Sexual behaviour and HPV in young women

The pre-vaccine era

Charlotte H. Lenselink

Sexual behaviour and HPV in young women

The pre-vaccine era

Een wetenschappelijke proeve op het gebied van
de Medische Wetenschappen

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. mr. S.C.J.J. Kortmann,
volgens besluit van het College van Decanen
in het openbaar te verdedigen op dinsdag 9 maart 2010
om 13.30 uur precies

door

Charlotte Henriette Lenselink
geboren op 10 maart 1978
te Amersfoort

Publication of this thesis was generously sponsored by:
GlaxoSmithKline B.V., Rovers Medical Devices B.V., Roche Diagnostics Nederland B.V.,
Sanofi Pasteur MSD, Medical Dynamics, Werkgroep Cervix Uteri

Cover design and lay out by In Zicht Grafisch Ontwerp, Arnhem
Printed by: Ipskamp Drukkers, Enschede

ISBN: 978-90-9025069-4

Promotor

Prof. dr. L.F.A.G. Massuger

Copromotores

Dr. R.L.M. Bekkers

Dr. W.J.G. Melchers

Manuscriptcommissie

Prof. dr. L.A.L.M. Kiemeney (voorzitter)

Prof. dr. A.L.M. Lagro-Janssen

Prof. dr. G.G. Kenter (AMC, VU medisch centrum, NKI-AVL)

Paranimfen

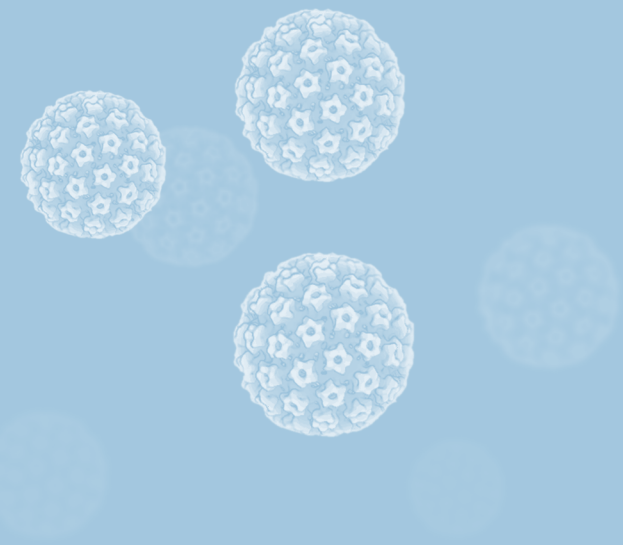
Drs. Wieteke M. Heidema

Drs. Stephanie E.M. Welschen-Wolleswinkel

Contents

Chapter 1

General introduction and outline of this thesis



Human Papillomavirus

The central aetiological role of the Human Papillomavirus (HPV) in the development of cervical cancer was discovered in the beginning of the 1980s.[1;2] Ever since, clinical, biological and epidemiologic studies have supported the causal role of infection with high-risk HPV (hr-HPV) in both cervical cancer and its high-grade non-invasive precursors.[1;3-6] However, it was not until 2005 that the World Health Organization indicated HPV as the primary cause of cervical cancer.[7]

HPVs are small non-enveloped, double-stranded DNA viruses of approximately 8.000 base pairs. The viral DNA consists of an early region, a late region, and a non-coding long control region. The non-coding control region contains regulatory elements. The early region open reading frames (ORFs), E1, E2, E4-E7, encode proteins that are expressed early in the viral life cycle. The E1 and E2 regulatory proteins modulate viral replication and transcription in the basal layers.[8] E2 negatively influences E6 and E7 expression. Three of the early ORFs are the oncogenes E5, E6, and E7, which are expressed in more distal layers. These ORFs encode multifunctional proteins that may control proliferation and transformation of the host cell. E4 encodes proteins affecting the mechanical stability of the keratin network which may facilitate viral particle release.[8] The two late region ORFs L1 and L2 encode the major and minor capsid proteins, respectively.

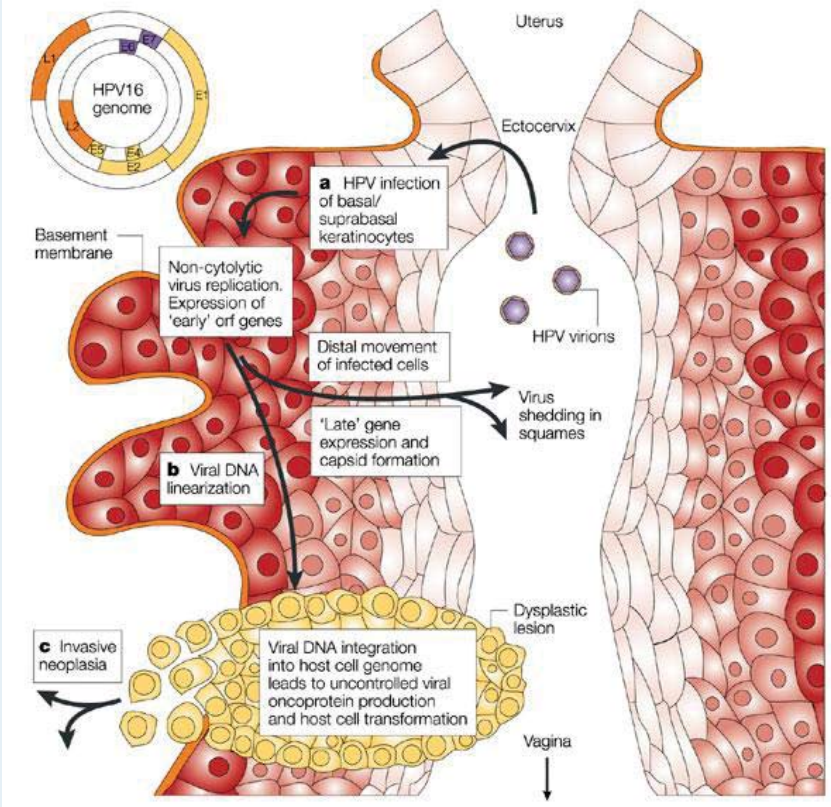
HPV can infect the epithelium when a micro-abrasion is present. Following infection, the early HPV ORFs E1, E2, E5, E6 and E7 are expressed and the viral DNA replicates. In the upper layers of the epithelium the viral genome is replicated further, E4 and the late ORFs L1 and L2 are expressed. The L1 and L2 proteins encapsidate the viral genome to form progeny virions in the nucleus. The shed virus can then initiate a new infection (Figure 1).[9] The viral life cycle is tightly linked to the epithelial differentiation programme.

The L1 ORF is the most conserved gene within the genome, and together with the E6 and E7 ORFs, it has been used to identify new papillomavirus types, subtypes and variants.[11] For the identification of a new type the L1, E6, and E7 ORFs should differ at least 10% from the closest type known. For the detection of a new subtype, a difference of 2-10% needs to be present, whereas a dissimilarity of maximally 2% indicates an intra-type variant.

Over 120 different HPV genotypes have been identified.[12-15] Approximately 40 HPV types are able to infect the genital epithelium.[16] These genotypes have been classified high risk or low risk according to their oncogenic potential.[3;14] Genotypes like HPV 6 and 11 are

Figure 1.

The biology of HPV infection. **a)** HPV virions infect basal cells of stratified mucosal epithelium at the transformation zone. In the basal layers, viral replication is accompanied by the expression of early ORFs E1 and E2. In the more distal layers, E6 and E7 are expressed promoting cell proliferation and delaying differentiation. When infected cells differentiate into squamous cells, the E4 protein, and late L1 and L2 proteins (which form the capsid) are expressed. Progeny virus is shed into the genital tract as cargo within desquamated epithelial cells. **b)** Rarely, the DNA of oncogenic HPVs linearises and integrates into the host cell genome leading to disruption of the viral E2 ORF. This induces over-expression of E6 and E7 proteins which in turn leads to host cell transformation (dysplasia). **c)** Invasive tumour ruptures the basement membrane and invades the sub-epidermal tissue. ORF: open reading frame. Adapted from R.T. Tindle (with permission).[10]



frequently detected in benign lesions like anogenital warts. These types are not related to cervical cancer development and are therefore termed low-risk (lr-HPV).[17] Whereas fourteen HPV genotypes are associated with cervical cancer development and are therefore called high-risk (hr-HPV). Of these hr-HPV genotypes, HPV 16 and HPV 18 account for approximately 70% of all cervical cancers worldwide.[14;18;19] Additionally, HPV 16 is one of the most common types found among women without a cervical abnormality.[14;20;21]

Fortunately, most HPV infections are transient and the majority of the women with a hr-HPV infection do not develop cervical cancer or premalignant lesions. In fact it is a relatively rare complication since 90 to 95% of the HPV infections are cleared by the immune-system and a large part of the premalignant lesions regress. However, women with persistent HPV infections have an increased risk of developing high-grade cervical intraepithelial neoplasia (CIN). If not treated, one third to 50% of these CIN lesions will progress to cervical cancer over a period of 10-15 years.[22]

HPV mediated carcinogenesis

The molecular pathogenesis of cancer caused by hr-HPV infections is not fully understood. Cervical carcinogenesis is a multi-step process requiring other events in addition to a persistent hr-HPV infection.[1;14] It is assumed that one of the key events of HPV–induced oncogenesis is the integration of viral DNA into the host genome.[8] It is a general assumption that this integration into the host genome leads to disruption of the E2 ORF of the virus, inducing over-expression of viral E6 and E7 proteins. Subsequently, the E6 and E7 oncoproteins interfere with two crucial mitosis regulating pathways of the host cell. The E6 protein targets the p53 protein, which normally induces growth arrest or apoptosis. The binding of E7 causes inactivation of the retinoblastoma protein (pRb) and subsequent release of host transcriptional factor E2F, which eventually disrupts cell cycle regulation. Inactivation of these two tumour suppressor pathways induces genomic instability and subsequent neoplastic transformation of the cell.[23] This may lead to an invasive tumour which ruptures the basement membrane and will invade the sub-epidermal tissue.[10]

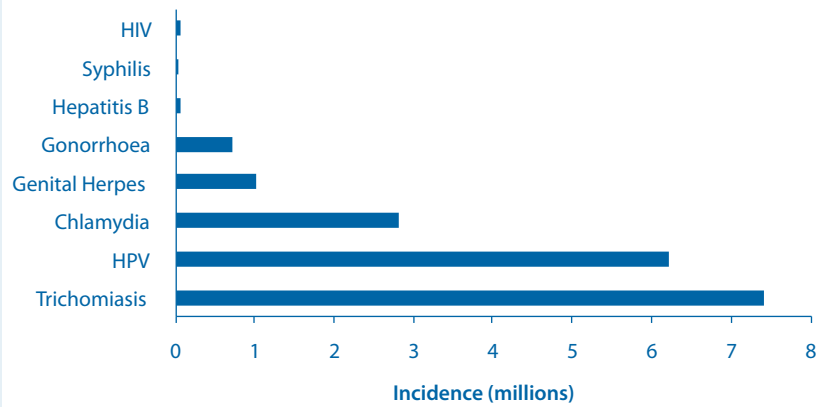
HPV epidemiology

Genital HPV is mainly but not only transmitted through sexual intercourse. HPV is well adapted to be transmitted by skin-to-skin contact and therefore transmissibility is several times higher than for other (viral) sexually transmitted diseases (STDs).[17;24;25] HPV is

commonly acquired shortly after onset of sexual activity and new genital infections are strongly associated with new and the total number of sexual partners.[26;27] As a result, genital HPV infection is one of the most common STDs among young sexually active women (Figure 2).[24]

Figure 2.

Estimated annual new cases of sexually transmitted diseases in the US in 2007 (sources: Centers for Disease Control and Prevention, www.cdc.gov, American Social health association, www.ashast.org).



