against anogenital warts [32], but these were not focused on specific low-risk types. Moreover, based on phylogenetic distance, (cross-) protection against HPV-6 and 11 is not expected [33]. Thus, this finding remains inconclusive and requires further research, for example on the possible biological mechanism underlying cross-protection to low-risk types from the 2vHPV vaccine.

Strengths of the current study include the longitudinal, population-based design. This is the first birth cohort from the Netherlands eligible for 2D vaccination according to protocol, which we were able to follow from a young age. Vaccinated and unvaccinated participants were comparable regarding sociodemographic and sexual characteristics, except for contraception use. This is in line with previous studies, providing no suggestion that HPV vaccination status does affect sexual (risk) behavior [34] and would as such, influence effectiveness of the program or confound VE estimates. Furthermore, our participants were comparable to the general Dutch population regarding sexual behavior, in which the median age for sexual intercourse was 17.5 years for girls in 2017 [35]. However, we also acknowledge some limitations. Due to ethical considerations related to the participants' age at vaccination, we were unable to start follow-up directly after vaccination and to select HPV-negative girls at time of vaccination. Consequently, we might have missed infections that were acquired and cleared between vaccination and the first measurement, and we attributed infections at year 1 to infection at that moment. Also, the rather young age of participants led to a still limited number of type-specific infections, hence decreasing power in type-specific VE estimates. Last, there was a relatively low response rate regarding participation to this study. This might affect generalizability of results to the general population if specific subgroups were more likely to be included. Although participants were

less likely to have a migration background compared to the general population [36], they were comparable regarding sexual behavior. Therefore, we think the bias of our estimates will be limited.

In conclusion, at 4 years postvaccination, 2 doses of 2vHPV vaccine were effective in the prevention of incident HPV-16/18 infections and additionally provided cross-protection against HPV-31/33/45. This is one of the first population-based observational studies investigating the 2D schedule in a regular immunization program setting, and indicates that protection is comparable to 3D schedules and to observations from RCTs. Our findings are promising regarding future clinical impact of reduced-dosing schedules.

Supplementary data

Supplementary data 1: Full models for pooled outcomes. Age, ethnicity, ever had sexual intercourse, and ever used contraception were used to adjust the estimates. The other characteristics that were associated with HPV were all sexual behavior related and were not included in the model as this resulted in non-convergence (to many variables in model).

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter estimate	Standard error	Chi-Square	Pr > ChiSq	Hazard ratio
HPV16/18	1	-1.83473	0.77432	5.6145	0.0178	0.160
Age (continuous)	1	0.59337	0.57338	1.0709	0.3007	1.810
Contraception use never (reference)						
Contraception use ever	1	0.96021	0.23204	17.1249	<.0001	2.612
Contraception use missing	1	0.93597	0.46803	3.9993	0.0455	2.550
Had sexual intercourse never - reference						
Had sexual intercourse ever	1	1.63644	0.19960	67.2140	<.0001	5.137
Had sexual intercourse missing	1	1.65293	0.43927	14.1596	0.0002	5.222
Ethnicity Dutch - reference						
Ethnicity non-Dutch	1	0.47857	0.21524	4.9438	0.0262	1.614
Ethnicity missing	1	-1.14118	0.80073	2.0311	0.1541	0.319

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter estimate	Standard error	Chi-Square	Pr > ChiSq	Hazard ratio
HPV16/18/31/33/45	1	-1.92163	0.54796	12.2983	0.0005	0.146
Age (continuous)	1	0.59338	0.57339	1.0709	0.3007	1.810
Contraception use never (reference)						
Contraception use ever	1	0.96020	0.23204	17.1243	<.0001	2.612
Contraception use missing	1	0.93597	0.46803	3.9993	0.0455	2.550
Had sexual intercourse never - reference						
Had sexual intercourse ever	1	1.63645	0.19961	67.2145	<.0001	5.137
Had sexual intercourse missing	1	1.65292	0.43927	14.1592	0.0002	5.222
Ethnicity Dutch - reference						
Ethnicity non-Dutch	1	0.47858	0.21524	4.9440	0.0262	1.614
Ethnicity missing	1	-1.14118	0.80074	2.0311	0.1541	0.319

Analysis of Maximum Likelihood Estimates						
Parameter	DF	Parameter estimate	Standard error	Chi-Square	Pr > ChiSq	Hazard ratio
HPV31/33/45	1	-2.00164	0.76519	6.8427	0.0089	0.135
Age (continuous)	1	0.59336	0.57338	1.0709	0.3007	1.810
Contraception use never (reference)						
Contraception use ever	1	0.96020	0.23203	17.1246	<.0001	2.612
Contraception use missing	1	0.93598	0.46803	3.9994	0.0455	2.550
Had sexual intercourse never - reference						
Had sexual intercourse ever	1	1.63644	0.19960	67.2141	<.0001	5.137
Had sexual intercourse missing	1	1.65291	0.43927	14.1592	0.0002	5.222
Ethnicity Dutch - reference						
Ethnicity non-Dutch	1	0.47858	0.21523	4.9441	0.0262	1.614
Ethnicity missing	1	-1.14119	0.80074	2.0311	0.1541	0.319

Analysis of Maximum Likelihood Estimates						
Parameter	DF	Parameter estimate	Standard error	Chi-Square	Pr > ChiSq	Hazard ratio
HPV6/11	1	-0.72768	0.38663	35.424	0.0598	0.483
Age (continuous)	1	0.59332	0.57337	1.0708	0.3008	1.810
Contraception use never (reference)						
Contraception use ever	1	0.96025	0.23204	17.1261	<.0001	2.612
Contraception use missing	1	0.93602	0.46802	3.9998	0.0455	2.550
Had sexual intercourse never - reference						
Had sexual intercourse ever	1	1.63645	0.19960	67.2151	<.0001	5.137
Had sexual intercourse missing	1	1.65297	0.43926	14.108	0.0002	5.222
Ethnicity Dutch - reference						
Ethnicity non-Dutch	1	0.47859	0.21523	4.9444	0.0262	1.614
Ethnicity missing	1	-1.14120	0.80072	2.0312	0.1541	0.319

Analysis of Maximum Likelihood Estimates						
Parameter	DF	Parameter estimate	Standard error	Chi-Square	Pr > ChiSq	Hazard ratio
HPV16/18/31/33/45/52/58	1	-1.04603	0.32950	10.0780	0.0015	0.351
Age (continuous)	1	0.59339	0.57339	1.0710	0.3007	1.810
Contraception use never (reference)						
Contraception use ever	1	0.96019	0.23203	17.1242	<.0001	2.612
Contraception use missing	1	0.93597	0.46803	3.9993	0.0455	2.550
Had sexual intercourse never - reference						
Had sexual intercourse ever	1	1.63643	0.19960	67.2138	<.0001	5.137
Had sexual intercourse missing	1	1.65293	0.43927	14.1595	0.0002	5.222
Ethnicity Dutch - reference						
Ethnicity non-Dutch	1	0.47854	0.21524	4.9432	0.0262	1.614
Ethnicity missing	1	-1.14116	0.80072	2.0311	0.1541	0.319

References

1. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. J Clin Virol 2005; 32:16–24.
2. Burchell AN, Winer RL, de Sanjosé S, Franco EL. Chapter 6: epidemiology and transmission dynamics of genital HPV infection. Vaccine 2006; 24:S52–61.
3. Committee for Medicinal Products for Human Use. Assessment report for Cervarix. London, UK: European Medicines Agency, 2013.
4. Pedersen C, Petaja T, Strauss G, et al. ; HPV Vaccine Adolescent Study Investigators Network. Immunization of early adolescent females with human papillomavirus type 16 and 18 L1 virus-like particle vaccine containing AS04 adjuvant. J Adolesc Health 2007; 40:564–71.
5. Romanowski B, Schwarz TF, Ferguson LM, et al. Immunogenicity and safety of the HPV-16/18 AS04-adjuvanted vaccine administered as a 2-dose schedule compared with the licensed 3-dose schedule: results from a randomized study. Hum Vaccin 2011; 7:1374–86.
6. Dobson SR, McNeil S, Dionne M, et al. Immunogenicity of 2 doses of HPV vaccine in younger adolescents vs 3 doses in young women: a randomized clinical trial. JAMA 2013; 309:1793–802.
7. Romanowski B, Schwarz TF, Ferguson LM, et al. Immune response to the HPV-16/18 AS04-adjuvanted vaccine administered as a 2-dose or 3-dose schedule up to 4 years after vaccination: results from a randomized study. Hum Vaccin Immunother 2014; 10:1155–65.
8. Iversen OE, Miranda MJ, Ulied A, et al. Immunogenicity of the 9-valent HPV vaccine using 2-dose regimens in girls and boys vs a 3-dose regimen in women. JAMA 2016; 316:2411–21.
9. Donken R, Dobson SR, Marty KD, et al. Immunogenicity of 2 and 3 doses of the quadrivalent HPV vaccine up to 120 months post-vaccination; follow-up of a randomized clinical trial. Clin Infect Dis 2020; 71.4:1022–29.
10. Donken R, Knol MJ, Bogaards JA, van der Klis FR, Meijer CJ, de Melker HE. Inconclusive evidence for non-inferior immunogenicity of two-compared with three-dose HPV immunization schedules in preadolescent girls: a systematic review and meta-analysis. J Infect 2015; 71:61–73.
11. International Agency for Research on Cancer HPV Working Group. Primary end-points for prophylactic HPV vaccine trials. Lyon, France: IARC, 2014.
12. Basu P, Bhatla N, Ngoma T, Sankaranarayanan R. Less than 3 doses of the HPV vaccine—review of efficacy against virological and disease end points. Hum Vaccin Immunother 2016; 12:1394–402.
13. Kavanagh K, Pollock KG, Cuschieri K, et al. Changes in the prevalence of human papillomavirus following a national bivalent human papillomavirus vaccination programme in Scotland: a 7-year cross-sectional study. Lancet Infect Dis 2017; 17:1293–302.
14. Johnson Jones ML, Gargano JW, Powell M, et al. ; HPV-IMPACT Working Group. Effectiveness of 1, 2, and 3 doses of human papillomavirus vaccine against high-grade cervical lesions positive for human papillomavirus 16 or 18. Am J Epidemiol 2020; 189:265–76.
15. Verdoodt F, Dehlendorff C, Kjaer SK. Dose-related effectiveness of quadrivalent human papillomavirus vaccine against cervical intraepithelial neoplasia: a Danish nationwide cohort study. Clin Infect Dis 2020; 70:608–14.
16. Markowitz LE, Naleway AL, Klein NP, et al. Human papillomavirus vaccine effectiveness against HPV infection: evaluation of one, two, and three doses. J Infect Dis 2020; 221:910–8.
17. Rodriguez AM, Zeybek B, Vaughn M, et al. Comparison of the long-term impact and clinical outcomes of fewer doses and standard doses of human papillomavirus vaccine in the United States: a database study. Cancer 2020; 126:1656–67.

18. Kreimer AR, Struyf F, Del Rosario-Raymundo MR, et al. Efficacy of fewer than three doses of an HPV-16/18 AS04-adjuvanted vaccine: combined analysis of data from the Costa Rica vaccine and PATRICIA Trials. Lancet Oncol 2015; 16:775–86.
19. D'Addario M, Redmond S, Scott P, et al. Two-dose schedules for human papillomavirus vaccine: systematic review and meta-analysis. Vaccine 2017; 35:2892–901.
20. De Melker HE, Conyn-Van Spaendonck MA, Boot HJ, Coutinho RA. Introduction to vaccination against cervical cancer [in Dutch]. Ned Tijdschr Geneeskd 2009; 153:658–61.
21. van Lier E, Oomen P, Giesbers H, et al. Vaccination rate and annual report: National Immunization Program Netherlands 2018 [in Dutch]. Bilthoven, the Netherlands: National Institute for Public Health and the Environment, 2019.
22. van Lier A, Oomen P, de Hoogh P, et al. Praeventis, the immunisation register of the Netherlands: a tool to evaluate the National Immunisation Programme. Euro Surveill 2012; 17:20153.
23. Lin DY, Dai L, Cheng G, Sailer MO. On confidence intervals for the hazard ratio in randomized clinical trials. Biometrics 2016; 72:1098–102.
24. Schurink-van "t Klooster TM, de Melker HE. The national immunisation programme in the Netherlands. Surveillance and developments in 2014–2015. Bilthoven, the Netherlands: National Institute for Public Health and the Environment, 2015.
25. Donken R, King AJ, Bogaards JA, Woestenberg PJ, Meijer CJLM, de Melker HE. High effectiveness of the bivalent human papillomavirus (HPV) vaccine against incident and persistent HPV infections up to 6 years after vaccination in young Dutch women. J Infect Dis 2018; 217:1579–89.
26. Woestenberg PJ, King AJ, van Benthem BHB, et al. ; Medical Microbiological Laboratories and the Public Health Services. Bivalent vaccine effectiveness against type-specific HPV positivity: evidence for cross-protection against oncogenic types among Dutch STI clinic visitors. J Infect Dis 2018; 217:213–22.
27. Schurink-van 't Klooster TM, Donken R, Schepp RM, van der Klis FRM, de Melker HE. Persistence of immune response following bivalent HPV vaccination: a follow-up study among girls routinely vaccinated with a two-dose schedule. Vaccine 2018; 36:7580–7.
28. Malagón T, Drolet M, Boily MC, et al. Cross-protective efficacy of two human papillomavirus vaccines: a systematic review and meta-analysis. Lancet Infect Dis 2012; 12:781–9.
29. Apter D, Wheeler CM, Paavonen J, et al. ; HPV PATRICIA Study Group. Efficacy of human papillomavirus 16 and 18 (HPV-16/18) AS04-adjuvanted vaccine against cervical infection and precancer in young women: final event-driven analysis of the randomized, double-blind PATRICIA trial. Clin Vaccine Immunol 2015; 22:361–73.
30. Szarewski A, Skinner SR, Garland SM, et al. Efficacy of the HPV-16/18 AS04-adjuvanted vaccine against low-risk HPV types (PATRICIA randomized trial): an unexpected observation. J Infect Dis 2013; 208:1391–6.
31. Latsuzbaia A, Arbyn M, Tapp J, et al. Effectiveness of bivalent and quadrivalent human papillomavirus vaccination in Luxembourg. Cancer Epidemiol 2019; 63:101593.
32. Woestenberg PJ, Guevara Morel AE, Bogaards JA, et al. Partial protective effect of bivalent HPV16/18 vaccination against anogenital warts in a large cohort of Dutch primary care patients. Clin Infect Dis 2020. 2021; 73:291–7.
33. Bogaards JA, van der Weele P, Woestenberg PJ, van Benthem BHB, King AJ. Bivalent human papillomavirus (HPV) vaccine effectiveness correlates with phylogenetic distance from HPV vaccine types 16 and 18. J Infect Dis 2019; 220:1141–6.
34. Donken R, Tami A, Knol MJ, et al. Changes in (risk) behavior and HPV knowledge among Dutch girls eligible for HPV vaccination: an observational cohort study. BMC Public Health 2018; 18:837.

35. de Graaf H, van den Borne M, Nikkelen S, Twisk D, Meijer S.Sex under 25: sexual health of young people in the Netherlands in 2017 [in Dutch]. Utrecht, the Netherlands: Rutgers/Soa AIDS, 2017.
36. Statistics Netherlands. StatLine. The Netherlands in figures. Available at: https://opendata.cbs.nl/statline/#/CBS/nl/. Accessed July 2020.

Chapter 7

Measuring vaccine effectiveness against persistent HPV infections: a comparison of different statistical approaches.

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ABSTRACT

Background: Persistent high-risk human papillomavirus (HPV) infection is endorsed by the World Health Organization as an intermediate endpoint for evaluating HPV vaccine effectiveness/efficacy. There are different approaches to estimate the vaccine effectiveness/efficacy against persistent HPV infections.

Methods: We performed a systematic literature search in to identify statistical approaches that have been used to estimate the vaccine effectiveness/efficacy against persistent HPV infections. We applied these methods to data of a longitudinal observational study to assess their performance and compare the obtained vaccine effectiveness (VE) estimates.

Results: Our literature search identified four approaches: the conditional exact test for comparing two independent Poisson rates using a binomial distribution, Generalized Estimating Equations for Poisson regression, Prentice Williams and Peterson total time (PWP-TT) and Cox proportional hazards regression. These approaches differ regarding underlying assumptions and provide different effect measures. However, they provided similar effectiveness estimates against HPV16/18 and HPV31/33/45 persistent infections in a cohort of young women eligible for routine HPV vaccination (range VE 93.7–95.1% and 60.4–67.7%, respectively) and seemed robust to violations of underlying assumptions.

Conclusions: As the rate of subsequent infections increased in our observational cohort, we recommend PWP-TT as the optimal approach to estimate the vaccine effectiveness against persistent HPV infections in young women. Confirmation of our findings should be undertaken by applying these methods after longer follow-up in our study, as well as in different populations.

Background

More than 30 types of the human papillomavirus (HPV) can infect the genital tract. Based on their oncogenic potential for cervical cancer, HPV types are divided into low- and high-risk (hrHPV) types. The majority of HPV infections are cleared by the immune system. However, remaining infections can persist within cells and progress to (pre)-cancerous lesions [1]. A persistent infection with HPV is the necessary cause for the development of cervical cancer. Beyond its role as etiological agent of cervical cancer, hrHPV is associated with other anogenital and oropharyngeal cancers in men and women [2]. Since 2006, three prophylactic vaccines have been licensed, and many countries have implemented HPV vaccination programs [3]. While these vaccines offer protection against two, four, or nine HPV types, all protect against hrHPV types 16 and 18. For the bivalent vaccine, cross-protection has been shown against additional types (HPV31, 33, 45) which are not included in the vaccine [4].

Given its role in the pathogenesis, persistent hrHPV infection is endorsed by the World Health Organization (WHO) as an intermediate endpoint for estimating HPV vaccine effectiveness/efficacy in cervical and anal cancer among 16–26 year olds [5]. In general, persistence is defined as presence of the same HPV type in consecutive measurements [6]. The use of persistent infections as an outcome for vaccine effectiveness/efficacy is more convenient than pre-cancerous lesion (e.g. cervical intraepithelial neoplasia), however it comes with several challenges. Besides uncertainties in the natural history of HPV infections, with possible viral latency and natural immunity after infection [7], difficulties in measuring vaccine effectiveness/efficacy might arise from longitudinal study designs with loss to follow-up and missing observations. In addition, clustered data can result from the possibility of infections with multiple HPV types (at once) and/or having recurrent detection (reinfection or reactivation) after a negative measurement. Additionally, the risk for recurrent detection might be higher than developing a first-time infection [8]. Another challenge is that rates of infection over time in young vaccinated cohorts might vary due to increasing sexual behavior in this age group [9]. This varying infection rate might influence which statistical approach is optimal for estimating vaccine efficacy/effectiveness in observational cohort studies.

In this paper, we identify and examine different approaches to estimate the vaccine effectiveness (VE) against persistent HPV infections from the literature, and determine whether the statistical assumptions of these approaches hold within data from an observational cohort study. Furthermore, we examine whether a violation of these statistical assumptions leads to bias in the estimation of the VE.

Methods

Literature search

A systematic literature search with no indicated start date till May 15, 2019 was performed in (detailed search strategy is in Additional file 1), to obtain insight into various methods used to estimate the vaccine effectiveness/efficacy against persistent HPV infections. Although vaccine efficacy and effectiveness vary in the conditions under which they are obtained, they both aim to measure the proportionate reduction in disease burden. Vaccine efficacy is studied under controlled circumstances, for example in a randomized controlled trial, while vaccine effectiveness is estimated from studies conducted under field circumstances [10]. Calculations of efficacy and effectiveness are comparable, especially in situations where the vaccine effectiveness aims to measure the direct effects by comparing the risk in vaccinated and unvaccinated participants [11]. Given our focus on observational studies, we will only use the abbreviation VE when vaccine effectiveness is described.

Papers were screened based on predefined inclusion criteria. Inclusion criteria covered original research papers estimating vaccine efficacy or effectiveness against persistent HPV infections (i.e. comparing different groups) for any prophylactic HPV vaccine, written in Dutch or English language. Data were extracted using a standardized data extraction form. Selection of papers and data-extraction were performed in duplicate by two researchers.

