Natural and vaccine derived immunity against the human papillomavirus

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CHAPTER 7

GENERAL DISCUSSION

HPV is the most common sexually transmitted infection worldwide, and its presence is a necessary condition for the development of cervical cancer. Besides cervical cancer, HPV-infections are also known to cause other cancers situated at the anogenital or head and neck region, resulting in approximately 5% of all cancer cases worldwide. Most HPV infections are transient, and only 3-5% will eventually lead to (cervical) cancer in a long period of several decades, when no therapeutic intervention is performed. HPV vaccination was implemented in the National Immunization Program of the Netherlands in 2009/2010 and in that of the Caribbean Netherlands three years later. Both with the primary goal of reducing cervical cancer. This thesis described a series of studies on the immunogenicity of natural HPV infections and vaccine induced immune responses in the Dutch population. Here, I will elaborate on the most important findings from this thesis, describe their potential value and impact as well as speculate on future perspectives.

SUMMARY OF MAIN FINDINGS

In **chapter 2,** we assessed the possible changes in HPV-seroprevalence among the HPV unvaccinated Dutch population due to HPV vaccination by comparing the HPV seroprevalence in the pre- and post-vaccination era. This revealed an increase of high-risk (hr) HPV types exposure in unvaccinated women and a rather stable seroprevalence in men. For HPV16 a decrease in seroprevalence was found among men, that is unlikely to be due to herd immunity as we measured this at a rather short time frame after vaccine introduction in a period with a suboptimal vaccine coverage.Both the incidence and the mortality of HPV-related diseases differs geographically. Information on the HPV serostatus on the Caribbean Netherlands is not available yet. We performed a representative sero-surveillance study on these islands and described the results in **chapter 3**. We provided insight into population-based HPV serostatus, on lifetime cumulative HPV exposure and in past infections. A three-fold higher prevalence of multiple hr-HPV types was found among the female population when compared to men.

We further focused on studying the immune response in HPV vaccinated individuals. In **chapter 4** we described that HPV-specific antibody levels remain high and persistent for both vaccine and non-vaccine types. We observed this up to nine years postvaccination of a three dose vaccination schedule with the bivalent HPV vaccine in Dutch girls vaccinated at the age of 16. Although high antibody levels are thought to be important in the protection against an HPV infection, a correlate level of protection is lacking. Our study on the longitudinal relationship between antibody levels and HPV infections revealed no consistent differences between these two.

In 2014, the HPV vaccination program changed from a three-dose to a two-dose schedule. In view of even further reduced dosing schedules, we studied the humoral and cellular immune response after just a single dose of the bivalent HPV vaccine and compared this with a two- and three-dose schedule in **chapter 5**. One-dose of the bivalent vaccine resulted in elevated HPV-specific antibody levels up to seven years post vaccination. The levels of antibodies, however, were lower when compared to those induced by the two- or three-dose schedules. This lower antibody response coincided with lower numbers of memory B- and T-cells in one-dose recipients. This possibly indicates that that girls receiving one-dose might be at higher risk for waning protection to HPV in the long-term.

Currently there are three prophylactic HPV vaccines on the market, and several studies show that the immunogenicity of the bivalent vaccine is higher when compared with the quadrivalent and nonavalent vaccine. In **chapter 6**, we studied the in-depth kinetics of innate and adaptive cellular responses directly upon vaccination for the first time in a head-to-head comparison between the bivalent and nonavalent HPV vaccine. We observed strong monocyte responses the first day after primary vaccination, which were most pronounced in the bivalent vaccinated women. Also a clear expansion in plasma cells was observed in both cohorts in the first week after primary vaccination. HPV-specific antibody levels and memory B- and T cell responses were higher in the bivalent vaccinated women, with the exception of HPV31 and-45 specific antibody levels.

GENERAL DISCUSSION

Population based HPV seroprevalence

At a population level sero-surveillance studies, provide us information on clustering of groups of persons susceptible for HPV infection by risk factors and the impact of bivalent vaccination on the prevalence on other HPV types and herd immunity in men and unvaccinated girls.

