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TIMING OF TWO VERSUS THREE DOSES OF QUADRIVALENT HPV VACCINE AND ASSOCIATED EFFECTIVENESS AGAINST CONDYLOMA IN SWEDEN: A NATIONWIDE COHORT STUDY.

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ABSTRACT

Objective

To assess incidence of condyloma after two doses of quadrivalent HPV (qHPV)-vaccine, by time since first vaccine dose, in girls and women initiating vaccination before age 20.

Design: Register-based nationwide open cohort study

Setting: Sweden

Participants: Girls and women initiating qHPV vaccination before age 20 between 2006 and 2012. The study cohort included 264 498 girls, of whom 72 042 had received two doses of qHPV vaccine and 185 456 had received all 3 doses.

Main outcome measure: Incidence rate ratios (IRRs) of condyloma estimated by time between first and second dose of qHPV in months (m) and age at vaccination, adjusted for attained age.

Results: For girls first vaccinated with two doses before the age of 17, the IRR of condyloma for 0-3m between first and second dose was 1.96 (95% CI 1.43 to 2.68) as compared to standard three-dose schedule. The IRRs were 1.27 (95% CI 0.63 to 2.58) and 4.36 (95% CI=2.05 to 9.28) after receipt of two doses with 4-7m and 8+m between doses, respectively. For women first vaccinated after the age of 17, vaccination with two doses of qHPV vaccine and 0-3m between doses was associated with an IRR of 2.12 (95% CI=1.62 to 2.77). For an interval of 4-7m between doses, the IRR did not statistically significantly differ to the standard three-dose schedule (IRR=0.81, 95% CI= 0.36 to 1.84). For women with 8+m between dose one and two the IRR was 3.16 (95% CI=1.40 to 7.14).

Conclusion

A two-dose schedule for qHPV vaccine with 4-7 months between first and second dose may be as effective against condyloma in girls and women initiating vaccination under 20 years as a three-dose schedule. Results from this nationwide study support immunogenicity data from clinical trials.

Strengths and limitations of this study

- We were able to link vaccination status to disease outcome on an individual level through use of high quality national register-based data.
- Observation studies such as this, are able to look at the pragmatic effectiveness of vaccination in a large population.
- We did not look at HPV disease outcomes other than condyloma.
- The majority of girls and women in the cohort had 0-3 months between first and second dose, which limited the power for other exposure groups in our study.
- A small proportion of condyloma cases may have been missed, as some patients will neither seek hospital care for condyloma nor receive prescription for treatment, and thus will not be included in the registers.

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Data sharing: The study utilises unique individual level Swedish register data, which cannot be shared in the public domain according to Swedish law. The individual-level data underlying the study will be available from the corresponding author upon request, given that appropriate ethical and legal requirements are met.

Contributors: FL, EH, AP, KS, IU, PS and LAD contributed to the design of the study; FL, EH, AP and PS analysed the data; FL drafted the manuscript; FL, EH, AP, KS, IU, PS and LAD critically reviewed the manuscript; FL, EH, AP, KS, IU, PS and LAD and prepared the manuscript for submission; LAD is the guarantor of the study.

INTRODUCTION

Human Papillomavirus (HPV) vaccines are subunit vaccines containing virus-like particles (VLPs), and typically require multiple doses to confer a sufficient immune response,[1] therefore, a three-dose schedule (0, 2, 6 months) was initially approved by the European Medicines Agency (EMA). As the immune response has been shown to be stronger in young girls 9-14 years of age compared to women 15-25 years of age, recommendations to reduce the number of doses to two have been put forward for the younger age groups, provided doses are optimally spaced.[2-6] Thus, in 2014, HPV vaccines were licensed in a two-dose schedule for girls aged between 9-14 years of age with doses at 0 and 6 months.[7, 8]

In Sweden, HPV vaccination was originally introduced as part of a subsidised three-dose schedule in 2007 for girls and women aged 13-17 years. Other ages could still be vaccinated, but were required to pay the full cost of the vaccine. In 2012, an organised national programme was initiated, with girls aged 10-12 routinely vaccinated as part of the childhood vaccination programme. Catch-up vaccinations were offered to girls aged 13-18 years. In January 2015, a two-dose schedule for girls aged 10-13 was implemented.

Several potential benefits may be conferred by such a reduced dosing schedule; including increased compliance, lower programme costs and improved logistics. However, the recommendation for a two-dose schedule was based on immunogenicity results and does not take into account the antibody threshold at which HPV diseases may be prevented – a threshold that has yet to be identified.[9] Therefore,

observational studies are necessary to ascertain effects of dose alterations in HPV vaccination on clinical endpoints. The use of condyloma as a marker for vaccine effectiveness is in this context timely, due to its considerably shorter latency period than precancerous cervical lesions and cancer. We here investigate whether optimal timing of two doses of qHPV vaccine could confer the same level of protection against condyloma as a standard three dose-schedule on a population level in Sweden.

METHODS

Study population

This study was a nationwide open cohort of girls and young women aged 10-27 and registered as living in Sweden between 1st January 2006 and 31st December 2012. Subjects entered the study cohort on the date of administration of the second dose of qHPV vaccine and were followed up for first occurrence of condyloma. The cohort of girls was sampled prior to the implementation of the two-dose schedule in Sweden i.e. girls and women were sampled during a three-dose schedule period.

To ensure only incident condyloma infection was measured, all individuals with condyloma diagnosis prior to follow up were excluded, as were individuals who emigrated or received bivalent HPV vaccine before follow up. Women that initiated qHPV vaccination over the age of 20 or turned 27 years of age before start of follow-up were also excluded (Figure 1). Women were censored during follow up if they died (n=58), received a condyloma diagnosis (n=619), emigrated (n=1037), were not resident in Sweden (N=4) or received the bivalent HPV vaccine (N=38).

Data sources

Data were collected using the Swedish national population registers and linked through use of unique personal identification numbers.[10] The Swedish HPV Vaccination Register (SVEVAC), a voluntary national HPV vaccination register initiated in 2006, was used for information on HPV vaccination exposure. Timing between doses was calculated using data from this register. In addition to SVEVAC, data was also collected from the Prescribed Drug Register (PDR), which contains information on all prescriptions handled at Swedish pharmacies since July 2005. The Patient Register and PDR were used to extract information on condyloma outcomes. The Patient Register contains data regarding all inpatient and outpatient visits in Swedish hospitals and specialist care since 1987 and 2001, respectively. Information regarding deaths was collected from the Cause of Death Register and emigration status was collected from the Migration Register.

Case definition

Condyloma cases were defined as a first diagnosis of condyloma in the Patient Register or a prescription for condyloma specific treatments in the PDR. In the Patient Register, all women that received a main or secondary diagnosis of condyloma were identified using the ICD10 code A63.0.[11] In the PDR, all women who received podophyllotoxin and imiquimod were identified using Anatomical Therapeutical Chemical Codes (ATC) D06BB04 and D06BB10 respectively.[12]

Vaccination status

SVEVAC was used to obtain bivalent and quadrivalent HPV vaccination dates and was complemented with prescription data collected from the PDR, using ATC codes J07BM01 and J07BM02, respectively.

Statistical analysis

Crude incidence rates (IRs) per 100 000 person-years were calculated as the number of cases of condyloma per accrued person-time, stratified by the time interval between first and second dose (0-3, 4-7, or 8+ months). As we have previously shown an effect of age at vaccination on vaccine effectiveness,[12, 13] girls and women were grouped into two age-at-first-vaccination categories (10-16 and 17-19 years), a divide reflecting the median age for sexual debut in Sweden at 16.5 years.[14]

Poisson regression was used to model IRs by time between first and second dose and age at first vaccination and adjusted for attained age. The time scale for individual follow-up was attained age, which was split into five intervals (10-13, 14-16, 17-19, 20-21 and 22+ years), to reflect increasing risk of infection and disease with increasing age. Vaccine dosage (three versus two doses) was handled as a time-varying exposure, so that women could contribute person-time to both dose categories. The effect of time between doses was allowed to vary by age at first vaccination via an interaction term. This model was then used to estimate incidence rate ratios (IRRs) and 95% confidence intervals (CIs) after two doses of qHPV relative to three sets of references groups: first, compared to women who had initiated vaccination at the same age and had received three doses of qHPV (0, 2 and 6 months); these IRRs measure effectiveness of a two-dose schedule with different timings between dose one and two relative to a standard three-dose schedule. Second,

compared to women who had initiated vaccination at the same age and had received three doses of qHPV with the same timing between first and second dose (two doses with 0-3 months vs three doses with 0-3 months etc.); this matched comparison addresses the question of how much extra protection is gained on average by a third dose for different timings for the first two. Third, compared to women who had initiated vaccination at the same age and had received three doses of qHPV with no restriction on the time between dose one and two or dose two and three; these IRRs measure effectiveness of a two dose schedule relative to a pragmatic three-dose schedule. IRs and IR differences (IRDs) with corresponding 95% CIs predicted by the models and averaged across levels of attained age in the study cohort were also reported. Furthermore, a sensitivity analysis was conducted restricting the time between dose one and two to 12 months, but as the IRRs were comparable, this cut off was not applied (data not shown).

Ethical Approval: Ethical approval for this study was granted by the Regional Ethical Review Board of Stockholm, Sweden, which determined that informed consent from the study participants was not required.

RESULTS

Study cohort

264 498 girls under the age of 20 were vaccinated with at least two doses of qHPV at the end of the study period. Of these, 79 042 (29.9%) received only two doses of qHPV vaccine and 185 456 (70.1%) received all three doses. The majority (n=154 440, 83.3%) of the individuals fully vaccinated followed the recommended

dosing schedule given at 0, 2, and 6 months. Mean time in follow-up was 682 days (range 1-2250 days).

Crude incidence rates

For girls initiating vaccination with qHPV before 17 years the IR after vaccination with two doses was 84 (95% CI 66 to 108), 95 (95% CI 48 to 190), and 351 (95% CI 168 to 737) per 100 000 person-years, when there were 0-3, 4-7 and 8+ months between dose one and two, respectively (Table 1).