Study population and design

To check the statistical assumptions and to compare the different statistical approaches, we used data of the HPV Amongst Vaccinated And Non-vaccinated Adolescents (HAVANA)-study. The study design of this observational cohort study has been described previously [12,13,14]. In brief, 29,162 girls born in 1993 or 1994 who were eligible for the catch-up campaign (three-doses of bivalent HPV vaccine) in 2009 and 2010 in the Netherlands were approached to participate in the study approximately one month before vaccination was offered. All participants provided written consent and the study was approved by the medical ethics committee (VU University Medical Center, Amsterdam). In total 1832 vaccinated and unvaccinated participants were included and asked to provide yearly follow-up with vaginal self-swabs and questionnaires. For current analyses, we included data up to eight years post-vaccination and study participants had to be negative for HPV16, 18, 31, 33 and 45 (vaccine and cross-protective types of the bivalent HPV vaccine) at baseline. Exposure was defined as having received the full recommended schedule of the bivalent HPV vaccine (three-doses at 0, 1 and 6 months) compared to unvaccinated women. Participants with an incomplete vaccination schedule were excluded from the analyses.

HPV DNA detection and genotyping

HPV DNA testing was done by SPF10-LIPA25 system, with storage of vaginal self-swabs and methods used for HPV DNA detection and genotyping described in detail elsewhere [12, 15].

Statistical analysis

We calculated the crude VE as 1 minus the hazard or rate ratio (*100%) using the different statistical approaches identified in the literature. Analyses were performed against a combined outcome of vaccine types HPV16/18 and cross-protective types HPV31/33/45. Persistence was defined on a type-specific level as being negative at baseline, followed by two consecutive positive rounds of testing. To be counted as a persistent case during follow-up, participants needed to have a persistent infection for at least one of these HPV types. In addition, at each time point a participant was evaluated to determine if they had a persistent infection based on previous time points. Person-time was counted from at least three consecutive rounds of participation, in order to be able to detect the endpoint of persistent infection based on three consecutive testing time points. Examples of calculating endpoints and person-time can be found in Additional file 2. Data analysis was performed using SAS 9.4 (SAS Institute Inc. 2010, USA).

Results

Literature search

The systematic literature search resulted in 425 s, of which after selection (title and abstract) 49 remained for full text screening. Of these, four were excluded because of the wrong publication type (e.g. comment or review), seven because a lack of an actual vaccine effectiveness/efficacy calculation, and in four studies a different outcome other than the one of interest was reported, leaving a total of 34 s (32 randomized controlled trials and 2 observational cohort studies) for inclusion [13, 14, 16,17,18,19,20,21,22 ,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46]. (Fig. 1) This resulted in 35 analyses regarding vaccine efficacy/effectiveness of persistent HPV infections. Four different analysis methods were observed. Two methods provided an estimate of rate ratios either via Generalized Estimating Equations (GEE) using a Poisson model (n = 2), or via direct comparison of independent incidence rates using the Conditional exact method (n = 31) [47, 48], which assumes that the number of events from one group, given the total number of events in both groups, follows a binomial distribution under the null hypothesis using identical Poisson processes in the vaccinated and unvaccinated group [49]. The other two methods provided an estimate of hazard ratios either via the Cox proportional hazards model (n = 2),

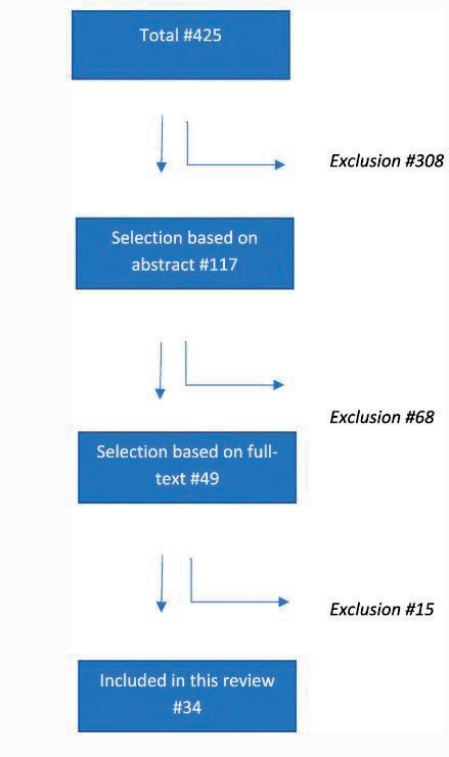


Figure 1: Flowchart of systematic literature search

or via the Prentice Williams Peterson total time (PWP-TT) approach (n = 1). In all papers vaccine efficacy/effectiveness was calculated as 1 minus the rate ratio, or hazard ratio, times hundred percent. The PWP-TT is a survival method for recurrent events taking into account total time at risk, assuming event-specific hazards, in which the hazard is allowed to differ for a subsequent event [50, 51]. The GEE Poisson approach counts multiple events per participant (either over time or at the same time point) considering person-time. Only the first event is counted in both the conditional exact method using the binomial distribution and the Cox method. The Cox approach uses time until first event. Studies using the conditional exact method for comparing two independent Poisson rates using a binomial distribution varied in the denominator of outcome variable, either being total number of participants or number of person years observed (Table 1). An important assumption of Cox regression is that the hazard ratio is constant over time (proportional hazard assumption), while for the GEE Poisson and Conditional exact method, a constant rate of events over time would give the most stable estimates [52, 53]. The four methods vary in how they handle missing data. An overview of the different methods and their assumptions is shown in Table 2.

Table 1: Methods used to evaluate the VE against persistent infections and analyses from included studies

Type of study	Definition of persistence	Duration of persistent infection	VE analysis method	Calculation of infection rates
- Observational [13, 14] -Experimental [13, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 43, 44, 45, 46]	- 2 consecutive measurements positive [13, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 42, 43, 44, 45, 46]. - 2 consecutive measurements: positive preceded by a negative measurement [14] - Sequence of positive measurements over a certain time span [18, 41]	- 6 months[a] [17, 20, 22, 25, 26, 28, 29, 30, 34, 35, 36, 37, 38, 39, 43, 44, 46] - 12 months[a] [13, 14, 27, 40] - 6/12 months[a] [16, 18, 19, 21, 23, 24, 31, 32, 33, 41, 42, 45]	- Conditional exact for comparing two independent Poisson rates using a binomial distribution (14, 15, 24–28 [39, 41–43] , 16, 35–44, 17–23) - GEE Poisson [13, 40] - Cox Proportional Hazard [22, 38] - Prentice Williams Peterson total time approach [14]	- Number of cases/number of participants [17, 18, 19, 21, 22, 25, 27, 31, 32, 33, 38, 41, 42, 43, 44] - Number of cases/ person years at risk [13, 14, 16, 20, 23, 24, 26, 28, 29, 30, 34, 35, 36, 37, 39, 40, 45, 46]

** Although it was stated as 6- or 12-month persistent infections authors specified durations varying between at least 4 to 6 months or 10 to 12 months respectively*

Table 2: Analysis methods for vaccine effectiveness against persistent HPV infections

	Conditional exact method for comparing two independent Poisson rates using a binomial distribution	**Cox proportional hazard**	**GEE Poisson**	**Prentice Williams Peterson-Total time**
Outcome	Rate ratio	Hazard ratio	Rate ratio	Hazard ratio
Assumption(s)	* Rate of events constant over time * Groups are considered to be equally exposed [52]	* Proportional hazard assumption (hazard ratio over time should be constant) * Independence assumption (estimate only for 1st event) [52]	* Rate of events constant over time [52] * Measurements are independent across subjects * Measurements may be correlated within subjects	* Event specific baseline hazard (baseline hazard for k^{th} event allowed to be different) [51]
Check assumptions in HAVANA	Assumption for constant rate over time violated among unvaccinated	Proportional hazard assumption not violated	Assumption for constant rate over time violated among unvaccinated	Assumption for event-specific hazard not violated

Assumptions

Data from the observational HAVANA-study [12,13,14] were used to check the assumptions and to calculate the VE estimates using the different methods. In total, 1615 participants were included in the current analyses. These participants provided a baseline sample and were negative at baseline for HPV16/18/31/33/45. Of these, 747 were unvaccinated and 868 were fully vaccinated (three doses at 0, 1 and 6 months), where vaccination occurred approximately one month after inclusion into the study. (Fig. 2) We checked whether assumptions regarding constancy of the hazard ratio, constancy in the rate of events and the event-specific hazard assumption hold in the HAVANA-study for persistent HPV16/18 and HPV31/33/45 infections. To check the proportional hazard assumption (Cox regression), we added the interaction between vaccination status and time to the Cox model. Based on the interaction term between vaccination and time, the proportional hazard assumption was not violated for both vaccine ($p = 0.19$) and cross-protective types ($p = 0.60$). To check for a constant event rate (GEE Poisson and Conditional exact method), we modeled the persistence rate as a function over time stratified for unvaccinated and vaccinated participants.

An increasing persistence rate ($p < 0.01$) for both vaccine types and cross-protective types over time was observed among unvaccinated, but not among vaccinated participants ($p = 0.14$ and $p = 0.17$ respectively). This indicates that the assumption of constant event rate was violated. (Table 3). In order to check whether there is an event specific hazard (PWP-TT), we estimated the persistence rate for each subsequent event number. The hazard for subsequent infections indeed seems to be different, as the persistence rate (PR) for a subsequent persistent infection in the total population was higher for the second infection compared to the first infection. The PR ratio (PRR) for the second infection was 7.38 95%CI 2.95–18.45 for HPV16/18 and 5.95 (95%CI 1.85–19.09) for HPV31/33/45 compared to the first infection (Table 4).
Vaccine effectiveness

We used the different methods found through the systematic literature search to calculate the VE against persistent infections (with an interval of at least twelve months) with vaccine or cross-protective types up to eight years post vaccination in the HAVANA-study. Definitions used for the analyses are shown in Table 5, with examples of calculations in Additional file 2. To estimate VE for the conditional exact method using a binomial distribution, whether a participant had a persistent infection during follow-up was used as the outcome (persistent e), assuming that the number of cases in each of the arms are independent Poisson random variables. For the PWP-TT participants with multiple simultaneous persistent infections, individuals were counted as having one persistent event at that specific time point. While in the GEE Poisson approach, all simultaneous infections for different HPV types were counted

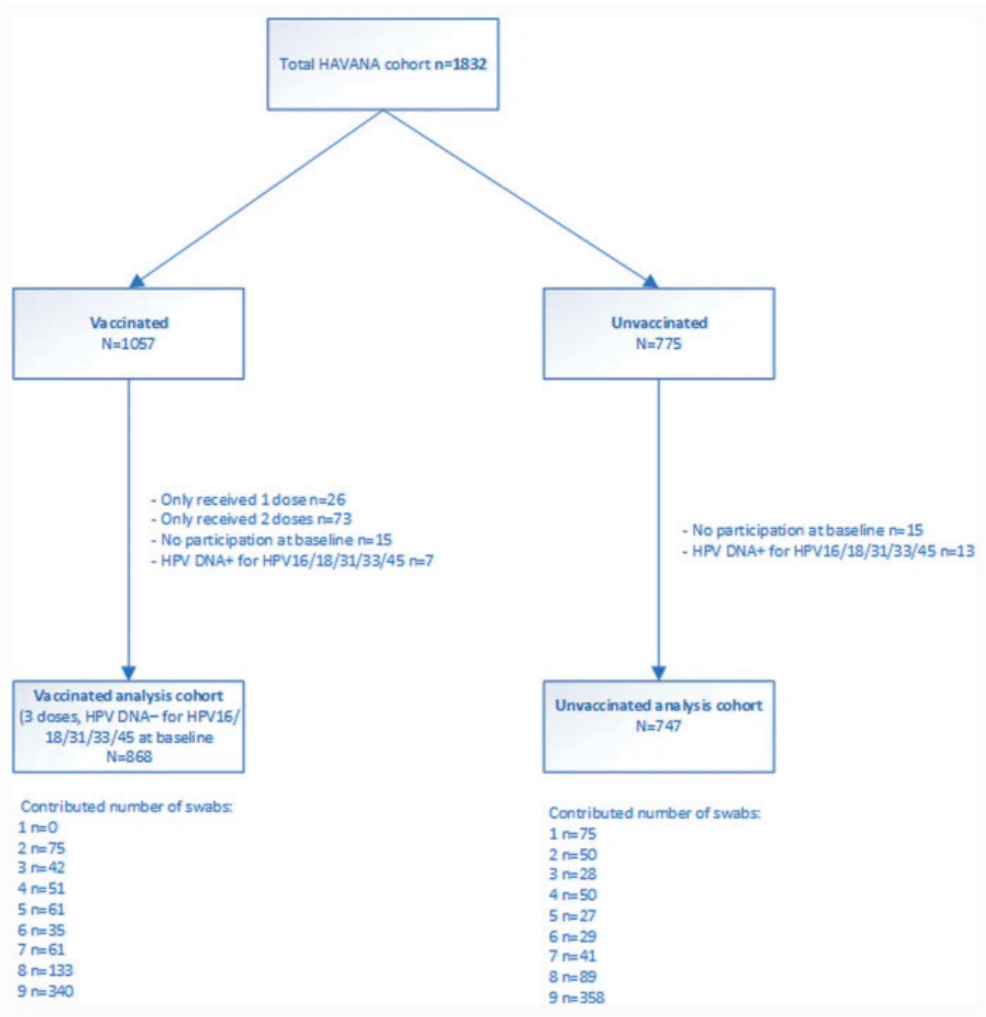


Figure 2: Flowchart of analysis population

and all subsequent events were counted as multiple events. For the Cox PH analysis only the first infection was used.

Through the model assumption checking, we found that the Cox model and the PWP-TT method were the only approaches for which the statistical assumptions were not violated using the HAVANA-study data. The PWP-TT takes into account the possibility of multiple infections during the follow-up time. Whereas the Cox model can only account for one event when using a pooled outcome of vaccine types or cross-protective types and multiple type infections occurring at the same moment.

Table 3: Persistence rates (PR) and persistence rate ratios (PRR) for HPV16/18 and HPV31/33/45 (vaccine and cross-protective types) over time, in years since vaccination

Yrs. Since vaccination	Vaccination status	N	Vaccine types (HPV16/18)			Cross-protective types (HPV31/33/45)		
			# infections	PR per 100 PY (95%CI)	PRR (95%CI)	# infections	PR per 100 PY (95%CI)	PRR (95%CI)
2	Unvaccinated	551	2	0.18 (0.05–0.73)		2	0.18 (0.05–0.73)	Ref
	Vaccinated	626	0	0.00 (0.00–0.59)		1	0.08 (0.01–0.57)	0.44 (0.04–4.85)
3	Unvaccinated	513	2	0.19 (0.05–0.78)		2	0.19 (0.05–0.78)	Ref
	Vaccinated	567	0	0.00 (0.00–0.65)		2	0.18 (0.04–0.71)	0.90 (0.13–6.42)
4	Unvaccinated	472	7	0.74 (0.35–1.56)		4	0.42 (0.16–1.13)	Ref
	Vaccinated	515	0	0.00 (0.00–0.72)		3	0.29 (0.09–0.90)	0.69 (0.15–3.074)
5	Unvaccinated	455	10	1.10 (0.59–2.04)		3	0.33 (0.11–1.02)	Ref
	Vaccinated	472	0	0.00 (0.00–0.78)		1	0.11 (0.01–0.75)	0.32 (0.03–3.09)
6	Unvaccinated	447	18	2.01 (1.27–3.20)	Ref	12	1.34 (0.76–2.36)	Ref
	Vaccinated	438	1	0.11 (0.02–0.81)	0.06 (0.01–0.42)	2	0.23 (0.06–0.91)	0.17 (0.04–0.76)
7	Unvaccinated	433	11	1.27 (0.70–2.99)	Ref	5	0.58 (0.24–1.39)	
	Vaccinated	448	2	0.22 (0.06–0.89)	0.18 (0.04–0.79)	0	0.00 (0.00–0.82)	
8	Unvaccinated	414	6	0.72 (0.33–1.61)		9	1.09 (0.57–2.09)	Ref
	Vaccinated	429	0	0.00 (0.00–0.86)		5	0.58 (0.24–1.40)	0.54 (0.18–0.60)

PR = persistence rate (with 95%CI), PRR = persistence rate ratio (with 95%CI), py = person years, Yrs = years

** Trend in persistence rate over time for HPV16/18 among unvaccinated, $p < 0.01$, among vaccinated $p = 0.14$*

*** Trend in persistence rate over time for HPV31/33/45 among unvaccinated, $p < 0.01$, among vaccinated $p = 0.17$*

Table 4: Persistence rates per event (event 1 is the first persistent infection with a vaccine /cross-protective type, event 2 is the second persistent infection, with at least one negative observation in between type-specific persistent infections, event 3 is the third persistent infection, with at least one negative observation in between type-specific infections)

Vaccination Status	Event	Cases	Person time	PR per 100 PY	PRR per 100 PY
Vaccine types (HPV16/18)					
Unvaccinated	1	51	3792	1.35 (1.02–1.77)	Ref
	2	5	95	5.26 (2.19–2.64)	3.91 (1.56–9.80)
	3	0	17		
Vaccinated	1	3	4100	0.07 (0.02–0.23)	
	2	0	4		
	3	0	0		
Cross-protective types (HPV31/33/45)					
Unvaccinated	1	34	3813	0.89 (0.64–1.25)	Ref
	2	3	60	5.00 (1.61–15.50)	5.61 (1.72–18.26)
	3	0	6		
Vaccinated	1	14	4082	0.34 (0.20–0.58)	
	2	0	60		
	3	0	0		

PR = persistence rate, PRR = persistence rate ratio, py = person years

Table 5: Definitions and analysis of cases and time at risk

Analysis Method	Case definition	Person-time definition
Conditional exact method for comparing two independent Poisson rates using a binomial distribution	Two consecutive measurements positive for the same HPV type. The participant is counted as a case if one or more persistent infections occur.	Data for two consecutive rounds counts as 1 person-year, each additional consecutive round adds another person-year. After a missing data point counting continues. Counting stops after event or at the end of follow-up.
Cox PH	Two consecutive measurements positive for the same HPV type. The participant is counted as a case if one or more persistent infections occur.	Data for two consecutive rounds counts as 1 person-year, each additional consecutive round adds another person-year. Person time is censored at event, loss to follow-up or end of follow-up; half-time censoring was applied.
GEE Poisson	Two consecutive measurements positive for the same HPV type. Multiple events can occur within one participant. In our study to be counted as next infection after at least one negative round was observed. The number of infections is counted.	Data for two consecutive rounds counts as 1 person-year, each additional consecutive round adds another person-year. After a missing data point counting continues. Counting stops at the end of follow-up.
PWP-TT	Two consecutive measurements positive for the same HPV type. Multiple events can occur within one participant, in our study to be counted as next infection; at least one negative round should be observed. The number of infections is counted. Analyses are stratified for sequential events.	Data for two consecutive rounds counts as 1 person-year. After a missing data point counting continues. Counting stops at the end of follow-up.

The estimated VE for vaccine types using the PWP-TT method was 93.7% (95%CI 79.7–98.0%) and for cross-protective types the VE was 63.2% (95%CI 28.6–81.0%). Despite observing small differences in estimates and confidence intervals with the other methodological approaches, the obtained VE estimates and corresponding 95%CI using any of the methods overlapped with the estimates obtained using the PWP-TT method. The VE against persistent HPV16/18 infections measured by the different methods varied between 93.7 and 95.1%, and for HPV31/33/45 between 60.4 and 67.7%, with the lowest point estimates given by the two methods for which the model assumptions were not violated. (Fig. 3).