HPV that enters the host by naturally occurring infection is capable of evading the immune system [1, 2], through the non-lytic life cycle of HPV and limited antigen presentation to lymph nodes. This results in limited but stable antibody production and seroconversion of only 40-60% of infected individuals [3-5]. HPV seroprevalence studies can be used to estimate the lifetime cumulative HPV exposure and experienced infections, whereas HPV DNA tests only detect clinical infections currently present. HPV seroprevalence studies are also easier to perform than HPV DNA studies by using vaginal DNA swabs. However, due to the limited immune response induced by HPV infection, HPV seroprevalence studies in naturally infected individuals do not represent an accurate measure of the number of infections, but actually underestimate them.
In **chapter 2**, we have observed that HPV seroprevalence among HPV unvaccinated women has increased in the Netherlands during the last decade. As we adjusted the results for demographic and/or sexual risk behavior, these factors cannot explain this increase. This may indicate that either the used questionnaire is not representative for the risk assessment for HPV seropositivity, or they were not answered in full honesty to intimate questions due to a social desirability bias, e.g. questions related to sexual behavior had the highest percentage of missing values. In men, HPV seroprevalence remained similar in this time period and for HPV16 even a decrease was found, which might be explained by herd immunity. However, in the specific age group (15-39 year old's) of men which most likely would benefit from girls-only HPV vaccination this decrease was not significant anymore, suggesting that herd immunity is unlikely. These findings were in agreement with observations in the United States, where just as in the Netherlands, there is a suboptimal vaccination coverage and no signs of herd immunity on the male population was observed after introduction of the HPV vaccine [6]. In contrast, the high vaccination coverage (>90%) in Australia resulted in herd immunity, impacting both (unvaccinated) males and females already at five years after the introduction of HPV vaccination [7, 8]. Thus the reason for a lack of herd immunity in the Dutch male population is probably due the suboptimal vaccination coverage of about ~ 50%, and in a lesser extent due to the short time period after introduction of HPV vaccination.

The incidence and mortality of HPV related diseases differ geographically [9], which can be largely explained by the presence of organized prevention programs, like screening and vaccination. In Caribbean countries, where vaccination and cervical cancer screening are mostly lacking, a higher incidence and mortality rate is observed when compared to the world average. The Caribbean Netherlands, comprised of the islands Bonaire, St. Eustatius and Saba (BES-islands) are public entities of the Netherlands. On the BES-islands, HPV-vaccination with the quadrivalent vaccine has been implemented in 2013 on St. Eustatius and Saba, and the bivalent vaccine on all three islands from 2015 onwards. So far, a population based screening program have not been introduced on these islands. As no data on protection against infectious diseases and associated risk factors were available for these islands, we have performed the Health Study Caribbean Neth-

erlands (HSCN) to monitor the National Immunization Program (NIP) (**chapter 3**). In **chapter 3**, we have described high HPV seroprevalence rates in the unvaccinated population, as described by other studies in the region [10], being highest in females in whom nearly three-times higher rates have been observed than found in men. These findings can be used as background information for future policy guidelines, since HPV vaccination is an outstanding method to be able to prevent cervical cancers on the long run. On the short term however, we speculate that cervical cancer screening has to be introduced on the BES islands, that certainly will reduce mortality due to cervical cancer.

To get a better understanding of the impact of the HPV-vaccination program, both in the Netherlands and on the BES-islands, it is of interest to continue the monitoring of the HPV seroprevalence and sexual behavior among the population every 10 year. In this way information can be generated about herd immunity, possible type replacement (HPV types not included in the vaccine) and changes in sexual behavior and which groups are at highest risk.

HPV seropositivity was higher in women than that in men in three consecutive population based studies (PIENTER) performed in 1995, 2006 and 2016 (**chapter 2 and 3**), that was in line with other population studies [11-15]. These differences between men and women are unlikely to be explained by different infection rates, since the similar infection rate between men and women was found in HPV DNA prevalence studies [16, 17]. The difference in HPV seropositivity between men and women is most likely caused by the different sites of virus infection. These are anatomically comprised of different epithelium types present at the penis and vulva/cervix, which are comprised out of either columnar or squamous and/or columnar epithelium respectively. The transformation zone in the cervix, where the squamous epithelium progressively undermines and replaces the columnar epithelium, displays metaplastic activity forming a risk for infection [18]. It is suggested that here HPV virions can easier reach the basement membrane and, therefore, are better capable of establishing a viral infection. The transformation zone also has the unique characteristic of facilitating pathogen recognition by the immune system, leading to a better HPV-specific immune response [19]. This could be an explanation to the observed differences in HPV seropositivity between men and women [3, 20-22]. This transformation has also been described in the anus, oropharynx and esophagus [23-25]. Therefore, if in the future questionnaires about sexual behavior would be extended with questions about vaginal, penile, anal and/or oral sex, we might get a better understanding of the role of the sites of entry of infection and corresponding seroconversion. It is important to note this information is accompanied with information about if this person is receptive and/or insertive during sexual intercourse.