Table 1. Number of individuals, cases, person-years, and crude IR by age at vaccination initiation and time between dose 1 and 2

Age at first vaccination	Number of doses	Time between dose 1 and 2 (months)	Individuals (n)	Condyloma cases (n)	Person-years	Crude IR, (95%CI)*
≤16yr	2 doses	0-3	204103	63	74611	84 (66;108)
		4-7	8095	8	8404	95 (48;190)
		8+	1894	7	1992	351 (168;737)
	3 doses	0-3	142046	222	275495	81 (71;92)
		4-7	2803	8	6619	121 (60;242)
		8+	919	2	1646	121 (30;486)
		Standard dosing schedule (0, 2, 6)	122425	182	231393	79 (68;91)
17-19yr	2 doses	0-3	46712	97	23750	408 (335;498)
		4-7	2965	6	3886	154 (69;344)

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		8+	615	6	995	603 (271;1343)
	3 doses	0-3	38705	197	93908	210 (182;241)
		4-7	808	3	2087	144 (46;446)
		8+	175	0	365	-
		Standard dosing schedule (0, 2, 6)	32015	146	76168	192 (163;225)

* IR reported per 100 000 person-years

Condyloma incidence after two-dose vaccination was higher in girls initiating vaccination after 17 years of age, with IRs of 408 (95% CI 335 to 498), 154 (95% CI 69 to 344), and 603 (95% CI 271 to 1343) per 100 000, when there were 0-3 months, 4-7 months and 8+ months between dose one and two, respectively (Table 1).

Incidence rate ratios comparing two doses versus standard three-dose vaccination

For girls initiating vaccination before the age of 17 there was a statistically significantly increased risk for condyloma when comparing two-dose vaccination 0-3 months apart (IRR 1.96, 95% CI 1.44 to 2.68) and 8+ months apart (IRR 4.36, 95% CI 2.05 to 9.28) to a standard three-dose schedule. No statistically significant association (IRR=1.27, 95% CI 0.63 to 2.58) was found after vaccination with two doses given 4-7 months apart. The IRDs predicted by the model were 59 (95% CI 25 to 92), 16 (95% CI -38 to 71) and 204 (95%CI=8 to 402) extra cases per 100 000 person-years for 0-3 months, 4-7 months and 8+ months between doses one and two, respectively (Table 2).

Table 2. IR, IRR, and IRD comparing 2 versus 3 dose vaccination by age at vaccination initiation and time between dose 1 and 2, adjusted for attained age

Age at first vaccination	Number of doses	Time between dose 1 and 2 (months)	IR, 95%CI*	P-value	IRR, 95%CI	P-value	IRD, 95%CI*	P-value
≤16yr	3 doses	Standard dosing schedule (0, 2, 6)	61 (52;70)	<0.001	Ref	Ref	Ref	Ref
	2 doses	0-3	119 (88;151)	<0.001	1.96 (1.44;2.68)	<0.001	59 (25;92)	0.001
		4-7	77 (24;131)	0.005	1.27 (0.63;2.58)	0.506	17 (-38;71)	0.551
		8+	265 (68;462)	0.008	4.36 (2.05;9.28)	<0.001	205 (8;402)	0.042
17-19yr	3 doses	Standard dosing schedule (0, 2, 6)	113 (90;135)	<0.001	Ref	Ref	Ref	Ref
	2 doses	0-3	239 (187;291)	<0.001	2.12 (1.62;2.77)	<0.001	126 (73;179)	<0.001
		4-7	91 (18;165)	0.015	0.81 (0.36;1.84)	0.615	-21 (-97;54)	0.580
		8+	355 (68;643)	0.015	3.16 (1.40;7.14)	0.006	243 (-44;530)	0.097

*IR, IRD reported per 100 000 person-years. Reference groups: ≤16yr with 3 doses of qHPV (0,2,6 months) and 17-19yr with 3 doses of qHPV (0,2,6 months)

A similar pattern is seen in girls and women initiating vaccination after turning 17, with increased risks for condyloma after two doses if given 0-3 months (IRR=2.12, 95%CI=1.62 to 2.77) or 8+ months (IRR=3.16 95%CI=1.40 to 7.14) apart was observed. No association was found when comparing two versus three doses with 4-7 months between dose one and two (IRR=0.81, 95%CI=0.36 to 1.84) (Table 2).

Incidence rate ratios comparing two dose versus matched three dose vaccination

Comparing two-dose vaccination, 0-3 months apart, versus three-dose vaccination with 0-3 months between doses one and two, results remained effectively unchanged both for girls initiating vaccination prior to age 17 (IRR=1.95, 95% CI=1.44 to 2.64) and girls initiating vaccination between 17 and 19 years (IRR=1.88, 96%CI=1.46 to 2.42) (Table 3)

Table 3. IR, IRR, and IRD comparing two-dose vaccination with varying time between dose 1 and 2 versus three-dose vaccination by age at vaccination initiation, adjusted for attained age

Age at first vaccination	Number of doses	Time between dose 1 and 2 (months)	IR, 95%CI*	P-value	IRR, 95%CI	P-value	IRD, 95%CI*	P-value
≤16yr	3 doses	0-3	63 (55;72)	<0.001	Ref	Ref	Ref	Ref
	2 doses	0-3	123 (90;156)	<0.001	1.95 (1.44;2.64)	<0.001	60 (26;94)	<0.001
≤16yr	3 doses	4-7	91 (28;154)	0.005	Ref	Ref	Ref	Ref
	2 doses	4-7	79 (24;133)	0.005	0.87 (0.33;2.32)	0.779	-12 (-95;71)	0.779
≤16yr	3 doses	8+	86 (-33;205)	0.158	Ref	Ref	Ref	Ref
	2 doses	8+	270 (70;470)	0.008	3.14 (0.65;15.09)	0.154	184 (-49;417)	0.122

17-19yr	3 doses	0-3	129 (107;150)	<0.001	Ref	Ref	Ref	Ref
	2 doses	0-3	242 (190;294)	<0.001	1.88 (1.46;2.42)	<0.001	114 (60;167)	<0.001
17-19yr	3 doses	4-7	88 (-12;189)	0.084	Ref	Ref	Ref	Ref
	2 doses	4-7	95 (19;172)	0.015	1.08 (0.27;4.31)	0.916	7 (-119;133)	0.915
17-19yr	3 doses	8+	0	-	Ref	Ref	Ref	Ref
	2 doses	8+	373 (72;675)	0.015	-	-	373 (72;675)	0.015

* IR, IRD reported per 100 000 person-years. Matched reference groups: ≤16yr with 3 doses of qHPV with 0-3 months between dose 1 and 2, ≤16yr with 3 doses of qHPV with 4-7 months between dose 1 and 2 and ≤16yr with 3 doses of qHPV with 8+ months between dose 1 and 2; 17-19yr with 3 doses of qHPV with 0-3 months between dose 1 and 2, 17-19yr with 3 doses of qHPV with 4-7 months between dose 1 and 2 and 17-19yr with 3 doses of qHPV with 8+ months between dose 1 and 2.

Comparing two versus three-dose vaccination with 4-7 and 8+ months between the first two doses for both schedules, we found non-significant associations with IRRs of 0.87 (95% CI=0.33 to 2.32) and 3.14 (95% CI=0.65 to15.09) respectively, for girls initiating vaccination prior to 17, with corresponding IRDs of -12 (95% CI=-95 to 71) and 184 (95% CI=-49 to 417) cases per 100 000 person-years (Table 3). For girls initiating vaccination between 17 and 19 years, no association was found for 4-7 months in between doses (IRR=1.08, 95%CI=0.27 to 4.31); no cases of condyloma were reported in fully vaccinated women initiating vaccination between 17 and 19 years (Table 3).

Incidence rate ratios comparing two doses versus pragmatic three dose vaccination

Changing the reference group to pragmatic three-dose vaccination did not materially affect the results. (See supplementary table).

DISCUSSION

Statement of principle findings

This population-based study investigates the incidence of condyloma after two doses of qHPV by time between first and second dose. Our results suggest that a two-dose regimen is similarly effective as a standard three-dose schedule if given 4-7 months apart. This is in line with the recommendations from the European Medicines Agency (EMA) and the World Health Organization Strategic Advisory Group of Experts (SAGE) and immunological results from clinical trials.[2, 3, 5, 6, 15-19]

In relation to other studies

The impact of HPV vaccines was first recognised for HPV infections, and HPV-related diseases with short incubation times following infection such as genital warts.[20] Studies have shown that three-dose schedules of qHPV vaccination have been effective in the prevention of genital warts at a population level.[21-24] In addition, observational studies assessing the effectiveness of qHPV against cervical abnormalities have been carried out.[25-28] A recent review by Garland et al. suggested that in successive birth cohorts that are beginning screening, there have been reductions in the number of low-grade cytological abnormalities and high-grade histology confirmed cervical lesions (approximately 45% and 85% respectively).[29]

Alternative dosing schedules on condyloma incidence have been investigated in Denmark and Sweden,[9, 13] with both studies showing that condyloma incidence was statistically significantly higher in women aged 19-24 years after two doses rather than three. However, receipt of two vaccine doses with optimum interval was reported as non-inferior to three doses in terms of condyloma reduction, a finding with which the present study concurs.

Strengths and weaknesses

This was a nationwide study including the entire vaccinated Swedish female population aged 10-27 years. The use of high quality national register-based data meant that we were able to link vaccination status to disease outcome on an individual level.

A limitation of our study is that a small proportion of patients will neither seek hospital care for condyloma nor receive prescription for treatment, and thus will not be included in the registers. This would result in an underestimation of the number of condyloma cases. We expect however, that this underestimation in the registers for the true number of condyloma cases [12] would be either non-differential with regards to vaccination exposure, or conservative in impact, based on the apparent health-seeking behavior of women who are vaccinated.[13]

It is also possible that individuals might have a prevalent HPV infection at time of vaccination, resulting in an underestimation of protective effect of the vaccine. We have attempted to control for this by excluding women who had a history of condyloma before the start of individual follow-up. Additionally, given that we start follow-up for condyloma incidence only after the second dose, we have the automatic benefit of a buffer period as used in.[13]

It is also of note that, the majority of women in the cohort had 0-3 months between first and second dose, which limited the power for other exposure groups in our study and resulted in wider confidence intervals, particularly in comparisons with the older age group and increasing time between doses.

Implications

Reducing the number of HPV vaccine doses from three to two could potentially lead to a number of positive effects, including lower costs, increased compliance and improved logistics of the vaccination programme. It is however key to remain vigilant with regards to follow-up of disease outcomes and supplement clinical trial data and

policy recommendations with real-life evidence, such as those presented here. The findings imply that the current recommendation of two dose-schedules is appropriate, but we reinforce the significance of optimal timing between doses.

