Dose-Related Differences in Effectiveness of Human Papillomavirus Vaccination Against Genital Warts: A Nationwide Study of 550 000 Young Girls

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Background. Reducing the number of doses in the human papillomavirus (HPV) vaccination regimen from 3 to 2 could increase coverage rates. In this cohort study, we assessed the risk of genital warts (GWs) according to timing and number of doses of quadrivalent HPV vaccine.

Methods. From population-based registries, we identified all girls in Denmark born during 1985–1999, for whom information on HPV vaccinations was retrieved. The cohort was followed for GW occurrence during 2006–2012. Incidence rate ratios (IRRs) were calculated by Poisson regression to determine differences in GW rates by number of vaccine doses.

Results. Of the 550 690 girls in the cohort, 361 734 had been vaccinated. Of these, 25.9% had been vaccinated twice and 58.8% 3 times. The risk of GWs decreased significantly with each additional dose of vaccine. For girls who received 2 doses, extension of the interval between doses reduced the incidence of GWs. In comparison with a 2-month interval, the incidence of GWs was reduced by 45% (95% confidence interval [CI], 20%–62%), 55% (95% CI, 35%–69%), and 63% (95% CI, 44%–75%), with an interval of 4, 5, and 6 months, respectively. The IRR of 2 vs 3 doses was close to 1, with an interval of about 6 months between the first 2 doses.

Conclusions. With the original vaccine schedule, completion of 3 doses seems to be required to obtain full protection against GWs. A 2-dose regimen may be as effective if the dosing interval is extended to around 6 months, although the long-term effectiveness of this regimen is unknown.

Keywords. genital warts; condyloma acuminata; human papillomavirus; vaccination; dose–response relationship.
as 3 [5], in 2014 the European Medicines Agency and the World Health Organization (WHO) Strategic Advisory Group of Experts recommended a 2-dose schedule for girls aged 9–13 years. Vaccination with <3 doses would reduce costs, be easier logistically, and facilitate the introduction of vaccination programs. Many previous studies of dose-related effects have been based on assessment of immunogenicity. As the antibody threshold at which HPV-related diseases are prevented has not been identified, studies with clinical endpoints are important. Because GWs have a shorter latency than precancerous lesions and cancer, the effect of immunization is first detectable from the occurrence of GWs. The aim of this study was to evaluate the effect of timing and number of doses of HPV vaccine on the incidence of GWs, focusing on the effects of 2 and 3 doses.

METHODS

Data Sources and Linkage of Data Among Nationwide Registers
The details of data collection have been described previously [6]. In brief, in Denmark, every citizen is allocated a unique personal identification number comprising information on sex and date of birth, which allows accurate linkage of information between registries. Using the personal identification number, we identified all women born in 1961–1999 from the Civil Registration System. This cohort was linked to the National Health Insurance Service Register to identify girls who had been vaccinated in the children’s vaccination or catch-up programs (service codes 8328, 8329, and 8330 for 1, 2, and 3 doses, respectively; and 8334, 8335, and 8336 for the second catch-up), and girls and women who had self-funded the vaccine were identified by linkage to the Prescription Registry (Anatomical Therapeutic Chemical [ATC] code J07BM01).

The number of doses and the dates of vaccination were collected for all vaccinated girls and women from October 2006 to December 2012. The earliest registered service code/ATC code was regarded as the first vaccination. We chose to include only birth cohorts with >10% coverage with at least 1 dose of vaccine, which was the case for the birth cohorts 1985–1999. Vaccinated and unvaccinated girls in these cohorts were linked to the National Patient Register and the Prescription Registry and followed for occurrence of GWs. Girls were considered to have had GWs if GWs were diagnosed at a private or public hospital (International Classification of Diseases, Tenth Revision, code A63.0 in the National Patient Register) and/or they had redeemed a prescription for podophyllotoxin (ATC code D06BB04 in the Prescription Registry). The girls were followed until a first episode of GWs, date of emigration, death, or 31 December 2012, whichever came first. Those with a diagnosis of GWs or a prescription for podophyllotoxin before vaccine licensure (October 2006) were excluded. As a measure of socioeconomic position, we adjusted for maternal educational level and disposable income at the start of follow-up by linkage with data from Statistics Denmark.