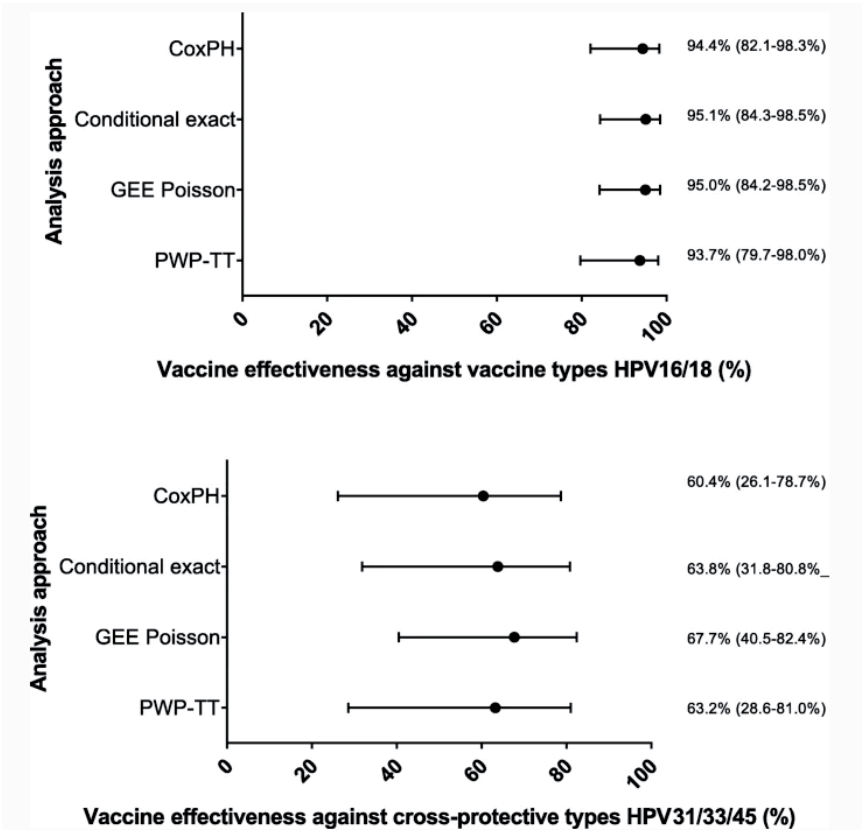


Figure 3: Crude vaccine effectiveness up to eight years post-vaccination against persistent HPV16/18 and HPV31/33/45 infections observed in the HAVANA-study using different statistical approaches

Discussion

Main findings

Our literature search identified four approaches for calculating the vaccine efficacy/effectiveness against persistent HPV infections. These different methods vary in their underlying assumptions and measures. Based on our observational study, the Cox Proportional hazard and PWP-TT method were the only ones whose assumptions were not violated in our observational cohort study data. In addition, the PWP-TT has the advantage that it uses information from the complete follow-up time, compared to a single event time used in the Cox model. Compared to the PWP-TT, the VE estimates against HPV16/18 and HPV31/33/45 calculated by the other methods were quite comparable, and seemed robust to violations of the underlying assumptions.

Statistical approaches

The four different methods found in our search vary in their underlying assumptions, but also in how they handle missing observations or loss-to-follow up. In our systematic search for methods to analyze VE against persistent HPV infections, we found both randomized controlled trials and observational studies. An important difference is that in randomized controlled trials there is no confounding, while in observational studies, adjustment for confounding is needed.

Assumptions

Using data from an observational cohort study we checked whether the assumptions of the various methods hold. The proportional hazard assumption for Cox models was not violated in our data. However, as follow-up time increases, the proportional hazard between vaccinated and unvaccinated might vary over time, for example, if vaccine protection might wane or gets boosted by exposure to the virus [53]. Malagon et al. suggested waning of HPV-cross-protection after five years post-vaccination [4]. However, recent studies did not show indications for waning of cross-protection [14, 54,55,56]. In our data, the assumption with regard to constancy of the event rate was violated in unvaccinated participants, which was to be expected based on existing literature about HPV prevalence over time. For example, Lenselink et al. have shown an increase in HPV prevalence till 22 years of age [9]. We also checked whether we found an event-specific hazard for subsequent infections.

In our study, observed follow-up for a second and third infection among vaccinated was small, hence interpretation of the findings in this group is difficult. Among unvaccinated, we clearly observed a higher rate of events amongst those who already had an event. In the literature so far, no clear consensus regarding the risk for a new infection after a previous infection has been reached [8, 57,58,59,60].

As analyses using PWP-TT are stratified by event number, and slightly wider confidence intervals are estimated, therefore the event-specific estimates could become unreliable if there are a limited number of events in a stratum [50].

A problem that might arise when using GEE Poisson models to estimate the VE is an excess of zero counts when the vaccine is highly effective, which leads to overdispersion. In the presence of overdispersion, the variance of the parameters within the model will be underestimated [61]. Based on the negative dispersion parameter [62], it seems that the observed variance within the data was higher than what was expected under the GEE Poisson model. However, estimating the VE using a negative binomial model showed comparable VE estimates, 95.0% (95%CI 84.1–98.5%) against vaccine types and 67.5% (38.2–82.9%) against cross-protective types, which may suggest robustness of the estimates despite the presence of overdispersion.

Vaccine effectiveness estimates

The obtained estimates from all the methods where assumptions were violated were quite comparable to the CoxPH and the PWP-TT methods, for which assumptions were not violated in our observational study. In addition, we observed comparable, or slightly higher point estimates, for the observed vaccine effectiveness against vaccine and cross-protective HPV-types in comparison to previous studies evaluating vaccine effectiveness against persistent infections after vaccination with the bivalent HPV vaccine in HPV naïve women [16, 24, 45, 63].

We did not find evidence that the vaccine effectiveness estimates were influenced due to a violation of the underlying model assumptions. However, as follow-up time and the number of persistent infections increases, significant differences between methods might develop. Difference between methods of calculating VE may also occur when these methods are applied in study populations at higher risk for HPV infections.

Although we found comparable estimates using different methods, we suggest the PWP-TT as a valid and preferable method to estimate the VE against persistent HPV infections in observational studies. This recommendation is based our findings regarding the violation of the model assumptions with respect to constant rates or ratios and common baseline hazard, combined with available literature, and our comparison analysis from complete follow-up data to calculate VE against persistent HPV infections in observational studies.

For our analyses, we used a combined endpoint of vaccine and cross-protective types to estimate the VE. An alternative for using combined endpoints would be measuring type-specific VEs and pooling these. A limitation of the PWP-TT method when using

a combined endpoint for multiple HPV types is that simultaneous infections cannot be counted separately, while infections for different types later in time are counted as separate events. However, running type-specific vaccine effectiveness models will overcome this potential limitation.

Conclusion

For the four methods used to calculate VE in our observational study, the estimates were comparable between those that did not violated statistical assumptions, the CoxPH and the PWP-TT methods, and those that did violate assumptions, GEE using a Poisson and conditional exact methods.

For monitoring the effectiveness of HPV vaccination in cohorts of young adolescents/adults with increasing HPV prevalence the PWP-TT approach seems is recommended as valid and preferable, as it considers the varying rates of events and uses data of the whole follow-up period. A limitation when using this method might occur when using combined endpoints for multiple HPV types, since this cannot be taken into account in the model. Further studies should focus on populations with higher HPV persistence rates in order to confirm our findings.

Supplementary data

Additional file 1: Search query
Search criteria for the Systematic literature search regarding vaccine effectiveness/efficacy against persistent HPV infections.

#1	Search **papillomavir*[tiab] or hpv[tiab] or hpv*[tiab] or papilloma*[tiab] or papillomaviridae[mh]**	#73441
#2	Search **vaccin*[tiab] or immunisat*[tiab] or immunizat*[tiab] or vaccines[mh] or vaccination[mh] or immunization[mh:noexp] or papillomavirus vaccines[mh]**	387929
#3	Search **persist*[tiab]**	446196
#4	Search **effectiv*[tiab] or effica*[tiab]**	387929
#5	#1 AND #2 AND #3 AND #4	425

Additional file 2: Examples of calculations for different approaches with regard to number of events and person time at risk
Only participants negative for HPV16/18/31/33/45 at baseline were included for these analyses.

Case nr.	Prevalence									Number of events				Person-time at risk			
	R0	R1	R2	R3	R4	R5	R6	R7	R8	CE	CoxPH	GEE Poisson	PWP-TT	CE	CoxPH	GEE Poisson	PWP-TT
1	NEG	POS	POS	NEG	POS	POS	NEG	NEG	NEG	1 case	1 case	2 inf	2 inf	2 yr	1.5 yrs	8 yrs	8 yrs
2	NEG	NEG	NEG	POS	POS	NEG	NEG	NEG	NEG	1 case	1 case	1 inf	1 inf	4 yrs	3.5 yrs	8 yrs	8 yrs
3	NEG	NEG	.	NEG	NEG	NEG	NEG	POS	POS	1 case	0 case	1 inf	1 inf	5 yrs	0 yrs	5 yrs	5 yrs
4	NEG	NEG	NEG	.	NEG	NEG	NEG	NEG	NEG	0 case	0 case	0 inf	0 inf	6 yrs	2.5 yrs	6 yrs	6 yrs

CE= conditional exact method for comparing two independent Poisson rates using a binomial distribution, Inf=Infections, PWP-TT= Prentice Williams Peterson-Total time, Yr(s)=year(s)

References

1. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. J Clin Virol. 2005;32:16–24. https://doi.org/10.1016/J.JCV.2004.12.008.
2. Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, et al. The biology and life-cycle of human papillomaviruses. Vaccine. 2012;30:F55–70. https://doi.org/10.1016/J.VACCINE.2012.06.083.
3. Cervical Cancer Action. Global Progress in HPV vaccination. http://www.cervicalcanceraction.org/comments/comments3.php. Accessed 9 Jul 2019.
4. Malagón T, Drolet M, Boily M-C, Franco EL, Jit M, Brisson J, et al. Cross-protective efficacy of two human papillomavirus vaccines: a systematic review and meta-analysis. Lancet Infect Dis. 2012;12:781–9. https://doi.org/10.1016/S1473-3099(12)70187-1.
5. IARC HPV Working Group. Primary end-points for prophylactic HPV vaccine trials. International Agency for Research on Cancer; 2014. http://www.ncbi.nlm.nih.gov//26468561. Accessed 9 July 2019.
6. Rositch AF, Koshiol J, Hudgens M, Razzaghi H, Backes DM, Pimenta JM, et al. Patterns of persistent genital human papillomavirus infection among women worldwide: a literature review and META-analysis. Int J Cancer. 2013;133:1271–85. https://doi.org/10.1002/ijc.27828.
7. Gravitt PE. The known unknowns of HPV natural history. J Clin Invest. 2011;121:4593–9. https://doi.org/10.1172/JCI57149.
8. Vaccarella S, Söderlund-Strand A, Franceschi S, Plummer M, Dillner J. Patterns of human papillomavirus types in multiple infections: an analysis in women and men of the high throughput human papillomavirus monitoring study. PLoS One. 2013;8:e71617. https://doi.org/10.1371/journal.pone.0071617.
9. Lenselink CH, Melchers WJG, Quint WGV, Hoebers AMJ, Hendriks JCM, Massuger LFAG, et al. Sexual behaviour and HPV infections in 18 to 29 year old women in the pre-vaccine era in the Netherlands. PLoS One. 2008;3:e3743. https://doi.org/10.1371/journal.pone.0003743.
10. Centers for Disease Control and Prevention. Lesson 3: Measures of Risk Section 6: Measures of Public Health Impact. 2019. https://www.cdc.gov/csels/dsepd/ss1978/lesson3/section6.html. Accessed 20 Apr 2020.
11. Halloran ME, Struchiner CJ, Longini IM. Study designs for evaluating different efficacy and effectiveness aspects of vaccines. Am J Epidemiol. 1997;146:789–803.
12. Mollers M, Scherpenisse M, van der Klis FRM, King AJ, van Rossum TGJ, van Logchem EM, et al. Prevalence of genital HPV infections and HPV serology in adolescent girls, prior to vaccination. Cancer Epidemiol. 2012;36:519–24. https://doi.org/10.1016/J.CANEP.2012.07.006.
13. Mollers M, King AJ, Knol MJ, Scherpenisse M, Meijer CJLM, van der Klis FRM, et al. Effectiveness of human papillomavirus vaccine against incident and persistent infections among young girls: results from a longitudinal Dutch cohort study. Vaccine. 2015;33:2678–83. https://doi.org/10.1016/J.VACCINE.2015.04.016.
14. Donken R, King AJ, Bogaards JA, Woestenberg PJ, Meijer CJLM, de Melker HE. High effectiveness of the bivalent human papillomavirus (HPV) vaccine against incident and persistent HPV infections up to 6 years after vaccination in young Dutch women. J Infect Dis. 2018;217:1579–89. https://doi.org/10.1093/infdis/jiy067.
15. Mollers M, Boot Hein J, Vriend Henrike J, King Audrey J, van den Broek Ingrid VF, van Bergen Jan EAM, et al. Prevalence, incidence and persistence of genital HPV infections in a large cohort of sexually active young women in the Netherlands. Vaccine. 2013;31:394–401. https://doi.org/10.1016/J.VACCINE.2012.10.087.

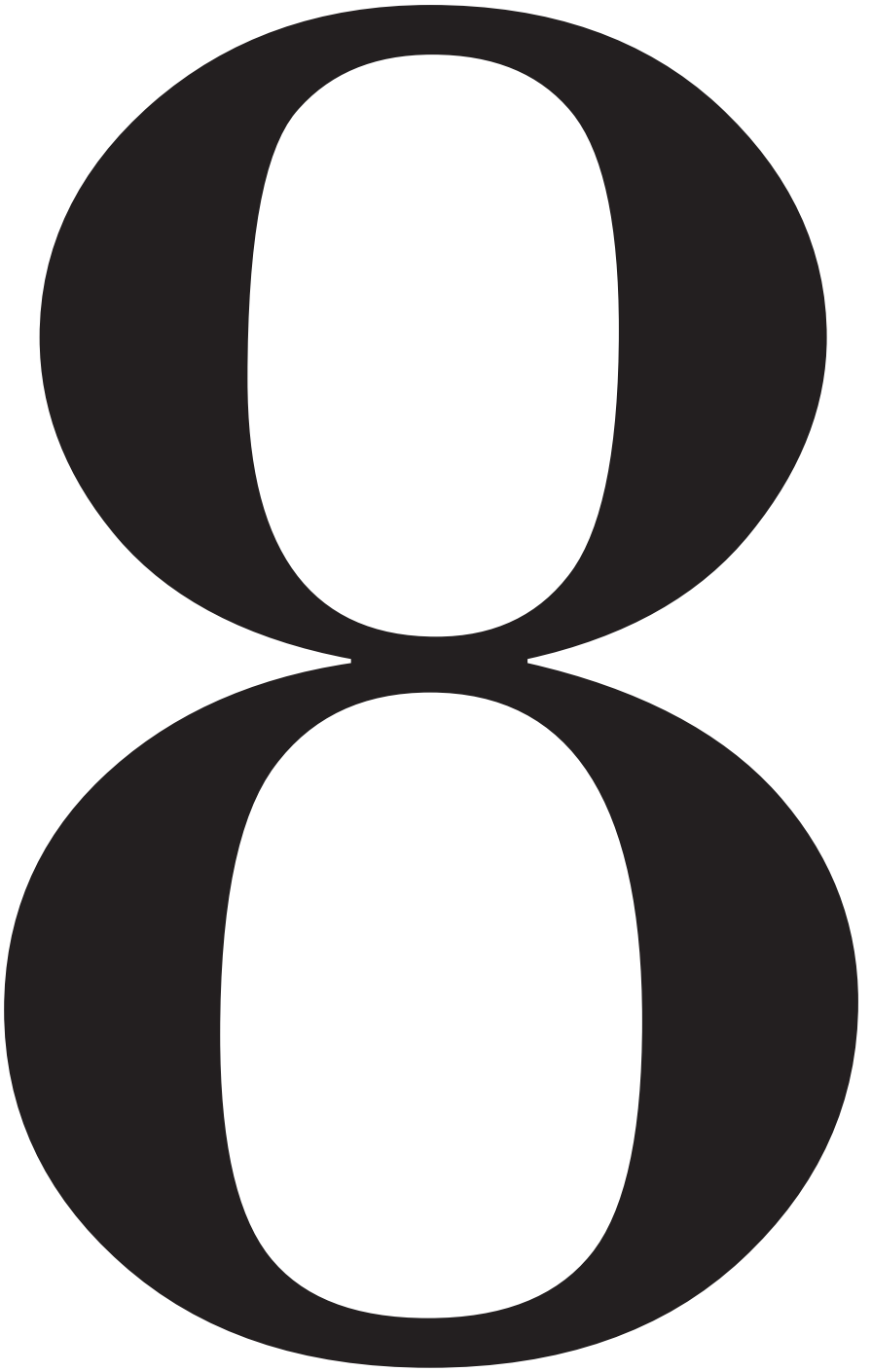
16. Apter D, Wheeler CM, Paavonen J, tellsagué X, Garland SM, Skinner SR, et al. Efficacy of human papillomavirus 16 and 18 (HPV-16/18) AS04-Adjuvanted vaccine against cervical infection and Precancer in young women: final event-driven analysis of the randomized. Double-Blind PATRICIA Trial Clin Vaccine Immunol. 2015;22:361–73. https://doi.org/10.1128/CVI.00591-14.
17. Castellsagué X, Muñoz N, Pitisuttithum P, Ferris D, Monsonego J, Ault K, et al. End-of-study safety, immunogenicity, and efficacy of quadrivalent HPV (types 6, 11, 16, 18) recombinant vaccine in adult women 24–45 years of age. Br J Cancer. 2011;105:28–37. https://doi.org/10.1038/bjc.2011.185.
18. De Carvalho N, Teixeira J, Roteli-Martins CM, Naud P, De Borba P, Zahaf T, et al. Sustained efficacy and immunogenicity of the HPV-16/18 AS04-adjuvanted vaccine up to 7.3 years in young adult women. Vaccine. 2010;28:6247–55.
19. Garland SM, Pitisuttithum P, Ngan HYS, Cho C-H, Lee C-Y, Chen C-A, et al. Efficacy, immunogenicity, and safety of a 9-Valent human papillomavirus vaccine: subgroup analysis of participants from Asian countries. J Infect Dis. 2018;218:95–108. https://doi.org/10.1093/infdis/jiy133.
20. Giuliano AR, Palefsky JM, Goldstone S, Moreira EDJ, Penny ME, Aranda C, et al. Efficacy of quadrivalent HPV vaccine against HPV infection and disease in males. N Engl J Med. 2011;364:401–11.
21. GlaxoSmithKline Vaccine HPV-007 Study. Sustained efficacy and immunogenicity of the human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine: analysis of a randomised placebo-controlled trial up to 6·4 years. Lancet. 2009;374:1975–85. doi:https://doi.org/10.1016/S0140-6736(09)61567-1.
22. Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A, et al. Efficacy of a bivalent L1 virus-like p vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. Lancet. 2004;364:1757–65. https://doi.org/10.1016/S0140-6736(04)17398-4.
23. Harper DM, Franco EL, Wheeler CM, Moscicki A-B, Romanowski B, Roteli-Martins CM, et al. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like p vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. Lancet (London, England). 2006;367:1247–55. doi:https://doi.org/10.1016/S0140-6736(06)68439-0.
24. Herrero R, Wacholder S, Rodriguez AC, Solomon D, Gonzalez P, Kreimer AR, et al. Prevention of persistent human papillomavirus infection by an HPV16/18 vaccine: a community-based randomized clinical trial in Guanate. Costa Rica Cancer Discov. 2011;1:408–19. https://doi.org/10.1158/2159-8290.CD-11-0131.
25. Konno R, Tamura S, Dobbelaere K, Yoshikawa H. Efficacy of human papillomavirus type 16/18 AS04-Adjuvanted vaccine in Japanese women aged 20 to 25 years: final analysis of a phase 2 double-blind, randomized controlled trial. Int J Gynecol Cancer. 2010;20:847–55. https://doi.org/10.1111/IGC.0b013e3181da2128.
26. Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, et al. A controlled trial of a human papillomavirus type 16 vaccine. N Engl J Med. 2002;347:1645–51. https://doi.org/10.1056/NEJMoa020586.
27. Kreimer AR, Rodriguez AC, Hildesheim A, Herrero R, Porras C, Schiffman M, et al. Proof-of-principle evaluation of the efficacy of fewer than three doses of a bivalent HPV16/18 vaccine. J Natl Cancer Inst. 2011;103:1444–51. https://doi.org/10.1093/jnci/djr319.
28. Mao C, Koutsky LA, Ault KA, Wheeler CM, Brown DR, Wiley DJ, et al. Efficacy of human Papillomavirus-16 vaccine to prevent cervical intraepithelial Neoplasia. Obstet Gynecol. 2006;107:18–27. https://doi.org/10.1097/01.AOG.0000192397.41191.fb.