Up to 80% of sexually active women have been genitally infected by one or more HPV types at some point in their life.[27;28] Estimates of single point prevalence of genital HPV infection among women worldwide vary from 2% up to 44%.[17;21;25;27-36] This wide variation may largely be explained by the sensitivity of the DNA assay used for the detection of HPV as well as by differences in age, geography or differences in other characteristics of the populations studied. The mean duration of a cervical infection in a healthy population is thought to vary between 8-13 months.[21] Most newly acquired infections are considered to be transient, at least when their duration is measured by how long the virus can be detected in cervical cytological samples.[37;38] Describing the average duration of an infection, in other words time till clearance, will be of great importance in establishing a clinically relevant definition of a persistent infection. Until now, the term "persistent infection" has often been defined loosely and in general has been described as a positive test on more than 2 occasions with intervals ranging from 2 months to 7 years, with a median of 6 months.[4;6;9;21] Definitions of HPV persistence are further complicated by differences in HPV detection methods, non-type-specific versus type-specific HPV persistence, and restriction to carcinogenic type persistence. Additionally, it is unknown whether persistent infections are characterized by the continuing detection of HPV or by a state of viral latency during which the virus remains undetectable so it can reappear later. A clear understanding of these issues is important for instance in order to effectively implement screening strategies that include HPV testing.[9]

Despite the various definitions of persistence, a persistent infection with a hr-HPV type is a major risk factor for the development of cervical abnormalities. As hr-HPV genotypes 16 and 18 together account for almost 70% of all cervical carcinomas and 55% of the high-grade cervical intraepithelial lesions, prophylactic vaccines against these two hr-HPV types have been developed.[19;39-47]

Worldwide mass vaccination with HPV vaccines will most certainly change HPV epidemiology. Monitoring these changes on a population level may prove crucial in assessing the effect of mass vaccination and overall HPV vaccine efficacy.

HPV vaccination

Currently, two prophylactic vaccines are registered and available in Europe; Gardasil (Merck, USA) and Cervarix (GlaxoSmithKline, Belgium). Gardasil is a quadrivalent vaccine containing L1 virus-like particles (VLPs) of the hr-HPV types 16 and 18, and VLPs of the lr-HPV types 6 and 11 and the classical aluminium hydroxyphosphate adjuvant (Table 1).[47] Cervarix is a bivalent HPV vaccine containing only the VLPs of HPV 16 and 18 as well as the new ASO4 adjuvant which consists of 3-deacylated monophosphoryl lipid A and aluminium hydroxide (Table 1).[40;41]

The prophylactic vaccines have shown to be highly effective in preventing persistent infections as well as related premalignant lesions.[39-44;47] However, it must be emphasized that these HPV vaccines are prophylactic, not therapeutic, and therefore, have no efficacy against existing HPV 16 or 18 infection or disease.[48;49]

The primary target group for vaccination consists of preadolescent girls as most of them (>95%) are not sexually active yet, and therefore have not been genitally infected with HPV. Presently, vaccination programs have started in many countries around the world,

Table 1. HPV vaccines

	HPV 6/11/16/18		HPV 16/18	
Manufacturer	**Merck**		**GlaxoSmithKline**	
Volume	Per dose	0.5 mL	Per dose	0.5 mL
Adjuvant	Aluminium salt	225 µg	ASO4:	
			$Al(OH)_3$	500 µg
			MPL®	50 µg
Antigens	L1 HPV 6	20 µg		
	L1 HPV 11	40 µg		
	L1 HPV 16	40 µg	L1 HPV 16	20 µg
	L1 HPV 18	20 µg	L1 HPV 18	20 µg
Expression system	Yeast		Hi-5 Baculovirus	
Schedule	Intramuscular	0,2,6 months	Intramuscular	0,1,6 months

primarily targeting 9 to 18 year old girls.[50-52] In the Netherlands, the HPV vaccine has been assimilated into the national vaccination programmes and catch-up vaccination of girls aged 13 to 16 years started in 2009 and vaccination of girls aged 12 years will start in 2010.

As women age they are more likely to have engaged in sexual activity resulting in exposure to HPV in general as well as vaccine specific types. Therefore, the clinical benefit of HPV vaccination afforded to older sexually active women is likely to be less than that of younger sexually naïve women. Nevertheless, extended vaccination of already sexually active women is under consideration in many countries in order to decrease cervical cancer incidence without a 15-20 years lag time.

Acceptability of the HPV vaccine may differ from other vaccines, as it could be seen as a vaccine against an STD. Most studies exploring HPV vaccine acceptability among young adults and students have been performed in the United States and the United Kingdom.[53-59] In these reports knowledge, number of sexual partners, educational level, and effectiveness of the vaccine were factors associated with vaccine acceptability. As vaccine acquisition costs are high, this may also be of influence. Hence, the decision to get vaccinated will be based on knowledge and balanced between personal benefit and costs. When the vaccine was implemented into the funded Dutch national vaccination programme early 2009, the coverage of catch-up vaccination among girls aged 13 to 16 years reached only 50%. The reasons for refusal of vaccination were mainly based on negative media attention, and, equally important, a lack of appropriate information to compensate the ghost stories about the vaccine and its long term effects. This underlines the need of educational campaigns along with vaccine introduction.

Mass vaccination of this young population will most certainly change HPV epidemiology. To correlate risk factors associated with HPV infection in the pre- and the post-vaccination era, baseline data are needed.

Risk factors for HPV

In order to get full insight in risk factors for acquiring genital HPV, results of HPV detection must be correlated to demographic characteristics and especially to past and present sexual behaviour. Until now, this has only been performed in a limited number of studies.[33;60-62]

Some studies mentioned sexual activity as the principle risk factor itself, whereas other studies discriminated between different aspects of sexual behaviour. Frequently-mentioned risk factors are total number of sexual partners, mixing sex with alcohol, being single, drug use, oral contraceptive use, current smoking, and age. The contribution of a history of STDs to HPV positivity has been challenged. It can be questioned whether having an STD other than HPV makes the cervix vulnerable for HPV infection or whether it is the other way around. Additionally, it is difficult to distinguish between the influence of an STD and the influence of the sexual risky behaviour itself.

Studying sexual behaviour may inform us about the risk of exposure to HPV. In order to correlate sexual behaviour to the present or future HPV status it is important to study the HPV status prospectively by performing HPV detection on a regular basis.

HPV detection

HPV cannot be grown in conventional cell cultures and serological assays are of limited value since they cannot distinguish between current and past infection, and not all infections induce measurable antibody levels. Therefore, accurate diagnosis of HPV infection relies on the detection of viral nucleic acid.[63;64] In the past decades new techniques have been developed and advances in existing techniques have been made, permitting large scale HPV testing.

In general, the HPV assays which are currently used widely are based on one of the two following principles.

The first is based on hybridisation of the target HPV DNA to labelled RNA probes in situ.[64] An example of this technique is the non-radioactive signal-amplification method Hybrid Capture II (hc2, Digene Corp., Gaithsburg, Maryland, USA). The hc2 is a commercially available test and is commonly used in screening. The hc2 test is the only FDA-approved screening assay. Unfortunately, as it only differentiates between an hr-HPV infection being present or not, it does not have the ability to identify individual genotypes nor infections harbouring multiple genotypes. This may be of importance in risk profiling and individual patient management.

The second is based on the principle of the consensus polymerase chain reaction (PCR). In order to detect HPV DNA in a single sample consensus primers should be used. The most widely used assays are the GP5+/6+ PCR system, the Roche Amplicor HPV Test (Roche Molecular Systems, Inc., Branchburg, NJ, USA), the PGMY primer set, and the SPF_{10} primer set. Several reports have assessed and compared different assays. Generally, the reports compare the ability to rapidly assess and/or genotype HPVs present in genital samples with high sensitivity and specificity.[64-71] The PCR assay is based on PCR amplification of the target HPV DNA directed by consensus or general primers that targets the highly conserved region of the L1 ORF.[64;68] Subsequent to the amplification of HPV DNA, reverse hybridisation of the amplicon to multiple oligonucleotides provides the possibility to simultaneously type up to 37 different HPV genotypes. The oligonucleotide probes which recognize the different genotypes are frequently tailed with poly(dT) and immobilised as parallel lines to membrane strips. The assay called line blot assay (LBA), line probe assay (LiPA), or linear array (LA) require only a little amount of PCR product.

As previously mentioned, the observed variations in HPV prevalence can be partly attributed to the properties of the HPV test used, i.e. its sensitivity and specificity. The risk-estimation for high-grade lesions following the outcome of the test is therefore also related to the assay used. The first application, the hc2, is aimed at identifying women at risk of developing cervical cancer, either in community-based screening programmes or in the clinical setting. The PCR based techniques, like the SPF_{10}Lipa, are highly sensitive in comparison to hybridization tests like the hc2. As a result, the terms "analytical" and "clinical" sensitivity have been introduced.[72] Clinically relevant hr-HPV infections can be distinguished from clinically irrelevant infections. Clinicians should be aware of these differences and should be able to translate the results into appropriate clinical treatment.

In contrast to the clinical application, highly sensitive and reproducible assays are required in vaccination trials, epidemiological and natural history studies, as the aim of these studies is to obtain a maximum of information about HPV status in populations and to monitor the course of infections in detail.[64;72]

Self-sampling

Regarding (hr-) HPV testing, material from vaginal lavages or self-sampling brushes has proven to be highly representative for the cervical (hr-) HPV status and have repetitively been proven to be as reliable as physician-taken samples.[73-78] However, it has been shown that self-sampling methods are not suitable for cytological analysis.[75;79] Several studies have shown that self-sampling for HPV testing was highly acceptable to and even favoured by the majority of women.[74;80] Self-sampling may be a less costly, a less invasive, and a timesaving alternative for the physician-based collection of cervico-vaginal material. Additionally, it is easy accessible as self-sampled material could be sent by mail, facilitating attempts to contact women who are not reached through present screening programmes or women living in rural settings, or with limited resources, i.e. health facilities. Several studies have shown that non-responders do actually take part in self-sampling studies.[73;75;79] Therefore, hr-HPV testing on self-sampled materials might be a promising opportunity to increase the efficiency of existing screening programmes as well as for establishing cervical cancer screening programs in developing countries.

Aim and outline of this thesis

Worldwide mass vaccination with HPV vaccines will most certainly change HPV epidemiology. Monitoring these changes on a population level may prove crucial in assessing overall HPV vaccine efficacy. To provide a basis for understanding possible future shifts in genotypes, as well as to provide insight in the HPV epidemiology of a target group for vaccination now and in the future, the prevalence, incidence and clearance rates of specific HPV types must be determined before vaccination takes place. Chapter 2 describes the Dutch situation of HPV prevalence and related risk factors among young adult women prior to mass vaccination. Furthermore, little is known about risk factors for acquiring new HPV infections as well as factors associated with clearance of HPV infections. These issues will be addressed in chapter 3.

As women age, they are more likely to have engaged in sexual activity resulting in exposure to HPV. Therefore, their clinical benefit of HPV vaccination is likely to be less than that of younger sexually naïve women. This may affect their acceptability of the HPV vaccine. Additionally, their vaccine acceptability may differ from other vaccines, as it could be seen as a vaccine against an STD. This view may also influence vaccine acceptability of parents of children in the target group for vaccination. Which in turn is very important, as these children are under age and parental consent for vaccination will be required. Assessing predictors of intention to receive the vaccine as well as assessing knowledge about HPV will be important to create effective vaccination campaigns and reach a high vaccine coverage. These issues are addressed in chapter 4 and 5.

To enhance vaccine acceptability, anticipation on factors influencing individual decision-making is needed. As vaccine acquisition costs are high, the decision to get vaccinated will be balanced between personal benefit and costs. Advising women on their personal benefit from vaccination will result in estimating ones individual risk of already being HPV 16 and 18 positive. To provide a guideline to estimate ones individual risk, a decision-aid based on young women's behavioural risk factors and prevalent HPV infections is designed and discussed in Chapter 6.

To meet the new post-vaccination screening requirements, the CSP may need transformation. Cervico-vaginal self-sampling may be an easy accessible, user-friendly, and timesaving alternative for the physician-based collection of cervico-vaginal material. Furthermore, it may be used to monitor women after the implementation of HPV vaccination. Chapter 7 reports the efficiency of HPV detection using a new method of sample storage, transportation and processing.

The results presented in the following chapters as well as future directions are generally discussed in Chapter 8 and summarised in Chapter 9.

Reference List

(1) Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999 Sep;189(1):12-9.

(2) zur Hausen H. Human papillomaviruses and their possible role in squamous cell carcinomas. Curr Top Microbiol Immunol 1977;78:1-30.

(3) Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. J Natl Cancer Inst 1995 Jun 7;87(11):796-802.

(4) Koshiol J, Lindsay L, Pimenta JM, Poole C, Jenkins D, Smith JS. Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. Am J Epidemiol 2008 Jul 15;168(2):123-37.

(5) Munoz N. Human papillomavirus and cancer: the epidemiological evidence. J Clin Virol 2000 Oct;19(1-2):1-5.

(6) Nobbenhuis MA, Walboomers JM, Helmerhorst TJ, Rozendaal L, Remmink AJ, Risse EK, et al. Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study. Lancet 1999 Jul 3;354(9172):20-5.

(7) Cogliano V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F. Carcinogenicity of human papillomaviruses. Lancet Oncol 2005 Apr;6(4):204.