Statistical Methods
To examine the effects of different doses of vaccine, we included number of doses as a time-varying covariate with the following 5 states: unvaccinated, vaccinated with first, second, and third dose, and GWs (absorbing state). Age was categorized into 6 age categories: ≤15, 16–17, 18–19, 20–21, 22–23, and 24–27 years. Each participant contributed person-time to each of the 24 combinations of number of doses and age group (absorbing state not included), corresponding to the time spent in each combination. To allow each dose to take effect, we included a buffer period of 28 days for all vaccine doses—that is, moving the dates of the first, second, and third dose 28 days forward in time.

We calculated the incidence of GWs per 100 000 person-years (PY) in each state. The incidence rates were estimated in a piecewise constant model; for example, the rate from unvaccinated to GWs was assumed to be constant over the age range 16–17 years. Estimates were made in a Poisson model, and likelihood ratio tests were used for testing. Incidence rate ratios (IRRs) were calculated to compare rates.

In the analysis of incidence rates after 2 and 3 doses, we further considered a model in which we took time between the first and the second dose (Δt) into account. We first considered the state corresponding to 2 doses, included Δt in a restricted cubic spline, and adjusted for attained age and calendar time. This showed a significant effect of Δt on the incidence rate (P < .0001), and adjusted IRRs were computed with 2 months as the reference. The same analysis was conducted for the state corresponding to 3 doses, which showed, however, an insignificant effect of Δt on the incidence rate (P = .5392).

We further considered the 2 states corresponding to the second and third doses in a combined model. We computed IRRs adjusted for age and calendar time for 2 vs 3 doses, with the incidence rate for 2 doses depending on Δt, by means of a restricted cubic spline. This was also done separately for ages <16 and ≥16 years at vaccination, under the assumption that the effect of vaccination early and later in life might differ.

We repeated all analyses after excluding girls with service codes that were not in consecutive order, but this had virtually no effect on the results (data not shown). We used R version 2.15.0 for all analyses and assumed a significance level of 5%. Data for the multistate model were processed with the Lexis framework in the Epi package in R [7–9].

RESULTS

Identification of the Cohort and Vaccination Status
After excluding 469 girls who had been vaccinated with the bivalent vaccine, we identified 555 473 girls born during 1985–1999,
of whom 374 248 (67.4%) had been given the quadrivalent vaccine between vaccine licensure (1 October 2006) and 31 December 2012. We excluded 4783 girls who had GWs before vaccine licensure (3026 were later vaccinated and 1757 unvaccinated), and 9488 vaccinated girls were registered as unvaccinated because they had GWs before vaccination (follow-up ended at the first diagnosis of GWs), leaving 361 734 vaccinated and 188 956 unvaccinated girls in the cohort. Table 1 lists the numbers of doses received by each birth cohort of vaccinated girls.

Table 1. Number of Girls Vaccinated Against Human Papillomavirus, 1 October 2006–31 December 2012, by Birth Cohort and Number of Doses

<table>
<thead>
<tr>
<th>Birth Cohort</th>
<th>Only 1 Dose</th>
<th></th>
<th></th>
<th>Only 2 Doses</th>
<th></th>
<th></th>
<th>Only 3 Doses</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>% of Total</td>
<td>% of Vaccinated</td>
<td>No. (%)</td>
<td>% of Total</td>
<td>% of Vaccinated</td>
<td>No. (%)</td>
<td>% of Total</td>
<td>% of Vaccinated</td>
</tr>
<tr>
<td>1985–1986</td>
<td>10 570</td>
<td>14.2</td>
<td>36.7</td>
<td>14 654</td>
<td>19.7</td>
<td>50.9</td>
<td>3539</td>
<td>4.8</td>
<td>12.3</td>
</tr>
<tr>
<td>1987–1988</td>
<td>11 218</td>
<td>14.5</td>
<td>35.6</td>
<td>15 749</td>
<td>20.4</td>
<td>49.9</td>
<td>4564</td>
<td>5.9</td>
<td>14.5</td>
</tr>
<tr>
<td>1989–1990</td>
<td>12 137</td>
<td>15.5</td>
<td>31.9</td>
<td>17 809</td>
<td>22.7</td>
<td>46.9</td>
<td>8052</td>
<td>10.3</td>
<td>21.2</td>
</tr>
<tr>
<td>1991–1992</td>
<td>11 746</td>
<td>15.8</td>
<td>27.1</td>
<td>16 098</td>
<td>21.6</td>
<td>37.2</td>
<td>15 431</td>
<td>20.7</td>
<td>35.7</td>
</tr>
<tr>
<td>1993–1994</td>
<td>2 743</td>
<td>3.8</td>
<td>4.3</td>
<td>8 894</td>
<td>12.3</td>
<td>13.9</td>
<td>52 213</td>
<td>72.0</td>
<td>81.8</td>
</tr>
<tr>
<td>1995–1996</td>
<td>2543</td>
<td>3.6</td>
<td>4.0</td>
<td>8 440</td>
<td>11.9</td>
<td>13.2</td>
<td>52 942</td>
<td>74.3</td>
<td>82.8</td>
</tr>
<tr>
<td>1997–1999</td>
<td>4709</td>
<td>4.6</td>
<td>5.1</td>
<td>11 875</td>
<td>11.6</td>
<td>12.9</td>
<td>75 808</td>
<td>73.8</td>
<td>82.1</td>
</tr>
</tbody>
</table>