We also observed HPV specific antibody levels in infants and young children, albeit being close to cut-off levels. In our and other population based studies [13, 15], these low levels were found up to the age of about nine years. There are various explanations for the presence of these antibodies in sexually naïve children. It is suggested that HPV-specific antibodies in infants are probably maternal IgG antibodies, however, since these antibodies wane in a few months after birth this explains it just partially [26, 27]. Infants could also be exposed to the virus via vertical transmission, for instance via the HPV infected genital tract of mothers during birth [28] or via an infected placenta or infected leukocytes cord blood [29]. Also horizontal transmission can occur, e.g. via breast-feeding [30] or oral/mucosal contacts [27, 31-33]. These data support the idea/

hypothesis that an HPV infection can be acquired early in life, possibly affecting an infection later in life. HPV-specific immunity in children is an unexplored area, as most focus is laid upon studying cervical infections in women. Just a few studies looked into the dynamics of HPV-specific immunity in children, showing that this was not related to the mothers HPV infection status. Especially oral infections were found to be most likely to occur in children [27, 34, 35]. This could lead to the induction of oral tolerance, or even a higher susceptibility for HPV exposure later in life but this still needs to be determined. However, in general the observed antibody levels in children are very low, and turned out to have no neutralizing capacities [36]. Presumably this will not interfere with vaccination later in life, as HPV vaccines are highly efficacious (~100%), meaning that this will also be the case in pre-exposed children.

HPV-seropositive associated risk factors were especially linked to sexual behavior, amplifying the need for prophylactic vaccination given before sexual debut. Our data supports the current advice of the Dutch Health council to vaccinate at an age of nine, as HPV seropositivity in the Dutch population begins to increase markedly after ten years of age.

In our studies we expressed antibody levels for HPV16 and 18 in international units (IU) per ml, according to an international reference serum for standardization of antibodies. This a prerequisite for comparisons and validation of various (population) serology studies, as currently different antibody detection techniques and associated cut off levels are being used. Adding of the current international reference serum for types 31, 33, 45, 52 and 58 is needed to make a broader comparison of differences in antibody levels to the several HPV-types in various laboratories worldwide and in follow up studies over time. The cut-off levels used in our studies (**chapter 2, 3 and 4**) to define seropositivity were determined by using a serum panel of children 1-10 years of age, assuming that they are predominantly HPV negative as they are sexually naïve. These cut-off levels are a fixed value per HPV-type, although the precise sensitivity and specificity is unknown by this method and could therefore be prone to misclassification bias. Other methodologies for instance by means of a mixture model could provide a more flexible approach, as it not defines one fixed cut-off value, until more is known of the serological response to HPV infection [37]. It is also argued to use sex-specific cut-off values [14, 38] as the infected epithelium differs. However, this only holds true if men would have only penile intercourse and not having anal intercourse. It has been described that the serological response upon anal infection is similar to that induced by a vaginal infection [20, 22]. Moreover, differences regarding HPV seropositivity that are present between men and women could be reduced and are perhaps not detectable anymore.

To get a better understanding in HPV-seropositivity, it is important to get a better insight in the immune response upon HPV infection. Most HPV infections are thought to be cleared or controlled to undetectable levels by the hosts immune response, whereas these infections persist in some individuals. A persistent (hr)-HPV infection is the major risk factor for the development of cervical cancer [39]. As described in **chapter 1**, HPV has several mechanisms to evade the host immune system, being an important step in persistence, but we do not know why this occurs just rarely. The first line of defense against infections is performed by innate immunity pathways and an efficient triggering of this innate response is a turning point between either viral clearance or virus persistence [40, 41]. Keratinocytes are a target for HPV infection and due to their expression of pattern-recognition receptors (PRRs) they can recognize microbial pathogens or damage

signals. PRRs include Toll-like-receptors (TLRs), which are capable of recognizing nucleic acids which are some of the microbial molecules that are accumulating during viral replication [42]. A high expression of TLR3, TLR7, TLR8 and TLR9 have been associated with HPV elimination, and are suggested to be predictors of HPV16 infection-clearance in women [40, 41]. Hr-HPV impairs important signaling pathways, like NF-kB and interferon-regulatory factor. This contributes to viral immune evasion and virus persistence [43-45]. In addition, polymorphisms in IL-1β, also affecting adaptive immunity [46], like IL-18 and inflammasome-related genes (NLR1 and NLR3) [47, 48] but not TLR9 polymorphisms, despite being a DNA sensor, were found to be associated with either viral clearance or persistence.

Thus, an inefficient innate immune response, thereby giving incorrect signals to the adaptive immune system, can lead to HPV viral persistence and eventually tumor progression. Why this innate immune response is different among individuals remains unknow so far. Explanations could lie in (epi)genetic changes, which have been observed for instance in transforming CIN lesions (reviewed in [49-52]), and deviations in host cell genes could accumulate over time being necessary for progression into cancer. Investment in studies to unravel this could help to elicit efficient therapies in HPV-related infections and tumors. On the other hand, the type of cells that are infected by HPV could be an important factor in progression towards lesions and cancer. Cells at the squamocolumnar junction (SJ) of the cervix have a unique gene-expression profile and biomarkers of these genes are highly present in high-grade CINs and cervical cancers[53]. Further research in exploiting the SJ phenotype can help us to better understand the risk in (early) cervical neoplasia.