Unanswered questions and future research

We did not consider HPV-related disease outcomes other than condyloma. More studies with longer follow-up time are needed to ascertain the effectiveness of a two-dose schedule for HPV-related disease outcomes such as cervical intraepithelial neoplasia or cervical cancer. As more countries implement two-dose schedules, the impact on transmission dynamics and herd immunity will also become clearer.[22] It should also be taken into account that the duration of protection for both the two-dose and three-dose schedule is not yet known and more time and data are required before conclusions can be drawn regarding the long-term effectiveness of these schedules, and a reduced-dose schedule can be recommended for girls older than 15.[2, 30]

Conclusion

For prevention of condyloma, a two-dose schedule of qHPV vaccine with 4-7 months between first and second dose may be as effective as standard three-dose vaccination, for women first vaccinated before the age of 20. The results from this nationwide observational study support immunogenicity findings from clinical trials.

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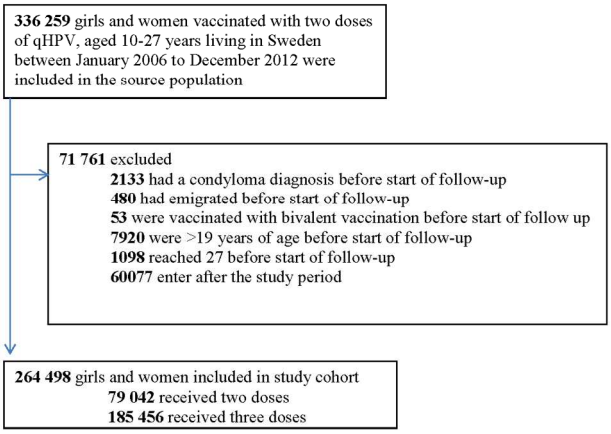
Figure 1. Details on study exclusions and the population analysed to investigate timing of two versus three doses of quadrivalent HPV vaccine and associated effectiveness against condyloma.



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Supplementary Table. IR, IRR, and IRD comparing 2 versus 3 dose vaccination by age at vaccination initiation and time between dose 1 and 2.

Age at first vaccination	Number of doses	Time between dose 1 and 2 (months)	IR, 95%CI*	P-value	IRR, 95%CI	P-value	IRD, 95%CI*	P-value
≤16yr	3 doses	Overall (0-3;4-7;8+)	64 (55;72)	<0.001	Reference	Reference	Reference	Reference
	2 doses	0-3	123 (90;156)	<0.001	1.92 (1.42;2.60)	<0.001	59 (25;93)	0.001
		4-7	79 (24;133)	0.005	1.23 (0.61;2.49)	0.562	15 (-40;70)	0.598
		8+	270 (70;470)	0.008	4.22 (1.99;8.94)	<0.001	206 (6;406)	0.044
17-19yr	3 doses	Overall (0-3;4-7;8+)	127 (106;149)	<0.001	Reference	Reference	Reference	Reference
	2 doses	0-3	242 (190;294)	<0.001	1.9 (1.48;2.45)	<0.001	115 (62;168)	<0.001
		4-7	95 (19;172)	0.015	0.75 (0.33;1.69)	0.484	-32 (-110;46)	0.422
		8+	374 (72;675)	0.015	2.93 (1.3;6.61)	0.009	246 (-54;547)	0.108

* IR, IRD reported per 100 000 person-years, reference groups: ≤16yr with 3 doses of qHPV (no time restriction between dose 1 and 1 and dose 2 and 3) and 17-19yrs with 3 doses of qHPV (no time restriction between dose 1 and 1 and dose 2 and 3).

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5, 6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6, 7, 8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6, 7, 8
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 7, 8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*7*
Bias	9	Describe any efforts to address potential sources of bias	6,
Study size	10	Explain how the study size was arrived at	6,
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8, 9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8, 9
		(*b*) Describe any methods used to examine subgroups and interactions	8, 9
		(*c*) Explain how missing data were addressed	N/A
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	9
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6, 9
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	6
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	9, 10
Outcome data	15*	Report numbers of outcome events or summary measures over time	9, 10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-18
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	18
Discussion			
Key results	18	Summarise key results with reference to study objectives	18
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	19, 20, 21
Generalisability	21	Discuss the generalisability (external validity) of the study results	20, 21
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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TIMING OF TWO VERSUS THREE DOSES OF QUADRIVALENT HPV VACCINE AND ASSOCIATED EFFECTIVENESS AGAINST CONDYLOMA IN SWEDEN: A NATIONWIDE COHORT STUDY.

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TIMING OF TWO VERSUS THREE DOSES OF QUADRIVALENT HPV VACCINE AND ASSOCIATED EFFECTIVENESS AGAINST CONDYLOMA IN SWEDEN: A NATIONWIDE COHORT STUDY.

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ABSTRACT

Objective

To assess incidence of condyloma after two doses of quadrivalent HPV (qHPV)-vaccine, by time since first vaccine dose, in girls and women initiating vaccination before age 20.

Design: Register-based nationwide open cohort study

Setting: Sweden

Participants: Girls and women initiating qHPV vaccination before age 20 between 2006 and 2012. The study cohort included 264 498 girls, of whom 72 042 had received two doses of qHPV vaccine and 185 456 had received all 3 doses.

Main outcome measure: Incidence rate ratios (IRRs) of condyloma estimated by time between first and second dose of qHPV in months (m) and age at vaccination, adjusted for attained age.

Results: For girls first vaccinated with two doses before the age of 17, the IRR of condyloma for 0-3m between first and second dose was 1.96 (95% CI 1.43 to 2.68) as compared to standard three-dose schedule. The IRRs were 1.27 (95% CI 0.63 to 2.58) and 4.36 (95% CI=2.05 to 9.28) after receipt of two doses with 4-7m and 8+m between doses, respectively. For women first vaccinated after the age of 17, vaccination with two doses of qHPV vaccine and 0-3m between doses was associated with an IRR of 2.12 (95% CI=1.62 to 2.77). For an interval of 4-7m between doses, the IRR did not statistically significantly differ to the standard three-dose schedule (IRR=0.81, 95% CI= 0.36 to 1.84). For women with 8+m between dose one and two the IRR was 3.16 (95% CI=1.40 to 7.14).

Conclusion

A two-dose schedule for qHPV vaccine with 4-7 months between first and second dose may be as effective against condyloma in girls and women initiating vaccination under 20 years as a three-dose schedule. Results from this nationwide study support immunogenicity data from clinical trials.

Strengths and limitations of this study

- We were able to link vaccination status to disease outcome on an individual level through use of high quality national register-based data.
- Observation studies such as this, are able to look at the pragmatic effectiveness of vaccination in a large population.
- We did not look at HPV disease outcomes other than condyloma.
- The majority of girls and women in the cohort had 0-3 months between first and second dose, which limited the power for other exposure groups in our study.
- A small proportion of condyloma cases may have been missed, as some patients will neither seek hospital care for condyloma nor receive prescription for treatment, and thus will not be included in the registers.

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Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; LAD has received research grants to her institution for other studies from MSD Sanofi Pasteur, Merck Sharp and Dohme, and GlaxoSmithKline. KS has received grants from Merck Sharp and Dohme for other studies on HPV vaccination in Sweden; no other relationships or activities that could appear to have influenced the submitted work.

Data sharing: The study utilises unique individual level Swedish register data, which cannot be shared in the public domain according to Swedish law. The individual-level data underlying the study will be available from the corresponding author upon request, given that appropriate ethical and legal requirements are met.

Contributors: FL, EH, AP, KS, IU, PS and LAD contributed to the design of the study; FL, EH, AP and PS analysed the data; FL drafted the manuscript; FL, EH, AP, KS, IU, PS and LAD critically reviewed the manuscript; FL, EH, AP, KS, IU, PS and LAD and prepared the manuscript for submission; LAD is the guarantor of the study.

INTRODUCTION

Human Papillomavirus (HPV) vaccines are subunit vaccines containing virus-like particles (VLPs), and typically require multiple doses to confer an immune response,[1] therefore, a three-dose schedule (0, 2, 6 months) was initially approved by the European Medicines Agency (EMA). As the immune response has been shown to be stronger in young girls 9-14 years of age compared to women 15-25 years of age, recommendations to reduce the number of doses to two have been put forward for the younger age groups, provided doses are optimally spaced.[2-6] Thus, in 2014, HPV vaccines were licensed in a two-dose schedule for girls aged between 9-14 years of age with doses at 0 and 6 months.[7, 8]

In Sweden, HPV vaccination was originally introduced as part of a subsidised three-dose schedule in 2007 for girls and women aged 13-17 years. Other ages could still be vaccinated, but were required to pay the full cost of the vaccine. In 2012, an organised national programme was initiated, with girls aged 10-12 routinely vaccinated as part of the childhood vaccination programme. Catch-up vaccinations were offered to girls aged 13-18 years. In January 2015, a two-dose schedule for girls aged 10-13 was implemented.

Several potential benefits may be conferred by such a reduced dosing schedule; including increased compliance, lower programme costs and improved logistics. However, the recommendation for a two-dose schedule was based on immunogenicity results and does not take into account the antibody threshold at which HPV diseases may be prevented – a threshold that has yet to be identified.[9] Therefore,

observational studies are necessary to ascertain effects of dose alterations in HPV vaccination on clinical endpoints. The use of condyloma as a marker for vaccine effectiveness is in this context timely, due to its considerably shorter latency period than precancerous cervical lesions and cancer. We here investigate whether optimal timing of two doses of qHPV vaccine could confer the same level of protection against condyloma as a standard three dose-schedule on a population level in Sweden.

METHODS

Study population

This study was a nationwide open cohort of girls and young women aged 10-27 and registered as living in Sweden between 1st January 2006 and 31st December 2012. Subjects entered the study cohort on the date of administration of the second dose of qHPV vaccine and were followed up for first occurrence of condyloma. The cohort of girls was sampled prior to the implementation of the two-dose schedule in Sweden i.e. girls and women were sampled during a three-dose schedule period.