(8) Munger K, Baldwin A, Edwards KM, Hayakawa H, Nguyen CL, Owens M, et al. Mechanisms of human papillomavirus-induced oncogenesis. J Virol 2004 Nov;78(21):11451-60.

(9) Woodman CB, Collins SI, Young LS. The natural history of cervical HPV infection: unresolved issues. Nat Rev Cancer 2007 Jan;7(1):11-22.

(10) Tindle RW. Immune evasion in human papillomavirus-associated cervical cancer. Nat Rev Cancer 2002 Jan;2(1):59-65.

(11) de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. Virology 2004 Jun 20;324(1):17-27.

(12) Stanley MA, Pett MR, Coleman N. HPV: from infection to cancer. Biochem Soc Trans 2007 Dec;35(Pt 6):1456-60.

(13) Jansen KU, Shaw AR. Human papillomavirus vaccines and prevention of cervical cancer. Annu Rev Med 2004;55:319-31.

(14) Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003 Feb 6;348(6):518-27.

(15) zur Hausen H. Papillomaviruses causing cancer: evasion from host-cell control in early events in carcinogenesis. J Natl Cancer Inst 2000 May 3;92(9):690-8.

(16) zur Hausen H. Roots and perspectives of contemporary papillomavirus research. J Cancer Res Clin Oncol 1996;122(1):3-13.

(17) Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine 2006 Mar 30;24 Suppl 1:S1-15.

(18) Bosch FX, Burchell AN, Schiffman M, Giuliano AR, de Sanjose S, Bruni L, et al. Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. Vaccine 2008 Aug 19;26 Suppl 10:K1-16.

(19) Clifford GM, Smith JS, Plummer M, Munoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. Br J Cancer 2003 Jan 13;88(1):63-73.

(20) Franco EL, Villa LL, Sobrinho JP, Prado JM, Rousseau MC, Desy M, et al. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. J Infect Dis 1999 Nov;180(5):1415-23.

(21) Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 1998 Feb 12;338(7):423-8.

(22) Sankaranarayanan R, Ferlay J. Worldwide burden of gynaecological cancer: the size of the problem. Best Pract Res Clin Obstet Gynaecol 2006 Apr;20(2):207-25.

(23) zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2002 May;2(5):342-50.

(24) Burchell AN, Richardson H, Mahmud SM, Trottier H, Tellier PP, Hanley J, et al. Modeling the sexual transmissibility of human papillomavirus infection using stochastic computer simulation and empirical data from a cohort study of young women in Montreal, Canada. Am J Epidemiol 2006 Mar 15;163(6):534-43.

(25) Burchell AN, Winer RL, de Sanjose S, Franco EL. Chapter 6: Epidemiology and transmission dynamics of genital HPV infection. Vaccine 2006 Aug 21;24 Suppl 3:S52-S61.

(26) Moscicki AB, Ma Y, Wibbelsman C, Powers A, Darragh TM, Farhat S, et al. Risks for Cervical Intraepithelial Neoplasia 3 Among Adolescents and Young Women With Abnormal Cytology. Obstet Gynecol 2008 Dec;112(6):1335-42.

(27) Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. J Clin Virol 2005 Mar;32 Suppl 1:S16-S24.

(28) Syrjanen K, Hakama M, Saarikoski S, Vayrynen M, Yliskoski M, Syrjanen S, et al. Prevalence, incidence, and estimated life-time risk of cervical human papillomavirus infections in a nonselected Finnish female population. Sex Transm Dis 1990 Jan;17(1):15-9.

(29) Auvinen E, Niemi M, Malm C, Zilliacus R, Trontti A, Fingerroos R, et al. High prevalence of HPV among female students in Finland. Scand J Infect Dis 2005;37(11-12):873-6.

(30) Auvinen E, Zilliacus R, Malm C, Karkkulainen T, Fingerroos R, Paavonen J. Repeated human papillomavirus DNA findings among female university students. Int J STD AIDS 2007 Dec;18(12):839-41.

(31) Bosch FX, de Sanjose S. Chapter 1: Human papillomavirus and cervical cancer--burden and assessment of causality. J Natl Cancer Inst Monogr 2003;(31):3-13.

(32) Castellsague X. Natural history and epidemiology of HPV infection and cervical cancer. Gynecol Oncol 2008 Sep;110(3 Suppl 2):S4-S7.

(33) Dunne EF, Unger ER, Sternberg M, McQuillan G, Swan DC, Patel SS, et al. Prevalence of HPV infection among females in the United States. JAMA 2007 Feb 28;297(8):813-9.

(34) Kjaer SK, Chackerian B, van den Brule AJ, Svare EI, Paull G, Walboomers JM, et al. High-risk human papillomavirus is sexually transmitted: evidence from a follow-up study of virgins starting sexual activity (intercourse). Cancer Epidemiol Biomarkers Prev 2001 Feb;10(2):101-6.

(35) Kjaer SK, Tran TN, Sparen P, Tryggvadottir L, Munk C, Dasbach E, et al. The burden of genital warts: a study of nearly 70,000 women from the general female population in the 4 Nordic countries. J Infect Dis 2007 Nov 15;196(10):1447-54.

(36) Tabora N, Zelaya A, Bakkers J, Melchers WJ, Ferrera A. Chlamydia trachomatis and genital human papillomavirus infections in female university students in Honduras. Am J Trop Med Hyg 2005 Jul;73(1):50-3.

(37) de Sanjose S, Bosch FX, Tafur LA, Nascimento CM, Izarzugaza I, Izquierdo A, et al. Clearance of HPV infection in middle aged men and women after 9 years' follow up. Sex Transm Infect 2003 Aug;79(4):348.

(38) Woodman CB, Collins S, Winter H, Bailey A, Ellis J, Prior P, et al. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. Lancet 2001 Jun 9;357(9271):1831-6.

(39) Future II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. N Engl J Med 2007 May 10;356(19):1915-27.

(40) Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. Lancet 2004 Nov 13;364(9447):1757-65.

(41) Harper DM, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, Roteli-Martins CM, et al. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. Lancet 2006 Apr 15;367(9518):1247-55.

(42) Harper DM. Impact of vaccination with Cervarix (trade mark) on subsequent HPV-16/18 infection and cervical disease in women 15-25 years of age. Gynecol Oncol 2008 Sep;110(3 Suppl 1):S11-S17.

(43) Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, et al. A controlled trial of a human papillomavirus type 16 vaccine. N Engl J Med 2002 Nov 21;347(21):1645-51.

(44) Paavonen J, Naud P, Salmeron J, Wheeler CM, Chow SN, Apter D, et al. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet 2009 Jul 25;374(9686):301-14.

(45) Schwarz TF. AS04-adjuvanted human papillomavirus-16/18 vaccination: recent advances in cervical cancer prevention. Expert Rev Vaccines 2008 Dec;7(10):1465-73.

(46) Schwarz TF, Spaczynski M, Schneider A, Wysocki J, Galaj A, Perona P, et al. Immunogenicity and tolerability of an HPV-16/18 AS04-adjuvanted prophylactic cervical cancer vaccine in women aged 15-55 years. Vaccine 2009 Jan 22;27(4):581-7.

(47) Villa LL, Costa RL, Petta CA, Andrade RP, Ault KA, Giuliano AR, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. Lancet Oncol 2005 May;6(5):271-8.

(48) Stanley M. Human papillomavirus vaccines versus cervical cancer screening. Clin Oncol (R Coll Radiol) 2008 Aug;20(6):388-94.

(49) Hildesheim A, Herrero R, Wacholder S, Rodriguez AC, Solomon D, Bratti MC, et al. Effect of human papillomavirus 16/18 L1 viruslike particle vaccine among young women with preexisting infection: a randomized trial. JAMA 2007 Aug 15;298(7):743-53.

(50) Brabin L, Roberts SA, Stretch R, Baxter D, Chambers G, Kitchener H, et al. Uptake of first two doses of human papillomavirus vaccine by adolescent schoolgirls in Manchester: prospective cohort study. BMJ 2008 May 10;336(7652):1056-8.

(51) Brotherton JM, Deeks SL, Campbell-Lloyd S, Misrachi A, Passaris I, Peterson K, et al. Interim estimates of human papillomavirus vaccination coverage in the school-based program in Australia. Commun Dis Intell 2008 Dec;32(4):457-61.

(52) Shefer A, Markowitz L, Deeks S, Tam T, Irwin K, Garland SM, et al. Early experience with human papillomavirus vaccine introduction in the United States, Canada and Australia. Vaccine 2008 Aug 19;26 Suppl 10:K68-K75.

(53) Boehner CW, Howe SR, Bernstein DI, Rosenthal SL. Viral sexually transmitted disease vaccine acceptability among college students. Sex Transm Dis 2003 Oct;30(10):774-8.

(54) Hoover DR, Carfioli B, Moench EA. Attitudes of adolescent/young adult women toward human papillomavirus vaccination and clinical trials. Health Care Women Int 2000 Jul;21(5):375-91.

(55) Kahn JA, Rosenthal SL, Hamann T, Bernstein DI. Attitudes about human papillomavirus vaccine in young women. Int J STD AIDS 2003 May;14(5):300-6.

(56) McClelland A, Liamputtong P. Knowledge and acceptance of human papillomavirus vaccination: perspectives of young Australians living in Melbourne, Australia. Sex Health 2006 May;3(2):95-101.

(57) Zimet GD, Mays RM, Winston Y, Kee R, Dickes J, Su L. Acceptability of human papillomavirus immunization. J Womens Health Gend Based Med 2000 Jan;9(1):47-50.

(58) Zimet GD. Improving adolescent health: focus on HPV vaccine acceptance. J Adolesc Health 2005 Dec;37(6 Suppl):S17-S23.

(59) Zimet GD, Perkins SM, Sturm LA, Bair RM, Juliar BE, Mays RM. Predictors of STI vaccine acceptability among parents and their adolescent children. J Adolesc Health 2005 Sep;37(3):179-86.

(60) Coupe VM, Berkhof J, Bulkmans NW, Snijders PJ, Meijer CJ. Age-dependent prevalence of 14 high-risk HPV types in the Netherlands: implications for prophylactic vaccination and screening. Br J Cancer 2008 Feb 12;98(3):646-51.

(61) Manhart LE, Holmes KK, Koutsky LA, Wood TR, Kenney DL, Feng Q, et al. Human papillomavirus infection among sexually active young women in the United States: Implications for developing a vaccination strategy. Sex Transm Dis 2006 Aug;33(8):502-8.

(62) Winer RL, Lee SK, Hughes JP, Adam DE, Kiviat NB, Koutsky LA. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. Am J Epidemiol 2003 Feb 1;157(3):218-26.

(63) Kleter B, van Doorn LJ, Schrauwen L, Molijn A, Sastrowijoto S, ter Schegget J, et al. Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. J Clin Microbiol 1999 Aug;37(8):2508-17.

(64) Molijn A, Kleter B, Quint W, van Doorn LJ. Molecular diagnosis of human papillomavirus (HPV) infections. J Clin Virol 2005 Mar;32 Suppl 1:S43-S51.

(65) Gravitt PE, Peyton CL, Apple RJ, Wheeler CM. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. J Clin Microbiol 1998 Oct;36(10):3020-7.

(66) Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Coutlee F, Hildesheim A, et al. Improved amplification of genital human papillomaviruses. J Clin Microbiol 2000 Jan;38(1):357-61.

(67) Kleter B, van Doorn LJ, ter Schegget J, Schrauwen L, van Krimpen K, Burger M, et al. Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses. Am J Pathol 1998 Dec;153(6):1731-9.

(68) Klug SJ, Molijn A, Schopp B, Holz B, Iftner A, Quint W, et al. Comparison of the performance of different HPV genotyping methods for detecting genital HPV types. J Med Virol 2008 Jul;80(7):1264-74.

(69) Melchers WJ, Bakkers JM, Wang J, de Wilde PC, Boonstra H, Quint WG, et al. Short fragment polymerase chain reaction reverse hybridization line probe assay to detect and genotype a broad spectrum of human papillomavirus types. Clinical evaluation and follow-up. Am J Pathol 1999 Nov;155(5):1473-8.

(70) van Ham MA, Bakkers JM, Harbers GK, Quint WG, Massuger LF, Melchers WJ. comparison of two commercial assays for detection of human papillomavirus (HPV) in cervical scrape specimens: validation of the Roche AMPLICOR HPV test as a means to screen for HPV genotypes associated with a higher risk of cervical disorders. J Clin Microbiol 2005 Jun;43(6):2662-7.

(71) van Hamont D, van Ham MA, Bakkers JM, Massuger LF, Melchers WJ. Evaluation of the SPF10-INNO LiPA human papillomavirus (HPV) genotyping test and the roche linear array HPV genotyping test. J Clin Microbiol 2006 Sep;44(9):3122-9.