29. Mikamo H, Yamagishi Y, Murata S, Yokokawa R, Han SR, Wakana A, et al. Efficacy, safety, and immunogenicity of a quadrivalent HPV vaccine in Japanese men: a randomized, phase 3, placebo-controlled study. Vaccine. 2019;37:1651–8. https://doi.org/10.1016/J.VACCINE.2019.01.069.
30. Naud PS, Roteli-Martins CM, De Carvalho NS, Teixeira JC, de Borba PC, Sanchez N, et al. Sustained efficacy, immunogenicity, and safety of the HPV-16/18 AS04-adjuvanted vaccine. Hum Vaccin Immunother. 2014;10:2147–62. https://doi.org/10.4161/hv.29532.
31. Roteli-Martins CM, Naud P, De Borba P, Teixeira JC, De Carvalho NS, Zahaf T, et al. Sustained immunogenicity and efficacy of the HPV-16/18 AS04-adjuvanted vaccine. Hum Vaccin Immunother. 2012;8:390–7. https://doi.org/10.4161/hv.18865.
32. Struyf F, Colau B, Wheeler CM, Naud P, Garland S, Quint W, et al. Post hoc analysis of the PATRICIA randomized trial of the efficacy of human papillomavirus type 16 (HPV-16)/HPV-18 AS04-Adjuvanted vaccine against incident and persistent infection with nonvaccine oncogenic HPV types using an alternative multiplex type-Sp. Clin Vaccine Immunol. 2015;22:235–44. https://doi.org/10.1128/CVI.00457-14.
33. Szarewski A, Poppe WAJ, Skinner SR, Wheeler CM, Paavonen J, Naud P, et al. Efficacy of the human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine in women aged 15-25 years with and without serological evidence of previous exposure to HPV-16/18. Int J Cancer. 2012;131:106–16. https://doi.org/10.1002/ijc.26362.
34. Szarewski A, Skinner SR, Garland SM, Romanowski B, Schwarz TF, Apter D, et al. Efficacy of the HPV-16/18 AS04-Adjuvanted vaccine against low-risk HPV types (PATRICIA randomized trial): an unexpected observation. J Infect Dis. 2013;208:1391–6. https://doi.org/10.1093/infdis/jit360.
35. Villa LL, Costa RLR, Petta CA, Andrade RP, Paavonen J, Iversen O-E, et al. High sustained efficacy of a prophylactic quadrivalent human papillomavirus types 6/11/16/18 L1 virus-like p vaccine through 5 years of follow-up. Br J Cancer. 2006;95:1459–66. https://doi.org/10.1038/sj.bjc.6603469.
36. Villa LL, Costa RLR, Petta CA, Andrade RP, Ault KA, Giuliano AR, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like p vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. Lancet Oncol. 2005;6:271–8. https://doi.org/10.1016/S1470-2045(05)70101-7.
37. Wheeler CM, Skinner SR, Del Rosario-Raymundo MR, Garland SM, Chatterjee A, Lazcano-Ponce E, et al. Efficacy, safety, and immunogenicity of the human papillomavirus 16/18 AS04-adjuvanted vaccine in women older than 25 years: 7-year follow-up of the phase 3, double-blind, randomised controlled VIVIANE study. Lancet Infect Dis. 2016;16:1154–68. https://doi.org/10.1016/S1473-3099(16)30120-7.
38. Wilkin TJ, Chen H, Cespedes MS, Leon-Cruz JT, Godfrey C, Chiao EY, et al. A randomized, placebo-controlled trial of the Quadrivalent human papillomavirus vaccine in human immunodeficiency virus-infected adults aged 27 years or older: AIDS Clinical Trials Group protocol A5298. Clin Infect Dis. 2018;67:1339–46. https://doi.org/10.1093/cid/ciy274.
39. Yoshikawa H, Ebihara K, Tanaka Y, Noda K. Efficacy of quadrivalent human papillomavirus (types 6, 11, 16 and 18) vaccine (GARDASIL) in Japanese women aged 18-26 years. Cancer Sci. 2013;104:465–72. https://doi.org/10.1111/.12106.
40. Hildesheim A, Gonzalez P, Kreimer AR, Wacholder S, Schussler J, Rodriguez AC, et al. Impact of human papillomavirus (HPV) 16 and 18 vaccination on prevalent infections and rates of cervical lesions after excisional treatment. Am J Obstet Gynecol. 2016;215:212.e1–212.e15. doi:https://doi.org/10.1016/J.AJOG.2016.02.021.
41. Zhu F-C, Chen W, Hu Y-M, Hong Y, Li J, Zhang X, et al. Efficacy, immunogenicity and safety of the HPV-16/18 AS04-adjuvanted vaccine in healthy Chinese women aged 18-25 years: results

from a randomized controlled trial. Int J Cancer. 2014;135:2612–22. https://doi.org/10.1002/ijc.28897.

42. Zhu F-C, Hu S-Y, Hong Y, Hu Y-M, Zhang X, Zhang Y-SY-J, et al. Efficacy, immunogenicity, and safety of the HPV-16/18 AS04-adjuvanted vaccine in Chinese women aged 18-25 years: event-triggered analysis of a randomized controlled trial. Cancer Med. 2017;6:12–25. https://doi.org/10.1002/cam4.869.
43. Skinner SR, Szarewski A, Romanowski B, Garland SM, Lazcano-Ponce E, Salmerón J, et al. Efficacy, safety, and immunogenicity of the human papillomavirus 16/18 AS04-adjuvanted vaccine in women older than 25 years: 4-year interim follow-up of the phase 3, double-blind, randomised controlled VIVIANE study. Lancet. 2014;384:2213–27. https://doi.org/10.1016/S0140-6736(14)60920-X.
44. Wei L, Xie X, Liu J, Zhao Y, Chen W, Zhao C, et al. Efficacy of quadrivalent human papillomavirus vaccine against persistent infection and genital disease in Chinese women: a randomized, placebo-controlled trial with 78-month follow-up. Vaccine. 2019;37:3617–24. https://doi.org/10.1016/J.VACCINE.2018.08.009.
45. Wheeler CM, tellsagué X, Garland SM, Szarewski A, Paavonen J, Naud P, et al. Cross-protective efficacy of HPV-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by non-vaccine oncogenic HPV types: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. Lancet Oncol. 2012;13:100–10. https://doi.org/10.1016/S1470-2045(11)70287-X.
46. Palefsky JM, Giuliano AR, Goldstone S, Moreira EDJ, Aranda C, Jessen H, et al. HPV vaccine against anal HPV infection and anal intraepithelial neoplasia. N Engl J Med. 2011;365:1576–85.
47. Dragalin V, Fedorov V, Cheuvart B. Statistical approaches to establishing vaccine safety. Stat Med. 2002;21:877–93. https://doi.org/10.1002/sim.1039.
48. Chan ISF, Bohidar NR. Exact power and sample size for vaccine efficacy studies. Commun Stat - Theory Methods. 1998;27:1305–22. https://doi.org/10.1080/03610929808832160.
49. Han C. Comparing two independent incidence rates using conditional and unconditional exact tests. Pharm Stat. 2008;:195–201. https://onlinelibrary.wiley.com/doi/pdf/10.1002/pst.289. .
50. Amorim LDAF, Cai J. Modelling recurrent events: a tutorial for analysis in epidemiology. Int J Epidemiol. 2015;44:324–33. https://doi.org/10.1093/ije/dyu222.
51. Kelly PJ, Lim LL. Survival analysis for recurrent event data: an application to childhood infectious diseases. Stat Med. 2000;19:13–33 http://www.ncbi.nlm.nih.gov//10623910. .
52. Kirkwood B, Sterne J. Essential Medical Statistics. 2nd edition. Blackwell Science Ltd. ; 2006. https://pdfs.semanticscholar.org/2de1/78e7e19a6641d48caa0ed935743ed07d409e.pdf. .
53. Halloran ME, Longini IM, Struchiner CJ. Design and interpretation of vaccine field studies. Epidemiol Rev. 1999;21:73–88. https://doi.org/10.1093/oxfordjournals.epirev.a017990.
54. Kavanagh K, Pollock KG, Cuschieri K, Palmer T, Cameron RL, Watt C, et al. No Title. 2017;17:1293–302. https://doi.org/10.1016/S1473-3099(17)30468-1.
55. Woestenberg PJ, King AJ, van Benthem BHB, Donken R, Leussink S, van der Klis FRM, et al. Bivalent vaccine effectiveness against type-specific HPV positivity: evidence for cross-protection against oncogenic types among Dutch STI clinic visitors. J Infect Dis. 2018;217:213–22. https://doi.org/10.1093/infdis/jix582.
56. Drolet M, Bénard É, Pérez N, Brisson M. Population-level impact and herd effects following the introduction of human papillomavirus vaccination programmes: updated systematic review and meta-analysis. Lancet. 2019. https://doi.org/10.1016/S0140-6736(19)30298-3.

57. Méndez F, Muñoz N, Posso H, Molano M, Moreno V, van den Brule AJC, et al. Cervical Coinfection with human papillomavirus (HPV) types and possible implications for the prevention of cervical Cancer by HPV vaccines. J Infect Dis. 2005;192:1158–65. https://doi.org/10.1086/444391.
58. Rousseau M, Pereira JS, Prado JCM, Villa LL, Rohan TE, Franco EL. Cervical Coinfection with human papillomavirus (HPV) types as a predictor of acquisition and persistence of HPV infection. J Infect Dis. 2001;184:1508–17. https://doi.org/10.1086/324579.
59. Vaccarella S, De Vuyst H, Mugo NR, Sakr SR, Plummer M, Heideman DAM, et al. Clustering patterns of human papillomavirus infections among HIV-positive women in Kenya. Infect Agent Cancer. 2013;8:50. https://doi.org/10.1186/1750-9378-8-50.
60. Skinner SR, Wheeler CM, Romanowski B, tellsagu X, Lazcano-Ponce E, Rowena M, et al. Progression of HPV infection to detectable cervical lesions or clearance in adult women: analysis of the control arm of the VIVIANE study. Int J Cancer. 2016;138:2428–38. https://doi.org/10.1002/ijc.29971.
61. Lee JH, Han G, Fulp WJ, Guiliano AR. Analysis of overdispersed count data: application to the human papillomavirus infection in men (HIM) study. Epidemiol Infect. 2012;140:1087–94. https://doi.org/10.1017/S095026881100166X.
62. SAS support, Assessing fit and overdispersion in categorical generalized linear models. http://support.sas.com/kb/22/630.html. Accessed 25 July 2019.
63. Lehtinen M, Dillner J. Clinical trials of human papillomavirus vaccines and beyond. Nat Rev Clin Oncol. 2013;10:400–10. https://doi.org/10.1038/nrclinonc.2013.84.



Chapter 8

High vaccine effectiveness persists for ten years after HPV16/18 vaccination among young Dutch women.

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ABSTRACT

The current study describes the long-term effectiveness of three-dose HPV16/18 vaccination among Dutch women who were eligible for vaccination during a catch-up campaign and were followed in an observational cohort study. Ten years post vaccination, vaccine effectiveness (VE) was estimated using generalized estimating equation models. VE against persistent infections with vaccine type infections (HPV16/18) was high at 95.8%. For cross protective type persistent infections (HPV31/33/45) this was 64.6%. There were no indications of waning of protection over time. This indicates solid long-term protection is provided by the vaccine and is promising with regard to the future clinical impact.

Introduction

Human papillomavirus (HPV) infections are common sexually transmitted infections, with an estimated lifetime risk of about 80 % in Western countries [1]. Although the majority of infections will be cleared spontaneously within 18 months, some infections may persist and cause disease [2]. Cervical cancer is the most common HPV associated cancer, with virtually all cases attributable to persistent high-risk (hr) HPV infections; Twelve hr types have been identified, of which hr types HPV16 and HPV18 are responsible for 70 % of all cervical cancers [3]. The bivalent HPV vaccine (2vHPV) targeting HPV16 and HPV18 has been included in the Dutch National Immunization Program (NIP) for 12-year-old girls since 2010. In 2009, a one-off catch-up campaign took place for girls aged 13 to 16 years (birth cohorts 1993–1996) [4].

Currently licensed HPV vaccines are prophylactic and should therefore be provided at young age, preferably before sexual debut. This explains why long-term protection following HPV vaccination is important, as many sexually active years should be bridged. Since disease progression is slow, the World Health Organization recommended monitoring of vaccine efficacy and effectiveness (VE) based on intermediate endpoints like persistent infection and precancerous stages, in particular high-grade cervical intraepithelial neoplasia (CIN2 +) [5]. Efficacy against persistent hr HPV infections or high-grade CIN related to vaccine HPV types has been shown to be high (>90 %) [6], but observational data are important to also evaluate the effectiveness of vaccination in the general population. In particular, protection against vaccine-targeted and non-targeted HPV types (cross protection) and the duration of protection determine the long-term impact of the vaccine.

The aim of the current study is to estimate the long-term direct vaccine effectiveness of the 2vHPV vaccine against incident and persistent HPV infections after a three-dose (3D) schedule in a population-based setting. We report VE over time up to ten years post-vaccination among Dutch women who were eligible for 3D HPV vaccination in a catch-up campaign, separately for vaccine types and cross protective types 31/33/45 for which consistent efficacy against 6-month persistent infection and CIN2 + was observed in the PATRICIA trial [7].

Methods and materials

Study design

The design of the study used for the current analyses has been described extensively before [8]. Briefly, a longitudinal cohort study was established for surveillance purposes following the catch-up campaign in 2009/2010 (HAVANA, birth cohort 1993–94). Vaccine-eligible girls were invited for participation and signed an informed consent form before inclusion in the study. Vaccination status of participants was acquired through the national vaccination registration system, Praeventis [9]. Yearly, participants filled out a web-based questionnaire and collected a vaginal self-sample (Viba-Brush; Rovers Medical Devices, Oss, the Netherlands). This study adhered to the tenets of the Declaration of Helsinki and was approved by the Medical Ethics Committee of the VU University Medical Center in Amsterdam (2009/022).

HPV DNA detection and genotyping

Self-collected vaginal samples were tested for HPV DNA using the SPF10–DEIA-LiPA25 platform (Labo Biomedical Products, Rijswijk, the Netherlands) as described before [8]. The assay is able to detect 25 HPV genotypes, including hr HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. Additionally, it can detect 12 low-risk (lr) HPV types: 6, 11, 34, 40, 42, 43, 44, 53, 54, 66, 70 and 74. HPV types 68, 73, and 97 could also be detected. However, no distinction between these types could be made in the assay, and they were therefore all classified as HPV68.

Statistical analysis

Only participants with a sample in round 0 (first study year comprising the baseline measurement pre-vaccination) and who completed a three dose schedule or were unvaccinated, were included for VE analysis. Missing follow-up data in the questionnaires was imputed using the last observation carried forward approach. Type-specific infections were determined among all participants who handed in a sample in that particular round. Type-specific incident infections were defined as being positive for a specific HPV type, preceded by a negative sample in the previous year. Persistent infections were defined as being HPV positive for the same HPV type in two consecutive years (12 month interval), preceded by a negative sample.

Sociodemographic characteristics and sexual behavior among vaccinated and unvaccinated girls were described per study year. The overall associations of these characteristics with vaccination status (and thus differences in characteristics between vaccinated and unvaccinated girls) were analyzed by a generalized estimating equation model (GEE) with an exchangeable correlation structure. Dichotomous outcomes were analyzed by a binomial model with logit link and continuous outcomes were analyzed

by a linear normal model. Additionally, we examined possible differences in trends over time between vaccinated and unvaccinated participants by adding an interaction term between time and vaccination status to the model. Sociodemographic and sexual characteristics that were statistically significantly associated with vaccination status or showed a significantly different trend over time were considered for inclusion as covariate in the adjusted VE analyses. P-values < 0.05 were considered significant.

Type-specific VE estimates against incident and persistent infections for all hr HPV types available in SPF10–DEIA-LiPA25 were calculated. Additionally, combined endpoints were constructed based on the following combinations of hr HPV types: vaccine types (HPV16/18), cross-protective types (HPV31/33/45), all hr HPV types, all hr HPV types included in the nonavalent vaccine (HPV16/18/31/33/45/52/58), and lr HPV types included in the quadrivalent or nonavalent HPV vaccine (HPV6/11). VE was estimated using the Prentice Williams Peterson Total time (PWP-TT) approach, which has been demonstrated as a valid approach in case persistent infections are modeled in the presence of recurrent events [10]. The PWP-TT is an extension of Cox regression that can accommodate recurrent events by considering an event-specific hazard for subsequent events. Crude and adjusted event-specific hazards were calculated, using age, urbanization degree, ever used contraception, and ever had sexual intercourse as time-varying covariates for the adjusted hazards. VE was calculated as 1 minus the hazard ratio times 100 %. Additionally, we analyzed the VE for the first period after vaccination (<5years) and from then onward (≥5 years) in order to study possible differences in protection over time. All statistical analyses were performed using SAS software package version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Study population HAVANA

At baseline, 1635 girls were included in the VE analyses up to 10 years post-vaccination. Table 1 describes the sociodemographic characteristics and sexual behavior characteristics per study round for unvaccinated and vaccinated participants separately. A significant association between vaccination status and age, urbanization degree, and the age of sexual debut was observed. Vaccinated participants were slightly younger, were less likely to live in low urbanization areas, and were less likely to have their sexual debut before 15 years of age compared to unvaccinated participants. Furthermore, a statistically significant interaction term between time and vaccination status was seen for ever using contraception and ever having sex, indicating that over time, vaccinated participants were more likely to ever have had sex or ever have used contraception compared to unvaccinated participants.

Table 1: Descriptive characteristics of the HAVANA cohort over time.