To ensure only incident condyloma infection was measured, all individuals with condyloma diagnosis prior to follow up were excluded, as were individuals who emigrated or received bivalent HPV vaccine before follow up. Women that initiated qHPV vaccination over the age of 20 or turned 27 years of age before start of follow-up were also excluded (Figure 1). Women were censored during follow up if they died (n=58), received a condyloma diagnosis (n=619), emigrated (n=1037), were not resident in Sweden (N=4) or received the bivalent HPV vaccine (N=38).

Data sources

Data were collected using the Swedish national population registers and linked through use of unique personal identification numbers.[10] The Swedish HPV Vaccination Register (SVEVAC), a voluntary national HPV vaccination register initiated in 2006, was used for information on HPV vaccination exposure. Timing between doses was calculated using data from this register. In addition to SVEVAC, data was also collected from the Prescribed Drug Register (PDR), which contains information on all prescriptions handled at Swedish pharmacies since July 2005. The Patient Register and PDR were used to extract information on condyloma outcomes. The Patient Register contains data regarding all inpatient and outpatient visits in Swedish hospitals and specialist care since 1987 and 2001, respectively. Information regarding deaths was collected from the Cause of Death Register and emigration status was collected from the Migration Register. Parents were identified from the Multigeneration Register and their highest education level nearest to the date of entry, as a proxy for socioeconomic status, was identified from the Education Register.

Case definition

Condyloma cases were defined as a first diagnosis of condyloma in the Patient Register or a prescription for condyloma specific treatments in the PDR. In the Patient Register, all women that received a main or secondary diagnosis of condyloma were identified using the ICD10 code A63.0.[11] In the PDR, all women who received podophyllotoxin and imiquimod were identified using Anatomical Therapeutical Chemical Codes (ATC) D06BB04 and D06BB10 respectively.[12]

Vaccination status

SVEVAC was used to obtain bivalent and quadrivalent HPV vaccination dates and was complemented with prescription data collected from the PDR, using ATC codes J07BM01 and J07BM02, respectively.

Statistical analysis

Crude incidence rates (IRs) per 100 000 person-years were calculated as the number of cases of condyloma per accrued person-time, stratified by the time interval between first and second dose (0-3, 4-7, or 8+ months). As we have previously shown an effect of age at vaccination on vaccine effectiveness,[12, 13] girls and women were grouped into two age-at-first-vaccination categories (10-16 and 17-19 years), a divide reflecting the median age for sexual debut in Sweden at 16.5 years.[14]

Poisson regression was used to model IRs by time between first and second dose and age at first vaccination and adjusted for attained age. The time scale for individual follow-up was attained age, which was split into five intervals (10-13, 14-16, 17-19, 20-21 and 22+ years), to reflect increasing risk of infection and disease with increasing age. Vaccine dosage (three versus two doses) was handled as a time-varying exposure, so that women could contribute person-time to both dose categories. The effect of time between doses was allowed to vary by age at first vaccination via an interaction term. This model was then used to estimate incidence rate ratios (IRRs) and 95% confidence intervals (CIs) after two doses of qHPV relative to three sets of references groups: first, compared to women who had initiated vaccination at the same age and had received three doses of qHPV (0, 2 and 6 months); these IRRs measure effectiveness of a two-dose schedule with different timings between dose one and two relative to a standard three-dose schedule. Second,

compared to women who had initiated vaccination at the same age and had received three doses of qHPV with the same timing between first and second dose (two doses with 0-3 months vs three doses with 0-3 months etc.); this matched comparison addresses the question of how much extra protection is gained on average by a third dose for different timings for the first two. Third, compared to women who had initiated vaccination at the same age and had received three doses of qHPV with no restriction on the time between dose one and two or dose two and three; these IRRs measure effectiveness of a two dose schedule relative to a pragmatic three-dose schedule. IRs and IR differences (IRDs) with corresponding 95% CIs predicted by the models and averaged across levels of attained age in the study cohort were also reported. Furthermore, two sensitivity analyses were carried out. First, to determine whether socioeconomic status was a confounder in our study, and second, a sensitivity analysis restricting the time between dose one and two to 12 months was conducted.

Ethical Approval: Ethical approval for this study was granted by the Regional Ethical Review Board of Stockholm, Sweden, which determined that informed consent from the study participants was not required.

RESULTS

Study cohort

264 498 girls under the age of 20 were vaccinated with at least two doses of qHPV at the end of the study period. Of these, 79 042 (29.9%) received only two doses of qHPV vaccine and 185 456 (70.1%) received all three doses. The majority

(n=154 440, 83.3%) of the individuals fully vaccinated followed the recommended dosing schedule given at 0, 2, and 6 months. Median time in follow up was 259 days [interquartile range 186 - 1271 days].

Crude incidence rates

For girls initiating vaccination with qHPV before 17 years the IR after vaccination with two doses was 84 (95% CI 66 to 108), 95 (95% CI 48 to 190), and 351 (95% CI 168 to 737) per 100 000 person-years, when there were 0-3, 4-7 and 8+ months between dose one and two, respectively (Table 1).

Table 1. Number of individuals, cases, person-years, and crude IR by age at vaccination initiation and time between dose 1 and 2

Age at first vaccination	Number of doses	Time between dose 1 and 2 (months)	Individuals (n)	Condyloma cases (n)	Person-years	Crude IR, (95%CI)*
≤16yr	2 doses	0-3	204103	63	74611	84 (66;108)
		4-7	8095	8	8404	95 (48;190)
		8+	1894	7	1992	351 (168;737)
	3 doses	0-3	142046	222	275495	81 (71;92)
		4-7	2803	8	6619	121 (60;242)
		8+	919	2	1646	121 (30;486)
		Standard dosing schedule (0, 2, 6)	122425	182	231393	79 (68;91)
17-19yr	2 doses	0-3	46712	97	23750	408 (335;498)
		4-7	2965	6	3886	154 (69;344)

						Lamb 12
		8+	615	6	995	603 (271;1343)
	3 doses	0-3	38705	197	93908	210 (182;241)
		4-7	808	3	2087	144 (46;446)
		8+	175	0	365	-
		Standard dosing schedule (0, 2, 6)	32015	146	76168	192 (163;225)

* IR reported per 100 000 person-years

Condyloma incidence after two-dose vaccination was higher in girls initiating vaccination after 17 years of age, with IRs of 408 (95% CI 335 to 498), 154 (95% CI 69 to 344), and 603 (95% CI 271 to 1343) per 100 000, when there were 0-3 months, 4-7 months and 8+ months between dose one and two, respectively (Table 1).

Incidence rate ratios comparing two doses versus standard three-dose vaccination

For girls initiating vaccination before the age of 17 there was a statistically significantly increased risk for condyloma when comparing two-dose vaccination 0-3 months apart (IRR 1.96, 95% CI 1.44 to 2.68) and 8+ months apart (IRR 4.36, 95% CI 2.05 to 9.28) to a standard three-dose schedule. No statistically significant association (IRR=1.27, 95% CI 0.63 to 2.58) was found after vaccination with two doses given 4-7 months apart. The IRDs predicted by the model were 59 (95% CI 25 to 92), 16 (95% CI -38 to 71) and 204 (95%CI=8 to 402) extra cases per 100 000 person-years for 0-3 months, 4-7 months and 8+ months between doses one and two, respectively (Table 2).

Table 2. IR, IRR, and IRD comparing 2 versus 3 dose vaccination by age at vaccination initiation and time between dose 1 and 2, adjusted for attained age

Age at first vaccination	Number of doses	Time between dose 1 and 2 (months)	IR, 95%CI*	P-value	IRR, 95%CI	P-value	IRD, 95%CI*	P-value
≤16yr	3 doses	Standard dosing schedule (0, 2, 6)	61 (52;70)	<0.001	Ref	Ref	Ref	Ref
	2 doses	0-3	119 (88;151)	<0.001	1.96 (1.44;2.68)	<0.001	59 (25;92)	0.001
		4-7	77 (24;131)	0.005	1.27 (0.63;2.58)	0.506	17 (-38;71)	0.551
		8+	265 (68;462)	0.008	4.36 (2.05;9.28)	<0.001	205 (8;402)	0.042
17-19yr	3 doses	Standard dosing schedule (0, 2, 6)	113 (90;135)	<0.001	Ref	Ref	Ref	Ref
	2 doses	0-3	239 (187;291)	<0.001	2.12 (1.62;2.77)	<0.001	126 (73;179)	<0.001
		4-7	91 (18;165)	0.015	0.81 (0.36;1.84)	0.615	-21 (-97;54)	0.580
		8+	355 (68;643)	0.015	3.16 (1.40;7.14)	0.006	243 (-44;530)	0.097

*IR, IRD reported per 100 000 person-years. Reference groups: ≤16yr with 3 doses of qHPV (0,2,6 months) and 17-19yr with 3 doses of qHPV (0,2,6 months)

A similar pattern is seen in girls and women initiating vaccination after turning 17, with increased risks for condyloma after two doses if given 0-3 months (IRR=2.12, 95%CI=1.62 to 2.77) or 8+ months (IRR=3.16 95%CI=1.40 to 7.14) apart was observed. No association was found when comparing two versus three doses with 4-7 months between dose one and two (IRR=0.81, 95%CI=0.36 to 1.84) (Table 2).

The first sensitivity analysis including socioeconomic status revealed no significant change to the point estimates (see supplementary table 1). In the second sensitivity analysis the IRRs were comparable, therefore the cut off at 12 months was not applied (data not shown).