(72) Snijders PJ, van den Brule AJ, Meijer CJ. The clinical relevance of human papillomavirus testing: relationship between analytical and clinical sensitivity. J Pathol 2003 Sep;201(1):1-6.

(73) Bais AG, van Kemenade FJ, Berkhof J, Verheijen RH, Snijders PJ, Voorhorst F, et al. Human papillomavirus testing on self-sampled cervicovaginal brushes: an effective alternative to protect nonresponders in cervical screening programs. Int J Cancer 2007 Apr 1;120(7):1505-10.

(74) Harper DM, Noll WW, Belloni DR, Cole BF. Randomized clinical trial of PCR-determined human papillomavirus detection methods: self-sampling versus clinician-directed--biologic concordance and women's preferences. Am J Obstet Gynecol 2002 Mar;186(3):365-73.

(75) Nobbenhuis MA, Helmerhorst TJ, van den Brule AJ, Rozendaal L, Jaspars LH, Voorhorst FJ, et al. Primary screening for high risk HPV by home obtained cervicovaginal lavage is an alternative screening tool for unscreened women. J Clin Pathol 2002 Jun;55(6):435-9.

(76) Ogilvie GS, Patrick DM, Schulzer M, Sellors JW, Petric M, Chambers K, et al. Diagnostic accuracy of self collected vaginal specimens for human papillomavirus compared to clinician collected human papillomavirus specimens: a meta-analysis. Sex Transm Infect 2005 Jun;81(3):207-12.

(77) Petignat P, Faltin DL, Bruchim I, Tramer MR, Franco EL, Coutlee F. Are self-collected samples comparable to physician-collected cervical specimens for human papillomavirus DNA testing? A systematic review and meta-analysis. Gynecol Oncol 2007 May;105(2):530-5.

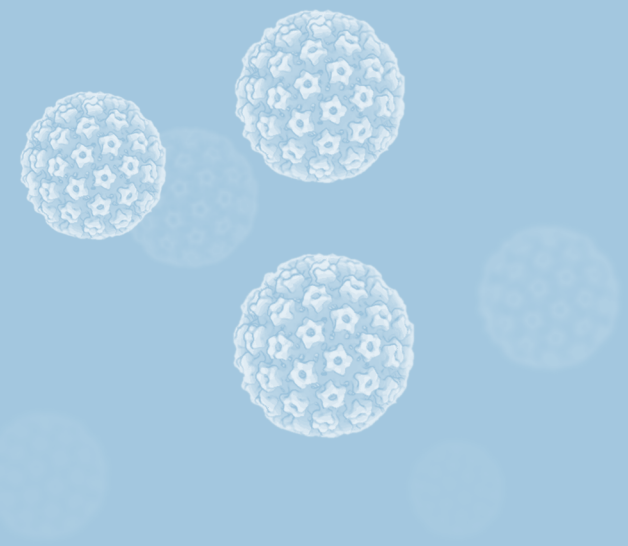
(78) Winer RL, Feng Q, Hughes JP, Yu M, Kiviat NB, O'Reilly S, et al. Concordance of self-collected and clinician-collected swab samples for detecting human papillomavirus DNA in women 18 to 32 years of age. Sex Transm Dis 2007 Jun;34(6):371-7.

(79) Brink AA, Meijer CJ, Wiegerinck MA, Nieboer TE, Kruitwagen RF, van Kemenade F, et al. High concordance of results of testing for human papillomavirus in cervicovaginal samples collected by two methods, with comparison of a novel self-sampling device to a conventional endocervical brush. J Clin Microbiol 2006 Jul;44(7):2518-23.

(80) Waller J, McCaffery K, Forrest S, Szarewski A, Cadman L, Austin J, et al. Acceptability of unsupervised HPV self-sampling using written instructions. J Med Screen 2006;13(4):208-13.

Chapter 2

Sexual behaviour and HPV infections in 18 to 29 year old women in the pre-vaccine era in the Netherlands



Charlotte H. Lenselink[1]
Willem J.G. Melchers[2]
Wim G.V. Quint[3]
Annelies M.J. Hoebers[2]
Jan C.M. Hendriks[4]
Leon F.A.G. Massuger[1]
Ruud L.M. Bekkers[1]

[1, 2, 4] Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands
[1] Department of Obstetrics & Gynaecology,
[2] Department of Medical Microbiology
[3] DDL Diagnostic Laboratory B.V., Voorburg, the Netherlands
[4] Department of Epidemiology, Biostatistics and HTA

PLoS ONE. 2008;3(11):e3743

Abstract

Infection with Human Papillomavirus (HPV) is a necessary event in the multi-step process of cervical carcinogenesis. Little is known about the natural history of HPV infection among unscreened young adults. As prophylactic vaccines are being developed to prevent specifically HPV 16 and 18 infections, shifts in prevalence in the post-vaccine era may be expected. This study provides a unique opportunity to gather baseline data before changes by nationwide vaccination occur. This cross-sectional study is part of a large prospective epidemiologic study performed among 2065 unscreened women aged 18 to 29 years. Women returned a self-collected cervico-vaginal specimen and filled out a questionnaire. All HPV DNA-positive samples (by SPF_{10} DEIA) were genotyped using the INNO-LiPA HPV genotyping assay. HPV point prevalence in this sample was 19%. Low and high risk HPV prevalence was 9.1% and 11.8%, respectively. A single HPV type was detected in 14.9% of all women, while multiple types were found in 4.1%. HPV types 16 (2.8%) and 18 (1.4%) were found concomitantly in only 3 women (0.1%). There was an increase in HPV prevalence till 22 years. Multivariate analysis showed that number of lifetime sexual partners was the most powerful predictor of HPV positivity, followed by type of relationship, frequency of sexual contact, age, and number of sexual partners over the past 6 months. This study shows that factors independently associated with HPV prevalence are mainly related to sexual behaviour. Combination of these results with the relative low prevalence of HPV 16 and/or 18 may be promising for expanding the future target group for catch-up vaccination. Furthermore, these results provide a basis for research on possible future shifts in HPV genotype prevalence, and enable a better estimate of the effect of HPV 16-18 vaccination on cervical cancer incidence.
Upcoming mass vaccination with Human Papillomavirus (HPV) vaccines will most certainly change HPV epidemiology. Monitoring these changes on population level may prove crucial in assessing the effect of mass vaccination and overall HPV vaccine efficacy. In the Netherlands, girls aged 12 years will be vaccinated as of September 2009, and the catch-up vaccination (girls aged 13 to 16 years) will probably start in the first part of 2009.

Introduction

Until now, only a limited number of large studies have investigated HPV epidemiology in female adolescents and young female adults. Even fewer studies have investigated HPV epidemiology in relation to past en present sexual behaviour.

Genital infection with HPV is the most common sexually transmitted disease (STD) among young sexually active women.[1] Most sexually active women (>50%) have been genitally infected by one or more HPV types at some point in their life.[2] Fourteen HPV genotypes are associated with cervical cancer development and are therefore called high-risk (hr-HPV). Of these hr-HPV genotypes, hr-HPV 16 and 18 are related to 70% of all cervical cancers. Therefore, prophylactic vaccines against these two HPV types have been developed. It has been estimated that the best results of prophylactic vaccination will be achieved by vaccinating women before they become genitally infected i.e. sexually active. Presently, vaccination programmes are being started in many countries around the world, targeting 9 to 16 year old girls.[3,4] Additionally, catch-up vaccination of already sexually active women is under consideration in many countries in order to get a faster decrease in cervical cancer incidence.

Estimates of HPV infections among asymptomatic women around the world range from 2% to 44%.[5-8] The wide variation in prevalence is largely explained by differences in sensitivity of the HPV-DNA assay used, differences in age, or differences in other characteristics of the populations studied.[2,7]

Additionally, little is known about risk factors for acquiring genital HPV in young female adults. Therefore, further assessment of risk factors like sexual behaviour is important. Knowledge of baseline, i.e. pre-vaccination, epidemiology of type specific HPV infections in relation to sexual behaviour is important in order to decide whether catch-up vaccination may be beneficial. After nationwide implementation of the prophylactic HPV vaccine, HPV epidemiology will most likely change due to expected decreases in HPV 16-18 prevalence and incidence, as well as possible changes in other types occurring due to cross-protection of the vaccine. Due to these shifts, prevalence and incidence of other HPV types may increase and therefore may change the oncogenicity of these types.

Therefore, this study, conducted before the nationwide introduction of HPV vaccines, provides a unique opportunity to determine baseline data on HPV prevalence in 18 to 29 year old women in the Netherlands. Additionally, no regular cervical cancer screening is

performed in this age group, as the Dutch Cervical Screening Programme starts at the age of 30 years. This study is part of a large prospective epidemiologic study conducted to study the dynamics of HPV infections, in particular HPV 16/18, and to get more insight in specific risk factors for acquiring genital HPV, like past and present sexual behaviour. These results provide a basis for understanding possible future shifts in genotypes, and presumably enable a better estimate of the effect of HPV 16/18 vaccination on cervical cancer incidence.

Methods

Study population and study design

This cross-sectional study is part of a large prospective epidemiologic study performed among 2065 unscreened women aged 18 to 29 years. Women were recruited between June and September 2007, using different advertisements, as well as active recruitment sites, and posters at general practices in the city regions of Arnhem, Nijmegen, and Den Bosch, the Netherlands. Furthermore, advertisement on the internet were used, which were accessible in the whole of the Netherlands. Of the 2297 women who responded to the advertisements, 2065 (89.9%) consented with the study, returned the cervico-vaginal swab specimens, and filled out the questionnaire. Written informed consent was obtained from all participants. This study was approved by the local Medical Ethics Committee.

Specimen Collection and Processing

All women were asked to fill out a questionnaire and to self-collect a cervico-vaginal sample in the privacy of their own home. Women received an explanatory letter, an informed consent form, a questionnaire, and a self-sample kit by mail. The self-sample kit contained a collection device (a small brush packaged in an individual sterile cover, Rovers® Viba-brush, Rovers Medical Devices B.V., Oss, the Netherlands), a collection tube containing medium (SurePath™, Tripath Imaging®, Inc., Burlington NC, USA), instructions how to perform the cervico-vaginal self-sample (written and in cartoon), and a return package consisting of a leak-proof seal bag, absorption sheet, and a reclosable plastic return envelope (Easyslider, Transposafe Systems Holland BV, Sassenheim, the Netherlands). In brief, participants were instructed to wash their hands before opening the brush cover, to hold the brush by the end of the handle, to insert the brush approximately 7 cm into the vagina (similar to inserting a tampon), to gently turn the brush 5 times, and to place the top of the brush in the collection tube. The collection tube was closed, and enclosed in the seal bag. Finally, the collection tube was placed in the return envelope, together with the questionnaire, and sent to the Department of Obstetrics and Gynaecology for further processing and HPV assessment. The samples were stored at room temperature.

Questionnaire

In this study we used a questionnaire consisting of two parts. The first part was composed of questions regarding socio-demographic variables like educational level, religion, smoking, medication use, contraceptive use, and ethnicity. Race and ethnicity were self-reported into different categories. The second part consisted of questions regarding sexual behaviour to gain insight in risk factors for acquiring genital HPV. Results of HPV detection were correlated to past and present sexual behaviour. Sex was defined as vaginal, oral, and/or anal sex. For women who had at least 1 lifetime sex partner, additional questions were asked on age at first sexual contact, age of first sex partner, number of sex partners before the age of sixteen, lifetime number of sex partners, number of sex partners in the past 6 months, gender of sex partners, frequency of sexual contact, condom use, and history of STDs.

HPV DNA Detection and Genotyping

Broad-spectrum HPV DNA amplification was performed using a short PCR fragment assay (SPF_{10}-LiPA HPV detection/genotyping assay, SPF_{10} system version 1, manufactured by Labo Biomedical Products BV, Rijswijk, the Netherlands). This assay amplifies a 65-bp fragment of the L1 open reading frame and allows detection of at least 43 different HPV types.[9-12] The SPF_{10} PCR was performed with a final reaction volume of 50 µl containing 10 µl of the isolated DNA sample, 10 mmol/liter Tris-HCl (pH 9.0), 50 mmol/liter KCl, 2.0 mmol/liter $MgCl_2$, 0.1% Triton X-100, 0.01% gelatin, 200 µmol/liter of each deoxynucleoside triphosphate, 15 pmol each of the forward and reverse primers tagged with biotin at the 5'end, and 1.5 U of AmpliTaq Gold (Perkin-Elmer). The mixture was incubated for 9 minutes at 94°C, 40 cycles of 45 s at 45°C, and 40 cycles of 45 s at 72°C, with a final extension of 5 minutes at 72°C. Each experiment was performed with a separate positive and negative PCR control. The presence of HPV DNA was determined by hybridization of SPF_{10} amplimers to a mixture of general HPV probes recognizing a broad range of HPV genotypes, in a microtiter plate format, as described previously.[9-12]

All HPV DNA-positive samples (by SPF_{10} DEIA) were genotyped using the INNO-LiPA HPV genotyping assays.

