CHAPTER 7

Persistence of immune response following bivalent HPV vaccination: a two-year follow-up study among girls routinely vaccinated with a two-dose schedule

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ABSTRACT

Background
In this cohort study, we examined antibody levels and avidity after a two-dose schedule (0, 6 months) of the bivalent HPV-vaccine in girls routinely vaccinated in the Dutch HPV-vaccination program, up to 2 years following vaccination.

Methods
A blood sample at 7, 12 and 24 months after the first dose and questionnaire data were collected (n=56). HPV type-specific antibody concentrations (IU/ml) against seven types (HPV16/18/31/33/45/52/58) were assessed using a validated virus-like particles (VLP) multiplex immunoassay. Avidity was tested using a modification of this assay.

Results
Seropositivity for vaccine types HPV 16 and 18 was 100% up to month 24, but declined for HPV-types 31/33/45/52/58, although not statistically significant for HPV45. All Geometric Mean Concentrations (GMCs) declined by months 12 and 24, but remained high for HPV16/18. Between month 7 and 12, GMCs declined more for other types. High avidity antibodies were induced up to 24 months for vaccine types, but less for other types.

Conclusion
For vaccine types HPV16/18 high antibody levels and avidity was observed after 24 months of follow-up. Antibody levels as well as avidity were considerably lower for HPV-types 31/33/45/52/58. Further follow-up of this cohort will provide insight into antibody and avidity kinetics over time.
BACKGROUND

Vaccination against human papillomavirus (HPV) has been available since 2006 for the prevention of HPV-related cancers. At that time, prophylactic HPV vaccination was introduced into the national immunization program of several countries around the world.[1] Currently, three vaccines which protect against HPV-related cancers are available.[2, 3] Initially the vaccines were licensed in a three-dose schedule. Later on, the European Medicines Agency (EMA) licensed a two-dose schedule for recipients through fourteen years of age for all three vaccines.[4-6] Following this licensure of the two-dose schedule, several countries have changed their schedule.[7]

Original licensure of the HPV-vaccines for adolescents, and later on of the two-dose schedule, was based on immune-bridging.[8, 9] Antibody concentrations in adolescents (9-13- or 9-14-years-old) who received a three- or two-dose schedule were compared with antibody concentrations in young women (15-25- or 16-26-year-old) who received a three-dose schedule, for which vaccine efficacy has been shown.[10-12] The International Agency for Research on Cancer (IARC) Working Group stated that immune-bridging is sufficient for extending licensure to other population groups and recommended that immunological non-inferiority is an appropriate endpoint in these kinds of evaluations.[13] Furthermore avidity of antibodies and the induction and maintenance of immunological memory may contribute to the ability to protect against HPV-associated cancers.[14, 15] Also post-licensure evaluation of long-term effectiveness and duration of protection would be especially important, given a lacking correlate of protection for HPV vaccination.[13]

In the Netherlands, the bivalent HPV16/18-vaccine was introduced in the national immunization program in 2009. Girls in the Netherlands are vaccinated in the year they turn thirteen.[16] In 2014, the program switched from a three- to two-dose schedule.[17] At that time, a cross-sectional study was carried out in which antibody responses after two- and three-dose schedules among young adolescents were compared up to 4½ years post-vaccination.[18] This study showed that geometric mean concentrations (GMCs) for HPV16/18 were not non-inferior for two- versus three-doses, except for HPV18, but for antibody avidity non-inferiority was shown, independent of antibody concentrations. In addition, a longitudinal study was initiated to examine the kinetics in antibody level and avidity after a
two-dose schedule (0, 6 months) of the HPV-vaccine in routinely vaccinated girls at 12 years of age. In this paper we describe the results of this study up to two years after the first dose.