	R0	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	Association vaccination	Interaction vaccination
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	status (p-value)	status and time (p-value)
Vaccination coverage													
n (%)	875 (54)	748 (54)	667 (53)	643 (53)	597 (52)	560 (52)	513 (51)	530 (52)	520 (53)	500 (53)	471 (52)		
Mean age ~ (range)												0.001	0.892
Unvaccinated	15 (14–17)	16 (15–18)	17 (16–19)	18 (17–20)	19 (18–21)	20 (19–22)	21 (20–23)	22 (21–24)	23 (22–25)	24 (23–26)	25 (24–27)		
Vaccinated	15 (14–16)	16 (15–17)	17 (16–18)	18 (17–19)	19 (18–20)	20 (19–21)	21 (20–22)	22 (21–23)	23 (22–24)	24 (23–25)	25 (24–26)		
Dutch Etnicity												0.354	0.072
Unvaccinated	665 (88)	573 (90)	532 (91)	528 (92)	493 (91)	466 (92)	452 (92)	431 (93)	413 (93)	398 (93)	389 (93)		
Vaccinated	753 (86)	655 (88)	585 (88)	569 (89)	531 (89)	496 (89)	464 (90)	452 (90)	446 (91)	431 (91)	409 (91)		
High education												0.816	0.195
Unvaccinated	417 (56)	394 (62)	372 (64)	365 (64)	367 (68)	370 (72)	368 (75)	371 (76)	360 (77)	356 (79)	351 (80)		
Vaccinated	490 (57)	471 (63)	433 (65)	434 (67)	414 (69)	406 (73)	387 (75)	389 (73)	394 (76)	371 (74)	364 (77)		
Low Urbanisation												<0.0001	0.220
Unvaccinated	227 (31)	202 (31)	177 (30)	173 (31)	145 (27)	118 (23)	114 (23)	104 (23)	99 (23)	96 (23)	99 (24)		
Vaccinated	114 (13)	95 (13)	83 (12)	82 (14)	72 (12)	61 (11)	54 (11)	49 (11)	46 (10)	47 (11)	40 (10)		
Current smoker												0.954	0.156
Unvaccinated	92 (37)	193 (32)	183 (34)	205 (38)	204 (39)	200 (40)	174 (37)	167 (34)	132 (29)	132 (29)	122 (28)		
Vaccinated	109 (41)	230 (32)	235 (36)	254 (41)	231 (40)	220 (41)	185 (37)	185 (35)	165 (32)	156 (31)	132 (28)		
Ever used contraception												0.166	<0.0001
Unvaccinated	314 (42)	370 (59)	420 (73)	467 (84)	477 (89)	462 (91)	447 (93)	454 (94)	439 (94)	426 (95)	413 (95)		
Vaccinated	332 (38)	460 (63)	515 (78)	558 (89)	550 (94)	528 (96)	489 (96)	517 (98)	509 (98)	493 (99)	464 (99)		
Ever had sex												0.466	0.031
Unvaccinated	210 (28)	275 (44)	331 (58)	398 (71)	424 (79)	427 (84)	420 (87)	426 (88)	423 (91)	410 (91)	399 (91)		
Vaccinated	187 (22)	316 (43)	397 (60)	439 (70)	480 (82)	479 (86)	450 (88)	478 (91)	481 (93)	471 (94)	444 (94)		

Table 1: Continued

	R0	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	Association vaccination	Interaction vaccination
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	status (p-value)	status and time (p-value)
Among sexually active participants:													
Age sexual debut before 15 years												0.038	0.907
Unvaccinated	104 (50)	87 (35)	76 (25)	79 (21)	78 (19)	76 (19)	73 (18)	72 (18)	70 (18)	67 (17)	66 (18)		
Vaccinated	93 (40	79 (29)	67 (19)	69 (16)	71 (15)	65 (14)	70 (16)	62 (14)	63 (14)	66 (15)	57 (14)		
Mean lifetime number of sexual partners												0.181	0.147
Unvaccinated	1.7 (1–20)	2.1 (1–15)	2.4 (1–15)	2.8 (1–18)	3.3 (1–22)	4.2 (1–50)	4.6 (1–50)	5.2 (1–45)	5.6 (1–50)	6.2 (1–70)	6.7 (1–70)		
Vaccinated	1.7 (1–16)	2.0 (1–15)	2.5 (1–20)	2.8 (1–20)	3.4 (1–31)	4.2 (1–34)	4.8 (1–34)	5.3 (1–45)	6.4 (1–35)	7.0 (1–40)	7.7 (1–80)		
New number of partners (mean, range) in previous 12 months												0.097	0.626
Unvaccinated		1.2 (0–10)	1.0 (0–6)	1.0 (0–7)	1.1 (0–12)	1.2 (0–31)	1.0 (0–17)	1.6 (0–10)	1.5 (0–10)	1.6 (0–20)	1.4 (0–15)		
Vaccinated		1.2 (0–7)	1.2 (0–12)	1.0 (0–13)	1.1 (0–11)	1.2 (0–16)	0.9 (0–6)	1.6 (0–13)	1.7 (0–12)	1.7 (0–20)	1.8 (0–40)		
Current partner												0.145	0.897
Unvaccinated	155 (73)	192 (76)	238 (79)	285 (74)	301 (77)	319 (79)	336 (82)	344 (86)	353 (89)	354 (92)	333 (87)		
Vaccinated	118 (63)	197 (67)	278 (76)	323 (76)	349 (75)	348 (79)	348 (79)	383 (81)	388 (86)	377 (87)	367 (89)		
Diagnosed with STI in the previous 12 months												0.229	0.834
Unvaccinated	2 (1)	5 (2)	5 (2)	6 (2)	7 (2)	22 (5)	14 (3)	22 (5)	17 (4)	11 (3)	11 (3)		
Vaccinated	2 (1)	3 (1)	8 (2)	19 (4)	3 (3)	22 (4)	24 (5)	27 (6)	24 (5)	20 (4)	15 (3)		

Vaccine effectiveness up to 10 years postvaccination

The VE analyses in the HAVANA cohort were performed comparing incident and persistent infections between vaccinated and unvaccinated participants up to 10 years post-vaccination, both on a type-specific level and for pooled outcomes. Type-specific rates of incident and persistent infections are reported in Supplementary Table 1. Age, urbanization degree, ever used contraception and ever had sex were included in the adjusted VE analyses. Type-specific vaccine effectiveness is reported in Fig. 1 (panel A and B) for incident and persistent infections and was statistically significant for HPV16, 18, 31, and 45 (the latter only against incident infections). This aligned well with the pooled outcomes (Fig. 1; panel C and D), where a significant VE against persistent vaccine types HPV16/18 infections was observed (VE: 95.8 %, 95 % confidence interval (CI) 86.6–98.7 %), as well as against persistent cross protective type infections (HPV31/33/45; VE 64.6 %, 95 % CI 37.6–79.9 %). No effect was observed against hr HPV type 33 or against the lr HPV types 6 and 11, the latter types being associated with genital warts and not targeted by the 2vHPV vaccine. Overall, VE was higher against persistent infections compared to incident infections and was also higher after adjustment for covariates. Short-term vaccine effectiveness (up to 5 years post-vaccination) and long-term VE (>5 years post-vaccination) were compared for vaccine types and cross protective types. No marked decline in effectiveness against persistent infections over time was observed (Supplementary Table 2).

Discussion

We studied vaccine effectiveness up to 10 years post bivalent HPV vaccination in a population-based cohort study including women eligible for the Dutch catch-up campaign. High VE was observed against 12-month persistent infection with vaccine types HPV16/18 and cross protective types HPV31/33/45 (both pooled outcomes), with no indication of waning over time. This indicates direct, long-term vaccine effects which will likely result in a substantial reduction of clinical disease over time.

The results from the current study are in line with previous findings from this cohort up to six years post-vaccination [8], which could now be extended to a ten-year follow-up period. Although observational data about the 2vHPV vaccine is limited, our results compare well with other real-world studies on protection against vaccine type HPV infections following 2vHPV vaccination; follow-up data on prevalent HPV infections in England has been collected up to eight years post-vaccination and indicated a VE of 82.0 % [11], while post-vaccination surveillance data in the Netherlands from a high-risk population indicated a VE of 89.9 % [12]. The Costa Rica Vaccine Trial has the longest follow-up for the bivalent vaccine of 11 years, and reported a cumulative

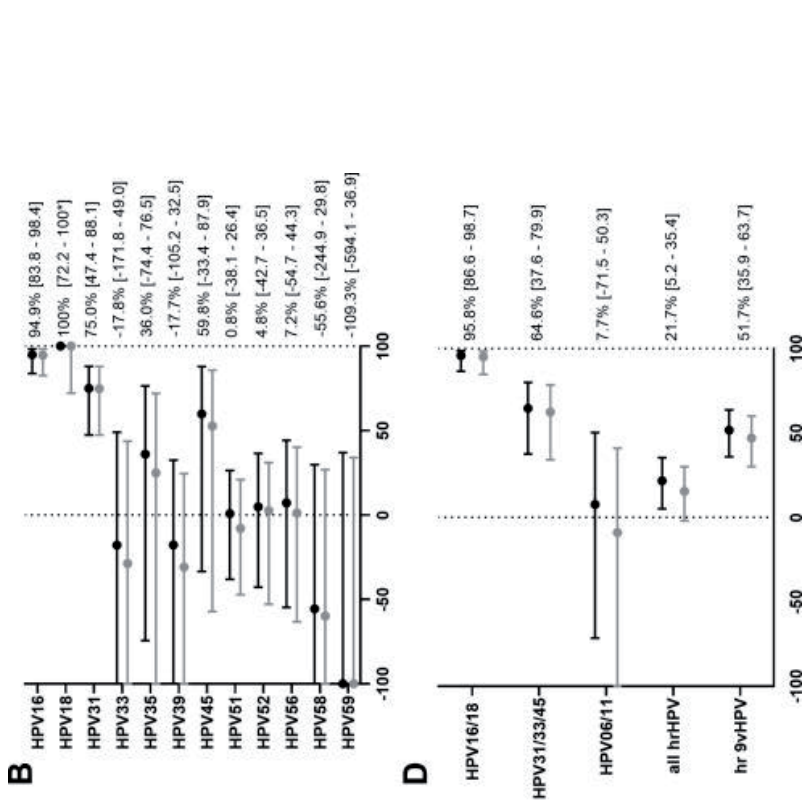

Figure 1: Type specific VE against incident infection (panel A) and persistent infections (panel B) with hrHPV types. Pooled outcomes are shown in panel C (incident infections) and panel D (persistent infections). Grey dots represent crude estimates, black dots were adjusted for age, urbanization degree, ever used contraception and ever had sex. All adjusted analyses are displayed with 95% confidence interval. *For HPV types with no infections among vaccinated participants, confidence estimates could only be included for the crude estimates (using the Peto estimator for the hazard ratio, based on the log-rank statistic).

vaccine efficacy against HPV 16/18-associated CIN2 + of 97.4 %, in accordance with our VE estimate for persistent HPV16/18 infections of 95.8 % [13]. This also indicates the correspondence between clinical trial and real-world data. Early evidence on the effect of vaccination on cancer is also emerging. Studies from the United Kingdom and Sweden indicated substantial risk reductions in cervical cancer among girls who were offered HPV vaccination compared to unvaccinated cohorts [14], [15]. Together with the long-term vaccination effects as shown in the current study, this further strengthens the impact of HPV vaccination.

Additionally, we observed long-term cross protection which contributes to the impact of the 2vHPV vaccine. A pooled analysis with four year follow-up data from the Costa Rica Vaccine Trial and the PATRICIA trial showed a vaccine efficacy of 67.6 % against persistent HPV31/33/45 infections, compared to 64.6 % in our study [16]. A main contribution of our study is that we showed that protection lasted for a long time, with no substantial differences in VE for the first 5 and next 5 years following vaccination. However, in the current analyses we did not observe protection on a type-specific level against HPV33 infections, nor did we observe a protective effect against persistent infections with HVP51 and HPV52, as indicated by some previous studies and EMA-EPAR documentation [7], [16], [17]. Our findings regarding these types might be related to the low sample sizes at the type-specific level, since the study-specific confidence intervals of the VEs for HPV33, HPV51, and HPV52 are overlapping. Continued monitoring of infections in the current study may increase sample sizes and lead to more robust and reliable effectiveness estimates regarding these HPV types.

Strengths of the current research includes the use of observational data from a longitudinal cohort and the length of follow-up. However, we do acknowledge some limitations. First, in our cohort, educational level was slightly higher as compared to the general Dutch population which might affect generalizability of the findings [18]. Second, the current cohort represents women who were eligible for a catch-up campaign and where therefore older compared to the girls in the routine program, increasing the likelihood of exposure to HPV before vaccination.

In conclusion, we observed high vaccine effectiveness of the 2vHPV vaccine against persistent vaccine type and cross protective type infections in a population-based observational study with long-term follow-up, indicating solid individual-level protection through the HPV vaccination program over time.

Supplementary data

Supplementary Table 1: Type-specific rates of incident and persistent infections as expressed per 1,000 person-years among vaccinated and unvaccinated participants from the HAVANA cohort up to 10 years post-vaccinations.

	Rate of incident infections				Rate of persistent infections			
	Unvaccinated (per 1,000 person-years)		Vaccinated (per 1,000 person-years)		Unvaccinated (per 1,000 person-years)		Vaccinated (per 1,000 person-years)	
HPV type	**Mean**	**95% CI**	**Mean**	**95% CI**	**Mean**	**95% CI**	**Mean**	**95% CI**
16	24.4	20.4 – 29.3	3.6	2.3 – 5.6	11.5	8.8 – 15.0	0.6	0.2 – 1.8
18	12.2	9.4 – 15.7	3.8	2.4 – 5.8	4.9	3.2 – 7.3	0.0	0.0 – 0.0
31	17.0	13.7 – 21.2	9.0	6.8 – 12.0	7.6	5.5 – 10.6	1.7	0.9 – 3.4
33	8.5	6.2 – 11.5	7.0	5.0 – 9.6	2.1	1.1 – 3.9	2.7	1.6 – 4.6
35	5.3	3.6 – 7.9	3.0	1.8 – 4.9	1.9	1.0 – 3.6	1.4	0.6 – 2.9
39	14.1	11.1 – 17.9	16.1	13.0 – 19.9	4.6	3.1 – 7.1	6.2	4.4 – 8.8
45	8.5	6.2 – 11.5	2.1	1.1 – 3.7	1.7	0.8 – 3.4	0.8	0.3 – 2.1
51	37.6	32.5 – 43.6	40.8	35.6 – 46.7	16.0	12.8 – 20.1	16.7	13.5 – 20.7
52	25.9	21.8 -30.9	22.8	19.1 – 27.3	10.0	7.5 – 13.3	10.2	7.7 – 13.3
56	20.8	17.1 – 25.3	21.1	17.5 – 25.5	6.6	4.6 – 9.3	6.2	4.4 – 8.8
58	7.8	5.7 – 10.8	10.0	7.6 – 13.1	2.1	1.1 – 4.0	3.3	2.1 – 5.4
59	7.0	5.0 – 9.8	11.5	9.0 – 14.8	0.8	0.3 – 2.2	1.7	0.9 – 3.4

Supplementary Table 2: Adjusted vaccine effectiveness by years since vaccination

	VE vaccine types (HPV16/18)	**VE cross-protective types (HPV31/33/45)**
Incident infections		
< 5yrs ago	76.9% (62.3-85.8%)	56.0% (31.0-72.0%)
≥ 5 yrs ago	81.9% (66.9-90.1%)	42.5% (12.9-62.0%)
Persistent infections		
< 5yrs ago	100% (72.2-100%)*	43.4% (-48.3-78.4%)
≥ 5 yrs ago	93.2% (78.1-97.9%)	71.9% (42.6-86.2%)

**For HPV types with no infections among vaccinated participants, confidence were calculated using the Peto estimator for the hazard ratio, based on the log-rank statistic).*

References

1. H.W. Chesson, E.F. Dunne, S. Hariri, L.E. Markowitz. The estimated lifetime probability of acquiring human papillomavirus in the United States. Sex Transm Dis, 41 (2014), p. 660
2. J.G. Baseman, L.A. Koutsky. The epidemiology of human papillomavirus infections. J Clin Virol, 32 (2005), pp. 16-24
3. J.M. Walboomers, M.V. Jacobs, M.M. Manos, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol, 189 (1999), pp. 12-19
4. H.E. De Melker, M.A. Conyn-Van Spaendonck, H.J. Boot, R.A. Coutinho. STAND VAN ZAKEN-Introductie van vaccinatie tegen baarmoederhalskanker. Ned Tijdschr Geneeskd, 153 (2009), p. 658
5. IARC HPV Working Group. Primary end-points for prophylactic HPV vaccine trials. International Agency for Research on Cancer; 2014.
6. D.M. Harper, L.R. DeMars. HPV vaccines - A review of the first decade. Gynecol Oncol, 146 (2017), pp. 196-204
7. Committee for Medicinal Products for Human Use (CHMP). Cervarix - EPAR: Product information; 2009.
8. R. Donken, A. King, J. Bogaards, P. Woestenberg, C. Meijer, H. de Melker. High Effectiveness of the Bivalent Human Papillomavirus (HPV) Vaccine Against Incident and Persistent HPV Infections up to 6 Years After Vaccination in Young Dutch Women. J Infect Dis, 217 (2018), pp. 1579-1589
9. A. van Lier, P. Oomen, P. de Hoogh, et al. Praeventis, the immunisation register of the Netherlands: a tool to evaluate the National Immunisation Programme. Euro Surveill, 17 (2012)
10. R. Donken, J. Hoes, M. Knol, et al. Measuring vaccine effectiveness against persistent HPV infections: a comparison of different statistical approaches. BMC Infect Dis, 20 (2020), pp. 1-11
11. D. Mesher, K. Panwar, S.L. Thomas, et al. The Impact of the National HPV Vaccination Program in England Using the Bivalent HPV Vaccine: Surveillance of Type-Specific HPV in Young Females, 2010–2016. J Infect Dis, 218 (2018), pp. 911-921
12. P.J. Woestenberg, A.J. King, B.H.B. van Benthem, et al. Bivalent Vaccine Effectiveness Against Type-Specific HPV Positivity: Evidence for Cross-Protection Against Oncogenic Types Among Dutch STI Clinic Visitors. J Infect Dis, 217 (2018), pp. 213-222
13. C. Porras, S.H. Tsang, R. Herrero, et al. Efficacy of the bivalent HPV vaccine against HPV 16/18-associated precancer: long-term follow-up results from the Costa Rica Vaccine Trial. Lancet Oncol, 21 (2020), pp. 1643-1652
14. M. Falcaro, A. Castañon, B. Ndlela, et al. The effects of the national HPV vaccination programme in England, UK, on cervical cancer and grade 3 cervical intraepithelial neoplasia incidence: a register-based observational study. Lancet, 398 (10316) (2021), pp. 2084-2092
15. J. Lei, A. Ploner, K.M. Elfström, et al. HPV vaccination and the risk of invasive cervical cancer. N Engl J Med, 383 (2020), pp. 1340-1348
16. J.E. Tota, F. Struyf, J.N. Sampson, et al. Efficacy of the AS04-Adjuvanted HPV16/18 Vaccine: Pooled Analysis of the Costa Rica Vaccine and PATRICIA Randomized Controlled Trials. J Natl Cancer Inst, 112 (2020), pp. 818-828
17. J.E. Tota, F. Struyf, M. Merikukka, et al. Evaluation of Type Replacement Following HPV16/18 Vaccination: Pooled Analysis of Two Randomized Trials. J Natl Cancer Inst, 109 (2017)
18. M. Mollers, M. Scherpenisse, F.R.M. van der Klis, et al. Prevalence of genital HPV infections and HPV serology in adolescent girls, prior to vaccination. Cancer Epidemiol, 36 (2012), pp. 519-524

9

Chapter 9

General discussion

This thesis continued the research activities related to monitoring of HPV vaccination within the National Immunization Program of the Netherlands. Focus was on vaccine effectiveness and immunogenicity, especially on the long-term effects following vaccination using intermediate endpoints. This is especially important since the time gap between implementation of the vaccination program and effects on clinical outcomes is considerable. Assessment of intermediate endpoints aid in timely evaluation of the program, which is important for public health. Our findings should be viewed within the larger picture of monitoring studies, which together have added to the growing pile of evidence of the highly beneficial public health effects of HPV vaccination in the real-world setting. In this general discussion the most important findings and implications from this thesis will be elaborated on, and future recommendations for research and conclusions will be shared.

Summary and discussion of main findings

Part 1: Vaccine and natural infection induced immune responses

Serological measurements are important in HPV research, since these can indicate (cumulative) previous exposure to the pathogen or vaccine. Vaccine induced and naturally induced immune responses (specifically antibody levels) can be distinguished by their levels; vaccine induced antibody levels are much higher compared to naturally induced levels. The induced immune response and following antibodies are mainly type-specific; HPV vaccines induce protection against specific targeted hr types, sometimes supplemented with cross protection. Also, after natural infection, protection against subsequent infections is assumed to be type-specific, if it is acquired at all.

In **chapter 2,** we explored seroprevalence of seven hr HPV types (HPV16/18/31/33/45/52/58) comparing a pre- (2006/2007) and post-vaccination (2016/2017) implementation time period among the unvaccinated Dutch population. It was shown that the overall seropositivity in the unvaccinated population was not affected by the recent introduction of the HPV vaccination program among teenage girls in the Netherlands. While herd effects among unvaccinated individuals may cause a shift in HPV seroprevalence, this effect was not yet observed. In fact, HPV seroprevalence even increased over the past decade among unvaccinated women, while it was stable among men. Results were adjusted for sociodemographic characteristics and sexual behavior (which might change over time), but this could not explain the observed changes in seroprevalence. Therefore, follow-up of the Pienter studies is recommended; since not everybody seroconverts after HPV infections, (shifts in) past exposure measured through serology takes a long time to become detectable. Combined with the suboptimal vaccination uptake in the Netherlands, shifts in seroprevalence, induced by herd immunity, might become detectable in the near future.

Furthermore, in **chapter 3,** the long-term follow-up of serological response following vaccination in a catch-up cohort was described for the same HPV types as mentioned above and compared to serological measurements among unvaccinated individuals from the same cohort. IgG antibody levels of vaccine HPV types remained high up till nine years past vaccination. Regarding cross protective HPV types, higher antibody levels were observed among vaccinated compared to unvaccinated individuals. Although antibody levels of cross-protective types were much lower than those of HPV16/18, this does indicate some cross-protective activity following vaccination. IgG antibody levels from vaccinated women who reported an HPV infection were compared to those without infection, but no significant difference in the antibody levels were observed the year before infection. Hence, no conclusions about a minimum level of IgG response (also known as a correlate of protection) can be drawn and

this suggests that other (immune or environmental) factors besides the antibody level determine final effectiveness of the vaccine.