The 28 oligonucleotide probes that recognize 25 different types were tailed with poly(dT) and immobilized as parallel lines to membrane strips (Labo Bio-Medical Products B.V., Rijswijk, the Netherlands). The HPV genotyping assay was performed as described previously.[9] Samples that tested positive using the DNA enzyme immunoassay but that showed no results on the LiPA strip were considered to be HPV X type, i.e. genotypes not available on the LiPA strip. Low-risk HPV (lr-HPV) types were defined as HPV type 6,11, 34, 40, 42, 43, 44, 53, 54, 55, 58, 66, 70, 74, and "X"; and hr-HPV types as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59, 68, 73, and 82.

Statistical Analysis

All women who completed the questionnaire and submitted a swab for HPV evaluation were included in the final analysis (n= 2065).

The Chi-Square test was used to test associations between demographic variables or behavioural characteristics and HPV. Differences of medians of continuous variables between the groups were analysed using non-parametric tests (Mann-Whitney).
In univariate and multivariate analysis, data of some variables were grouped due to small numbers and/or to gain a better overview. We grouped ethnicity into two groups: Dutch and not Dutch. Lifetime number of partners and number of partners in the past six months were divided into four categories, and frequency of sexual contact was grouped into five categories. Years of being sexually active (i.e. sexual age) ranged from 0 to 23 years, the category "0" years consisted of women who became sexually active in the past year. Because of the small numbers, 0 and 1 year were combined as well as 13 to 23 years. Chlamydia, genital warts, Syphilis, Gonorrhoea, Genital Herpes, and HIV were defined as STD. In further statistical analysis previous STDs were defined as yes or no.
Variables found to be significantly related to HPV infection by univariate analyses were entered into a multiple logistic regression model with forward selection procedures to identify variables that contributed independently to the probability of HPV prevalence.
Participants with missing data on variables included in the multivariate analysis were excluded. In all tests, p values < 0.05 were regarded statistically significant.
Statistical analyses were performed using SAS 8.0 (SAS Institute Inc., Cary, NC, USA) and SPSS 14.1 (Chicago, Illinois, USA).

Results

Socio-demographic characteristics

The age distribution and socio-demographic characteristics of the 2065 participants are summarised in table 1. Many women attended higher vocational training or University in past or presence (n=1545, 75.6%). Of all women, 622 (30.3%) were single and 1431 (69.7%) were involved in a relationship. Only 69 women (3.4%) reported an ethnicity other than Dutch, including "other European" 1.1% (n=23), Caribbean 0.7% (n=15), Turkish 0.2% (n=4), Asian 0.6% (n=12), African 0.2% (n=3), and "other" 0.6% (n=12). Because of these small numbers, they were divided into two groups: Dutch (96.6%, n= 1981), and "other" (3.4%, n=69).

Additionally, the mean age at first sexual contact was 16.7 years, and the mean sexual age (i.e. years of being sexually active) was 6.8 years. Women who were not sexually active yet were significantly more often living with their parents (11.5%, n=41 versus 4.4%, n=75, p=<0.001, data not shown).

Prevalence of HPV infection

Of the 2065 adequate specimens, 19% (n=393) tested positive for one or more HPV genotypes. Age-specific prevalence is shown in table 1. There was an overall increase in HPV prevalence with age till 22 years, afterwards a plateau phase was reached. Prevalence of HPV infection showed a decrease at 23 years and a peak among women aged 27 years (13%, n=24, and 31%, n=52, respectively). However, as the 95% confidence interval was overlapping with adjacent age groups, the differences were considered accidental findings (Figure 1).

The overall prevalence of hr-HPV types was 11.8% and of lr-HPV types 9.1%, including co-infections. Prevalence of both hr- and lr-HPV types showed an almost similar age-distribution (Figure 2A).

Prevalence of specific HPV genotypes

A single HPV-type was detected in 14.9% of all women, while multiple types were found in 4.1% (21.6% of all HPV-positive women). We identified 25 different genotypes, most common types detected were HPV type 16 (2.8%, n=57), HPV type 51 (2.5%, n=51), and HPV type 52 (2.5%, n=52). HPV types 18, 6, and 11, were detected in 1.4% (n=28), 0.6%

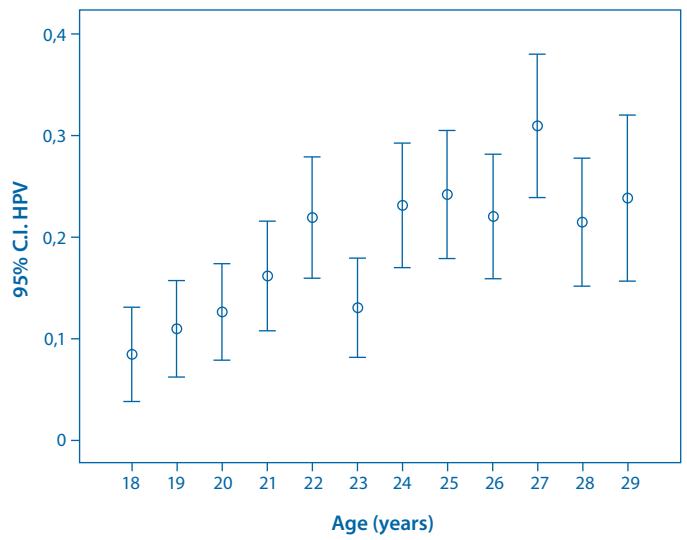
Table 1. HPV prevalence by demographic variables among all women

	Sample size (n)	HPV Prevalence (n)	p
Overall	2065	393 (19.0%)	-
Age (in years)			<0.001*
18	142	12 (8.5%)	
19	173	19 (11.0%)	
20	190	24 (12.6%)	
21	185	30 (16.2%)	
22	187	41 (21.9%)	
23	185	24 (13.0%)	
24	186	43 (23.1%)	
25	182	44 (24.2%)	
26	186	41 (22.0%)	
27	168	52 (31.0%)	
28	172	37 (21.5%)	
29	109	26 (23.9%)	
Ethnicity**	2050		0.73*
Dutch	1981	378 (19.1%)	
Other	69	12 (17.4%)	
Education***	2044		0.424*
Lower secondary / Lower vocational training	71	13 (18.3%)	
Higher Secondary / Vocational training	428	72 (16.8%)	
Higher vocational training / University	1545	303 (19.6%)	
Current smoking	2054		<0.001*
Yes	406	114 (28.1%)	
No	1648	277 (16.8%)	
Using OCC	2061		0.758*
Yes	1459	275 (18.8%)	
No	602	117 (19.4%)	
Living with parents	2052		0.008*
Yes	357	59 (14.0%)	
No	1695	340 (20.1%)	
Relationship	2053		<0.001*
Married	125	7 (5.6%)	
Living together	483	73 (15.1%)	
LAT	823	177 (21.5%)	
Single	622	134 (21.5%)	
Sexual activity ever			<0.001*
Yes	1947	389 (20%)	
No	116	4 (3.4%)	

HPV+: HPV positive if one or more genotypes (high-risk as well as low-risk) are detected simultaneously.
Sample sizes change because of missing values of the questionnaire.
n: number
p: p-value
-: not applicable
*by Chi-square test
**ethnicity was self-reported
***type of education: group of lower secondary education includes 2 women who reported only primary/ no education
LAT: living apart together
OCC: oral contraceptives

Figure 1.

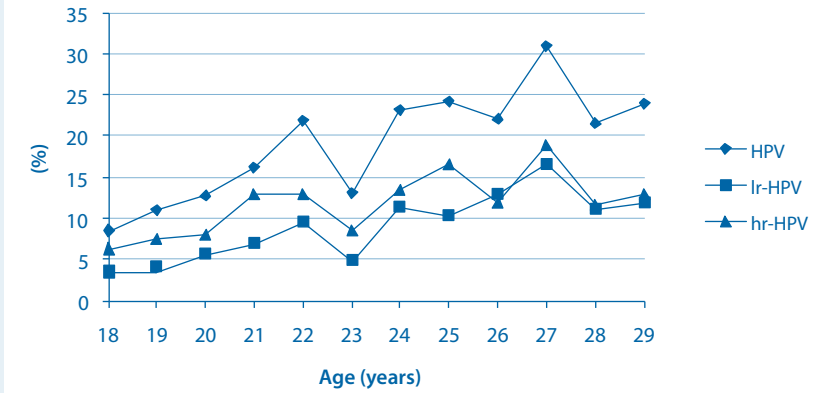
95% Confidence interval of HPV prevalence by age (n=2065).



There was an overall increase in HPV prevalence with age till 22 years, afterwards a plateau phase was reached. A decrease is shown at 23 years and a peak among women aged 27 years, however, as the 95% confidence interval (95% C.I.) is overlapping with adjacent age groups, the differences were considered accidental findings.

Figure 2A.

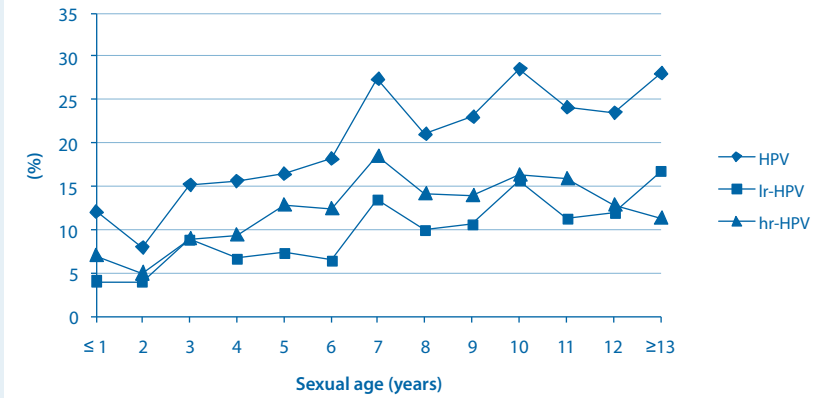
Prevalence of low-risk and high-risk types by age (n=2065).



Prevalence of overall and both high-risk (hr-) and low-risk (lr-) HPV types showed an almost similar age-distribution. In some women both hr- and lr-types were detected.

Figure 2B.

Prevalence of low-risk and high-risk types by sexual age (n=1943).

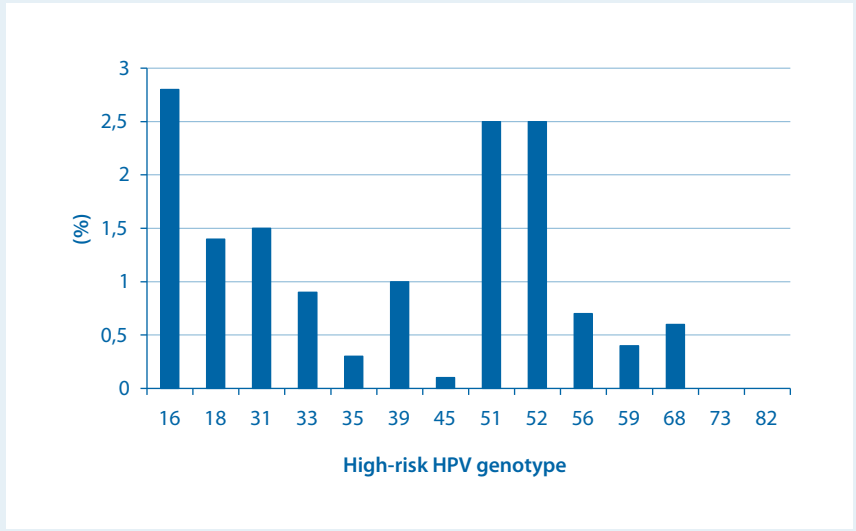


Only sexually active women were selected (n=1943). Overall HPV prevalence, as well as high-risk (hr-) and low-risk (lr-) HPV prevalence, showed an increase with rising sexual age. However, hr-HPV prevalence decreased from a sexual age of 10 years. In some women both hr- and lr-types were detected.