METHODS

Study population and procedures
For this cohort study, the Dutch national vaccination registry Praeventis [19] was used to select a sample of girls who were routinely vaccinated with a two-dose schedule of the bivalent HPV-vaccine in 2014 (girls born in 2001). For sample size calculations we assumed that at 24 months of follow-up the observed geometric mean concentration (GMC) should still be above the plateau observed in clinical trials after a three-dose schedule. We estimated that at 24 months at least 14 participants were needed. Adjusting for an estimated participation rate of 10%, loss-to-follow-up of 10% per round and uncertainty about the complete vaccination status at the time of inclusion, we invited 198 girls. Only girls from a middle-sized municipality in the center of the Netherlands (Amersfoort) were invited for logistical reasons, however within this municipality invitations were send randomly. Girls received an invitation letter with an informed consent form by mail. In order to participate, both the girl and her parent(s) or guardian(s) were required to provide written consent. Participants were asked to fill out an online questionnaire, with questions regarding socio-demographics and sexual behavior, and to give a blood sample by venipuncture at 7, 12 and 24 months after the first dose of HPV-vaccine. Blood was drawn by use of a serum tube (VACUETTE®, Greiner Bio-one, North Carolina, USA). In case the venipuncture failed or the girl did not show up for venipuncture, they were be offered the possibility to draw a self-sample of finger prick blood at home (using the Whatman 903 Protein Saver Card, GE Healthcare, Cardiff, United Kingdom). In a previous study (data not published) we validated the interchangeable use of venipuncture or finger prick blood. After each sampling moment, participants received an incentive of €25 gift card following blood drawing and completion of the questionnaire.

Serological measurements
HPV type-specific antibody concentrations against HPV types 16, 18, 31, 33, 45, 52, 58 were assessed using a validated virus-like particles (VLP) multiplex
immunoassay, which is previously described by Scherpenisse et al. Hereby, HPV specific IgG antibodies were analysed using a Bioplex system 200 with Bioplex software (Bio-Rad Laboratories, Hercules, CA). The median fluorescent intensity (MFI) for each analyte was converted to Luminex Units/ml (LU/ml) by using a twofold serial diluted reference standard (IVIG, lot LE12H227AF, Baxter) and interpolating the MFI data through a 5-parameter curve-fitting algorithm. Cutoffs for seropositivity had been determined at 9, 13, 27, 11, 19, 14 and 31 LU/ml for HPV 16, 18, 31, 33, 45, 52 and 58, respectively. The procedure for serological analysis of the protein saver cards, in case of self-sampling of finger prick blood, is also previously described. In summary, the dry blood spot (DBS) samples were stored at -20°C until analysis. Samples were diluted 200 and 5000 fold in assay buffer and determined simultaneously within one assay run using the HPV multiplex assay.

Avidity of IgG specific antibodies was tested using a modification of the VLP multiplex immunoassay. In summary, sera were diluted to an HPV antibody concentration of 0.05-0.75 LU/ml. After incubation with VLP conjugated beads for one hour at room temperature and three washes with PBS, 50 μl of a 2.5 M ammonium thiocyanate solution (NH4SCN; Sigma-Aldrich, St.Louis, Missouri, USA) in PBS was added and incubated at room temperature for 10 minutes. Following three washes, residual bound antibodies were measured. The avidity index (AI) was defined as the percentage of remaining IgG levels in presence of ammonium thiocyanate in comparison with the IgG levels after addition of PBS.

Statistical analysis
Socio-demographic characteristics of the study population were described at 7, 12 and 24 months after the first dose. Seroprevalences and geometric mean concentrations (GMCs) with corresponding 95% confidence intervals (CI) for HPV types 16, 18, 31, 33, 45, 52, 58 were determined at months 7, 12 and 24. Also, HPV type-specific antibody avidity indexes (AI) were calculated. The vaccination status obtained from the vaccination registry was used for the main analyses. In addition, sensitivity analysis was performed on participants with consistent vaccination status in both the vaccination registry and the questionnaire (self-reported). All data were analysed using SAS software package 9.3 (SAS Institute INC., Cary, NC, USA).
Ethical approval
The study protocol was approved by the medical ethics committee of VU University Medical Center (NL48754.029.14, protocol number 2014.230), Amsterdam, the Netherlands and was conducted in adherence to the Declaration of Helsinki. The study is registered in the Dutch Trial Registry (NTR 4719).