In **chapter 4** we focused on long-term immune responses following the different HPV vaccines and dosing schedules. The longest follow-up of 14 years was reported for the three-dose schedule of the bivalent vaccine, while serology data for the one and two-dose schedule and the nonavalent vaccine had a much shorter follow-up . Furthermore, the similarities and differences between the vaccines regarding cross protection were discussed, indicating that the bivalent vaccine showed highest immune responses against non-vaccine types. Importantly, both real-world data and model predictions for the three-dose schedule did not show waning antibody levels against vaccine HPV types over time; this is an important factor to consider as (life)long protection adds to the health benefits of HPV vaccination.

In general, serology measurements are informative concerning previous exposure to HPV infection or vaccination. Serology is an easy and accessible tool for monitoring population-based shifts as compared to DNA status and can provide insight into both increases and decreases of HPV types; seroprevalence shifts in (nonvaccine) HPV types can be informative about HPV circulation among the unvaccinated population [1]. Over time, a population-based decrease in serology of vaccine types is expected in the unvaccinated Dutch population, although that was not yet observed in the current study. Our findings on the seroprevalence in the population after vaccination introduction are in line with those from the United States, where suboptimal vaccination uptake is a problem as well and no signs of herd immunity on the seroprevalence in the (male) population was observed [2]. In countries with higher uptake a shift in seroprevalence is already noticeable, indicating that herd effects (measurable through seroprevalence) in the Netherlands might develop in the near future[3].

We also observed persisting antibody responses against HPV16,18,31,33,45,52 and 58 up to nine years post vaccination. These findings were in line with previous trials and other observational studies examining the immunogenicity of the bivalent vaccine [4, 5]. Nevertheless, serology measurements remain complicated in their interpretation on the individual level, both among vaccinated and unvaccinated individuals; a correlate of protection would simplify these evaluations. To clarify, if a certain level of antibodies is required for protection, identification of individuals who have not reached sufficient protection following vaccination or previous exposure would be much simpler [6]. For program evaluation purposes a correlate of protection would also be useful; long-term protection, new vaccines, and reduced dosing schedules can then easily be assessed based on a target threshold. However, it is assumed that even at low antibody levels, protection can be reached and indications for breakthrough

infections would be difficult to identify. Therefore, it is likely that besides antibody levels also other factors are involved in the immune response and final success of HPV vaccination [7].

Research efforts on defining a correlate of protection have been limited. Established clinical trials with long follow up could, theoretically, focus on identifying vaccine failures or breakthrough infections among their participants and evaluate past immunological measurements in these individuals. If these individuals show deviating serological responses (i.e. lower antibody levels from the start, no response to the vaccine, waning antibody levels) then that could aid in the search for a correlate of protection. However, practically this type of research is difficult to conduct, because of the very limited number of breakthrough infections as a result of the high efficacy of the vaccines and the high immune responses.

Part 2: Effects of HPV vaccination on genital HPV infection

Data on infections at the individual- and population-level provide an important source of information for the evaluation of (HPV) vaccines. While the former can be used to assess the direct effect of a vaccine, the latter indicates whether effects are also observed outside the targeted population. In the case of HPV, we are aiming for protection against clinical disease caused by HPV but this may take very long to develop. Therefore, incident, persistent and prevalent infections have their own value in research, although all of them are intermediate endpoints.

In **chapter 5**, prevalent infections were assessed among sexual health clinic visitors over time, as an indicator of population-level effects. We observed declining trends of vaccine HPV types not only among vaccinated, young women, but also among unvaccinated women and men from the same age. This indicates that herd protection becomes measurable in the population, with clearer results as time since the introduction of the vaccination program progresses. Since the effects took some time to become apparent, further follow up of this study seems useful; effects on other (cross protective) HPV types might take longer to develop. Besides, also the (inconsistent) increase in the number of HPV types is important to notice, since this may be indicative of type replacement. Both observations are valuable and can be used in policy recommendations and vaccination campaigns.

In **chapter 6,** vaccine effectiveness against incident HPV infections following a regular two-dose schedule was assessed among adolescent girls. We showed a significant vaccine effectiveness against vaccine types and cross protective types up to three years after vaccination. This is important because mainly immunobridging studies were used to justify the switch from three to two doses for young girls. Further research

should determine the long-term effectiveness of a two-dose schedule and include a comparison to the three-dose schedule. Additionally, the long-term cross protective effects should be further evaluated. Since the first two-dose results do not show differences in protection compared with the three-dose schedule, use of a two-dose schedule seems justified. Notably, some trials already showed encouraging results for one-dose vaccination and prolonged data on two-dose vaccination, which may further simplify the HPV vaccination program.

In **chapter 7**, different methods to study vaccine effectiveness were collected, compared and assessed. Especially longitudinal data contain missing observations and repeated events. It is important to use an analysis method that can handle this in the right way, without compromising the data or losing valuable information. The Prentice Williams and Peterson total time (PWP-TT) approach was selected as suitable for the current data, since all measurements (also recurrent events) could be included in the model and the model assumptions were not violated. However, the different statistical methods that were identified and evaluated yielded similar estimates of the VE. Therefore, the main value of the study was to provide an overview of different methods and indicate the importance of selecting the right method for the data. Differences between VE estimates may become larger when data is collected over a longer period of time and the impact of violation of assumptions, such as a constant event rate, becomes more important. The choice of the method may also become more important with an increased number of missing follow-up observations and recurrent events.

Finally, in **chapter 8,** the long-term follow up of the three-dose schedule was assessed using the method described in chapter 7. Data from the HAVANA cohort were used, which includes vaccinated and unvaccinated women from the catch-up cohort (1993/1994). This 10-year follow-up showed no indications for waning protection over time, as the VE against persistent vaccine type infections remained very high. This was also the case for cross protective types. Concerning the three-dose schedule and evaluation of the long-term HPV vaccine effectiveness, clinical outcomes are becoming measurable as the first women who were eligible for vaccination are approaching the screening start age. Showing the (long-term) protection against pre-stages and actual cancer development in screening data is very important and may positively influence the HPV vaccination uptake.

HPV DNA data form an important source of information to bridge the gap between vaccination implementation and clinical disease. We were able to show effects of the vaccination program on the population-level: after two and three-dose schedules, the vaccine effectiveness against vaccine types was high, in line with findings from other clinical trials and observational studies in countries using the bivalent vaccine [8, 9].

For the vaccine types, declining trends were observed among the general population (vaccinated and unvaccinated women, and heterosexual men) [10]. This indicates the proper working mechanisms of the vaccine, the beneficial effects of which spread through the population in place and time.

Besides the bridging function, studying HPV infections has other advantages; for instance, type-specific information can be used to calculate vaccine effectiveness against vaccine types and cross protective types separately or combined, providing detailed information on the effects of the vaccine. In our studies, cross protection was observed for non-vaccine targeted HPV types, mainly in line with previous studies [11, 12]. Also, for population-level impact type-specific information is important, as HPV prevalence might not only decrease among vaccinated individuals but also among unvaccinated individuals. It is important to take all effects of HPV vaccination into account, direct effects to vaccine types, cross-protective effects, and indirect herd effects, as this provides a more complete picture of the vaccine effects. These might also include specific HPV type increases as experienced in both our and other studies, although consistent patterns could not be shown [13, 14].

While long-term data on the infection level were shown in this thesis, we still await the first clinical study outcomes from the Dutch screening program concerning protection of HPV vaccination against cervical lesions and malignancies. However, a study on opportunistic screening indicated positive effects on HPV positivity and cytological abnormalities among vaccinated young women [15]. Also research from abroad is reassuring on this matter [16] and our current studies all indicate consistently high effectiveness of HPV vaccination against genital HPV infections and high vaccine induced immune responses. Therefore, these findings are convincing regarding future clinical impact and beneficial long-term effects of the vaccine.

Reflection - Methodological considerations

In this thesis observational data was used, available from (repeated) cross-sectional studies (Pienter and PASSYON) and prospective cohort studies (HAVANA and HAVANA2). Observational data has several drawbacks as compared to experimental data, but at the same time provides a very important information source; they are representative for real world data collected in population-based studies instead of clinical trials. Therefore, circumstances are less ideal and pre-existing differences between participants might affect the outcomes, something that is accounted for by randomization in clinical studies. However, the effect of vaccination is something that should be studied in the real world after the clinical trial phase in order to observe the final effects, since nonoptimal circumstances are part of the real world [17].

Challenges in (repeated) cross-sectional studies include the differences in the population over time and how these differences are captured, in our studies through questionnaires on demographic information and sexual behavior, which can be prone to recall bias or socially desirable answers. While this was assessed as thoroughly as possible, residual confounding could not be ruled out (i.e., information that cannot be corrected for, as it is not known or not available). Challenges in the design of prospective cohort studies include lack of information about the expected number of events that would occur during the study period. That was observed in both HAVANA and HAVANA2, where VE estimates for combined end-points were reported because of lack of power at the type-specific level.

Another methodological challenge from (mainly) the HAVANA studies was that measurements were taken with a one year interval. This means that we may have missed infections that occurred and cleared between two measurements. If these undetected infections occur more often among vaccinated than among unvaccinated individuals, then the reported vaccine effectiveness estimate overestimates the effect of the vaccine on the HPV infection rate. However, we think that if an infection occurs and clears within a 12-month period this may indicate that the clinical relevance of this infection is low.

A final methodological challenge is that infections may also have been missed due to technical reasons, for instance because the viral load remains below the detection limit. We used the SPF10-DEIA-Lipa platform for assessing infections in our cohort studies, which is a very sensitive method and therefore a useful tool in epidemiological research. Note that this test is not recommended for assessing clinically relevant infections in screening, as these require tests with a high specificity (like the GP5/6+ test, amongst others). Thus, although we may have missed a few short-lived infections, we think that only a small proportion of the reported infections has the potential to persist and progress to CIN2/3 [18].

Study design is very important in HPV vaccination evaluation in general. The study population, endpoints that are used, and frequency of visits affect the findings. For example, in a trial with a short interval between measurements, slowly progressive lesions will be picked up as CIN2 and rapidly progressive lesions will be picked up as CIN3. If vaccine type infections progress more rapidly than non-vaccine type infections, vaccine efficacy against CIN3 will be higher than against CIN2. It is plausible that this holds for HPV16, but it may also apply to the cross-protected HPV types. Although cross protection against HPV31/33/45 (and some other types to a lesser extent) is broadly acknowledged for bivalent HPV vaccination [19], how the cross protective effect is empirically established is important to consider: when CIN2 is

treated once detected, that will be in favor of rapidly progressive type infections such as HPV 16, 31, and 33 infections [20]. This effect leads to a relatively high total effect for rapidly progressive HPV type infections and an optimistic view on cross protection against CIN3 for 2vHPV vaccination. Thus, establishing efficacy, effectiveness and cross protection and comparing it between studies and (maybe even more important) between vaccines, largely depends on the research design and requires studies to be conducted exactly alike in order to make a proper comparison.

Future perspective and research

Monitoring

In most research and monitoring, samples are collected for HPV DNA testing by a vaginal brush or cervical smear. Alternatively, other (monitoring) tools could also be considered. For example, some Scandinavian studies have already focused on urine collection as a method to detect HPV infections. Collection of urine is easy and not invasive and can also be done in males. There is still uncertainty about the standardization and sensitivity of urine-based tests, which hampers the implementation of urine sampling as a monitoring tool[21]. Nevertheless, in research settings urine sampling could simplify the collection and analysis method and an increased frequency or sample size could be achieved against limited efforts. Both would result in increased power for the vaccine efficacy or effectiveness estimates. Further research could focus on the comparison between urine testing and established (monitoring) methods in order to further explore its applicability.

Changes to the Dutch immunization program

Recently, changes to the existing vaccination program have been implemented in the Netherlands after an advice of the Health Council in 2019 [22]. These include lowering of the age of vaccination to nine years (in the year children turn ten) and adding boys to the regular program in order to further lower the total HPV related disease burden. Moreover, a catch-up campaign was conducted for all young people (boys and girls) up to the age of 18 years in 2022. This campaign continues in 2023, with an additional opportunity to get vaccinated for everyone from 18 to 26 years old. Sex neutral vaccination is offered in an increasing number of countries, including Australia, Canada, the USA and Austria.

Non-cervical HPV associated disease

The new HPV vaccination strategy has several consequences. First, the preventive impact of the vaccination program on disease is likely to increase, since boys will be added to the program resulting in better prevention of disease burden of (non-

cervical) (pre-) cancers among this group; the age is lowered which increases the opportunity to vaccinate adolescents before sexual debut (which decreases the chance of HPV exposure pre-vaccination). Furthermore, vaccinating boys along with girls will lead to both direct protection among this group and further reduction of HPV transmission in the population (protecting unvaccinated girls through increasing herd effects). This effect might even be stronger than the direct effect for boys [23]. Additionally, by offering the vaccine to all boys, future men who have sex with men will be reached as well. This is a high-risk group that has not been targeted nor reached by the program so far, as MSM do not benefit from the herd effects that are established in the heterosexual population [24]. Together, this will likely lead to an increased population-level impact. The expected reduction in disease burden in the Netherlands depends on the final vaccine uptake, but with sufficient uptake a substantial part of HPV-related cancer can be prevented. This will shift the focus from a cervical cancer vaccine towards a broader perspective including prevention of all HPV associated disease, which is already the intent of the sex neutral vaccination campaign.

Vaccination shortage, is 1D the solution?

A consequence of the sex-neutral vaccination strategy is an increased need of vaccines in the Netherlands. This is an international problem; the continuing high global demand for HPV vaccines has created significant challenges. Due to the introduction of the vaccine in countries around the world, there has been a vaccine supply shortage although suppliers are expected to better meet the high demand in the near future. In a time where HPV vaccines are scarce and expensive, questions about the use of HPV one-dose schedules seem legitimate. Besides, an increased vaccine demand in the developed world might even slow down implementation of HPV vaccination programs in low and middle income countries, something that should be avoided as the disease burden is disproportionately high in these countries [25]. The case for a one-dose schedule is therefore important. Several studies including post-hoc analyses from trial data (the first one-dose trial with 18 months follow-up), and real-world effectiveness already show non-inferior effectiveness from one dose of HPV vaccination against HPV16/18 [26-29]. Antibody levels were lower after a one-dose schedule compared to two or three doses, but still substantially higher compared to natural infection and robust over time [26]. Moreover, the high effectiveness that is observed following the (reduced) two-dose schedule, does not indicate any signs of diminished protection compared to the three-dose schedule. Hence, a further reduction from two to one dose deserves further consideration. Besides, the accelerated use of a single-dose schedule is suggested as (part of the) solution for the implementation of HPV vaccination across the world, which is falling behind in LMIC [30].

Considering the increasing pile of evidence, the Strategic Advisory Group of Experts on Immunization (SAGE) recently advised the WHO on reduced dosing schedules; a one or two-dose schedule is recommended for the primary target of girls aged 9-14 and for young women aged 15-20. For women older than 21, two doses with a 6-month interval are recommended [31]. Also the Joint Committee on Vaccination and Immunisation (JCVI) advised a one-dose schedule for the routine adolescent program before 25 years of age to the UK government [32]. Also the Dutch Health Council has updated the vaccination advice, according to which all invitees are now offered two vaccine doses, instead of three doses for people >15 years of age [33]. Likely, these advices will lead to accelerated implementation of reduced dosing schedules in the upcoming years globally; hopefully this will be encouraged both in LMIC and in developed countries, in order to ensure better access to prevention around the globe. Also, in the Netherlands introduction of a one-dose schedule might have beneficial effects, for instance for the vaccine uptake, although this was not yet included in the most recent advice.

Improvement of vaccine uptake

From the start, HPV vaccine uptake has fallen behind compared to other vaccines in the Dutch NIP, although recent numbers do show an increase. This phenomenon is also observed in other countries and likely has several explanations: the connection with sexual activity while the vaccine is ideally provided before sexual debut, may cause discomfort among parents. Furthermore, the long incubation time between HPV infection and disease development may lead to diminished perceived individual risk. On the other hand, the perceived risk of experiencing side effects of HPV vaccination are high; although VLP vaccines are among the safest there are in use, questions about safety of HPV vaccination remain an issue, leading to vaccine hesitancy and lower vaccine uptake. These parental concerns may be can be amplified by lobbyist groups (as seen in Ireland) and lead to doubts about HPV vaccination [34]. Although multiple studies have shown the safety of HPV vaccines, the targeted age group complicates this statement; among adolescent girls, hormonal changes are causing discomfort in general, which can then incorrectly be assumed to be causally related to the vaccine. Together, these factors are likely to be contributing to a lower uptake of the HPV vaccine compared to other NIP vaccines, where the uptake reaches up to 95%.

The changes to the HPV vaccination program concerning age and gender provide an opportunity to promote the vaccine again and to improve the uptake. It is important that parents are able to make an informed decision about vaccination, thus sufficient, understandable information is necessary and might require a change in the communication strategy. The shift in communication from a ‘HPV vaccine’ to an ‘Six cancers

vaccine' already indicates the changing perspective. Perhaps this should be combined with more information on (study) results from abroad in order to convince people of the safety and impact of HPV vaccination. Moreover, the lower age and sex-neutrality of the program on itself might already lead to an improved uptake, as it unlinks the vaccine and (the start of) sexual activity. Furthermore, research has shown that logistic aspects (like school based vaccination instead of mass vaccination) could also be considered as factors that can aid in an improved vaccine uptake [35]. Perhaps strategies from abroad can also be used to improve the Dutch program; for example, in Ireland a vaccination alliance was established following an HPV vaccination crisis in order to raise awareness of HPV vaccination. Combined with an active social media campaign this aided in the improved uptake [34, 36]. Although the Netherlands already has a vaccination alliance, its impact may still be improved.

Besides logistic and information aspects, general awareness of the threats of infectious diseases can also be important in the willingness to accept vaccination. The Covid-19 pandemic has led to structural disruptions of regular immunization programs all over the world, including HPV vaccination. Moreover, this may have delayed the introduction of HPV vaccination in LMIC. Also, in Europe and other Western countries vaccination and screening activities have been interrupted due to the pandemic. In the Netherlands, invitations for the second HPV vaccine dose were delayed, as was the case for the cervical cancer screening program invitation. Hence, it is important to consider some lessons to be learned from this crisis: For instance, Arbyn et al suggest that the COVID-19 pandemic might lead to more efficient prevention through improved use of resources, for example by using evidence-based protocols among women who are at high-risk and by extended use of self-sampling [37]. Moreover, the Covid-19 pandemic might have led to increased awareness about the threats of infectious diseases and the possibilities that vaccination offers. It is important to closely monitor the uptake of NIP vaccines in the upcoming period to observe whether the pandemic causes positive or negative shifts in vaccination acceptance.

Integration of prevention strategies

Prevention strategies of (cervical) cancer can include screening besides vaccination. However, with the expansion of HPV vaccination programs, proper integration of these two strategies is required. For example, in the Netherlands the first vaccinated cohort is entering the national screening program in 2023. What is the residual need for screening? Should vaccinated and unvaccinated women be offered different screening strategies in order to prevent 'over' screening among vaccinated or 'under' screening among unvaccinated women? Due to the rather low vaccine uptake in the Netherlands in the last decade, herd effects may not yet be strong enough to reduce the screening frequency for all women. This suggests that if the same strategy is offered

to vaccinated and unvaccinated women, vaccinated women will probably get more screens than necessary as the residual risk after vaccination is very low (but dependent on vaccine type and duration of protection [38]). Further improvement of the vaccine uptake could result in changes to the screening program for the population as a whole, indicating the importance to evaluate and integrate these prevention strategies. A broader scope could be taken when discussing prevention strategies; HPV vaccination is a primary prevention strategy and may lower psychosocial screening harms, follow-up procedures in HPV-positive women, and subsequent treatments with associated risks, such as fertility problems and obstetric complications. Therefore, the combination of vaccination early on in life with de-intensified screening later in life should be pursued. Previous and future modeling and cost-effectiveness studies can aid in designing the optimal integrated strategy with respect to the type of vaccine, the age at which screening initiates and the number of lifetime screening visits [39].