Incidence rate ratios comparing two dose versus matched three dose vaccination

Comparing two-dose vaccination, 0-3 months apart, versus three-dose vaccination with 0-3 months between doses one and two, results remained effectively unchanged both for girls initiating vaccination prior to age 17 (IRR=1.95, 95% CI=1.44 to 2.64) and girls initiating vaccination between 17 and 19 years (IRR=1.88, 96%CI=1.46 to 2.42) (Table 3)

Table 3. IR, IRR, and IRD comparing two-dose vaccination with varying time between dose 1 and 2 versus three-dose vaccination by age at vaccination initiation, adjusted for attained age

Age at first vaccination	Number of doses	Time between dose 1 and 2 (months)	IR, 95%CI*	P-value	IRR, 95%CI	P-value	IRD, 95%CI*	P-value
≤16yr	3 doses	0-3	63 (55;72)	<0.001	Ref	Ref	Ref	Ref
	2 doses	0-3	123 (90;156)	<0.001	1.95 (1.44;2.64)	<0.001	60 (26;94)	<0.001
≤16yr	3 doses	4-7	91 (28;154)	0.005	Ref	Ref	Ref	Ref
	2 doses	4-7	79 (24;133)	0.005	0.87 (0.33;2.32)	0.779	-12 (-95;71)	0.779
≤16yr	3 doses	8+	86 (-33;205)	0.158	Ref	Ref	Ref	Ref
	2 doses	8+	270 (70;470)	0.008	3.14 (0.65;15.09)	0.154	184 (-49;417)	0.122

17-19yr	3 doses	0-3	129 (107;150)	<0.001	Ref	Ref	Ref	Ref
	2 doses	0-3	242 (190;294)	<0.001	1.88 (1.46;2.42)	<0.001	114 (60;167)	<0.001
17-19yr	3 doses	4-7	88 (-12;189)	0.084	Ref	Ref	Ref	Ref
	2 doses	4-7	95 (19;172)	0.015	1.08 (0.27;4.31)	0.916	7 (-119;133)	0.915
17-19yr	3 doses	8+	0	-	Ref	Ref	Ref	Ref
	2 doses	8+	373 (72;675)	0.015	-	-	373 (72;675)	0.015

* IR, IRD reported per 100 000 person-years. Matched reference groups: ≤16yr with 3 doses of qHPV with 0-3 months between dose 1 and 2, ≤16yr with 3 doses of qHPV with 4-7 months between dose 1 and 2 and ≤16yr with 3 doses of qHPV with 8+ months between dose 1 and 2; 17-19yr with 3 doses of qHPV with 0-3 months between dose 1 and 2, 17-19yr with 3 doses of qHPV with 4-7 months between dose 1 and 2 and 17-19yr with 3 doses of qHPV with 8+ months between dose 1 and 2.

Comparing two versus three-dose vaccination with 4-7 and 8+ months between the first two doses for both schedules, we found non-significant associations with IRRs of 0.87 (95% CI=0.33 to 2.32) and 3.14 (95% CI=0.65 to15.09) respectively, for girls initiating vaccination prior to 17, with corresponding IRDs of -12 (95% CI=-95 to 71) and 184 (95% CI=-49 to 417) cases per 100 000 person-years (Table 3). For girls initiating vaccination between 17 and 19 years, no association was found for 4-7 months in between doses (IRR=1.08, 95%CI=0.27 to 4.31); no cases of condyloma were reported in fully vaccinated women initiating vaccination between 17 and 19 years (Table 3).

Incidence rate ratios comparing two doses versus pragmatic three dose vaccination

Changing the reference group to pragmatic three-dose vaccination did not materially affect the results. (See supplementary table 2).

DISCUSSION

Statement of principle findings

This population-based study investigates the incidence of condyloma after two doses of qHPV by time between first and second dose. Our results suggest that a two-dose regimen is similarly effective as a standard three-dose schedule if given 4-7 months apart. This is in line with the recommendations from the European Medicines Agency (EMA) and the World Health Organization Strategic Advisory Group of Experts (SAGE) and immunological results from clinical trials.[2, 3, 5, 6, 15-19]

In relation to other studies

The impact of HPV vaccines was first recognised for HPV infections, and HPV-related diseases with short incubation times following infection such as genital warts.[20] Studies have shown that three-dose schedules of qHPV vaccination have been effective in the prevention of genital warts at a population level.[21-24] In addition, observational studies assessing the effectiveness of qHPV against cervical abnormalities have been carried out.[25-28] A recent review by Garland et al. suggested that in successive birth cohorts that are beginning screening, there have been reductions in the number of low-grade cytological abnormalities and high-grade histology confirmed cervical lesions (approximately 45% and 85% respectively).[29]

Alternative dosing schedules on condyloma incidence have been investigated in Denmark and Sweden,[9, 13] with both studies showing that condyloma incidence was statistically significantly higher in women aged 19-24 years after two doses rather than three. However, receipt of two vaccine doses with optimum interval was reported as non-inferior to three doses in terms of condyloma reduction, a finding with which the present study concurs.

Strengths and weaknesses

This was a nationwide study including the entire vaccinated Swedish female population aged 10-27 years. The use of high quality national register-based data meant that we were able to link vaccination status to disease outcome on an individual level.

A limitation of our study is that a small proportion of patients will neither seek hospital care for condyloma nor receive prescription for treatment, and thus will not be included in the registers, resulting in an underestimation of the true number of condyloma cases. [12] However, we expect this to be negligible in our study, as a) vaccinated women have been found to have higher screening uptake than unvaccinated women and can thus also be assumed not to be less prone to access healthcare [30] b) it would only inflate the estimated effect of the two-dose regimens if the subjects less willing to complete the three-dose regimens would be substantially more likely to see healthcare for genital warts than those who complete three doses.

Another potential limitation is that SVEVAC was a voluntary register for the period 2006-2010, with only 80-85% coverage. To avoid an underestimation of vaccination exposure, we complemented missing data using the Prescribed Drug Register. This method has been used previously in a study by Herweijer et al, who found unique vaccination dose dates for 99.6% of the vaccinated girls and women in the cohort. [13]

It is also possible that individuals might have a prevalent HPV infection at time of vaccination, resulting in an underestimation of protective effect of the vaccine. We have attempted to control for this by excluding women who had a history of condyloma before the start of individual follow-up. Additionally, given that we start follow-up for condyloma incidence only after the second dose, we have the automatic benefit of a buffer period as used in.[13]

It is also of note that, the majority of women in the cohort had 0-3 months between first and second dose, which limited the power for other exposure groups in our study and resulted in wider confidence intervals, particularly in comparisons with the older age group and increasing time between doses. While we did not find socioeconomic status as a confounder in our study and we hypothesise that this is because we only follow subjects from the second dose forwards, so there has already been a large degree of self-selection with regard to the role of socioeconomic factors in our study participants.

Implications

Reducing the number of HPV vaccine doses from three to two could potentially lead to a number of positive effects, including lower costs, increased compliance and improved logistics of the vaccination programme. It is however key to remain vigilant with regards to follow-up of disease outcomes and supplement clinical trial data and policy recommendations with real-life evidence, such as those presented here. The findings imply that the current recommendation of two dose-schedules is appropriate, but we reinforce the significance of optimal timing between doses.

Unanswered questions and future research

We did not consider HPV-related disease outcomes other than condyloma. More studies with longer follow-up time are needed to ascertain the effectiveness of a two-dose schedule for HPV-related disease outcomes such as cervical intraepithelial neoplasia or cervical cancer. As more countries implement two-dose schedules, the impact on transmission dynamics and herd immunity will also become clearer.[22] It should also be taken into account that the duration of protection for both the two-dose

and three-dose schedule is not yet known and more time and data are required before conclusions can be drawn regarding the long-term effectiveness of these schedules, and a reduced-dose schedule can be recommended for girls older than 15.[2, 31]

The finding that the 8+ months between doses was less protective that the 4-7 month group was unexpected as for one-dose priming schedules it is often better with a longer interval between doses. Since this is an observational study, we cannot exclude that our finding was due to an unmeasured confounding factor however, with some (unknown) underlying reason why these girls had a longer time to dose three and high incidence/exposure. While we can only speculate about this higher risk in the 8+ month group, it has highlighted the need for further studies with a longer follow up time investigating the upper time limit between doses and vaccine effectiveness.

Conclusion

For prevention of condyloma, a two-dose schedule of qHPV vaccine with 4-7 months between first and second dose may be as effective as standard three-dose vaccination, for women first vaccinated before the age of 20. The results from this nationwide observational study support immunogenicity findings from clinical trials.

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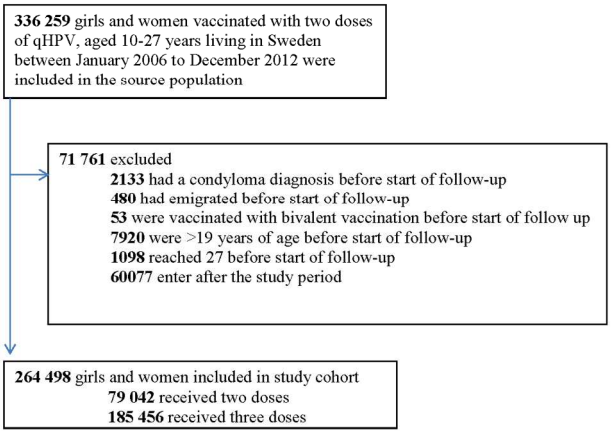
Figure 1. Details on study exclusions and the population analysed to investigate timing of two versus three doses of quadrivalent HPV vaccine and associated effectiveness against condyloma.



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Supplementary Table 1. IR, IRR, and IRD comparing 2 versus 3 dose vaccination by age at vaccination initiation and time between dose 1 and 2, adjusted for attained age and education level¶.

Age at first vaccination	Number of doses	Time between dose 1 and 2 (months)	IR, 95%CI*	P-value	IRR, 95%CI	P-value	IRD, 95%CI*	P-value
≤16yr	3 doses	Standard dosing schedule (0, 2, 6)	62 (53;72)	<0.001	Ref	Ref	Ref	Ref
	2 doses	0-3	122 (89;155)	<0.001	1.96 (1.43;2.70)	<0.001	60 (25;95)	0.001
		4-7	73 (19;128)	0.008	1.17 (0.55;2.51)	0.669	11 (-44;66)	0.692
		8+	250 (49;450)	0.015	4.02 (1.78;9.07)	0.001	188 (13;388)	0.067
17-19yr	3 doses	Standard dosing schedule (0, 2, 6)	113 (90;135)	<0.001	Ref	Ref	Ref	Ref
	2 doses	0-3	244 (189;299)	<0.001	2.15 (1.63;2.84)	<0.001	131 (75;186)	<0.001
		4-7	100 (19;181)	0.015	0.88 (0.39;2.00)	0.767	-13 (-95;69)	0.754
		8+	383 (73;694)	0.016	3.39 (1.50;7.68)	0.003	270 (-39;579)	0.087

*IR, IRD reported per 100 000 person-years. Reference groups: ≤16yr with 3 doses of qHPV (0,2,6 months) and 17-19yr with 3 doses of qHPV (0,2,6 months).

¶ Highest education level of either parent, nearest to the date of entry, was used as a proxy for socioeconomic status. Individuals with educational information (n=252 768).