(n=12), and 0.2% (n=4), respectively (Figure 3A and 3B). In 3.5% of the women the HPV type could not be specified and was named Lipa X (n=72). A simultaneous presence of HPV 16 and 18 only occurred in 3 women (0.1%). HPV DNA was detected in 4 women who reported never having had sex. It concerned single infections with HPV type 33, two times HPV type 16, and a co-infection with HPV type 66 and 52.

Figure 3A.

Prevalence of high-risk HPV types.



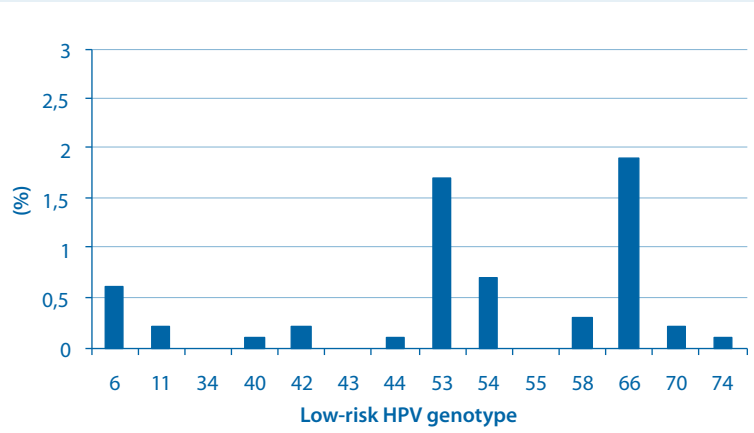
Most common types detected were HPV type 16 (2.8%, n=57), HPV type 51 (2.5%, n=51), and HPV type 52 (2.5%, n=52). In some women both low-risk and high-risk types were detected.

Sexually active women

When univariate analysis was restricted to sexually active women, factors significantly associated with HPV prevalence were increasing age, current smoking, number of partners in the past 6 months, and years of being sexually active (i.e. sexual age) (Table 2 and Figure 2B). Sexual age was defined as time interval in years between age at first sexual contact and current age. Furthermore, a higher number of lifetime sexual partners, was significantly associated with overall HPV prevalence as well as hr-HPV prevalence (Table 2 and Figure 4). Women without an HPV infection tended to be married or living together with their partner. Age at first sexual contact did not show a significant relationship with current HPV

Figure 3B.

Prevalence of low-risk HPV types.



In some women both low-risk and high-risk types were detected. HPV genotype 53 and 66 may also be considered as possible high-risk types.

prevalence (Table 2). HPV prevalence, as well as hr- and lr-HPV prevalence, showed an increase with rising sexual age (Table 2 and Figure 2B). However, hr-HPV prevalence decreased from a sexual age of 10 years (Figure 2B). Oral contraceptive (OCC) use could not be defined as a risk factor for HPV positivity.

After logistic regression, age, smoking, number of sexual partners (lifetime and in past 6 months), type of relationship, living with parents, and sexual age were significantly associated with HPV prevalence. Additionally, HPV prevalence was lower among women without a previous STD (Odds Ratio (OR) 0.355, p<0.001, Table 2), but there was no significant difference between the type of STDs. Women who reported to be non-smokers tested significantly less often positive for HPV than women who reported to be current smokers (17.8% versus 28.3%, OR 0.551, p <0.001, Table 2). Women not living with their parents tested significantly more often positive for HPV than women who were living with their parents (20.9% versus 15.2% , OR 1.468, 95% C.I. 1.055;2.041, p=0.02).

Age at first sexual intercourse was not significantly related to HPV prevalence, whereas sexual age was (p= 0.053, and p< 0.001, respectively).

The analysis was concluded by completing a multivariate regression analysis on all factors that showed a significant relation with HPV in the univariate analysis. The factors

Table 2. HPV prevalence and Odds Ratio's for HPV prevalence among sexually active women using univariate analysis and logistic regression.

	n	HPV+ n (%) / Median (range)	p	OR	(95% C.I.)	p
Age (years)	**1947**	25 (18-29)	<0.001^	1.097	(1.059;1.136)	<0.001
Current smoking	**1936**		<0.001*			
No	1536	274 (17.8%)		0.551	(0.428;0.711)	<0.001
Yes	400	113 (28.3%)		1	(ref)	
Using OCC	**1944**		0.272*			
No	528	114 (21.6%)		1.148	(0.898;1.467)	0.272
Yes	1416	274 (19.4%)		1	(ref)	
Living with parents	**1934**		0.022*			
No	1619	398 (20.9%)		1.468	(1.055;2.041)	0.023
Yes	315	48 (15.2%)		1	(ref)	
Relationship	**1935**		<0.001*			
Married	125	7 (5.6%)		0.216	(0.099;0.471)	<0.001
Living together	483	73 (15.1%)		0.647	(0.480;0.874)	0.004
Single	511	131 (25.6%)		1.254	(0.967;1.625)	0.088
LAT¤	816	176 (21.6%)		1	(ref)	
Age at first intercourse** (years)	**1944**		0.053*			
≤ 13	45	13 (28.8%)		1.517	(0.719;3.204)	0.274
14-16	935	203 (21.7%)		1.036	(0.688;1.560)	0.866
17-19	803	139 (17.3%)		0.782	(0.514;1.190)	0.251
≥ 20	161	34 (21.1%)		1	(ref)	
Lifetime sex partners (number)	**1938**		<0.001*			
1	403	17 (4.2%)		0.044	(0.025;0.077)	<0.001
2-5	924	136 (14.7%)		0.173	(0.125;0.239)	<0.001
6-10	397	127 (32%)		0.470	(0.334;0.662)	<0.001
>10	214	107 (50%)		1	(ref)	
Gender of sex partner(s)	**1939**		<0.001*			
Male	1829	349 (19.1)		0.393	(0.260;0.594)	<0.001
Female	6	0 (0%)		0.000	(0.000; .)	0.999
Both	104	39 (37.5%)		1	(ref)	

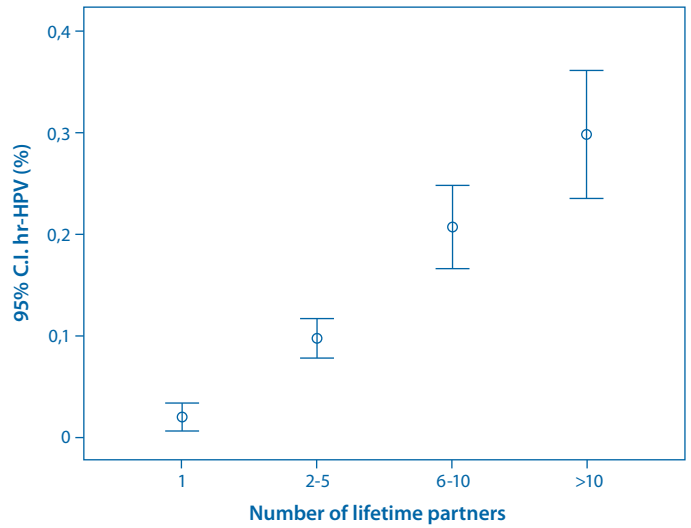
Table 2. Continued

	n	HPV+ n (%) / Median (range)		p	OR	(95% C.I.)	p
Sex partners in past 6 months (number)	**1939**			<0.001*			
0	170	22	(12.9%)		0.140	(0.077;0.254)	<0.001
1	1485	249	(16.8%)		0.190	(0.125;0.288)	<0.001
2	188	66	(35.1%)		0.509	(0.310;0.835)	0.008
>2	99	51	(51.1%)		1	(ref)	
Sexual contact in past 6 months (frequency)	**1886**			<0.001*			
0	146	19	(13%)		0.461	(0.274;0.775)	0.003
1-6	221	66	(29.9%)		1.312	(0.927;1.857)	0.125
7-24	239	47	(19.7%)		0.754	(0.519;1.096)	0.139
25-54	729	111	(15.2%)		0.553	(0.418;0.732)	<0.001
>54	551	135	(25.4%)		1	(ref)	
Ever diagnosed an STD?	**1940**			<0.001*			
No	1755	315	(17.9%)		0.355	(0.257;0.488)	<0.001
Yes	186	71	(38.2%)		1	(ref)	
Condom use	**1938**			<0.001*			
Never (0%)	924	142	(15.4%)		1.005	(0655;1.541)	0.983
Sometimes (0-50%)	499	134	(26.9%)		2.031	(1.313;3.143)	0.001
Most of times (50-100%)	318	82	(25.8%)		1.923	(1.210;3.055)	0.006
Always (100%)	197	30	(15.2%)		1	(ref)	
Sexual age (years)***	**1943**	8	(1-13)	<0.001^	1.096	(1.061;1.133)	<0.001

n: number
Sample sizes change because of missing values of the questionnaire
HPV+: HPV positive if one or more genotypes are detected simultaneously
p: p-value, OR: Odds Ratio
95% C.I.: 95% Confidence Interval
^Mann Whitney
*by Chi-square test
**below the age of 10 years several cases of sexual abuse were reported
***Sexual age in years with 0 and 1 combined as well as sexual age higher than 13
ref: reference, OCC: oral contraceptives, LAT: living apart together, STD, sexually transmitted disease

Figure 4.

Number of lifetime sexual partners by high-risk HPV.



A higher number of lifetime sexual partners was significantly associated with overall HPV prevalence as well as hr-HPV prevalence.
95% C.I.: 95% Confidence Interval.

living together and having a relationship but living apart. This was followed by frequency of sexual contact (p=0.001), age (p<0.001), and number of sexual partners in past 6 months (p=0.018), with a protective effect of having a single partner. Sexual age (p=0.022) was also independently associated with HPV prevalence. Additionally, condom use was not defined as an independent risk factor as it was dependent on age, type of relationship, frequency of sexual contact, and number of sexual partners in the past six months.

Table 3. Adjusted Odds Ratio's for HPV prevalence among sexually active women using multivariate logistic regression (n=1820).

	Adj. OR	(95% C.I.)	p
Age (years)	1.160	(1.081;1.246)	<0.001
Relationship			<0.001
Married	0.227	(0.098;0.525)	0.001
Living together	0.565	(0.397;0.801)	0.001
Single	1.037	(0.685-1.570)	0.864
LAT	1	(ref)	
Lifetime sex partners (number)			<0.001
1	0.061	(0.031;0.117)	<0.001
2-5	0.208	(0.139;0.313)	<0.001
6-10	0.512	(0.350;0.748)	<0.001
>10	1	(ref)	
Sex partners in past 6 months (number)			0.018
0	0.153	(0.009;2.723)	0.201
1	0.467	(0.278;0.784)	0.004
2	0.674	(0.389;1.169)	0.160
>2	1	(ref)	
Sexual contact in past 6 months (frequency)			0.001
0	1.218	(0.073;20.232)	0.886
1-6	0.701	(0.431;1.142)	0.160
7-24	0.541	(0.345;0.848)	0.008
25-54	0.513	(0.375;0.703)	<0.001
>54	1	(ref)	
Sexual age (years)*	0.917	(0.851;0.988)	0.022

n: number
Adj. OR: Adjusted Odds Ratio
95% C.I.: 95% Confidence Interval
p: p-value
LAT: living apart together
ref: reference
*Sexual age in years with 0 and 1 combined as well as sexual age higher than 13 years

Discussion

This is the first Dutch HPV epidemiological study conducted among unscreened women aged 18 to 29 years. The point prevalence of HPV DNA in this sample was 19%. Lr- and hr-HPV prevalence were 9.1% and 11.8%, respectively. Hr-HPV types 16 (2.8%) and 18 (1.4%) were found concomitantly in only 3 women (0.1%). These results are comparable with recent studies among young women, although different sampling methods were used.[8;13-15] In this large study, self-collected cervico-vaginal samples were used. Material from self-sampling brushes or vaginal lavages has been proven to be highly representative for the cervical HPV status.[16] HPV point prevalence was linked to sexual behaviour by using questionnaires. As the questionnaires were only provided with a study number, they could be considered as fairly anonymous, inducing high credibility. The number of sexual partners, as well as the type of current relationship were significantly associated with HPV positivity. Several international studies confirm sexual behaviour and a high number of sexual partners as the most important risk factors to contract STDs.[5;15;17-22] In this study not only the number of sexual partners in the past six months but also number of lifetime sexual partners was independently associated with a higher risk for HPV prevalence. An active HPV infection is likely to be dependent on recent sexual activity and may therefore be acquired recently, whereas latent or persistent infection could be influenced by past sexual behaviour. A higher number of lifetime sexual partners increases the risk of getting infected with one or more HPV types in time. Every HPV infection has its type dependent clearance which takes 8 to 14 months on average. Women, who have not been sexually active recently, i.e. in the past six months, may test positive for HPV. Another explanation for the influence of the sexual past is that latent infections are detected. Detecting a latent infection is dependent on the sensitivity of the technique used. In this study the highly sensitive HPV genotyping test SPF_{10}-LIPA is used, which could make it difficult to discriminate between active (i.e. chronic productive infections) and latent infections because of its low threshold value. Therefore, results of this study, showing point prevalence of HPV infections, may be a mixture of latent and active or persistent infections.