Exemplary for the integration of prevention methods is the HPV-FASTER project, in which the extension of routine vaccination to women up to 30 years of age (and to the 45-50 years in some settings), paired with at least one HPV screening test at age 30 years or older is suggested [40]. The expansion of vaccination to older age groups opens research possibilities and encourages further integration of preventive measurements, although its cost-effectiveness, which does not always have a favorable profile, should be taken into account as well [41]. A related idea is the HPV EVEN FASTER project where the reproductive rate is used to more specifically identify age groups to target with FASTER vaccination and screening [42].

Another potential target group are HPV positive women who were surgically treated for a precancerous cervical lesion. Research showed a significant risk reduction of developing recurrent CIN after surgical excision combined with HPV vaccination, as compared to surgical excision only [43]. Future investigation could explore how and in which circumstances this specific target group should be vaccinated for optimal cost-effective health benefits and how screening should be used in post-treatment follow-up management.

Current and future vaccines on the market

Currently, three prophylactic vaccines are on the market (Cervarix, Gardasil, Gardasil9), with the addition of a more recently developed vaccine from China: Cecolin, which just recently received WHO prequalification. With this vaccine, the aim is to increase availability for low- and middle-income countries because of a more affordable price (especially in time of supply shortage). Cecolin targets HPV16/18 and can be offered in a two-dose schedule [44]. Another Indian vaccine also just recently got licensed for use in India (CERAVANAC qHPV), which was developed in collabora-

tion with the International Agency for Research on Cancer (IARC). Like Cecolin, it aims to provide an accessible and low-cost alternative to marketed vaccines [45].

Vaccine choice is an important issue, as the available vaccines differ in price and in targeted genotypes. All offer protection against oncogenic types HPV16 and HPV18, while the quadri- and nonavalent vaccine also offer protection against genital wart types HPV6 and HPV11. Currently, the bivalent and nonavalent vaccine are often considered the two most important vaccines, because of their established broad spectrum protection through cross-protection (bivalent) or by vaccine design (nonavalent) and strong population-level impact [46]. However, direct comparisons between these vaccines are scarce. The choice of countries between vaccines largely depends on the affordability and the degree of perceived or estimated protection; a study on the most favorable vaccine for GAVI countries indicated that, although the nonavalent vaccine averts more cases of cervical cancer, the bivalent vaccine with favorable cross-protection is also considered a high-value vaccine [47]. This topic requires further investigation which should also incorporate the total population-level disease- burden and how this can best be minimized; HPV-related disease burden allocation to HPV types is different in men (mainly HPV16/18) and women (additional burden from HPV31/33/35/45/51/52/56/58/59) [25] and might therefore require a different view on how to achieve the largest population-level impact.

Another important research direction is the development of therapeutic vaccines, which are aimed at generating cell-mediated immunity rather than humoral immune responses, as these are important for the clearance of established infections. Although numerous studies have been conducted and many lessons have been learned, to date, no therapeutic vaccine has been approved for use in the treatment of HPV infections and related malignancies in humans. However, research during the last years has resulted in several vaccine candidates that are currently in phase III clinical trials, showing that there is promise of a therapeutic vaccine in the near future [48]. This would add possibilities to the currently established HPV vaccination strategies and lead to faster decline in the cancer incidence. This is especially important for LMIC where the burden of HPV related cancer is very high and screening and treatment options are limited.

WHO goals for elimination of cervical cancer

Globally, one woman dies of cervical cancer approximately every two minutes. Around 90% of these deaths occur in low- and middle-income countries, while many deaths could be prevented using HPV vaccination and screening. Following a Call to Action in 2018 from the World Health Organization (WHO) Director-General, several steps in the Global Strategy to Accelerate the Elimination of Cervical Cancer have been

made. This led to three targets in the strategy to reach this goal: by the year 2030, all countries should achieve 90% HPV vaccination uptake (1), 70% screening uptake (2), and 90% access to treatment for cervical pre-cancer and cancer (3), including access to palliative care [49]. However, according to Bruni et al. it is unlikely that this target will actually be reached by 2030; only 6% of all WHO member states had reached a vaccination uptake over 90% for the final dose in 2019 [50]. Furthermore, only one out of three women between 30 and 49 years of age had ever been screened for cervical cancer by 2021 [51]. Thus, in order to reach the WHO targets by 2030, accelerated implementation and improved uptake is required, but financial and infrastructural problems often hamper this.

Concluding remarks

Continued monitoring of the HPV vaccination program in the Netherlands has shown the importance of the different surveillance aspects within the Dutch population. No disturbing findings concerning effectiveness or safety have come to light, supporting evidence on the solid protection of HPV vaccination over time and the importance to ensure a high vaccine uptake in the coming years. Moreover, the Dutch NIP is one of the few that has been consistently using the bivalent vaccine, providing scarce population-based evidence on the impact of this vaccine for long-term effects. Both immune responses on individual and population-level provide information on the exposure to HPV within the country. On the other hand, studying genital infections in different cohorts indicated the proper working of the vaccine in both the two- and three-dose schedule, and the resulting herd effects that are unfolding in the population. This also shows the importance of continued surveillance in the coming years, when the addition of boys and catch-up campaigns within the program will likely lead to new shifts and insights. Relevant research opportunities within the field are optimization of vaccine uptake, estimation of the clinical effects from vaccination within the screening program and integration of these two programs, and the opportunities of further reducing the number of doses without compromising on (long-term) effectiveness. Considering the currently available evidence, a great impact on HPV associated cancer and disease reduction through vaccination programs can and should be realized in the coming years.

References

1. Mollers M, Vossen JM, Scherpenisse M, van der Klis FR, Meijer CJ, de Melker HE. Current knowledge on the role of HPV antibodies after natural infection and vaccination: implications for monitoring an HPV vaccination programme. Journal of medical virology 2013; 85:1379-85.
2. Brouwer AF, Eisenberg MC, Carey TE, Meza R. Trends in HPV cervical and seroprevalence and associations between oral and genital infection and serum antibodies in NHANES 2003-2012. BMC infectious diseases 2015; 15:575.
3. Pillsbury AJ, Quinn HE, Evans TD, McIntyre PB, Brotherton JM. Population-level herd protection of males from a female human papillomavirus vaccination program: evidence from Australian serosurveillance. Clinical Infectious Diseases 2017; 65:827-32.
4. Artemchuk H, Eriksson T, Poljak M, et al. Long-term Antibody Response to Human Papillomavirus Vaccines: Up to 12 Years of Follow-up in the Finnish Maternity Cohort. The Journal of infectious diseases 2018:582-9.
5. Naud PS, Roteli-Martins CM, De Carvalho NS, et al. Sustained efficacy, immunogenicity, and safety of the HPV-16/18 AS04-adjuvanted vaccine: final analysis of a long-term follow-up study up to 9.4 years post-vaccination. Human vaccines & immunotherapeutics 2014; 10:2147-62.
6. Pinto LA, Dillner J, Beddows S, Unger ER. Immunogenicity of HPV prophylactic vaccines: Serology assays and their use in HPV vaccine evaluation and development. Vaccine 2018; 36:4792-9.
7. Stanley MA, Sudenga SL, Giuliano AR. Alternative dosage schedules with HPV virus-like p vaccines. Expert review of vaccines 2014; 13:1027-38.
8. Cuschieri K, Kavanagh K, Moore C, Bhatia R, Love J, Pollock KG. Impact of partial bivalent HPV vaccination on vaccine-type infection: a population-based analysis. British journal of cancer 2016; 114:1261-4.
9. Skinner SR, Apter D, De Carvalho N, et al. Human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine for the prevention of cervical cancer and HPV-related diseases. Expert review of vaccines 2016; 15:367-87.
10. Garnett GP. Role of herd immunity in determining the effect of vaccines against sexually transmitted disease. The Journal of infectious diseases 2005; 191:S97-S106.
11. Kavanagh K, Pollock KG, Cuschieri K, et al. Changes in the prevalence of human papillomavirus following a national bivalent human papillomavirus vaccination programme in Scotland: a 7-year cross-sectional study. The Lancet Infectious diseases 2017; 17:1293-302.
12. Lehtinen M, Luostarinen T, Vanska S, et al. Gender-neutral vaccination provides improved control of human papillomavirus types 18/31/33/35 through herd immunity: Results of a community randomized trial (III). International journal of cancer 2018; 143:2299-310.
13. Mesher D, Soldan K, Lehtinen M, et al. Population-level effects of human papillomavirus vaccination programs on infections with nonvaccine genotypes. Emerging infectious diseases 2016; 22:1732.
14. Gray P, Palmroth J, Luostarinen T, et al. Evaluation of HPV type-replacement in unvaccinated and vaccinated adolescent females—Post-hoc analysis of a community-randomized clinical trial (II). International journal of cancer 2018; 142:2491-500.
15. Tessa M. Schurink-van 't Klooster et al. Early effect of bivalent HPV vaccination on cytology outcomes in cervical samples among young women, 2022.
16. Falcaro M, tañon A, Ndlela B, et al. The effects of the national HPV vaccination programme in England, UK, on cervical cancer and grade 3 cervical intraepithelial neoplasia incidence: a register-based observational study. The Lancet 2021.

17. Weinberg GA, Szilagyi PG. Vaccine epidemiology: efficacy, effectiveness, and the translational research roadmap. The Journal of infectious diseases 2010; 201:1607-10.
18. Snijders PJ, van den Brule AJ, Meijer CJ. The clinical relevance of human papillomavirus testing: relationship between analytical and clinical sensitivity. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland 2003; 201:1-6.
19. Committee for Medicinal Products for Human Use. Cervarix - EPAR: Product information, 2009.
20. Vintermyr OK, Andersland MS, Bjørge T, et al. Human papillomavirus type specific risk of progression and remission during long-term follow-up of equivocal and low-grade HPV-positive cervical smears. International journal of cancer 2018; 143:851-60.
21. Enerly E, Olofsson C, Nygård M. Monitoring human papillomavirus prevalence in urine samples: a review. Clinical epidemiology 2013; 5:67-79.
22. Gezondheidsraad. advies Vaccinatie tegen HPV, 2019.
23. Qendri V, Bogaards JA, Berkhof J. Who Will Benefit From Expanding HPV Vaccination Programs to Boys? JNCI Cancer Spectrum 2018; 2:pky076.
24. Bogaards JA, Mooij SH, Xiridou M, Schim van der Loeff MF. Potential effectiveness of prophylactic HPV immunization for men who have sex with men in the Netherlands: A multi-model approach. PLoS medicine 2019; 16:e1002756.
25. de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. International journal of cancer 2017; 141:664-70.
26. Kreimer AR, Sampson JN, Porras C, et al. Evaluation of Durability of a Single Dose of the Bivalent HPV Vaccine: The CVT Trial. Journal of the National Cancer Institute 2020; 112:1038-46.
27. Barnabas RV, Brown ER, Onono MA, et al. Efficacy of single-dose human papillomavirus vaccination among young African women. NEJM Evidence 2022; 1:EVIDoa2100056.
28. Basu P, Malvi SG, Joshi S, et al. Vaccine efficacy against persistent human papillomavirus (HPV) 16/18 infection at 10 years after one, two, and three doses of quadrivalent HPV vaccine in girls in India: a multicentre, prospective, cohort study. The Lancet Oncology 2021; 22:1518-29.
29. Brotherton JM, Budd A, Rompotis C, et al. Is one dose of human papillomavirus vaccine as effective as three?: A national cohort analysis. Papillomavirus research (Amsterdam, Netherlands) 2019; 8:100177.
30. Toh ZQ, Russell FM, Garland SM, Mulholland EK, Patton G, Licciardi PV. Human papillomavirus vaccination after COVID-19. JNCI cancer spectrum 2021; 5:pkab011.
31. SAGE. Highlights from the Meeting of the Strategic Advisory Group of Experts (SAGE) on Immunization, 2022.
32. Joint Committee on Vaccination and Immunisation. JCVI statement on a one-dose schedule for the routine HPV immunisation programme, 2022.
33. Gezondheidsraad. Aanpassing doses HPV-vaccinatie, 2022.
34. Corcoran B, Clarke A, Barrett T. Rapid response to HPV vaccination crisis in Ireland. The Lancet 2018; 391:2103.
35. Mollema L, Antonise-Kamp L, van Vliet J, de Melker H. Organisatorische en communicatieve interventies die de opkomst voor HPV-vaccinatie kunnen verhogen. JGZ Tijdschrift voor jeugdgezondheidszorg 2019; 51:101-5.
36. Hansen PR, Schmidtblaicher M, Brewer NT. Resilience of HPV vaccine uptake in Denmark: decline and recovery. Vaccine 2020; 38:1842-8.
37. Arbyn M, Bruni L, Kelly D, et al. Tackling cervical cancer in Europe amidst the COVID-19 pandemic. Lancet Public Health 2020; 5.

38. Inturrisi F, Lissenberg-Witte BI, Veldhuijzen NJ, et al. Estimating the direct effect of human papillomavirus vaccination on the lifetime risk of screen-detected cervical precancer. International journal of cancer 2021; 148:320-8.
39. Naber SK, Matthijsse SM, Rozemeijer K, Penning C, de Kok IM, van Ballegooijen M. Cervical cancer screening in partly HPV vaccinated cohorts–A cost-effectiveness analysis. PloS one 2016; 11:e0145548.
40. Bosch FX, Robles C, Diaz M, et al. HPV-FASTER: broadening the scope for prevention of HPV-related cancer. Nature reviews Clinical oncology 2016; 13:119-32.
41. Kim JJ, Simms KT, Killen J, et al. Human papillomavirus vaccination for adults aged 30 to 45 years in the United States: A cost-effectiveness analysis. PLoS medicine 2021; 18:e1003534.
42. J Dillner ME, I Baussano. The EVEN FASTER concept for cervical cancer elimination. Available at: www.HPVWorld.com.
43. Jentschke M, Kampers J, Becker J, Sibbertsen P, Hillemanns P. Prophylactic HPV vaccination after conization: a systematic review and meta-analysis. Vaccine 2020; 38:6402-9.
44. Qiao Y-L, Wu T, Li R-C, et al. Efficacy, safety, and immunogenicity of an Escherichia coli-produced bivalent human papillomavirus vaccine: an interim analysis of a randomized clinical trial. JNCI: Journal of the National Cancer Institute 2020; 112:145-53.
45. Gavi. 2022. Millions to benefit from Indian-made cervical cancer vaccine. Accessed 09 January 2023. Millions to benefit from Indian-made cervical cancer vaccine | Gavi, the Vaccine Alliance
46. Beyer WE, Osterhaus AD. Bivalent AS04-adjuvanted HPV vaccine provides optimal cancer prevention for HPV types not included in the vaccine. Vaccine 2020; 38:7414-6.
47. Burger EA, Portnoy A, Campos NG, Sy S, Regan C, Kim JJ. Choosing the optimal HPV vaccine: the health impact and economic value of the nonavalent and bivalent HPV vaccines in 48 Gavi-eligible countries. International journal of cancer 2021; 148:932-40.
48. Chabeda A, Yanez RJ, Lamprecht R, Meyers AE, Rybicki EP, Hitzeroth II. Therapeutic vaccines for high-risk HPV-associated diseases. Papillomavirus research 2018; 5:46-58.
49. Worl Health Organization. Draft: Global strategy towards eliminating cervical cancer as a public health problem, 2020.
50. Bruni L, Saura-Lázaro A, Montoliu A, et al. HPV vaccination introduction worldwide and WHO and UNICEF estimates of national HPV immunization coverage 2010–2019. Preventive medicine 2021; 144:106399.
51. Bruni L, Serrano B, Roura E, et al. Cervical cancer screening programmes and age-specific coverage estimates for 202 countries and territories worldwide: a review and synthetic analysis. The Lancet Global Health 2022; 10:e1115-e27.

Supplementary

Summary in English

Human papillomavirus is one of the most common sexually transmittable infections. The majority of infections are asymptomatic and pass transiently, but in some cases an infection with a hr HPV type can persist and cause the development of malignancies on the anogenital site and in the head and neck region. To strongly reduce the transmission of HPV and development of (cervical) cancer, prophylactic, bivalent HPV vaccination was introduced into the Dutch NIP in 2009 as a girls-only vaccine, preventing the most oncogenic HPV types 16 and 18. A catch-up campaign was performed offering vaccination to girls born between 1993 and 1996, followed by routine immunization for 12-year old girls from 2010 onwards. At first a three-dose schedule was offered (0, 1 and 6 months), but since 2014 a two-dose schedule has been used (0, 6 months). As of 2022, the HPV vaccination is offered to boys and girls at ten years of age to prevent not only cancer of the cervix but also of the anus, oropharynx, vagina, vulva, and penis.

Monitoring of the NIP is important and is based on several pillars, including vaccine uptake, immunosurveillance, adverse events monitoring, and disease (or pathogen) surveillance. The current thesis describes monitoring of the routine HPV vaccination program within the Netherlands using intermediate endpoints, given the large gap between occurrence of HPV infection and development of cancer. This thesis is based on observational studies, with focus on long term effects regarding antibody level development after vaccination and vaccine effectiveness against (persistent) hr type HPV infections. of the vaccine. **Chapter 1** is a general introduction about HPV, disease progression, epidemiology and effects of HPV vaccination.

Part 1: Vaccine and infection induced immune responses

Serological measurements are important in vaccination research and evaluation, since they can provide information on the responsiveness to a vaccine. Moreover, serological measurements also provide a tool to monitor previous exposure to infection and naturally induced immune responses. Therefore, in **chapter 2,** the population-based changes in seroprevalence of unvaccinated individuals were evaluated over a ten-year time period, during which HPV vaccination was implemented in the Netherlands. Population-based seroepidemiological data from the Dutch Pienter studies (2006/2007 and 2016/2017) was used. IgG antibody levels to seven hr HPV types (HPV16/18/31/33/45/52/58) as induced by natural infection showed that seroprevalence increased over the past decade among unvaccinated women, while it was stable among men. A lower seroprevalence among young women or herd effects among men, which may follow from the recent introduction of the HPV vaccination program among teenage girls in the Netherlands, was not yet observed. The continued moni-

toring of seroprevalence among unvaccinated individuals is important, as declines in seroprevalence are expected for vaccine types.

In **chapter 3**, the focus was on vaccine derived immune responses. Within the HAVANA cohort, antibody levels against the seven earlier described hr HPV types (including vaccine types and cross protective types) among both vaccinated and unvaccinated participants were studied. Antibody levels remained high up till nine years past vaccination (three doses), both for vaccine types and to some extent for cross protective types. Immune responses from vaccinated women who presented with an HPV infection were compared to immune responses from women without infection, but the difference was not significant in the year before infection. This indicates that an immune correlate of protection, i.e. a threshold that should be reached in order to be protected, cannot be easily determined. This was also described in **chapter 4**, where we provided a review of the currently available information about immunological responses following vaccination with three different HPV vaccines, with special attention to long-term effects and dosing schedules. Examples of possible (follow-up) research include the question whether lifelong protection can be established by vaccination and if a correlate of protection should be identified.

Part 2: Effects of HPV vaccination on genital HPV infection

Genital infections are useful intermediate endpoints in the evaluation of HPV vaccination; the ultimate aim of HPV vaccination is of course prevention of (pre)cancerous lesions, but as cancer takes a long time to develop, evaluation of protection against infections is a robust and faster alternative. Both on individual and on population level this is an important source of information for the assessment of direct and indirect effectiveness of the vaccine. In **chapter 5** we used data from sexual health clinic visitors (Passyon study) to assess trends of type-specific HPV prevalence over time since the introduction of HPV vaccination. Both among vaccinated women, heterosexual men, and unvaccinated women, declining trends of vaccine HPV types 16 and 18 were observed. This indicates that the population-level impact of a girls-only HPV vaccination program extends beyond the targeted group, inducing first- and second-order herd effects. For cross-protective types HPV31, HPV33, and HPV45 an early decline was seen, although not (yet) among unvaccinated women. Increases in specific HPV types were also observed, indicating the importance of continued monitoring over time.

Chapter 6 focused on the vaccine effectiveness of two doses of routinely provided HPV vaccination. To support the switch from a three- to a two-dose schedule, mainly immunobridging studies were used. Therefore, clinical and observational data remain important to confirm the high vaccine effectiveness in the target population eligible

for a two-dose schedule. Genital infections among vaccinated (two doses) and unvaccinated participants from the HAVANA2 cohort were compared and indicated high, significant vaccine effectiveness against vaccine type infections (HPV16/18) and cross protective type infections (HPV31/33/45) up to four years after vaccination. Further research is required to determine the long term effectiveness of this schedule and include the formal comparison to the three-dose schedule.