Current Dutch vaccination program: bivalent vaccine in a two-dose schedule

The Netherlands has implemented HPV vaccination in their NIP in 2010 for 12 year old girls, together, with a catch up campaign which was offered to girls born between 1993 and 1996 in 2009. From 2014 onwards, the vaccination scheme changed from a three-dose schedule to a two-dose schedule. After implementation of a new vaccine into the NIP, its impact on the population is being monitored. Several factors can influence this impact, like 1) vaccination coverage, 2) the duration of the vaccine induced protection, 3) the hr-HPV types present in the vaccine, 4) rate of cross-protection and 5) the potential type replacement.

In the Netherlands, several studies are ongoing to monitor the effects on effectiveness and/or immunogenicity of the HPV vaccination program. In **chapter 4**, we describe persisting antibody responses against HPV16,18,31,33,45,52 and 58 up to nine years post vaccination. These findings were in line [54-56] with previous trials and other observational studies examining the immunogenicity of the bivalent vaccine. These high antibody responses also showed high vaccine effectiveness against incident persistent infections with HPV16,18,31,33 and 45 up to six years post vaccination [57]. No waning immunity to either vaccine type and cross-protective HPV types was observed. In several other countries observational studies also have shown a high vaccine effectiveness against infections and CIN lesions [58-61], as most of these countries start their cervical cancer screening at a younger age. In the Netherlands screening starts from 30 years onwards, consequently the first HPV vaccinated women will enter the cervical cancer screening program in the Netherlands in 2023. Cost-effectiveness models suggest that when HPV-screening is used as a primary tool, three life-time screens for vaccinated women are optimal [62]. All histological and cytological outcomes in the Netherlands are registered by the PALGA (Pathologisch- Anatomisch Landelijk Geautomatiseerd Archief) database and its linkage to the vaccine registration register (Praeventis) could be used to determine the vaccine effectiveness. This linkage is currently being done, and preliminary results showed that fully vaccinated women had a lower risk of developing atypical squamous cells of undetermined significance (ASC-US) and (H)SIL than unvaccinated women of the same age (Schurink-van 't Klooster; manuscript in preparation). The currently used HPV-based screening will still be the best method to detect cervical cancer cases, as it is not influenced by HPV prevalence in population [63].

Determining vaccine effectiveness by means of measuring persistent infections comes with several challenges. In the case of a natural infection our immune system is an important regulator in controlling HPV associated disease. The immune system either clears the infection or controls it by keeping it at a low copy number, thereby becoming latent [64]. Viral latency can be caused by an infection that did not reach a sufficient amount of viral load to be able to trigger the immune system. Latency can also be represented by an infection that is detected by the immune system, but subsequently not completely cleared. When the immune pressure subsides, the virus can reactivate. An infection could also become intermittently positive due to detection of a certain genotyping assay, while a more sensitive assay could detect a consistent persistent infection [19, 65]. Therefore it is difficult to differentiate between HPV infections that are latent or those that are simply below the level of detection of a certain test. Clinically validated genotyping assays, like the GP5+/6+ broad-spectrum PCR, consider the infections below their level of detection as not clinically relevant. This underlies the need that effectiveness measured with regard to CIN

and (cervical) cancer cases is of importance and must be awaited until 'real' effectiveness of the HPV vaccines to be established. Another challenge in determining effectiveness of HPV vaccination is with respect to other HPV related cancers, as mostly only cervical smears are sampled and not anal or oral samples. Efficacy of vaccination against anal intraepithelial neoplasia has been determined, and is as high as observed in CIN lesions [66, 67].

Measuring persistent infections requires longitudinal studies with standardized participation and follow-up, which is difficult to establish. For instance in the Netherlands, we achieved participation rates of about 10-20% for these type of studies. In the HAVANA study, written in **chapter 3**, a loss to follow-up was approximately 40%, being the biggest in the first two years of the study. Afterwards this follow-up became more stable with almost no loss of participation in the past years of follow-up. Although this possibly could lead to selection and/or habituation bias.

Male vaccination

Although still most of the HPV disease burden is caused by cervical cancer, HPV is also related to other morbidities affecting both men and women. These are less common than cervical cancer, but still result in various degrees of morbidity, mortality and costs. Even if all women were immunized, the transmission of HPV would still occur and be maintained among men who have sex with men (MSM).