RESULTS

Socio-demographic characteristics
A total of 56 girls (28.3%) participated in the study. Of them, 49 (87.5%) completed all three samples. The median time between both doses of HPV-vaccine was 5.8 (range 5.7-11.7) months. Sociodemographic characteristics of the participants per sampling moment are presented in Table 1. At all time points none of the girls reported to ever have had sex. One girl used immunosuppressive medication at the time of the first blood sampling.

Seroprevalence
Seropositivity for the vaccine types 16 and 18 was 100% up to month 24. For HPV types 31, 33, 45, 52 and 58 seropositivity ranged between 89.1% and 96.4% in month 7, between 70.4% and 90.7% in month 12, and between 30.8% and 80.8% in month 24. (Figure 1) Statistically significant declines in seroprevalence were observed between month 7 and 12 for HPV52 and between month 12 and 24 for HPV types 31, 33 and 58. For HPV45, a small decline was seen in seroprevalence over time, however, this was not statistically significant.

Level of antibodies
Geometric Mean Concentrations (GMCs) were high for vaccine types 16 and 18 (Figure 2) and at least 20 to 30 fold lower for the non-vaccine HPV types 31, 33, 45, 52 and 58. For all types, the GMCs were lower in months 12 and 24 compared to month 7. This decline seems more pronounced for HPV types 31, 33, 45, 52 and 58 (GMC ratio month 24 vs. months 7 ranged between 0.14 (95%CI 0.09-0.21) and 0.28 (95%CI 0.19-0.42) than for vaccine types (GMC ratio for HPV16 0.34 (95%CI 0.24-0.49) and for HPV18 0.36 (95%CI 0.25-0.51)).
Table 1. Socio-demographic characteristics of the participants per sampling moment.

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<th>Month 7</th>
<th>Month 12</th>
<th>Month 24</th>
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<td>29  52.7</td>
<td>29  53.7</td>
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<tr>
<td>Middle</td>
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<td>21  38.9</td>
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<td>6  10.9</td>
<td>4  7.4</td>
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<td>5  9.1</td>
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<td>2  3.7</td>
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<td>49  90.7</td>
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<td>5  3.6</td>
<td>3  5.6</td>
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<td>0  0.0</td>
</tr>
<tr>
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<td>53  96.4</td>
<td>54  98.2</td>
<td>51  94.4</td>
</tr>
<tr>
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<td>1  1.8</td>
<td>3  5.6</td>
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<td>51  94.4</td>
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<td>9  16.4</td>
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<td>12 (10-14)</td>
<td>12 (10-14)</td>
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</tbody>
</table>

* In questionnaire asked for the following illnesses: immune disorder, kidney disease, lung disease, cardiac disorder, thyroid disorder, liver disease, gastrointestinal disorder, metabolism disorder, malignant disease or cancer, neurological disorder and other disease (free text).
~ Low = primary or lower general vocational secondary education; Middle = intermediate vocational secondary education; High=higher vocational/general secondary education, (pre)university education
Figure 1. Seroprevalence of HPV types 16/18/31/33/45/52/58 following a two-dose schedule (0, 6 months) at months 7, 12 and 24 after the first dose.

*Statistically significant different compared with the previous sampling.

Figure 2. Geometric Mean Concentrations (GMC; lu/ml) of HPV types 16/18/31/33/45/52/58 following a two-dose schedule (0, 6 months) at months 7, 12 and 24 after the first dose.
Figure 3. Antibody avidity of HPV types 16/18/31/33/45/52/58 following a two-dose schedule (0, 6 months) at months 7, 12 and 24 after the first dose.

The lines indicate the mean antibody avidity index with 95% confidence interval.
Persistence of two-dose immune response

**Antibody avidity**
Antibodies with highest avidity were induced up to 24 months for both vaccine types 16 and 18. (Figure 3) For both types the AI increased significantly over time, i.e. 75.0%, 78.4% and 81.6% for HPV16 and 75.0%, 76.8% and 81.4% for HPV18 at months 7, 12 and 24, respectively. Avidity of antibodies against HPV-types 31, 33, 45, 52 and 58 was considerably lower, i.e. 16-29% at month 7, 20-32% at month 12 and 19-32% at month 24, however avidity for these non-vaccine types was stable over time.

