

SUMMARY

Human Papillomavirus (HPV) is the most common sexually transmittable infection worldwide and is involved in approximately 5% of all cancers worldwide. In over 99% of the squamous cell carcinomas of the cervix a persistent infection with oncogenic or high-risk HPV types is the causative agent. Since 2006, prophylactic HPV vaccines have been introduced in order to prevent HPV-related diseases. Many countries have introduced the HPV vaccine in their National Immunization Program. In 2010, the Netherlands has introduced routine vaccination for 12-year old girls with the bivalent HPV16/18 vaccine (against HPV16 and 18). In 2009 a catch-up campaign was performed offering vaccination to girls born between 1993 and 1996. Originally the vaccine was given in three-dose schedule at 0, 1 and 6 months. From 2014 onwards a two-dose schedule was implemented (0 and 6 months). This thesis describes research on monitoring activities with regard to the effectiveness and immunogenicity of the Dutch HPV vaccination program. **Chapter 1** gives a general introduction about HPV, its role in carcinogenesis, epidemiology and preventive strategies, with a focus on HPV vaccination.

Part 1: Effects of HPV vaccination from a monitoring perspective

Alongside introduction, the Dutch Health Council advised for close monitoring of the HPV vaccination program. Several studies have been performed since, of which some are addressed in this thesis. Important question at the implementation of HPV vaccines in immunization programs was the long-term population effectiveness of the vaccines. Part I of this thesis covers studies among girls born between 1993 and 1996 eligible for the three-dose catch-up campaign in 2009/2010. The HPV Amongst Vaccinated And Non-vaccinated Adolescents (HAVANA) cohort study aims to follow-up the immunogenicity and effectiveness of the three-dose HPV vaccination schedule among girls eligible for the catch-up HPV vaccination program. In **Chapter 2**, using data from the HAVANA study, we estimated the vaccine effectiveness against incident and 12-month persistent infections up to six years post-vaccination. Nearly full protection (97.7% (95%CI 83.5-99.7%) against HPV16/18 persistent infections of was found. Also, an effectiveness against HPV31/33/45 of 61.8% (95%CI 16.7-82.5%) was observed. No indications were found for waning of protection up to six years post-vaccination for both vaccine and cross-protective types (HPV 31/33/45).

Concerns that HPV vaccination might lead to riskier sexual behavior were raised at the implementation of HPV vaccination. **Chapter 3** describes results from a longitudinal questionnaire study among girls eligible for the catch-up vaccination with a follow-up of two-years. Overall, we did not find indications that vaccination influenced sexual behavior differentially between HPV-vaccinated and unvaccinated girls during our two year follow-up. The few differences found may be related to existing disparities in the socio-demographic characteristics of this young population. Our results also point to the importance (and improvement) of education with regard to safe sex practices. Our findings do not suggest that vaccination status is associated with changes in sexual risk behavior and thus it is unlikely that this might influence the effectiveness of the vaccination program.

Part 2: Monitoring the introduction of the two-dose HPV vaccination schedule

Based on recommendations of the European Medicines Agency, the Dutch Minister of Health, Welfare and Sports decided to change to a two-dose vaccination schedule in January 2014. The two-dose schedule (0,6 months) was directly implemented. Studies evaluating and monitoring the two-dose HPV vaccination schedule are covered in Part II of this thesis. At the time of implementation available evidence on immunogenicity of two- compared with three-doses was systematically reviewed, this review is described in **Chapter 4**. This systematic review showed non-inferior antibody concentrations of two- compared with three-doses for the quadrivalent vaccine up to 36 months and up to 48 months for the bivalent vaccine comparing two-doses in girls to three-doses in young women. However, comparing girls within their own age group not always resulted in non-inferior immunogenicity. We confirmed these findings using Dutch data from the HPV2D-study in **Chapter 5**. Girls, eligible for three-dose vaccination (birth cohorts 1997-2000), who had received three- or two-doses (0,6 months) of the bivalent vaccine up to 4 ½ years previously, were compared on antibody concentrations and avidity. Non-inferior antibody concentrations for the vaccine and cross-protective types, for HPV18/31/33/45 were only found at 2-3 years post-vaccination. Non-inferior antibody avidity for vaccine and cross-protective types was observed comparing three- with two-doses up to 4 ½ years vaccination, independent of antibody concentrations. **Chapter 6**, a review up to the fall of 2015, shows comparable results with regard to antibody levels and avidity in two- and three-doses vaccinated girls as in Chapter 4 and 5. Data with regard to T-cell formation after a two-dose schedule (0,6 months) was inconclusive. B-cell

memory seems to be mainly influenced by age and not by dose. Comparable B-cell responses were found in girls 9-14 years of age receiving either two- (0,6 months) or three-doses of vaccine. For HPV18 a better B-cell response was observed among the younger recipients in general. Effectiveness studies on genital warts, HPV infections and cervical lesions performed so far, indicated in some cases a slightly lower effectiveness for a two-dose schedule. However, results so far arise from cohorts eligible for three-dose vaccination and methodological challenges arise when using these studies. Vaccinated girls from the first birth cohort (2001) eligible for routine vaccination with the two-dose schedule in 2014 were followed up with regard to immunogenicity. In **Chapter 7**, high antibody levels and high avidity against vaccine types were shown up to 24 months after vaccination.

Part III: Methodological challenges in monitoring of HPV vaccination

Part III of this thesis covers the methodological challenges encountered when monitoring the HPV vaccination program. At this moment, no clear guidelines are available for the use of non-inferiority margins in vaccine studies. By means of the systematic review described in **Chapter 8**, it was found that most studies used the implicitly recommended margins of a difference of 10%, or a geometric mean concentration/titer of 1.5/2.0. Explanation with regard to the margin was lacking in most articles reporting on non-inferiority vaccine studies. A framework for defining the non-inferiority margin in vaccine studies was suggested, as it is debatable whether different vaccines should all use the same non-inferiority margin.

Various statistical approaches are available to analyze data from longitudinal studies evaluating effectiveness against persistent HPV infections. In **Chapter 9**, we have examined these different statistical methods. The aim was to get insight into differences between available statistical methods. Also, the impact on VE estimations was compared by applying these methods to data of the HAVANA study. Although the underlying assumptions between these methods differed, quite comparable estimations for the effectiveness against HPV16/18/31/33/45 persistent infections in young women eligible for routine HPV vaccination were found. Based on the satisfaction of assumptions and the use of all available data the Prentice Williams Peterson Total-Time approach was suggested as most valid approach.

Part IV: Future perspective

Since introduction of HPV vaccination, knowledge on the role of HPV in non-cervical cancers has increased. In **Chapter 10**, the role of including non-cervical HPV-associated diseases in cost-effectiveness analyses for HPV vaccination was explored. Decreases up to almost three (girls only programs) or four (gender-neutral programs) times were found when including these diseases in the Incremental Cost-Effectiveness Ratio (ICER). These lower ICERs make it more likely for programs to fall beneath the cost-effectiveness threshold, enlarging the possibilities for large scale (gender-neutral) vaccination programs.

Chapter 11 is the general discussion of this thesis. In this discussion the most important findings from this thesis are elaborated, in relation to the current Dutch vaccination program and future perspectives. Important considerations with regard to HPV vaccination in the upcoming years will be the possibility of gender-neutral vaccination, the challenge of suboptimal vaccination coverage, whether one dose of vaccine might be enough and the confluence of vaccinated women into the cervical cancer screening program.