Immunization of the male population will, besides to direct protection in males, result in a reduction of the risk of females being infected, through herd immunity. Sex-restricted vaccination demonstrated lower effectiveness, compared to universal vaccination [68]. Cost-effectiveness studies have shown that male vaccination is most cost-effective when female coverage is low. In contrast, a Dutch modeling study suggested that the most effective reduction of HPV infection is through increasing the uptake among girls, which is currently ~50% in the Netherlands, rather than including boys in existing programs [69]. Although this might be true, various approaches in communication strategies in the Netherlands have been conducted over the past years and numbers of vaccinated women are only slightly rising. Therefore, the introduction of male vaccination could benefit the Netherlands and significantly reduce disease burden because this might have a positive effect on the uptake by girls, as the focus would shift more towards HPV-related cancers than just cervical cancer. Target vaccination of only MSM, which will be at an older age, is likely to be less effective than vaccinating boys, as the prevalence of HPV among MSM is already high, especially at the anal site, at the time of vaccination [70] thereby being less effective. The MSM group will not benefit of female HPV vaccination. These data collectively argue for sex neutral vaccination. The Dutch Health Council advised to implement this from 2021 onwards [71].

In the Netherlands, potential side effects and sexual health aspects, believing that the girls were too young, of the vaccine were predominated in the HPV vaccine hesitance [72][73]. Other factors that could help to further increase the vaccination coverage is communicating honest about uncertainties and risks of vaccines and being transparent about how decisions are made within the NIP[74], enables parents to make an informed decision. Plain language must be used throughout communication to the public, as its wording, structure and design are so clear that the intended audience can easily find what they need, understand it and use it [75].

Monitoring of this introduction of sex-neutral vaccination is of high importance, as it is clear that vaccination can prevent cervical and anal cancer. If such a vaccination program would also prevent other HPV-associated cancers, like OPC, is still unknown as this has not been determined yet. Most OPCs are caused by HPV16. This type is present in all vaccines and effectively prevented anogenital diseases. It is suggested that this works just as efficient in OPCs, as in HPV vaccinated women a decrease in detection of HPV types is observed in the oral cavity [76-78]. However, clinical trials evaluating vaccine efficacy have been hampered by lack of data regarding incidence and clearance rates of oral HPV infections [79]. Persistent HPV infections result less often and slower to OPCs than cervical cancers. Therefore, more people and a longer follow-up time is needed to study the efficacy against OPC, making these trials highly expensive thereby hampering their initiation. The lack of an early clinical endpoint, since there are no well-defined oropharyngeal precancer lesions, also is a problem. However, initial studies suggest that vaccination might be effective in OPC [79, 80]. The presence of HPV16 E6 antibodies up to 10 years before cancer diagnosis is now suggested to be a biomarker, and could perhaps be used as an early marker to determine vaccine efficacy against OPC, as no precursor lesions are currently known [81].

One dose

Efficacy

Reduced dose HPV-vaccination schedules are of great interest in respect to the global health HPV burden, as this will reduce costs and simplify logistics. It will then become easier to reach women who are at the greatest lifetime risk of cervical cancer, and who are currently not being vaccinated [82]. Post-hoc analyses of original vaccine trials and population effectiveness studies among women who did not complete the full HPV vaccination scheme have suggested that one dose of the HPV vaccine is effective [83-86]. The protective effect against HPV16/18 infections was comparable, both for the bivalent and quadrivalent vaccine [59, 83, 85, 86]. However, cross-protection was observed after two and three doses with the bivalent vaccine, but not after one injection [59, 83], suggesting that an one-dose vaccination has a more limited efficacy to cervical cancer in general compared with two-or three doses.

Biological plausibility

The biological plausibility that HPV vaccines could be effective given in single dose is both supported by immunologic and virologic factors. The antibody levels detected against the vaccine targeted types are higher in most one dose recipients when compared to naturally infected individuals (**chapter 5**). This immunogenicity is largely attributed to the structure of the HPV vaccine antigen. HPV VLPs are composed of 360 ordered protein subunits forming a repetitive array of epitopes of 55nm on their surface. The interaction of these repetitive elements with B cell receptors on naïve B cells is exceptionally strong and leads to a consistent activation of memory B cells and long lived plasma cells (LLPCs) continuously producing antibodies for many years. Epitope spacing of 50 to 100Å appears critical for this, together with efficient trafficking to lymph nodes and efficient phagocytosis by antigen presenting cells. This leads to a potent and long lasting immune response, more closely resembling an acute virus infection rather than a simple subunit vaccine. In addition, as an infection is characterized by slow kinetics, the vaccine-induced anti-

bodies have more time to neutralize invading HPV virus [87]. In mice experiments, the transfer of HPV-specific antibodies at levels being even 100-fold lower than detectable in *in vitro* assays of vaccinated human beings, still was sufficient to protect against a HPV genital infection in this mice model [88]. It was therefore envisioned that antibody levels after a single dose with the HPV vaccine, although antibody concentrations are approximately 4-fold lower than two- and three dose vaccinated individuals, would not impair the efficacy of the HPV vaccines [87].