Supplementary Table 2. IR, IRR, and IRD comparing 2 versus 3 dose vaccination by age at vaccination initiation and time between dose 1 and 2.

Age at first vaccination	Number of doses	Time between dose 1 and 2 (months)	IR, 95%CI*	P-value	IRR, 95%CI	P-value	IRD, 95%CI*	P-value
≤16yr	3 doses	Overall (0-3;4-7;8+)	64 (55;72)	<0.001	Reference	Reference	Reference	Reference
	2 doses	0-3	123 (90;156)	<0.001	1.92 (1.42;2.60)	<0.001	59 (25;93)	0.001
		4-7	79 (24;133)	0.005	1.23 (0.61;2.49)	0.562	15 (-40;70)	0.598
		8+	270 (70;470)	0.008	4.22 (1.99;8.94)	<0.001	206 (6;406)	0.044
17-19yr	3 doses	Overall (0-3;4-7;8+)	127 (106;149)	<0.001	Reference	Reference	Reference	Reference
	2 doses	0-3	242 (190;294)	<0.001	1.9 (1.48;2.45)	<0.001	115 (62;168)	<0.001
		4-7	95 (19;172)	0.015	0.75 (0.33;1.69)	0.484	-32 (-110;46)	0.422
		8+	374 (72;675)	0.015	2.93 (1.3;6.61)	0.009	246 (-54;547)	0.108

* IR, IRD reported per 100 000 person-years, reference groups: ≤16yr with 3 doses of qHPV (no time restriction between dose 1 and 1 and dose 2 and 3) and 17-19yrs with 3 doses of qHPV (no time restriction between dose 1 and 1 and dose 2 and 3).

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5, 6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6, 7, 8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6, 7, 8
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 7, 8, 9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*7*
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8, 9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8, 9
		(*b*) Describe any methods used to examine subgroups and interactions	8, 9
		(*c*) Explain how missing data were addressed	N/A
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	9
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6, 9, 10
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	6
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	9, 10
Outcome data	15*	Report numbers of outcome events or summary measures over time	9, 10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-18
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	15, 18
Discussion			
Key results	18	Summarise key results with reference to study objectives	18
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	19, 20, 21, 22
Generalisability	21	Discuss the generalisability (external validity) of the study results	20, 21, 22
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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TIMING OF TWO VERSUS THREE DOSES OF QUADRIVALENT HPV VACCINE AND ASSOCIATED EFFECTIVENESS AGAINST CONDYLOMA IN SWEDEN: A NATIONWIDE COHORT STUDY.

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TIMING OF TWO VERSUS THREE DOSES OF QUADRIVALENT HPV VACCINE AND ASSOCIATED EFFECTIVENESS AGAINST CONDYLOMA IN SWEDEN: A NATIONWIDE COHORT STUDY.

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ABSTRACT

Objective

To assess incidence of condyloma after two doses of quadrivalent HPV (qHPV)-vaccine, by time since first vaccine dose, in girls and women initiating vaccination before age 20.

Design: Register-based nationwide open cohort study

Setting: Sweden

Participants: Girls and women initiating qHPV vaccination before age 20 between 2006 and 2012. The study cohort included 264 498 girls, of whom 72 042 had received two doses of qHPV vaccine and 185 456 had received all 3 doses.

Main outcome measure: Incidence rate ratios (IRRs) of condyloma estimated by time between first and second dose of qHPV in months (m) and age at vaccination, adjusted for attained age.

Results: For girls first vaccinated with two doses before the age of 17, the IRR of condyloma for 0-3m between first and second dose was 1.96 (95% CI 1.43 to 2.68) as compared to standard three-dose schedule. The IRRs were 1.27 (95% CI 0.63 to 2.58) and 4.36 (95% CI=2.05 to 9.28) after receipt of two doses with 4-7m and 8+m between doses, respectively. For women first vaccinated after the age of 17, vaccination with two doses of qHPV vaccine and 0-3m between doses was associated with an IRR of 2.12 (95% CI=1.62 to 2.77). For an interval of 4-7m between doses, the IRR did not statistically significantly differ to the standard three-dose schedule (IRR=0.81, 95% CI= 0.36 to 1.84). For women with 8+m between dose one and two the IRR was 3.16 (95% CI=1.40 to 7.14).

Conclusion

A two-dose schedule for qHPV vaccine with 4-7 months between first and second dose may be as effective against condyloma in girls and women initiating vaccination under 20 years as a three-dose schedule. Results from this nationwide study support immunogenicity data from clinical trials.

Strengths and limitations of this study

- We were able to link vaccination status to disease outcome on an individual level through use of high quality national register-based data.
- Observation studies such as this, are able to look at the pragmatic effectiveness of vaccination in a large population.
- We did not look at HPV disease outcomes other than condyloma.
- The majority of girls and women in the cohort had 0-3 months between first and second dose, which limited the power for other exposure groups in our study.
- A small proportion of condyloma cases may have been missed, as some patients will neither seek hospital care for condyloma nor receive prescription for treatment, and thus will not be included in the registers.

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Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; LAD has received research grants to her institution for other studies from MSD Sanofi Pasteur, Merck Sharp and Dohme, and GlaxoSmithKline. KS has received grants from Merck Sharp and Dohme for other studies on HPV vaccination in Sweden; no other relationships or activities that could appear to have influenced the submitted work.

Data sharing: The study utilises unique individual level Swedish register data, which cannot be shared in the public domain according to Swedish law. The individual-level data underlying the study will be available from the corresponding author upon request, given that appropriate ethical and legal requirements are met.

Contributors: FL, EH, AP, KS, IU, PS and LAD contributed to the design of the study; FL, EH, AP and PS analysed the data; FL drafted the manuscript; FL, EH, AP, KS, IU, PS and LAD critically reviewed the manuscript; FL, EH, AP, KS, IU, PS and LAD and prepared the manuscript for submission; LAD is the guarantor of the study.

INTRODUCTION

Human Papillomavirus (HPV) vaccines are subunit vaccines containing virus-like particles (VLPs), and typically require multiple doses to confer an immune response,[1] therefore, a three-dose schedule (0, 2, 6 months) was initially approved by the European Medicines Agency (EMA). As the immune response has been shown to be stronger in young girls 9-14 years of age compared to women 15-25 years of age, recommendations to reduce the number of doses to two have been put forward for the younger age groups, provided doses are optimally spaced.[2-6] Thus, in 2014, HPV vaccines were licensed in a two-dose schedule for girls aged between 9-14 years of age with doses at 0 and 6 months.[7, 8]

In Sweden, HPV vaccination was originally introduced as part of a subsidised three-dose schedule in 2007 for girls and women aged 13-17 years. Other ages could still be vaccinated, but were required to pay the full cost of the vaccine. In 2012, an organised national programme was initiated, with girls aged 10-12 routinely vaccinated as part of the childhood vaccination programme. Catch-up vaccinations were offered to girls aged 13-18 years. In January 2015, a two-dose schedule for girls aged 10-13 was implemented.

Several potential benefits may be conferred by such a reduced dosing schedule; including increased compliance, lower programme costs and improved logistics. However, the recommendation for a two-dose schedule was based on immunogenicity results and does not take into account the antibody threshold at which HPV diseases may be prevented – a threshold that has yet to be identified.[9] Therefore,

observational studies are necessary to ascertain effects of dose alterations in HPV vaccination on clinical endpoints. The use of condyloma as a marker for vaccine effectiveness is in this context timely, due to its considerably shorter latency period than precancerous cervical lesions and cancer. We here investigate whether optimal timing of two doses of qHPV vaccine could confer the same level of protection against condyloma as a standard three dose-schedule on a population level in Sweden.

METHODS

Study population

This study was a nationwide open cohort of girls and young women aged 10-27 and registered as living in Sweden between 1st January 2006 and 31st December 2012. Subjects entered the study cohort on the date of administration of the second dose of qHPV vaccine and were followed up for first occurrence of condyloma. The cohort of girls was sampled prior to the implementation of the two-dose schedule in Sweden i.e. girls and women were sampled during a three-dose schedule period.

To ensure only incident condyloma infection was measured, all individuals with condyloma diagnosis prior to follow up were excluded, as were individuals who emigrated or received bivalent HPV vaccine before follow up. Women that initiated qHPV vaccination over the age of 20 or turned 27 years of age before start of follow-up were also excluded (Figure 1). Women were censored during follow up if they died (n=58), received a condyloma diagnosis (n=619), emigrated (n=1037), were not resident in Sweden (N=4) or received the bivalent HPV vaccine (N=38).

Data sources

Data were collected using the Swedish national population registers and linked through use of unique personal identification numbers.[10] The Swedish HPV Vaccination Register (SVEVAC), a voluntary national HPV vaccination register initiated in 2006, was used for information on HPV vaccination exposure. Timing between doses was calculated using data from this register. In addition to SVEVAC, data was also collected from the Prescribed Drug Register (PDR), which contains information on all prescriptions handled at Swedish pharmacies since July 2005. The Patient Register and PDR were used to extract information on condyloma outcomes. The Patient Register contains data regarding all inpatient and outpatient visits in Swedish hospitals and specialist care since 1987 and 2001, respectively. Information regarding deaths was collected from the Cause of Death Register and emigration status was collected from the Migration Register. Parents were identified from the Multigeneration Register and their highest education level nearest to the date of entry, as a proxy for socioeconomic status, was identified from the Education Register.

Case definition

Condyloma cases were defined as a first diagnosis of condyloma in the Patient Register or a prescription for condyloma specific treatments in the PDR. In the Patient Register, all women that received a main or secondary diagnosis of condyloma were identified using the ICD10 code A63.0.[11] In the PDR, all women who received podophyllotoxin and imiquimod were identified using Anatomical Therapeutical Chemical Codes (ATC) D06BB04 and D06BB10 respectively.[12]

Vaccination status

SVEVAC was used to obtain bivalent and quadrivalent HPV vaccination dates and was complemented with prescription data collected from the PDR, using ATC codes J07BM01 and J07BM02, respectively.