**Sensitivity analyses**
We observed disagreement between the vaccination status obtained from the vaccination registry and the self-reported vaccination status (number of doses) in the questionnaire in three participants (5.4%). Although not significantly different, the point estimates for the GMCs were slightly higher when only participants with a concordant vaccination status were included. However, the same pattern over time was comparable. Comparable results were found regarding the avidity.

**DISCUSSION**
This population-based cohort study was designed to assess the kinetics in antibody levels and antibody avidity against HPV-types 16, 18, 31, 33, 45, 52 and 58 in routinely vaccinated girls at 12 years of age with a two-dose schedule (0, 6 months) of the bivalent HPV16/18 vaccine. Girls included in this study were one of the first cohorts globally to be eligible for two-dose vaccination. [21] Here, results up to 24 months after vaccination showed that antibody levels and avidity remained high for vaccine types 16 and 18. For HPV-types 31, 33, 45, 52 and 58, antibody levels as well as avidity were lower. Antibody levels for all types were highest at 7 months after the first dose, and declined thereafter (months 12 and 24). Antibody avidity over time increased for the vaccine-types and remained stable for the non-vaccine types.

Our findings with regard to lower antibody concentrations up to 24 months compared to those at 7 months are in line with previous trials on the immunogenicity of the two-dose schedule (0, 6 months) of the bivalent vaccine in 9-14-year-old girls. Both these trials have at present a longer follow-up than our observational...
study, up to 36 and 60 months respectively. These studies showed that antibody responses peaked at month 7 and thereafter gradually declined between months 18 and 24 to reach a plateau, sustaining for at least up to 60 months. [22-24] A review by Donken et al. concluded that GMCs for different age groups and different dosing schedules showed a fast decline after the first year and further declined although at a very small rate for at least up to four years after the first dose. [25]

The pattern and GMCs after two-doses in 9-14-year olds were comparable with those observed in women 15-25 years of age who received a three-dose schedule. [23] Lazcano-Ponce et al. found non-inferior levels of vaccine types HPV16/18 for girls 9-10 years of age compared to a three-dose group being 18-24 years up to 21 months post-vaccination. However, comparing two- versus three-dose schedules in girls 9-10 years of age showed non-inferior antibody concentrations, although concentrations were higher after three-doses. [26] In trials of the bivalent, quadrivalent vaccine and nonavalent vaccine, the same kinetics in antibody levels were found, and non-inferiority of a two-dose schedule in girls compared to the three-dose schedule in young women was demonstrated up to a maximum of 6 years after the last dose. [27-30] In addition, a review of D’Addario et al., which included the bivalent and quadrivalent vaccine, concluded that studies have had mixed results, but none has found consistently poorer responses following a two-dose schedule than following a three-dose schedule. [31] The comparable patterns of two- versus three-doses as shown in previous studies [23-25, 28] and the high GMCs for vaccine types HPV16 and 18 found in our study are reassuring with regard to long-term sustainability of antibodies generated after a two-dose schedule.

To our knowledge, this is the first longitudinal evaluation of immunogenicity against non-vaccine types HPV31/33/45/52/58 after vaccination with a two-dose schedule. As expected, antibody concentrations for non-vaccine types HPV31/33/45/52/58 in our study were much lower than those found for the vaccine types. A comparable pattern of lower antibody responses for non-vaccine compared to vaccine types was also observed in a Dutch cohort study among three-dose routinely vaccinated girls. [32] Although the three-dose girls were slightly older when vaccinated (catch-up campaign), in our study we observed comparable or higher antibody concentrations after two-doses than what was observed in that study of three-dose recipients. Given the lack of a correlate of
Persistence of two-dose immune response

...it is unknown how antibody concentrations translate into effectiveness. [33] However, in the three-dose cohort at three years post-vaccination, significant cross-protection was shown against incident and persistent HPV infections with HPV31/45. [34] Also, post-hoc analyses of clinical trials demonstrated that among women who received the second dose of their two-dose schedule at 6 months, comparable cross-protection with regard to incident infections with a three-dose schedule was observed against HPV-31/33/45 up to seven years post-vaccination. [35-37] Comparable antibody concentrations for non-vaccine types between three- and two-doses in combination with the shown efficacy against HPV infections by cross-protective types is reassuring, as these concentrations seem protective.