Furthermore, multivariate analysis provided insight in the independent risk factors for prevalent HPV infection. The independent risk factors were all related to sexual behaviour, with the exception for age. We found that HPV prevalence increased with age. Studies often show HPV prevalence decreasing towards 30 years. This may be explained by the fact that the Dutch Cervical Screening Programme starts at the age of 30 years, and therefore these women are unscreened. Furthermore, we did not study women above the age of 29, and we did not combine different age groups, which may provide another perspective by levelling the differences. Other explanations could be the techniques used or differences in the population studied. The use of contraceptive methods like condoms was influenced by type of relationship. Results of several studies on condom use have been inconsistent partly owing to the fact that different populations have been studied.[7;15;22;23] Furthermore, our multivariate analysis showed no significant relation

between HPV positivity and smoking. Results of studies regarding the effect of smoking and HPV have also been inconsistent.[8;21;24]

No correlation of educational level with HPV prevalence was seen. However, the over-representation of women attending university college possibly limits generalisation of these findings, and may be influenced by the method of recruitment. Nevertheless, this study provides a unique sample with an equal distribution of women over the age groups of 18 to 29 years, using one of the last opportunities to gather baseline i.e. pre-vaccine data. These baseline data enable future study on HPV dynamics. HPV epidemiology will most likely change after vaccination due to expected decreases in HPV 16-18 prevalence and incidence, as well as decreases in other types due to cross-protection of the vaccine. These decreases in type specific prevalence and incidence may be substituted by increases in other HPV genotypes. The relative low point prevalence of HPV 16 and 18, and co-infection with both types in only 0.1%, combined with independent predictors of prevalent HPV infection, may be promising for future catch-up vaccination. These results suggest that it may be possible to expand the future target group for catch-up vaccination by including women with a higher age or by targeting women with a low risk profile.

This study shows that sexual behaviour, especially the number of sexual partners as well as type of current relationship, remain the dominant and individual risk factors for HPV positivity i.e. HPV point prevalence. These unique baseline epidemiological data on HPV prevalence in combination with knowledge of sexual behaviour provide a basis for research on possible future shifts in HPV genotype prevalence, and enable a better estimate of the effect of nationwide HPV 16-18 vaccination on cervical cancer incidence.

Acknowledgements

The authors would like to thank Onno Schaap for the technical support critical to this study. The authors are also grateful to Fanny van den Brand, Annette van der Geest, and Channa Schmeink for their logistical support.

Reference List

(1) Burchell AN, Richardson H, Mahmud SM, Trottier H, Tellier PP, Hanley J, et al. Modeling the sexual transmissibility of human papillomavirus infection using stochastic computer simulation and empirical data from a cohort study of young women in Montreal, Canada. Am J Epidemiol 2006 Mar 15;163(6):534-43.

(2) Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. J Clin Virol 2005 Mar;32 Suppl 1:S16-S24.

(3) Brabin L, Roberts SA, Stretch R, Baxter D, Chambers G, Kitchener H, et al. Uptake of first two doses of human papillomavirus vaccine by adolescent schoolgirls in Manchester: prospective cohort study. BMJ 2008 May 10;336(7652):1056-8.

(4) Markowitz LE, Dunne EF, Saraiya M, Lawson HW, Chesson H, Unger ER. Quadrivalent Human Papillomavirus Vaccine: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2007 Mar 23;56(RR-2):1-24.

(5) Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 1998 Feb 12;338(7):423-8.

(6) Tabora N, Zelaya A, Bakkers J, Melchers WJ, Ferrera A. Chlamydia trachomatis and genital human papillomavirus infections in female university students in Honduras. Am J Trop Med Hyg 2005 Jul;73(1):50-3.

(7) Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine 2006 Mar 30;24 Suppl 1:S1-15.

(8) Winer RL, Lee SK, Hughes JP, Adam DE, Kiviat NB, Koutsky LA. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. Am J Epidemiol 2003 Feb 1;157(3):218-26.

(9) Kleter B, van Doorn LJ, Schrauwen L, Molijn A, Sastrowijoto S, ter Schegget J, et al. Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. J Clin Microbiol 1999 Aug;37(8):2508-17.

(10) Melchers WJ, Bakkers JM, Wang J, de Wilde PC, Boonstra H, Quint WG, et al. Short fragment polymerase chain reaction reverse hybridization line probe assay to detect and genotype a broad spectrum of human papillomavirus types. Clinical evaluation and follow-up. Am J Pathol 1999 Nov;155(5):1473-8.

(11) van Ham MA, Bakkers JM, Harbers GK, Quint WG, Massuger LF, Melchers WJ. comparison of two commercial assays for detection of human papillomavirus (HPV) in cervical scrape specimens: validation of the Roche AMPLICOR HPV test as a means to screen for HPV genotypes associated with a higher risk of cervical disorders. J Clin Microbiol 2005 Jun;43(6):2662-7.

(12) van Hamont D, van Ham MA, Bakkers JM, Massuger LF, Melchers WJ. Evaluation of the SPF10-INNO LiPA human papillomavirus (HPV) genotyping test and the roche linear array HPV genotyping test. J Clin Microbiol 2006 Sep;44(9):3122-9.

(13) Coupe VM, Berkhof J, Bulkmans NW, Snijders PJ, Meijer CJ. Age-dependent prevalence of 14 high-risk HPV types in the Netherlands: implications for prophylactic vaccination and screening. Br J Cancer 2008 Feb 12;98(3):646-51.

(14) Dempsey AF, Gebremariam A, Koutsky LA, Manhart L. Using risk factors to predict human papillomavirus infection: implications for targeted vaccination strategies in young adult women. Vaccine 2008 Feb 20;26(8):1111-7.

(15) Manhart LE, Holmes KK, Koutsky LA, Wood TR, Kenney DL, Feng Q, et al. Human papillomavirus infection among sexually active young women in the United States: Implications for developing a vaccination strategy. Sex Transm Dis 2006 Aug;33(8):502-8.

(16) Stewart DE, Gagliardi A, Johnston M, Howlett R, Barata P, Lewis N, et al. Self-collected samples for testing of oncogenic human papillomavirus: a systematic review. J Obstet Gynaecol Can 2007 Oct;29(10):817-28.

(17) Fenton KA, Hughes G. Sexual behaviour in Britain: why sexually transmitted infections are common. Clin Med 2003 May;3(3):199-202.

(18) Fenton KA, Mercer CH, Johnson AM, Byron CL, McManus S, Erens B, et al. Reported sexually transmitted disease clinic attendance and sexually transmitted infections in britain: prevalence, risk factors, and proportionate population burden. J Infect Dis 2005 Feb 1;191 Suppl 1:S127-S138.

(19) Jonsson M, Karlsson R, Rylander E, Gustavsson A, Wadell G. The associations between risk behaviour and reported history of sexually transmitted diseases, among young women: a population-based study. Int J STD AIDS 1997 Aug;8(8):501-5.

(20) Karlsson R, Jonsson M, Edlund K, Evander M, Gustavsson A, Boden E, et al. Lifetime number of partners as the only independent risk factor for human papillomavirus infection: a population-based study. Sex Transm Dis 1995 Mar;22(2):119-27.

(21) Kjaer SK, van den Brule AJ, Bock JE, Poll PA, Engholm G, Sherman ME, et al. Determinants for genital human papillomavirus (HPV) infection in 1000 randomly chosen young Danish women with normal Pap smear: are there different risk profiles for oncogenic and nononcogenic HPV types? Cancer Epidemiol Biomarkers Prev 1997 Oct;6(10):799-805.

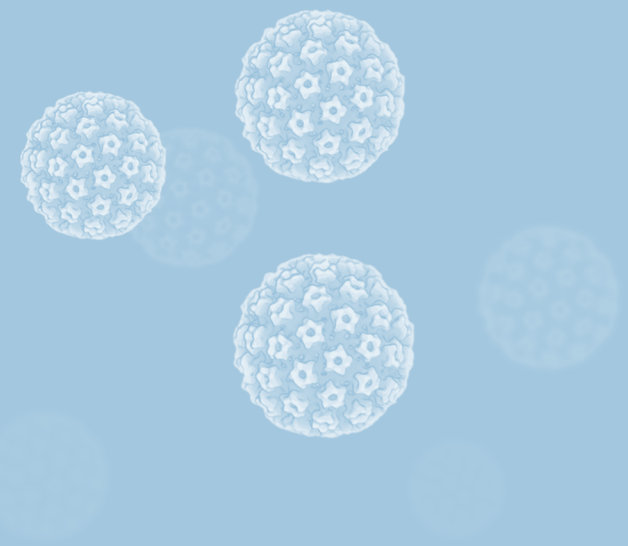
(22) Vaccarella S, Franceschi S, Herrero R, Munoz N, Snijders PJ, Clifford GM, et al. Sexual behavior, condom use, and human papillomavirus: pooled analysis of the IARC human papillomavirus prevalence surveys. Cancer Epidemiol Biomarkers Prev 2006 Feb;15(2):326-33.

(23) Winer RL, Hughes JP, Feng Q, O'Reilly S, Kiviat NB, Holmes KK, et al. Condom use and the risk of genital human papillomavirus infection in young women. N Engl J Med 2006 Jun 22;354(25):2645-54.

(24) Wiley DJ, Wiesmeier E, Masongsong E, Gylys KH, Koutsky LA, Ferris DG, et al. Smokers at higher risk for undetected antibody for oncogenic human papillomavirus type 16 infection. Cancer Epidemiol Biomarkers Prev 2006 May;15(5):915-20.

Chapter 3

Factors influencing HPV incidence and clearance in young women in the pre-vaccine era; a prospective study



Charlotte H. Lenselink[1]
Channa E. Schmeink[1]
Wim G.V. Quint[2]
Nel Roeleveld[3]
Leon F.A.G. Massuger[1]
Willem J.G. Melchers[4]
Ruud L.M. Bekkers[1]

[1, 3, 4] Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands
[1] Department of Obstetrics & Gynaecology
[2] DDL Diagnostic Laboratory, Voorburg, the Netherlands
[3] Department of Epidemiology, Biostatistics and HTA
[4] Department of Medical Microbiology

In preparation

Abstract

Infection with Human Papillomavirus (HPV) is a necessary event in the multi-step process of cervical carcinogenesis. Most sexually active women have been genitally infected by HPV at some point in their life. The natural dynamics of HPV acquisition, clearance, and persistence, may be influenced by viral, host, and environmental factors. Only a few studies investigated the natural course of HPV in healthy unscreened young women. The aim of this study is to obtain more insight into the dynamics of high-risk (hr) HPV infections and specific risk factors of incidence and clearance of genital HPV among young unscreened women.

This prospective epidemiologic study analyses the results of HPV detection in 1812 women aged 18 to 29 years. Women provided three consecutive cervico-vaginal self-samples with a 6 month interval and filled out accompanying questionnaires. Hr-HPV prevalence at study entry was 11.8% (n= 213). During the follow up hr-HPV incidence in sexually active women was 6.3% (n=218). The most commonly acquired hr-HPV type was HPV 16 (2.3%, n=80). The risk of hr-HPV acquisition increased with being single, change in current type of relationship, as well as change in number of sexual partners 3 months prior to sampling, and sexual age at study entry. Hr-HPV clearance was significantly associated with current type of relationship as well as total number of sexual partners (lifetime). This study showed that hr-HPV incidence as well as clearance were related to past and present sexual behaviour. These results suggest that some infections were newly acquired whereas others were acquired in the past and remained latent below detection level and could be considered as accidental pick-ups. As HPV infections are very common, it is difficult to discriminate separate risk factors for HPV dynamics. Our results indicate that sexual behaviour itself, i.e. being sexually active, is the most important determinant, and that certain aspects of sexual behaviour may be of particular interest when looking at genotypes separately.

Introduction

Genital infection with Human Papillomavirus (HPV) is the most common sexually transmitted disease (STD) among young sexually active women.[1] Most sexually active women have been genitally infected by one or more HPV types at some point in their life.[2] Infection with HPV is a necessary event in the multi-step process of cervical carcinogenesis.[3-6] Fortunately, HPV infections are usually transient without causing any symptoms or abnormalities. Estimates of HPV point prevalence among asymptomatic women around the world range from 2% to 44%.[7-9] The wide variation in prevalence is largely explained by differences in age, geography or differences in other characteristics of the populations studied, as well as by differences in sensitivity of the HPV-DNA assay used.[2;9]

The natural dynamics of HPV acquisition, clearance and persistence, may be influenced by viral, host, and environmental factors.[10;11] Still, little is known about these factors, and until recently, only a few large studies investigated the natural course of HPV in female adolescents and young women. Even fewer studies have prospectively investigated the natural course in relation to past and present sexual behaviour in a young and unscreened population.