Statistical analysis

Crude incidence rates (IRs) per 100 000 person-years were calculated as the number of cases of condyloma per accrued person-time, stratified by the time interval between first and second dose (0-3, 4-7, or 8+ months). As we have previously shown an effect of age at vaccination on vaccine effectiveness,[12, 13] girls and women were grouped into two age-at-first-vaccination categories (10-16 and 17-19 years), a divide reflecting the median age for sexual debut in Sweden at 16.5 years.[14]

Poisson regression was used to model IRs by time between first and second dose and age at first vaccination and adjusted for attained age. The time scale for individual follow-up was attained age, which was split into five intervals (10-13, 14-16, 17-19, 20-21 and 22+ years), to reflect increasing risk of infection and disease with increasing age. Vaccine dosage (three versus two doses) was handled as a time-varying exposure, so that women could contribute person-time to both dose categories. The effect of time between doses was allowed to vary by age at first vaccination via an interaction term. This model was then used to estimate incidence rate ratios (IRRs) and 95% confidence intervals (CIs) after two doses of qHPV relative to three sets of references groups: first, compared to women who had initiated vaccination at the same age and had received three doses of qHPV (0, 2 and 6 months); these IRRs measure effectiveness of a two-dose schedule with different timings between dose one and two relative to a standard three-dose schedule. Second,

compared to women who had initiated vaccination at the same age and had received three doses of qHPV with the same timing between first and second dose (two doses with 0-3 months vs three doses with 0-3 months etc.); this matched comparison addresses the question of how much extra protection is gained on average by a third dose for different timings for the first two. Third, compared to women who had initiated vaccination at the same age and had received three doses of qHPV with no restriction on the time between dose one and two or dose two and three; these IRRs measure effectiveness of a two dose schedule relative to a pragmatic three-dose schedule. IRs and IR differences (IRDs) with corresponding 95% CIs predicted by the models and averaged across levels of attained age in the study cohort were also reported. Furthermore, two sensitivity analyses were carried out. First, to determine whether socioeconomic status was a confounder in our study, and second, a sensitivity analysis restricting the time between dose one and two to 12 months was conducted.

Ethical Approval: Ethical approval for this study was granted by the Regional Ethical Review Board of Stockholm, Sweden, which determined that informed consent from the study participants was not required.

RESULTS

Study cohort

264 498 girls under the age of 20 were vaccinated with at least two doses of qHPV at the end of the study period. Of these, 79 042 (29.9%) received only two doses of qHPV vaccine and 185 456 (70.1%) received all three doses. The majority

(n=154 440, 83.3%) of the individuals fully vaccinated followed the recommended dosing schedule given at 0, 2, and 6 months. Median time in follow up was 259 days [interquartile range 186 - 1271 days].

Crude incidence rates

For girls initiating vaccination with qHPV before 17 years the IR after vaccination with two doses was 84 (95% CI 66 to 108), 95 (95% CI 48 to 190), and 351 (95% CI 168 to 737) per 100 000 person-years, when there were 0-3, 4-7 and 8+ months between dose one and two, respectively (Table 1).

Table 1. Number of individuals, cases, person-years, and crude IR by age at vaccination initiation and time between dose 1 and 2

Age at first vaccination	Number of doses	Time between dose 1 and 2 (months)	Individuals (n)	Condyloma cases (n)	Person-years	Crude IR, (95%CI)*
≤16yr	2 doses	0-3	204103	63	74611	84 (66;108)
		4-7	8095	8	8404	95 (48;190)
		8+	1894	7	1992	351 (168;737)
	3 doses	0-3	142046	222	275495	81 (71;92)
		4-7	2803	8	6619	121 (60;242)
		8+	919	2	1646	121 (30;486)
		Standard dosing schedule (0, 2, 6)	122425	182	231393	79 (68;91)
17-19yr	2 doses	0-3	46712	97	23750	408 (335;498)
		4-7	2965	6	3886	154 (69;344)

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		8+	615	6	995	603 (271;1343)
	3 doses	0-3	38705	197	93908	210 (182;241)
		4-7	808	3	2087	144 (46;446)
		8+	175	0	365	-
		Standard dosing schedule (0, 2, 6)	32015	146	76168	192 (163;225)

* IR reported per 100 000 person-years

Condyloma incidence after two-dose vaccination was higher in girls initiating vaccination after 17 years of age, with IRs of 408 (95% CI 335 to 498), 154 (95% CI 69 to 344), and 603 (95% CI 271 to 1343) per 100 000, when there were 0-3 months, 4-7 months and 8+ months between dose one and two, respectively (Table 1).

Incidence rate ratios comparing two doses versus standard three-dose vaccination

For girls initiating vaccination before the age of 17 there was a statistically significantly increased risk for condyloma when comparing two-dose vaccination 0-3 months apart (IRR 1.96, 95% CI 1.44 to 2.68) and 8+ months apart (IRR 4.36, 95% CI 2.05 to 9.28) to a standard three-dose schedule. No statistically significant association (IRR=1.27, 95% CI 0.63 to 2.58) was found after vaccination with two doses given 4-7 months apart. The IRDs predicted by the model were 59 (95% CI 25 to 92), 16 (95% CI -38 to 71) and 204 (95%CI=8 to 402) extra cases per 100 000 person-years for 0-3 months, 4-7 months and 8+ months between doses one and two, respectively (Table 2).

Table 2. IR, IRR, and IRD comparing 2 versus 3 dose vaccination by age at vaccination initiation and time between dose 1 and 2, adjusted for attained age

Age at first vaccination	Number of doses	Time between dose 1 and 2 (months)	IR, 95%CI*	P-value	IRR, 95%CI	P-value	IRD, 95%CI*	P-value
≤16yr	3 doses	Standard dosing schedule (0, 2, 6)	61 (52;70)	<0.001	Ref	Ref	Ref	Ref
	2 doses	0-3	119 (88;151)	<0.001	1.96 (1.44;2.68)	<0.001	59 (25;92)	0.001
		4-7	77 (24;131)	0.005	1.27 (0.63;2.58)	0.506	17 (-38;71)	0.551
		8+	265 (68;462)	0.008	4.36 (2.05;9.28)	<0.001	205 (8;402)	0.042
17-19yr	3 doses	Standard dosing schedule (0, 2, 6)	113 (90;135)	<0.001	Ref	Ref	Ref	Ref
	2 doses	0-3	239 (187;291)	<0.001	2.12 (1.62;2.77)	<0.001	126 (73;179)	<0.001
		4-7	91 (18;165)	0.015	0.81 (0.36;1.84)	0.615	-21 (-97;54)	0.580
		8+	355 (68;643)	0.015	3.16 (1.40;7.14)	0.006	243 (-44;530)	0.097

*IR, IRD reported per 100 000 person-years. Reference groups: ≤16yr with 3 doses of qHPV (0,2,6 months) and 17-19yr with 3 doses of qHPV (0,2,6 months)

A similar pattern is seen in girls and women initiating vaccination after turning 17, with increased risks for condyloma after two doses if given 0-3 months (IRR=2.12, 95%CI=1.62 to 2.77) or 8+ months (IRR=3.16 95%CI=1.40 to 7.14) apart was observed. No association was found when comparing two versus three doses with 4-7 months between dose one and two (IRR=0.81, 95%CI=0.36 to 1.84) (Table 2).

The first sensitivity analysis including socioeconomic status revealed no significant change to the point estimates (see supplementary table 1). In the second sensitivity analysis the IRRs were comparable, therefore the cut off at 12 months was not applied (data not shown).

Incidence rate ratios comparing two dose versus matched three dose vaccination

Comparing two-dose vaccination, 0-3 months apart, versus three-dose vaccination with 0-3 months between doses one and two, results remained effectively unchanged both for girls initiating vaccination prior to age 17 (IRR=1.95, 95% CI=1.44 to 2.64) and girls initiating vaccination between 17 and 19 years (IRR=1.88, 96%CI=1.46 to 2.42) (Table 3)

Table 3. IR, IRR, and IRD comparing two-dose vaccination with varying time between dose 1 and 2 versus three-dose vaccination by age at vaccination initiation, adjusted for attained age

Age at first vaccination	Number of doses	Time between dose 1 and 2 (months)	IR, 95%CI*	P-value	IRR, 95%CI	P-value	IRD, 95%CI*	P-value
≤16yr	3 doses	0-3	63 (55;72)	<0.001	Ref	Ref	Ref	Ref
	2 doses	0-3	123 (90;156)	<0.001	1.95 (1.44;2.64)	<0.001	60 (26;94)	<0.001
≤16yr	3 doses	4-7	91 (28;154)	0.005	Ref	Ref	Ref	Ref
	2 doses	4-7	79 (24;133)	0.005	0.87 (0.33;2.32)	0.779	-12 (-95;71)	0.779
≤16yr	3 doses	8+	86 (-33;205)	0.158	Ref	Ref	Ref	Ref
	2 doses	8+	270 (70;470)	0.008	3.14 (0.65;15.09)	0.154	184 (-49;417)	0.122

17-19yr	3 doses	0-3	129 (107;150)	<0.001	Ref	Ref	Ref	Ref
	2 doses	0-3	242 (190;294)	<0.001	1.88 (1.46;2.42)	<0.001	114 (60;167)	<0.001
17-19yr	3 doses	4-7	88 (-12;189)	0.084	Ref	Ref	Ref	Ref
	2 doses	4-7	95 (19;172)	0.015	1.08 (0.27;4.31)	0.916	7 (-119;133)	0.915
17-19yr	3 doses	8+	0	-	Ref	Ref	Ref	Ref
	2 doses	8+	373 (72;675)	0.015	-	-	373 (72;675)	0.015

* IR, IRD reported per 100 000 person-years. Matched reference groups: ≤16yr with 3 doses of qHPV with 0-3 months between dose 1 and 2, ≤16yr with 3 doses of qHPV with 4-7 months between dose 1 and 2 and ≤16yr with 3 doses of qHPV with 8+ months between dose 1 and 2; 17-19yr with 3 doses of qHPV with 0-3 months between dose 1 and 2, 17-19yr with 3 doses of qHPV with 4-7 months between dose 1 and 2 and 17-19yr with 3 doses of qHPV with 8+ months between dose 1 and 2.

Comparing two versus three-dose vaccination with 4-7 and 8+ months between the first two doses for both schedules, we found non-significant associations with IRRs of 0.87 (95% CI=0.33 to 2.32) and 3.14 (95% CI=0.65 to15.09) respectively, for girls initiating vaccination prior to 17, with corresponding IRDs of -12 (95% CI=-95 to 71) and 184 (95% CI=-49 to 417) cases per 100 000 person-years (Table 3). For girls initiating vaccination between 17 and 19 years, no association was found for 4-7 months in between doses (IRR=1.08, 95%CI=0.27 to 4.31); no cases of condyloma were reported in fully vaccinated women initiating vaccination between 17 and 19 years (Table 3).