Immunogenicity

Immunogenicity studies show that antibody levels after a one dose schedule are lower when compared to two or three doses of either the bivalent or quadrivalent vaccine [84, 85], but still higher than after natural infection [84]. These findings are in line with what we find in **chapter 4**. Here, we also observed that these lower antibody responses coincided with lower production of T helper cytokines and lower memory T cell numbers, confirming results of Toh *et al.*[89]. Moreover, we showed for the first time that an one dose vaccination can induce HPV-specific memory B cells up to six years post vaccination, albeit that more doses resulted in a higher number of HPV-specific memory B cells. Follow-up data should clarify whether this lower immune response is also of clinical relevance. The lack of a correlate between the levels of HPV-specific antibodies and protection against HPV hampers further discussion on the minimum levels required to protect against infection. Randomized controlled trials, designed to determine the efficacy and/or immunogenicity of a one dose HPV vaccination are currently ongoing; in Costa-Rica (ESCUDDO; NCT03180034), Kenya (KEN-SHE; NCT03675256), the Gambia (HANDS; NCT03832049) and Tanzania (DoRIS; NCT02834637)[90]. In all these trials the nonavalent vaccine is also incorporated and more data concerning the efficacy against the five additional hr-types after just a single dose is expected soon. For the bivalent vaccine it was suggested that multiple doses are necessary to get an effective cross-protection against the non-16/18 HPV types, implying that a one dose strategy would not be an option. The nonavalent HPV vaccine on the other hand, generates antibody responses against all nine hr HPV types in one single dose. Thereby also closing the gap in costs, as at the moment the nonavalent is about twice as expensive as the bivalent vaccine.

Global cancer elimination

In 2018, the WHO Director General called a Draft Strategy to eliminate cervical cancer as a public health problem. This was approved by the World Health Assembly's in May 2020. The strategy outlines that cervical cancer is eliminated if there are less than 4 cases per 100,000 women and the timeline is that this should happen within the lifetime of today's young girls [91]. One of the prominent ways to achieve this is by increasing the vaccination uptake worldwide, ideally with a catch up in adults (sex neutral) to expand the proportion of immune individuals and thereby decreasing transmission.

There are several hurdles that hamper this. There is currently a HPV vaccine shortage, which is expected to be unsolved in the next five years. Nowadays, around 52% of all countries have implemented a HPV vaccination program, corresponding to the vaccination of 30% of 9-14 year old girls [91]. Eighteen percent of the global demand is currently used for males, and this number is only rising as more and more countries are implementing sex neutral vaccination. This percentage is for instance equal to no implementation in twelve LIC/LMIC. Therefore the Strategic Advisory Group of Experts (SAGE) of the WHO advised to postpone vaccination programs of

boys/men from 15 year old's onwards, thereby relieving the supply constraints in the short term and enable allocation in the countries with the highest HPV disease burden. Indeed, I agree with this that in the current situation of vaccine shortage, priority must be given to vaccinate women in LIC//LMICs with the highest burden of disease and most lives to be saved.

Another hurdle is the current global delivery infrastructure, especially in Sub-Saharan countries, making it not possible to vaccinate everyone and everywhere or at very high costs. HPV has for instance to compete with infectious diseases, like malaria and polio, for the global (research) funds.

Solutions to relief the current vaccine shortage, could be pausing the sex-neutral vaccination and catch up campaigns. This must however be done with extreme care, as it could help anti-vax audience to grab this as an opportunity to claim that there is something wrong with the vaccine. Therefore a delay in the introduction of a sex-neutral vaccination, rather than to pause a current program, might be better. HIC could argue that the HPV-related diseases in their countries, which is for instance the case in the United States is equal between men and women. This would lead to inequity on a country level, but not on a global level. As still 85% of all HPV-related diseases concerns cervical cancer. In addition to HPV vaccinations to prevent cervical cancer, cervical cancer screening programs also add to the prevention of cervical cancer by screening and treatment of pre-cancerous lesions. If we would only use the strategy of vaccinations, this would result in a 0.1% reduction in cervical cancer mortality in 2030 [91]. Combining both strategies, by scaling up of screening and treatment of pre-cancerous lesions would speed up this process. This would result in a reduction in mortality by cervical cancer of over 30%, in the same period of time [92]. In my view HIC are also better capable of affording and arranging a screening program, giving LIC/LLMC priority to vaccinations.