Figure 1. Incidence of genital warts (GWs) in the study population according to vaccination state. Crude incidence rates (and confidence intervals [CIs]) of GWs were calculated per 100 000 person-years in each state on the basis of a Poisson model with log (person-time) as offset.
The up program (cohorts 1993–1999), >80% completed the vaccination regimen (Table 1). As the second catch-up program was initiated only 5 months before the end of the follow-up, completion of 3 doses was markedly lower (22.3%) for the birth cohorts 1985–1992. The interval between the first and second dose was 2 months for 70% of the girls, up to 3 months for 89% of girls, and up to 4 months for 93% of girls.

Dose-Related Risk of Genital Warts

GWs were diagnosed in 14,540 unvaccinated girls (2,217,764 PY for 550,690 girls), in 370 girls after the first dose (111,005 PY for 357,536 girls), in 261 girls after the second dose (178,319 PY for 304,236 girls), and in 398 girls after all 3 doses (591,832 PY for 212,551 girls). Figure 1 shows the crude incidence rates of GWs with each vaccination state for all ages combined. We found that GWs occurred significantly less frequently with each additional dose (IRR1vs0, 0.51 [95% CI, 0.46–0.56], P < .001; IRR2vs1, 0.44 [95% CI, 0.37–0.51], P < .001; IRR3vs2, 0.46 [95% CI, 0.39–0.54], P < .001). The results for 2 vs 3 doses in all age groups are shown in Table 2. Adjustment for age at vaccination, maternal educational level, disposable income, and calendar time changed the estimates to some degree, but the incidence rate after 3 doses was still significantly lower than after 2 doses. The change in IRR was primarily caused by the effect of calendar time.

Consequences of Changing the Interval Between the First and Second Doses

We then considered the importance of the time between the first and second dose. After 2 doses, the incidence of GWs decreased with increasing time between doses (Figure 2). With a 2-month interval as reference, the incidence of GWs was statistically significantly reduced by 27% (95% CI, 4%–45%), 45% (95% CI, 20%–62%), 55% (95% CI, 35%–69%), and 63%

### Table 2. Incidence Rate Ratios of Genital Warts After Vaccination With 2 Doses of Quadrivalent Human Papillomavirus Vaccine Versus 3 Doses, 1 October 2006–31 December 2012

<table>
<thead>
<tr>
<th>Attained Age, y</th>
<th>2 Doses</th>
<th>3 Doses</th>
<th>IRR (95% CI)</th>
<th>P Value</th>
<th>IRRb (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>146.4</td>
<td>67.2</td>
<td>2.18 (1.86–2.54)</td>
<td>&lt;.001</td>
<td>1.88 (1.60–2.21)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>≤15</td>
<td>13.7</td>
<td>2.6</td>
<td>5.38 (2.19–13.20)</td>
<td>&lt;.001</td>
<td>3.04 (1.17–7.90)</td>
<td>&lt;.002</td>
</tr>
<tr>
<td>16–17</td>
<td>137.4</td>
<td>30.9</td>
<td>4.44 (3.00–6.57)</td>
<td>&lt;.001</td>
<td>2.84 (1.87–4.31)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>18–19</td>
<td>299.8</td>
<td>101.7</td>
<td>2.95 (2.09–4.16)</td>
<td>&lt;.001</td>
<td>2.09 (1.47–2.98)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>20–21</td>
<td>900.0</td>
<td>315.1</td>
<td>2.86 (2.09–3.90)</td>
<td>&lt;.001</td>
<td>2.05 (1.48–2.84)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>22–23</td>
<td>950.6</td>
<td>531.0</td>
<td>1.79 (1.27–2.53)</td>
<td>.001</td>
<td>1.70 (1.20–2.41)</td>
<td>.003</td>
</tr>
<tr>
<td>24–27</td>
<td>672.8</td>
<td>447.0</td>
<td>1.51 (0.98–2.31)</td>
<td>.061</td>
<td>1.80 (1.17–2.78)</td>
<td>.008</td>
</tr>
</tbody>
</table>