Incidence rate ratios comparing two doses versus pragmatic three dose vaccination

Changing the reference group to pragmatic three-dose vaccination did not materially affect the results. (See supplementary table 2).

DISCUSSION

Statement of principle findings

This population-based study investigates the incidence of condyloma after two doses of qHPV by time between first and second dose. Our results suggest that a two-dose regimen is similarly effective as a standard three-dose schedule if given 4-7 months apart. This is in line with the recommendations from the European Medicines Agency (EMA) and the World Health Organization Strategic Advisory Group of Experts (SAGE) and immunological results from clinical trials.[2, 3, 5, 6, 15-19]

In relation to other studies

The impact of HPV vaccines was first recognised for HPV infections, and HPV-related diseases with short incubation times following infection such as genital warts.[20] Studies have shown that three-dose schedules of qHPV vaccination have been effective in the prevention of genital warts at a population level.[21-24] In addition, observational studies assessing the effectiveness of qHPV against cervical abnormalities have been carried out.[25-28] A recent review by Garland et al. suggested that in successive birth cohorts that are beginning screening, there have been reductions in the number of low-grade cytological abnormalities and high-grade histology confirmed cervical lesions (approximately 45% and 85% respectively).[29]

Alternative dosing schedules on condyloma incidence have been investigated in Denmark and Sweden,[9, 13] with both studies showing that condyloma incidence was statistically significantly higher in women aged 19-24 years after two doses rather than three. However, receipt of two vaccine doses with optimum interval was reported as non-inferior to three doses in terms of condyloma reduction, a finding with which the present study concurs.

Strengths and weaknesses

This was a nationwide study including the entire vaccinated Swedish female population aged 10-27 years. The use of high quality national register-based data meant that we were able to link vaccination status to disease outcome on an individual level.

A limitation of our study is that a small proportion of patients will neither seek hospital care for condyloma nor receive prescription for treatment, and thus will not be included in the registers, resulting in an underestimation of the true number of condyloma cases. [12] However, we expect this to be negligible in our study, as a) vaccinated women have been found to have higher screening uptake than unvaccinated women and can thus also be assumed not to be less prone to access healthcare [30] b) the estimated effect of the two-dose schedule would only be inflated if girls less willing to complete the three-dose schedule would have been more likely to seek healthcare for condyloma than those going on to complete three doses.

Another potential limitation is that SVEVAC was a voluntary register for the period 2006-2010, with only 80-85% coverage. To avoid an underestimation of vaccination exposure, we complemented missing data using the Prescribed Drug Register. This method has been used previously in a study by Herweijer et al, who found unique vaccination dose dates for 99.6% of the vaccinated girls and women in the cohort. [13]

It is also possible that individuals might have a prevalent HPV infection at time of vaccination, resulting in an underestimation of protective effect of the vaccine. We have attempted to control for this by excluding women who had a history of condyloma before the start of individual follow-up. Additionally, given that we start follow-up for condyloma incidence only after the second dose, we have the automatic benefit of a buffer period as used in.[13]

It is also of note that, the majority of women in the cohort had 0-3 months between first and second dose, which limited the power for other exposure groups in our study and resulted in wider confidence intervals, particularly in comparisons with the older age group and increasing time between doses. While we did not find socioeconomic status as a confounder in our study and we hypothesise that this is because we only follow subjects from the second dose forwards, so there has already been a large degree of self-selection with regard to the role of socioeconomic factors in our study participants.

Implications

Reducing the number of HPV vaccine doses from three to two could potentially lead to a number of positive effects, including lower costs, increased compliance and improved logistics of the vaccination programme. It is however key to remain vigilant with regards to follow-up of disease outcomes and supplement clinical trial data and policy recommendations with real-life evidence, such as those presented here. The findings imply that the current recommendation of two dose-schedules is appropriate, but we reinforce the significance of optimal timing between doses.

Unanswered questions and future research

We did not consider HPV-related disease outcomes other than condyloma. More studies with longer follow-up time are needed to ascertain the effectiveness of a two-dose schedule for HPV-related disease outcomes such as cervical intraepithelial neoplasia or cervical cancer. As more countries implement two-dose schedules, the impact on transmission dynamics and herd immunity will also become clearer.[22] It should also be taken into account that the duration of protection for both the two-dose

and three-dose schedule is not yet known and more time and data are required before conclusions can be drawn regarding the long-term effectiveness of these schedules, and a reduced-dose schedule can be recommended for girls older than 15.[2, 31]

The finding that the 8+ months between doses was less protective that the 4-7 month group was unexpected as for one-dose priming schedules it is often better with a longer interval between doses. Since this is an observational study, we cannot exclude that our finding was due to an unmeasured confounding factor however, with some (unknown) underlying reason why these girls had a longer time to dose three and high incidence/exposure. While we can only speculate about this higher risk in the 8+ month group, it has highlighted the need for further studies with a longer follow up time investigating the upper time limit between doses and vaccine effectiveness.

Conclusion

For prevention of condyloma, a two-dose schedule of qHPV vaccine with 4-7 months between first and second dose may be as effective as standard three-dose vaccination, for women first vaccinated before the age of 20. The results from this nationwide observational study support immunogenicity findings from clinical trials.

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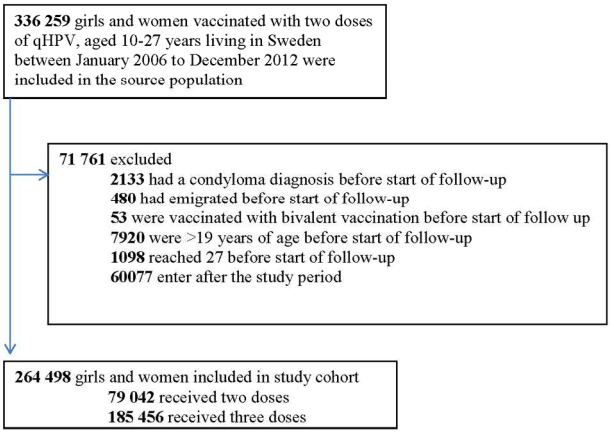
Figure 1. Details on study exclusions and the population analysed to investigate timing of two versus three doses of quadrivalent HPV vaccine and associated effectiveness against condyloma.



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Supplementary Table 1. IR, IRR, and IRD comparing 2 versus 3 dose vaccination by age at vaccination initiation and time between dose 1 and 2, adjusted for attained age and education level¶.

Age at first vaccination	Number of doses	Time between dose 1 and 2 (months)	IR, 95%CI*	P-value	IRR, 95%CI	P-value	IRD, 95%CI*	P-value
≤16yr	3 doses	Standard dosing schedule (0, 2, 6)	62 (53;72)	<0.001	Ref	Ref	Ref	Ref
	2 doses	0-3	122 (89;155)	<0.001	1.96 (1.43;2.70)	<0.001	60 (25;95)	0.001
		4-7	73 (19;128)	0.008	1.17 (0.55;2.51)	0.669	11 (-44;66)	0.692
		8+	250 (49;450)	0.015	4.02 (1.78;9.07)	0.001	188 (13;388)	0.067
17-19yr	3 doses	Standard dosing schedule (0, 2, 6)	113 (90;135)	<0.001	Ref	Ref	Ref	Ref
	2 doses	0-3	244 (189;299)	<0.001	2.15 (1.63;2.84)	<0.001	131 (75;186)	<0.001
		4-7	100 (19;181)	0.015	0.88 (0.39;2.00)	0.767	-13 (-95;69)	0.754
		8+	383 (73;694)	0.016	3.39 (1.50;7.68)	0.003	270 (-39;579)	0.087

*IR, IRD reported per 100 000 person-years. Reference groups: ≤16yr with 3 doses of qHPV (0,2,6 months) and 17-19yr with 3 doses of qHPV (0,2,6 months).

¶ Highest education level of either parent, nearest to the date of entry, was used as a proxy for socioeconomic status. Individuals with educational information (n=252 768).

Supplementary Table 2. IR, IRR, and IRD comparing 2 versus 3 dose vaccination by age at vaccination initiation and time between dose 1 and 2.

Age at first vaccination	Number of doses	Time between dose 1 and 2 (months)	IR, 95%CI*	P-value	IRR, 95%CI	P-value	IRD, 95%CI*	P-value
≤16yr	3 doses	Overall (0-3;4-7;8+)	64 (55;72)	<0.001	Reference	Reference	Reference	Reference
	2 doses	0-3	123 (90;156)	<0.001	1.92 (1.42;2.60)	<0.001	59 (25;93)	0.001
		4-7	79 (24;133)	0.005	1.23 (0.61;2.49)	0.562	15 (-40;70)	0.598
		8+	270 (70;470)	0.008	4.22 (1.99;8.94)	<0.001	206 (6;406)	0.044
17-19yr	3 doses	Overall (0-3;4-7;8+)	127 (106;149)	<0.001	Reference	Reference	Reference	Reference
	2 doses	0-3	242 (190;294)	<0.001	1.9 (1.48;2.45)	<0.001	115 (62;168)	<0.001
		4-7	95 (19;172)	0.015	0.75 (0.33;1.69)	0.484	-32 (-110;46)	0.422
		8+	374 (72;675)	0.015	2.93 (1.3;6.61)	0.009	246 (-54;547)	0.108

* IR, IRD reported per 100 000 person-years, reference groups: ≤16yr with 3 doses of qHPV (no time restriction between dose 1 and 1 and dose 2 and 3) and 17-19yrs with 3 doses of qHPV (no time restriction between dose 1 and 1 and dose 2 and 3).

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5, 6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6, 7, 8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6, 7, 8
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 7, 8, 9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*7*
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8, 9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8, 9
		(*b*) Describe any methods used to examine subgroups and interactions	8, 9
		(*c*) Explain how missing data were addressed	N/A
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	9
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6, 9, 10
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	6
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	9, 10
Outcome data	15*	Report numbers of outcome events or summary measures over time	9, 10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-18
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	15, 18
Discussion			
Key results	18	Summarise key results with reference to study objectives	18
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	19, 20, 21, 22
Generalisability	21	Discuss the generalisability (external validity) of the study results	20, 21, 22
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.