Another solution tackling both hurdles could be implementation of an one dose vaccination, as described in **chapter 5**, which will not contribute to further vaccine shortage. In my view this best can first be implemented in a HIC, with the argument stated above that here better monitoring and screening programs are in place, providing a 'safety net' if breakthrough infections would occur. If efficacy is then determined, one dose vaccination could simplify logistics and reduce financial costs.

We could also think of a way to save antigens and thereby relieving current supply constraints, by delivering the HPV vaccines in another way. All the HPV vaccines are currently administered by an intramuscular injection, but an intradermal injection might could save antigens, about to 1/5 up to 1/1000 of the current dose, which for instance has been done for the yellow fever and influenza vaccine [93-95].

So is this call for global cancer elimination rather a political statement instead of being realistic? My viewpoint is that as long as there is a vaccine shortage, there is not one perfect way to tackle all HPV related diseases. The global community therefore first has to decide with what aim we vaccinate; do we protect the individual or do we want to protect the population? Therefore to declare elimination seems a plan which is currently far from possible. Nevertheless, this does not mean there is no time for a combined action of vaccination and cervical cancer screening.

The perfect HPV vaccine

All currently licensed HPV vaccines, Cervarix, Gardasil and Gardasil 9, are very immunogenic and demonstrated to be highly effective [96, 97]. For all three VLP-based vaccines, formulation with adjuvants is essential to generate an effective immune response [98]. Adjuvants, like the classical aluminum salts are used in all three HPV vaccines and the AS04 adjuvant is also added in the bivalent vaccine. AS04 has the ability to stimulate TLR4, claiming to enhance APC maturation and a Th-1 mediated response [99]. For vaccine type-specific antibody levels, AS04 has proven the ability to induce higher responses than formulations only containing aluminum salt (**chapter 6**) [100, 101]. In **chapter 6**, we observed stronger HPV16 and HPV45-specifcic memory CD4 Th1 cell responses after vaccination with a AS04 adjuvanted vaccine. This may explain why the use of the bivalent vaccine results in higher antibody levels, as the interaction between HPV-specific CD4 Th cells HPV-specific B cells is required for B cell expansion and plasma cell formation. Plasma cells are responsible for the production of antibodies.

It is debated whether the AS04 adjuvant is responsible for the observed cross-protection in bivalent vaccinated individuals. Although this is observed in several studies [102, 103], the underlying mechanism remains unclear.
Another difference between the bivalent and the quadrivalent/nonavalant vaccine is the different L1 expression systems that are used to produce the L1 proteins for the HPV vaccines. A baculovirus expression vector system is used to produce the bivalent vaccine VLPs. These VLPs display important conformation-dependent neutralizing epitopes, such as U4, V5 and J4, thereby resembling the native virions in a close manner[104, 105]. Also the shape of the VLPs were found to be more consistent [106], when compared to the quadrivalent and nonavalent vaccine VLPs which are produced using a yeast expression system [107]. Potentially, this forms an alternative or additional explanation for the high antibody levels observed when the bivalent vaccine is used. It would therefore be interesting to study whether the VLPs formed in a yeast expression system together with the AS04 adjuvant would give the same immune response, as the VLPs formed in a baculovirus with AS04. At the same time, it is interesting to study which immune response is formed if the VLPs formed in a baculovirus are only adjuvanted with aluminum. In this manner, the impact of the adjuvants on the observed cross-protection of the vaccine may be revealed.

The limited amount of targeted HPV types in the bivalent and quadrivalent vaccine, is currently almost completely tackled by the nonavalent vaccine. Anogenital warts also have a large influence on the quality of life, impacting emotional well-being and sexual health [108] and its prevalence has been increasing in the Netherlands. Even after treatment, recurrences are high, leading to high treatment costs [109]. It is even stated that introducing a vaccine including type 6 and 11 in the NIP would lead to a more favorable cost-effectiveness of the vaccine [110]. Literature regarding the effect of the bivalent HPV vaccination against HPV6 and 11 infections and anogenital warts has been equivocal. There is no definitive answer as some studies find evidence for an effect [111-115], while others do not [116-120]. Although anogenital warts are not life threatening, considering to also include the protection of genital warts in the NIP could significantly decrease the economic burden and increase the quality of life.