In **chapter 7**, methodological challenges regarding vaccine effectiveness estimates were described, specifically the selection of the right method. Different statistical methods as identified in the literature were described, compared, and applied to the HAVANA data in order to identify the most robust method for VE estimates from observational cohort data. When using longitudinal data, the selected method of analysis should be able to correctly use all information without compromising the data. The evaluated methods were compared regarding underlying assumptions and regarding how they calculated person-years and events. Although deviations in the calculated VE against persistent HPV16/18/31/33/45 infections were limited, the PWP-TT approach was selected as preferable for the current data since all measurements (also recurrent events) could be included and underlying assumptions were not violated. Differences between methods were small at the time of study, but are important to re-evaluate when more data is collected. The method of choice was applied in **chapter 8**, where we studied the long-term protection from the three-dose schedule up to 10 years after vaccination using HAVANA data. No indication of waning protecting over time was observed, as the VE against persistent vaccine type infections remained very high. This was also the case for cross protective HPV types. These findings are in line with observational research from other countries and suggests that vaccination will likely result in solid protection against clinical disease.

In **chapter 9** the general discussion was described. The most important findings from this thesis were summarized and discussed, and future recommendations for research and monitoring of HPV vaccination within the NIP were made. Focus in the coming years should be on the recently introduced sex-neutral vaccination, the improvement of vaccine uptake, the identification of optimal dosing schedules and the long-term effects, also in relation to other preventive strategies such as screening. Clinically relevant outcomes will become available and can increasingly be used to advocate fast implementation of HPV vaccination worldwide so HPV related disease can be reduced to a minimum in the near future.

Samenvatting in het Nederlands

Het humaan papillomavirus (HPV) is een van de meest voorkomende seksueel overdraagbare infecties. De meeste infecties zijn asymptomatisch en van voorbijgaande aard, maar in sommige gevallen kan een infectie met een hoog-risico HPV-type persisteren en de ontwikkeling van maligniteiten in de anogenitale zone en in het hoofd-halsgebied veroorzaken. Om de transmissie van HPV en het ontstaan van (baarmoederhals)kanker sterk terug te dringen, is in 2009 profylactische, bivalente HPV-vaccinatie opgenomen in het Rijksvaccinatieprogramma (RVP) voor meisjes, om hen te beschermen tegen de meest oncogene HPV-typen 16 en 18. Eerst werd een inhaalcampagne uitgevoerd om meisjes geboren tussen 1993 en 1996 te vaccineren; vanaf 2010 werd HPV vaccinatie opgenomen in het reguliere programma voor 12-jarige meisjes. In eerste instantie werd een drie doses schema aangeboden (0, 1 en 6 maanden), maar in 2014 werd dit vervangen door een schema van twee doses (0, 6 maanden). Vanaf 2022 wordt de HPV-vaccinatie aangeboden aan jongens en meisjes van tien jaar om niet alleen kanker van de baarmoederhals maar ook van de anus, orofarynx, vagina, vulva en penis te voorkomen.

Monitoring van het RVP is belangrijk en is gebaseerd op verschillende pijlers, waaronder de vaccinatiegraad, immuun surveillance, monitoring van bijwerkingen en surveillance van ziekten (of ziekteverwekkers). Het huidige proefschrift beschrijft de monitoring van het routinematige HPV-vaccinatieprogramma in Nederland met behulp van intermediaire eindpunten, gezien de grote kloof tussen het optreden van een HPV-infectie en de ontwikkeling van kanker. Dit proefschrift is gebaseerd op observationele studies, met specifieke focus op langetermijneffecten aangaande het beloop van antistoffen na vaccinatie en vaccin effectiviteit tegen (persisterende) hoogrisico typen HPV infecties. **Hoofdstuk 1** betreft een algemene inleiding over HPV, ziekteprogressie, epidemiologie en effecten van HPV-vaccinatie.

Deel 1: Vaccin-geïnduceerde en natuurlijke immuunreacties

Serologische metingen zijn belangrijk bij het evalueren en onderzoeken van vaccins, omdat ze informatie kunnen geven over de respons op een vaccin. Bovendien bieden serologische metingen ook een tool om eerdere blootstelling aan infecties en natuurlijk geïnduceerde immuun responsen te volgen. Daarom werden in **hoofdstuk 2** de veranderingen in seroprevalentie onder de niet-gevaccineerde bevolking geëvalueerd gedurende een tien-jaar periode waarin HPV-vaccinatie in Nederland werd geïmplementeerd. Er werd gebruik gemaakt van populatie gebaseerde seroepidemiologische gegevens uit de Nederlandse Pienter-onderzoeken (2006/2007 en 2016/2017). IgG antistoffen opgewekt door natuurlijke infectie tegen zeven HPV-typen (HPV16/18/31/33/45/52/58) toonden aan dat de seroprevalentie de afgelopen

tien jaar toenam bij niet-gevaccineerde vrouwen, terwijl deze bij mannen stabiel was. Lagere seroprevalentie onder jonge vrouwen of groepsbescherming bij mannen, wat gevolgen kunnen zijn van de recente introductie van het HPV-vaccinatieprogramma onder tienermeisjes in Nederland, werden nog niet waargenomen. Het blijven monitoren van de seroprevalentie bij niet-gevaccineerde personen is belangrijk aangezien een afname van de seroprevalentie wordt verwacht, in ieder geval voor vaccintypen.

In **hoofdstuk 3** lag de focus op vaccin-geïnduceerde immuunrespons na vaccinatie. Binnen het HAVANA-cohort werden antilichaamniveaus tegen de zeven eerder beschreven HPV-typen (inclusief vaccintypen en kruisbeschermende typen) bij zowel gevaccineerde als niet-gevaccineerde deelnemers onderzocht. De antilichamen bleven hoog tot negen jaar na vaccinatie (drie doses), zowel voor vaccintypen als tot op zekere hoogte voor kruisbeschermende typen. De immuunrespons van gevaccineerde vrouwen die een HPV-infectie hadden doorgemaakt, werd vergeleken met de immuunrespons van gevaccineerde vrouwen zonder infectie, maar er was geen significant verschil in het jaar vóór infectie. Dit duidt op een specifieke uitdaging in HPV-vaccinonderzoek; de identificatie van een immuun correlaat van bescherming, oftewel een drempel die moet worden bereikt om beschermd te zijn. Dit werd ook beschreven in **hoofdstuk 4**, waar we een overzicht gaven van de momenteel beschikbare informatie over immunologische reacties na vaccinatie met drie verschillende HPV-vaccins, met speciale aandacht voor langetermijneffecten en doseringsschema's. Dit overzicht laat zien wat de huidige stand van zaken en kennis is en welke aspecten nog in aanmerking kunnen komen voor verder onderzoek. Voorbeelden van mogelijke onderwerpen voor (vervolg) studies zijn of levenslange bescherming kan worden bereikt door vaccinatie en of een correlaat van bescherming zou moeten worden geïdentificeerd.

Deel 2: Effecten van HPV-vaccinatie op genitale HPV-infectie

Genitale infecties zijn nuttige tussentijdse eindpunten bij de evaluatie van HPV-vaccinatie; het uiteindelijke doel van HPV-vaccinatie is uiteraard het voorkomen van (voorstadia van) kanker, maar aangezien de ontwikkeling hiervan lang kan duren, is evaluatie van de bescherming tegen infecties een robuust en sneller alternatief. Zowel op individueel als op populatieniveau is dit een belangrijke informatiebron voor de beoordeling van de directe en indirecte effectiviteit van het vaccin. In **hoofdstuk 5** gebruikten we gegevens van bezoekers van seksuele gezondheidscentra (Passyon-studie) om de trends van type specifieke HPV-prevalentie in de tijd sinds de introductie van HPV-vaccinatie te beoordelen. Zowel bij gevaccineerde vrouwen, heteroseksuele mannen als niet-gevaccineerde vrouwen werden dalende trends van vaccin HPV-typen 16/18 waargenomen. Dit geeft een indicatie van de impact van het HPV-vaccinatieprogramma op populatieniveau, welke verder reikt dan de beoogde groep en leidt tot groepseffecten van de eerste en tweede orde. Voor kruisbeschermende

typen HPV31/33/45 werden enkele eerste dalingen gezien, hoewel (nog) niet bij niet-gevaccineerde vrouwen. Er werden ook enkele toenamen van specifieke HPV-typen opgemerkt, wat aangeeft dat het belangrijk is om in de loop van de tijd de trends te blijven monitoren.

Hoofdstuk 6 richtte zich op de vaccineffectiviteit van het twee doses HPV-vaccinatie programma. Bij de omschakeling van een drie- naar een twee-doses schema werd voornamelijk gebruik gemaakt van immunobridging studies. Daarom zijn klinische en observationele gegevens belangrijk om de hoge vaccineffectiviteit te bevestigen bij de doelpopulatie die in aanmerking komt voor dit schema. Genitale infecties onder gevaccineerde (twee doses) en niet-gevaccineerde deelnemers uit het HAVANA2-cohort werden vergeleken en wezen op een hoge, significante werkzaamheid van het vaccin tegen vaccintype infecties (HPV16/18) en kruisbeschermende type infecties (HPV31/33/45) tot vier jaar na vaccinatie. Verder onderzoek blijft nodig om de effectiviteit van dit schema op lange termijn vast te stellen en om de formele vergelijking met het drie-doses schema uit te voeren.

In **hoofdstuk 7** werden methodologische uitdagingen met betrekking tot de effectiviteitsschattingen van vaccins beschreven, met name het selecteren van de juiste methode. Verschillende statistische methoden zoals geïdentificeerd in de literatuur werden beschreven, vergeleken en toegepast op de HAVANA-gegevens om de meest robuuste methode voor VE-schattingen uit observationele cohortgegevens te bepalen. Bij gebruik van longitudinale data moet de gekozen analysemethode alle informatie correct kunnen gebruiken zonder de data te compromitteren. De geëvalueerde methoden werden vergeleken op onderliggende aannames en op de manier waarop ze persoonsjaren en events berekenden. Hoewel de verschillen in de berekende VE tegen persistente HPV16/18/31/33/45 infecties beperkt waren, werd de PWP-TT-benadering gekozen als de voorkeursmethode voor de huidige gegevens, aangezien alle metingen (ook herhaalde events) konden worden meegenomen en de onderliggende aannames niet werden geschonden. De verschillen tussen de methoden waren klein op het moment van onderzoek, maar het is belangrijk om deze opnieuw te evalueren wanneer er meer data beschikbaar is. De gekozen methode werd toegepast in **hoofdstuk 8**, waar we met behulp van HAVANA-gegevens de lange termijn bescherming van het drie-dosesschema tot 10 jaar na vaccinatie bestudeerden. Er werden geen aanwijzingen voor afnemende bescherming in de loop van de tijd waargenomen, aangezien de VE tegen persistente vaccintype-infecties zeer hoog bleef. Dit was ook het geval voor kruisbeschermende HPV-typen. Deze bevindingen zijn in lijn met observationeel onderzoek uit andere landen en zullen naar alle waarschijnlijkheid leiden tot robuuste bescherming tegen klinische uitkomsten.

In **hoofdstuk 9** werd de algemene discussie beschreven. De belangrijkste bevindingen uit dit proefschrift werden samengevat en besproken, en toekomstige aanbevelingen voor onderzoek en monitoring van HPV-vaccinatie binnen het RVP werden gedaan. De focus in de komende jaren zou moeten liggen op de recent geïmplementeerde gender neutrale vaccinatie, de verbetering van de vaccinatiegraad, het bepalen van optimale doseringsschema's en de langetermijneffecten, ook in relatie tot andere preventieve strategieën zoals screening. Klinisch relevante resultaten zullen in toenemende mate beschikbaar komen en moeten worden gebruikt om te pleiten voor snelle implementatie van HPV-vaccinatie wereldwijd, zodat HPV-gerelateerde ziekten in de nabije toekomst tot een minimum kunnen worden beperkt.

List of abbreviations

2D: 2-dose
3D: 3-dose
2vHPV: Bivalent vaccine
4vHPV: quadrivalent vaccine
9vHPV: nonavalent vaccine
AGW: Anogenital warts
CFS: chronic fatigue syndrome
CIN: cervical intraepithelial lesion
CRPS: complex regional pain syndromes
EMA: European medicines agency
HAVANA(2): Human Papillomavirus among vaccinated and nonvaccinated adolescents
HPV: Human Papillomavirus
Hr: high-risk
IARC: International Agency for Research on Cancer
IgG: Immunoglubuline G
JCVI: Joint Committee on Vaccination and Immunisation
LMIC: low- and middle-income countries
Lr: low-risk
NIP: National Immunization Program
PASSYON: Papillomavirus Surveillance among STI clinic Youngsters in the Netherlands
Pienter: Peiling Immunisatie Effect Nederland ter Evaluatie van het Rijksvaccinatieprogramma
POTS: postural orthostatic tachycardia syndrome
PV: Papillomavirus
PWP-TT: Prentice Williams and Peterson total time
RCT: Randomized clinical trial
SAGE: Strategic Advisory Group of Experts on Immunization
STI: sexually transmitted infection
VE: Vaccin effectiveness
VLP: virus like particle
WHO: World Health Organization

Dankwoord

It always seems impossible until it's done. – Nelson Mandela

Wellicht niet de meest originele quote, maar voor promovendi in het algemeen en mijzelf in het bijzonder wel een hele toepasselijke. De berg die bij vlagen niet te beklimmen leek, is getrotseerd. Het is nu dan toch echt zover: Mijn proefschrift is af! Ik ben van het aantal onderzoekspakketten wat daarvoor moest worden ingepakt de tel kwijt geraakt, maar het zullen er gedurende de afgelopen jaren zeker meer dan 10.000 zijn geweest. Gelukkig heb ik dat niet allemaal alleen hoeven doen en waren er altijd mensen bereid om te helpen. Hetzelfde geldt voor het schrijven van een proefschrift, weet ik nu. Dat doe je niet alleen; daarbij werk je samen met, leer je van en word je ondersteund door een heleboel mensen. Hen wil ik hier graag bedanken.

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De promotiecommissie die de tijd heeft genomen om mijn proefschrift zorgvuldig te beoordelen. Prof. dr. Schim-van der Loeff, Prof. dr. Kenter, Prof. dr. Sanders, dr. Feltkamp en dr. Buiskamp, bedankt voor de tijd die u heeft gestoken in het lezen van mijn proefschrift en de bereidheid om plaats te nemen in de oppositie.

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List of publications

Publications included in this thesis

1. Pasmans, H., Hoes, J., Tymchenko, L., de Melker, H. E., & van der Klis, F. R. (2020). Changes in HPV Seroprevalence from an unvaccinated toward a girls-only vaccinated population in the Netherlands. Cancer Epidemiology, Biomarkers & Prevention, 29(11), 2243-2254.
2. Hoes, J., Pasmans, H., Knol, M. J., Donken, R., van Marm-Wattimena, N., Schepp, R. M., ... & de Melker, H. E. (2020). Persisting antibody response 9 years after bivalent human papillomavirus (HPV) vaccination in a cohort of Dutch women: immune response and the relation to genital HPV infections. The Journal of infectious diseases, 221(11), 1884-1894.
3. Donken, R., Hoes, J., Knol, M. J., Ogilvie, G. S., Dobson, S., King, A. J., ... & De Melker, H. E. (2020). Measuring vaccine effectiveness against persistent HPV infections: a comparison of different statistical approaches. BMC Infectious Diseases, 20(1), 1-11.
4. Hoes, J., Woestenberg, P. J., Bogaards, J. A., King, A. J., de Melker, H. E., Berkhof, J., ... & van Benthem, B. H. (2021). Population impact of girls-only human papillomavirus 16/18 vaccination in The Netherlands: cross-protective and second-order herd effects. Clinical Infectious Diseases, 72(5), e103-e111.
5. Hoes, J., Pasmans, H., Schurink-van't Klooster, T. M., van der Klis, F. R. M., Donken, R., Berkhof, J., & de Melker, H. E. (2022). Review of long-term immunogenicity following HPV vaccination: Gaps in current knowledge. Human Vaccines & Immunotherapeutics, 18(1), 1908059.
6. Hoes, J., King, A. J., Klooster, T. M. S. V. T., Berkhof, J., Bogaards, J. A., & de Melker, H. E. (2022). Vaccine Effectiveness Following Routine Immunization With Bivalent Human Papillomavirus (HPV) Vaccine: Protection Against Incident Genital HPV Infections From a Reduced-Dosing Schedule. The Journal of infectious diseases, 226(4), 634-643.
7. Hoes, J., King, A. J., Berkhof, J., & de Melker, H. E. (2023). High vaccine effectiveness persists for ten years after HPV16/18 vaccination among young Dutch women. Vaccine, 41(2), 285-289.

Other publications

8. Van der Maas, N. A. T., Hoes, J., Sanders, E. A. M., & de Melker, H. E. (2017). Severe underestimation of pertussis related hospitalizations and deaths in the Netherlands: A capture-recapture analysis. Vaccine, 35(33), 4162-4166.
9. Hoes, J., Boef, A. G., Knol, M. J., de Melker, H. E., Mollema, L., van der Klis, F. R., ... & van Baarle, D. (2018). Socioeconomic status is associated with antibody levels against vaccine preventable diseases in the Netherlands. Frontiers in public health, 6, 209.
10. Hoes, J., Knol, M. J., Mollema, L., Buisman, A., De Melker, H. E., & Van der Klis, F. R. M. (2019). Comparison of antibody response between boys and girls after infant and childhood vaccinations in the Netherlands. Vaccine, 37(32), 4504-4510.
11. Hoes, J., Schurink, T., van't Klooster, H. P., van der Weele, P., van Eer, K., Woestenberg, P., ... & de Melker, H. HPV- vaccinatie in Nederland 10 jaar in het Rijksvaccinatieprogramma, een overzicht. themanummer Vaccinaties (2) NTMM 4 2019
12. Schurink-van't Klooster, T. M., Kemmeren, J. M., van der Hilgersom, W. J. A., Hoes, J., & de Melker, H. E. (2019). Surveillance van mogelijke bijwerkingen na HPV-vaccinatie: het vaccin is veilig. Infectieziekten Bulletin 2019; 30(3)
13. Wijstma, E. S., Jongen, V. W., Alberts, C. J., de Melker, H. E., Hoes, J., & Schim van der Loeff, M. F. (2022). Approaches to estimating clearance rates for Human Papillomavirus groupings: a systematic review and real data examples. Epidemiology, 34(1), 119-130.

14. Schurink-van't Klooster, T. M., Siebers, A. G., Hoes, J., van Kemenade, F. J., Berkhof, J., Bogaards, J. A., & de Melker, H. E. (2023). Early effect of bivalent human papillomavirus vaccination on cytology outcomes in cervical samples among young women in the Netherlands. Cancer Medicine, 12(10), 11786-11794.

PhD Portfolio

Courses

Scientific Writing in English – Vrije Universiteit - 2016
Startcursus Questback gebruik – RIVM - 2017
Introductie WMO en GCP – RIVM - 2017
Mixed Models – Universiteit Utrecht - 2018
Course on scientific integrity – Vrije Universiteit - 2019
Gevorderde Epidemiologische Methoden – Universiteit Leiden - 2019
Survival Analyses -Universiteit Utrecht - 2020
eBROK - NFU - 2020
Missing data: consequences and solutions – Vrije Universiteit - 2021
Systematische reviews en meta-analyse - Vrije Universiteit – 2021
Begin-R and Vis-R cursus – RIVM - 2021

Oral presentations at (international) conferences and other scientific meetings

EUROGIN International Multidisciplinary HPV Congress - Lisbon, Portugal - 2018
International Papillomavirus Conference - virtual – 2020
EUROGIN International Multidisciplinary HPV Congress – virtual - 2021
2de Nederlandse HPV onderzoeksdag – RIVM - 2019

Other academic activities

7^{th} – 9^{th} RVP onderzoeksdag - 2017 - 2020
Symposium on HPV-induced cancers: new developments in prevention and treatment - 2019
Organising HAPEEVEE meetings – RIVM – 2017 - 2021

Education

Teaching activities within the NSPOH program 'doctor society and health' and within the Universiteit Utrecht bachelor program 'infection and protection' – 2018- 2020
Supervising master internship Health Sciences - 2020