Vaccination and age were included as time-varying exposures. The same women could contribute person-time to 1 or both vaccination states (2 doses and 3 doses) and 1 or more age groups according to the time spent in each of these states. Attained age is therefore the age attained in each vaccination state. The incidence rates were estimated in a piecewise constant model; the rate from vaccinated with, eg, 2 doses to genital warts was assumed to be constant over the age range of 16–17 years. Estimates are based on Poisson regression with log (person-time) as offset. IRRs were calculated as incidence rate2 doses/incidence rate3 doses.

Abbreviations: CI, confidence interval; IRR, incidence rate ratio.

a Per 100,000 person-years.
b Adjusted for age at vaccination, maternal educational level, disposable income, and calendar time.

Figure 2. Influence of interval between the first and second dose with 2 human papillomavirus vaccinations. Incidence rate ratios (IRRs) of genital warts (GWs) were calculated as the ratio between the incidence rate after 2 doses with a variable interval between the first and second doses and the incidence rate after 2 doses with 2 months between the first and the second dose. The estimates were based on Poisson regression, adjusted for age and calendar time, and 2 months between the first and the second dose was used as reference. Dashed lines and numbers in parentheses indicate 95% confidence limits, and the horizontal line corresponding to IRR = 1 indicates similar incidence rates of GWs after 2 doses with a variable interval and 2 doses with a 2-month interval.
(95% CI, 44%–75%) with a dosing interval of 3, 4, 5, and 6 months, respectively.

We further compared the incidence of GWs after a 3-dose regimen with that after 2 doses with increasing interval between the first and second dose. For a short interval between the first 2 doses, 3 doses provided greater protection against GWs than 2 doses (IRRs >1), but the difference between 3 and 2 doses decreased (IRR approached 1) with increasing time between the first 2 doses (data not shown). When the data were analyzed separately for girls aged <16 and ≥16 years at vaccination, although the underlying risk differed, we observed the same overall pattern in the 2 groups (Figure 3). No statistically significantly different effect of 2 and 3 doses was found with an interval of about 4 months between the first and second doses, and the IRR was close to 1 with an interval of about 6 months.

**DISCUSSION**

In this nationwide study of some 550,000 young girls, of whom about 360,000 received the quadrivalent HPV vaccine, we observed a significantly lower incidence of GWs with each additional dose of vaccine in the currently recommended schedule of vaccination at intervals of 0, 2, and 6 months. The incidence of GWs after 2 doses decreased significantly when the interval between doses was extended, and the effect of 2 doses approached that of 3 doses when the 2 doses were received at longer intervals.

Evidence for the optimal HPV vaccination schedule was recently reviewed in a background paper prepared for the WHO Strategic Advisory Group of Experts on Immunization [5]. The group concluded that a 2-dose schedule with at least 6 months between the first and second dose could have similar protective efficacy as a 3-dose schedule. This conclusion is supported by information about the time needed for memory B cells to mature and differentiate into high-affinity B cells, which is 4–6 months [10]. Studies of immunogenicity indicate that a 2-dose schedule at 0 and 6 months is not inferior to the standard regimen, in agreement with our results. Nevertheless, the relation between noninferiority (often defined as the lower limit of the 95% CI of the geometric mean antibody titer ratio [2/3 doses] >0.5) and clinical relevance in public programs is not well understood. Furthermore, immunological studies often seek immune responses in young girls that are not inferior to those in clinically protected women [11, 12]. Young girls are known to react with higher antibody levels to vaccination than adult women; therefore, when girls in the same age strata are compared, noninferiority may not be reached for all HPV types in the 2-dose schedule at the end of follow-up. This was the case for HPV type 18 by month 18 and for HPV type 6 by month 36 for the quadrivalent vaccine [12], and for HPV type...
type 16 by month 24 for the bivalent vaccine [13]. These findings may potentially cast doubt on the long-term effectiveness of 2 doses of vaccine. The minimum level of antibodies necessary for preventing GWs and precancerous lesions has not, however, yet been determined, and strong memory antibody responses have been demonstrated [14].