Efforts are currently underway to design second-generation vaccines. Second-generation vaccines are aimed at generating a more broad, also mucosal, immune response in a more conve-

nient delivery mode. Currently, vaccines that currently have been tested in published clinical trials include purified VLPs delivered in the upper respiratory tract [121] with the aim to induce both serum IgG and secretory IgA in the female genital tract [122]. Commercial interest for this method, however, is limited as the delivery method still needs to improve to compete with subcutaneous injections. Preclinical studies with novel approaches are still at the preclinical phase. Other approaches have focused on L1 proteins expressed by existing live microbial vaccines. Cadila has generated HPV16 L1 recombinant of the Moraten Berna vaccine strain of measles virus, showing in mice induction of similar levels of measles virus antibodies as the parental vaccine strain and HPV16 antibody levels comparable to those induced after injection of purified VLPs [123]. This might lead to the addition of HPV to the current measles-mumps-rubella vaccination, which is already widely implemented, even in low-resource settings. Also an attempt with a recombinant *Salmonella* typhi vaccine is made, with hopeful results in mice [124]. These attempts hold promise for low-cost production and efficient delivery.

Vaccines based on the L2 protein, which is the minor capsid protein, are also under development. Antibodies to some L2 epitopes display a remarkable cross-neutralizing efficacy against a wide array of mucosal and cutaneous HPV types. Mouse and rabbit studies show that this broad neutralization is not only an *in vitro* artefact [125-127]. Sanofi Pasteur, together with its subsidiary Shanta Biotechnics, is initiating a clinical evaluation of a multimeric L2 peptide vaccine [128]. This holds promise for a broad protection against mucosal and cutaneous HPV infections by a relative inexpensive vaccine.

CONCLUSION

In this thesis, we showed that the use of HPV serology in big population studies is of importance for monitoring the HPV seroprevalence and the effects of HPV vaccination over time. The HPV antibody seroprevalence among women has increased in a 10-year time period in the Netherlands. Among males, seroprevalence remained similar and even a decrease for HPV16 was seen. Due to the short time after introduction combined with suboptimal coverage, this effect is unlikely to already be attributable to herd immunity. In the Caribbean Netherlands, there is a high seroprevalence of multiple hr-HPV types, especially among women. This indicates that there is a relative high risk of (precursors of) HPV-related cancers, thereby underlying the need to consider routine cervical cancer screening in Caribbean Netherlands. HPV antibody seroprevalence is increasing from ten years of age onwards in both the Dutch and the CN population, justifying the recent advice of the Health Council to lower the age at vaccination from 12 to 9 years of age. There is also a significant seroprevalence among the male populations albeit being lower than in women. This together with the lack of a clear induction of herd immunity by the girls-only vaccination justifies the advice of the Health Council to implement a sex-neutral vaccination program in the Netherlands.

We also showed that the bivalent HPV vaccine is highly effective and induces robust antibody responses up to nine years post-vaccination. Having a hr-HPV type infection was however not associated with HPV antibody levels before infection, thereby suggesting that likely also other (immunological) factors are of importance in determining the correlate of protection. A hr-HPV infection was associated with sexual risk behavior and smoking one year before infection.
In view of further reduction of HPV vaccination schedules, we studied both humoral and cellular antibody responses after different doses of the bivalent HPV vaccine. We found that the one-dose schedule induces detectable immunity up to seven years post-vaccination, but resulted in fewer B- and T-cell numbers and considerable lower antibody levels compared to two- or three doses. This might implicate that some of the girls receiving only one dose are at higher risk for unprotective immunity to HPV in the long term. However, a single dose vaccination is believed to significantly reduce the global cervical cancer disease burden, thereby also simplifying logistics and reducing costs which are of great importance for developing countries. A single dose would also not further constrain current vaccine shortages. To get a better understanding in the potential implications of the innate and adaptive immune response on the long-term responses, we studied the immune responses direct upon vaccination with the bivalent and nonavalent vaccine. Here we especially observed strong monocyte responses upon primary vaccination, being most potent in the bivalent vaccinated women. A clear expansion in plasma cells was observed in both vaccinated groups, and coincided with high long term antibody levels. HPV-specific antibody levels and memory B- and T cell responses were higher in the bivalent vaccinated women, with the exception of HPV31 and-45 specific antibody levels. This could be an explanation for the stronger cross-protection of the bivalent vaccine.

Finally, in the coming years important changes are expected regarding HPV screening and vaccination. The generation of vaccinated girls will enter the cervical cancer screening program and ultimate efficacy data will be available. The effectiveness of the one-dose schedule will become clear as clinical trials come to an end. In the Netherlands, a sex-neutral vaccination will have been

implemented in the near future. These changes will need to be monitored to provide scientific answers about the effectiveness and immunogenicity. For the current girls-only routine vaccination program, which is very effective, and efforts to try to increase its coverage are needed to generate higher health benefit for the total population. This thesis contains a variety of information about the natural and vaccine induced immunity against the human papillomavirus, follow up of the studies used in this thesis should be continued to get a better understanding of the 'real-world' evidence of HPV vaccination.

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