Scherpenisse et al. suggested that in addition to antibody concentrations, antibody avidity be used as possible immune correlate to distinguish vaccine derived from naturally derived immunity. This study has shown that the avidity of vaccine-derived HPV-specific antibodies was 3 times higher than that of antibodies induced by HPV infection. [20] Avidity indicates the strength of the binding between antibodies and antigen. A low concentration of antibodies with high avidity may be sufficient to provide protection. In this study, we found that high-avidity antibodies of HPV16 and HPV18 were induced after a two-dose schedule. These also increased slightly over time. Although for non-vaccine types HPV31/33/45/52/58 antibody avidity was lower, over time these were avidity indexes remained stable. For HPV31/33/45 infections in previous studies, consistent cross-protective efficacy has been shown after three-doses of HPV vaccine [38]. In comparison to HPV52 and HPV58, for these cross-protective types we observed slightly higher antibody avidity; however the exact implication of this finding is unknown.

The lack of correlate of protection [4, 5] emphasizes the need for monitoring the (long-term) effectiveness of a two-dose schedule against virological and disease endpoints. A review of Basu et al. concluded that studies embedded in the population-based screening programs of different countries indicated reduced efficacy of two doses against virological and disease endpoints. [7] However, in these studies the interval between the first and second dose in the two-dose schedules was not taken into account. In contrast, similar effectiveness against genital warts after two doses compared to three doses of the quadrivalent vaccine has been shown with at least four months between the two-doses. [39-41] It should be
taken into consideration that these observational studies so far, have been carried out among women who were eligible for a three-dose vaccination schedule, but received two-doses. Monitoring of routinely two-dose HPV vaccinated cohorts with regard to effectiveness is currently ongoing or in design in several countries. Results from these studies will give insight into the impact of two-dose schedules.

We observed disagreement between the vaccination status obtained from the vaccination registry and the self-reported vaccination status (number of doses) in the questionnaire by three participants. Previous studies have shown that registry-based vaccination status is the most reliable.[42, 43] Therefore, we based the vaccination status of the participant on the vaccination registry in the main analysis. However, to explore the influence of possible errors in vaccination status sensitivity analyses were performed, in which only participants with consistent vaccination status in both the vaccination registry and the questionnaire (self-reported) were included. This sensitivity analysis showed slightly higher GMCs, but with the same pattern over time. Comparable results were found regarding the avidity.

In this cohort of girls who were members of the first cohort routinely vaccinated with a two-dose schedule of the bivalent HPV vaccine in the Netherlands, results of 24 months of follow-up are available at this time. Up to this sampling moment, none of the participants have dropped out and 49 out of the 56 girls (88%) participated completely in all three samples. This strengthens the further follow-up of this first cohort eligible for two-dose HPV vaccination in the Netherlands, which is planned up to at least 60 months.

CONCLUSION AND RECOMMENDATIONS

High antibody levels, with high avidity, against vaccine-types HPV16/18 were observed up to 24 months of follow-up in girls routinely vaccinated with a two-dose schedule. The high antibody avidity increased over the two years. In combination with other immunogenicity and effectiveness studies, our results are reassuring with regard to long-term immunogenicity of the two-dose schedule against vaccine types. Antibody levels as well as avidity were considerable lower for HPV-types 31, 33, 45, 52 and 58, although the avidity remained stable over time.
However, implications of these findings in absence of a correlate of protection are unknown, in post-hoc analyses effectiveness against infections by HPV31/33/45 are comparable to a three-dose schedule. Further follow-up of this cohort will provide insight into antibody and avidity kinetics over a longer time-period to ensure long-term protection.
REFERENCES

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