The effectiveness of 2 and 3 doses of quadrivalent HPV vaccine against cervical abnormalities was assessed in 2 observational studies in Australia [15, 16], and Herweijer et al [17] assessed the dose-related incidence of GWs after quadrivalent HPV vaccination in Sweden. Consistent with our findings, all of these studies showed that 2 doses provided some, but lower, protection than 3 doses. Unlike the former studies, however, we were able to assess differences in risk with 2 vs 3 doses according to the interval between doses. In these studies and in the present study, girls who received 2 doses might not be comparable to those who completed the standard 3-dose schedule in terms of high-risk sexual behavior and potential prevalent HPV infection (self-selection bias); however, in the multistate model used in this and the Swedish study, girls who received 3 doses contributed person-time to the group that received 2 doses, reducing the risk or the magnitude of self-selection bias. We further minimized the possibility of self-selection bias in our analysis of 2 vs 3 doses by calculating the incidence rates of GWs in girls aged <16 years at vaccination, who had very high vaccination and completion rates. We found the same trend for girls both <16 and ≥16 years at first vaccination: the IRRs decreased almost equally with increasing interval between the 2 doses (Figure 3).

In a trial of the bivalent vaccine in Costa Rica, Kreimer et al [18] found that 2 doses given at months 0 and 1 (n = 802) were as effective in preventing incident, persistent HPV type 16/18 infection as the standard 3-dose schedule (n = 5967) in women aged 18–25 years. The results of this and other studies of the bivalent vaccine [5, 11, 19] may be compared with those of the quadravalent vaccine, but, in interpreting the results, it is important to remember the differences in valence, dose, production system, adjuvant, and laboratory tests used to measure antibody levels with the 2 vaccines.

In August 2014, Denmark changed the dosing schedule in the nationwide immunization program for 12- to 13-year-old girls to 2 doses. Although this and other studies indicate that 2 doses of HPV vaccine are as effective as 3 doses, there is insufficient data to draw conclusions about the sustainability of protection after 2 doses. If future randomized trials show equal long-term efficacy with 2 and 3 doses, certain population groups (eg, immunocompromised individuals) should be followed to identify any difference in antibody response or protection. In addition, cross-protection against other HPV types should be analyzed. For example, cross-protection against HPV type 31 appeared to be poorer after 2 than 3 doses in a recent study of antibody responses [19].

A strength of this study is its considerable size, with 360 000 vaccinated girls, which we achieved because we could identify almost all vaccinated girls in the country. In addition, we had practically no loss to follow-up owing to the accuracy and completeness of the nationwide registries, and we were able to adjust for socioeconomic status. As described earlier, the study was limited by nonrandomization, which may have resulted in differences in risk behavior related to GWs between groups, potentially biasing the comparison between numbers of doses received. This potential limitation was partly adjusted for by use of the multistate model. Although most GW cases caused by prevalent infection should have been excluded by starting GW registration 28 days after the first dose of vaccine, some prevalent cases may still have been included, as the time between HPV infection and visible GWs is often longer. This problem would affect mainly the risk estimates after the first and potentially the second vaccination, resulting in underestimation of the effects of 1 and 2 doses, and it would apply mainly to older women, some of whom might have been vaccinated because of perceived risk behavior. An extension of the buffer period to 3 months decreased the incidence of GWs after the second dose, but the trends in Figure 3 did not change. Another potential limitation of our study is the fact that most girls received the first 2 doses 2–4 months apart. Therefore, the results of a 6-month interval between doses are based on a limited number and should be interpreted with caution. In addition, a minority of HPV vaccine doses and GW cases could have been misclassified, because vaccines bought outside the country or directly through the doctor were not registered (<10% of self-paid vaccinations), and GWs diagnosed outside the hospitals with treatment other than podophyllotoxin were not included. However, podophyllotoxin is the first-line treatment against GWs in Denmark, so we believe that the vast majority of girls with GWs are included. Finally, although our results on GWs may predict a similar outcome for infection with HPV types 16/18, studies on the number of doses required in the prevention of HPV type 16/18 infection or cervical premalignant changes are needed.

In conclusion, this nationwide study indicates that, with the current interval between doses, completion of a 3-dose schedule offers better protection against GWs than a 2-dose schedule. Two doses appear to be as effective as 3, however, if the dosing interval is extended (to about 6 months). This was indicated by immunological studies and is supported by this study with a clinical outcome. We must await the results of randomized clinical trials with disease endpoints to establish the long-term significance of omitting the third dose of vaccine.

**Notes**

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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