This study presents the descriptive epidemiological results on the dynamics of high- risk (hr) HPV infections in general as well as hr-HPV type specific infections. It provides an unique insight into specific risk factors for acquisition and clearance of genital HPV infections among young unscreened women before mass vaccination affects the natural history of HPV.

Materials and Methods

Study population and study design

This prospective epidemiologic study was performed among 2065 unscreened women aged 18 to 29 years. Women were recruited between June and September 2007, using different advertisements, as well as active recruitment sites, and posters at general practices in the city regions of Arnhem, Nijmegen, and Den Bosch, the Netherlands. Furthermore, advertisements on the internet were used, which were accessible throughout the Netherlands. Of the 2297 women who responded to the advertisements, 2065 (89.9%) consented with the study, returned the cervico-vaginal swab specimens, and filled out the questionnaire at baseline (time point 0 months, i.e. T0).

The study consisted of 3 sequential test-moments with a 6 month interval (mean 5.8 months, SD 0.63 months). From the 2065 participants at study entry, a total of 253 (12.3%) women were excluded from further analyses. These women became pregnant (n=63, 3.0%), got vaccinated against HPV (n=9, 0.4%), or were lost to follow-up (n=181, 8.8%). This resulted in a final number of 1812 (87.7%) participating women, of whom 1703 reported to be sexually active at study entry. An additional 26 women became sexually active during the follow up, i.e. 13 every 6 months. Results of these women were used for further analyses.

Written informed consent was obtained from all participants. This study was approved by the Local Medical Ethics Committee.

Specimen Collection and Processing

All women were asked to fill out a questionnaire and to self-collect a cervico-vaginal sample in the privacy of their own home at 0, 6, and 12 months. Women received an explanatory letter, an informed consent form, a questionnaire, and a self-sample kit by mail. The self-sample kit contained a collection device (a small brush packaged in an individual sterile cover, Rovers® Viba-brush, Rovers Medical Devices B.V., Oss, the Netherlands), a collection tube containing medium (SurePath™, Tripath Imaging®, Inc., Burlington, NC, USA), instructions how to perform the cervico-vaginal self-sample (written and in cartoon), and a return package consisting of a leak-proof seal bag, absorption sheet, and a reclosable plastic return envelope (easyslider, Transposafe Systems Holland B.V., Sassenheim, the Netherlands).

The self sample was taken and processed as described earlier.[12] In brief, participants were instructed to wash their hands before opening the brush cover, to hold the brush by the end of the handle, to insert the brush approximately 7 cm into the vagina (similar to inserting a tampon), to gently turn the brush 5 times, and to place the top of the brush in the collection tube. The collection tube was closed, and enclosed in the seal-bag. Finally, the collection tube was placed in the return envelope, together with the questionnaire, and sent to the Department of Obstetrics and Gynaecology for further processing and HPV assessment. The samples were stored at room temperature.

Questionnaire

In this study, a questionnaire consisting of two parts was used. The first part was composed of questions regarding socio-demographic variables like educational level, religion, smoking, medication use, oral contraceptive use (OCC), and ethnicity. Race and ethnicity were self-reported into different categories. The second part consisted of questions regarding sexual behaviour. Results of HPV detection were correlated to past and present sexual behaviour. Sex was defined as vaginal, oral, and/or anal sex. For women who had at least 1 lifetime sex partner, additional questions were asked on age at first sexual contact, age of first sex partner, number of sex partners before the age of sixteen, lifetime number of sex partners, number of sex partners in the three months prior to sampling, gender of sex partners, frequency of sexual contact, condom use, and history of STDs.

HPV DNA Detection and Genotyping

As the aim of this study was to obtain a maximum of information about the HPV status in the study population and to monitor the course of infections in detail, the highly sensitive broad-spectrum HPV DNA amplification was performed using a short PCR fragment assay (SPF_{10}-LiPA HPV detection/genotyping assay, SPF_{10} system version 1, manufactured by Labo Biomedical Products BV, Rijswijk, the Netherlands). This assay amplifies a 65-bp fragment of the L1 open reading frame and allows detection of at least 43 different HPV types.[13-15] The SPF_{10} PCR was performed with a final reaction volume of 50 µl containing 10 µl of the isolated DNA sample, 10 mmol/liter Tris-HCl (pH 9.0), 50 mmol/liter KCl, 2.0 mmol/liter $MgCl_2$, 0.1% Triton X-100, 0.01% gelatin, 200 µmol/liter of each deoxynucleoside triphosphate, 15 pmol each of the forward and reverse primers tagged with biotin at the 5'end, and 1.5 U of AmpliTaq Gold (Perkin-Elmer). The mixture was incubated for 9 minutes at 94°C, 40 cycles of 45 s at 45°C, and 40 cycles of 45 s at 72°C, with a final extension of 5 minutes at 72°C. Each experiment was performed with a separate positive and negative PCR control. The presence of HPV DNA was determined by hybridization of SPF_{10} amplimers to a mixture of general HPV probes recognizing a broad range of HPV genotypes, in a microtiter plate format, as described previously.[13-15]

All HPV DNA-positive samples (by SPF_{10} DEIA) were genotyped using the LiPA HPV genotyping assay.

The 28 oligonucleotide probes that recognize 25 different types were tailed with poly(dT) and immobilized as parallel lines to membrane strips (Labo Bio Medical Products BV, Rijswijk, the Netherlands). The HPV genotyping assay was performed as described previously.[13] Samples that tested positive using the DNA enzyme immunoassay but that showed no results on the LiPA strip were considered to be HPV X type, i.e. genotypes not available on the LiPA strip. Low-risk HPV (lr-HPV) types were defined as HPV type 6,11, 34, 40, 42, 43, 44, 53, 54, 55, 58, 66, 70, 74, and "X"; and hr-HPV types as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59, 68, 73, and 82.

Statistical Analysis

Results were used for further analyses when women completed the questionnaires and submitted the swab for HPV evaluation at all three time points (T0-T6-T12) and when they were not pregnant or vaccinated against HPV during the follow up (n= 1812).
Acquisition was defined as transition from an HPV-negative state to an HPV-positive state, i.e. HPV positive for a genotype that had not been detected in the previous self-sample. Clearance of infection was defined as the absence of one or multiple HPV types that had been present in the previous self-sample. This introduces two possible moments of acquisition and clearance, namely, between T0 and T6 and between T6 and T12. For statistical analysis of clearance, the results of clearance were compared to women not clearing their HPV infection in that transition moment. For HPV acquisition and clearance only sexually active women were included. This resulted in 3445 transition moments (T0 →T6 1703 + 13 newly sexually actives= 1716 + T6→T12 1716 + 13 newly sexually actives = 1716 + 1716 + 13 = 3445 transition moments).
The Chi-Square test was used to test associations between demographic variables or behavioural characteristics and HPV incidence. In univariate and multivariate analysis, data of some variables were grouped due to small numbers and/or to gain a better overview. We grouped ethnicity into two groups: Dutch and not Dutch. Lifetime number of partners and number of partners in the past months were divided into four and three categories, respectively. Frequency of sexual contact was grouped into four categories. Years of being sexually active at study entry (i.e. sexual age) ranged from 0 to 23 years, the category "0" years consisted of women who became sexually active in the year prior to the start of the study. Because of the small numbers, 0 and 1 year were combined as well as 13 to 23 years. For the same reason, having both female and male sex partners were grouped together with having male sex partners only. Chlamydia, genital warts, Syphilis, Gonorrhoea, Genital Herpes, and HIV were defined as STD. In further statistical analysis previous STDs were defined as yes or no.
Some questions like age, smoking, age at first intercourse, and lifetime number of sex partners, were only asked at study entry. Other questions were asked at all time points. At each transition moment we measured if there was a change in the existing answer i.e. variable. For example, stop or start OCC use or change in type of relationship. This way a new variable was created consisting of "change yes/no". Variables significantly related to HPV incidence or clearance by univariate analyses were entered into a multiple logistic regression model with forward selection procedures to identify variables that contributed independently to the probability of HPV incidence or clearance. Participants with missing data on variables included in the multivariate analysis were excluded.
In all tests, p values < 0.05 were regarded statistically significant. Statistical analyses were performed using SPSS 16.0 (Chicago, Illinois, USA).

Results

Baseline Characteristics

Of the 2065 participants at study entry, 253 women (12.3%) were excluded because of pregnancy, vaccination against HPV, or because they did not return all three samples. In general, the demographic characteristics and sexual behaviour at study entry were similar for the excluded women (n= 253, 12.3%) and the included women (n=1812, 87.7%). However, smoking, OCC use and type of relationship differed between the included and excluded women. Analysis showed that the excluded group consisted of significantly more smokers (28.3% versus 18.6%, p=<0.01), less OCC users (57.4% versus 72.7%, p=<0.01), and more women who were married or cohabiting. However, type of relationship as well as OCC use was confounded by women who were pregnant and therefore excluded (data not shown). Most important, the HPV prevalence at study entry was not significantly different between included and excluded women.
The results of the remaining 1812 women were used for further analyses. Their mean age at baseline was 23.2 years (SD 3.3). Age distribution and socio-demographic characteristics are summarised in Table 1. Many women attended higher vocational training or University in past or presence (n=1384, 76.7%). Of all women, 557 (30.8%) were single and 1249 (69.2%) were involved in a relationship. Only 53 women (2.9%) reported an ethnicity other than Dutch. Because of these small numbers, they were divided into two groups: Dutch (97.1%, n= 1750), and "other" (2.9%, n=53).
At study entry the mean age at first sexual contact was 16.7 years, and the mean sexual age (i.e. years of being sexually active) was 6.4 years. Furthermore, 107 (5.9%) women reported not to be sexually active in past or presence. Of these women, 26 became sexually active during follow up.

Prevalence of HPV infection

Of the 1812 women included in this analysis, 343 women (18.9%) were positive for one or more HPV infections at study entry, of whom 3 women reported not to be sexually active in past or presence. Lr- and hr-HPV prevalence were 8.9% (n=161) and 11.8% (n=213), respectively. Five most frequent hr-HPV types were HPV 16 (2.8%, n=51), HPV 18 (1.4%, n=25), HPV 31 (1.4%, n=25), HPV 51 (2.5%, n=45), and HPV 52 (2.3%, n=41). During this year of follow up, 1218 (67.2%) of the 1812 women remained HPV negative at all time points.

Table 1. Baseline characteristics of all 1812 women (including non-sexually active women)

	n	(%)
Age (in years)	1812	
18	126	(7.0)
19	159	(8.8)
20	180	(9.9)
21	170	(9.4)
22	168	(9.3)
23	156	(8.6)
24	164	(9.1)
25	160	(8.8)
26	157	(8.7)
27	149	(8.2)
28	134	(7.4)
29	89	(4.9)
Ethnicity*	1803	
Dutch	1750	(97.1)
Other	53	(2.9)
Education	1804	
Lower secondary / Lower vocational training	56	(3.1)
Higher Secondary / Vocational training	356	(19.7)
Higher vocational training / University	1384	(76.7)
Other	8	(0.4)
Current smoking	1803	
Yes	335	(18.6)
No	1468	(81.4)
Using OCC	1810	
Yes	1315	(72.7)
No	495	(27.3)
Living with parents	1802	
Yes	317	(17.6)
No	1485	(82.4)
Relationship	1806	
Married	78	(4.3)
Living together	417	(23.1)
LAT	754	(41.7)
Single	557	(30.8)
Sexual activity at study entry	1810	
Yes	1703	(94.1)
No	107	(5.9)

Sample sizes change because of missing values of the questionnaire
n: number
* ethnicity was self-reported
LAT: living apart together
OCC: oral contraceptives

Incidence of HPV infections among sexually active women

Transition from an HPV-negative state to an HPV-positive state, i.e. HPV incidence, could occur between T0 and T6 and between T6 and T12. Among sexually active women this resulted in 3445 transition moments. Hr-HPV incidence was 6.3% (n=218) (Table 2).
For hr-HPV types 16 and 18 the acquisition rate were 2.3% (n=80) for HPV 16 and 1.2% (n=40) for HPV 18 (Table 2).

Table 2. Incidence and clearance of HPV infections among sexually active women

	Incidence (n)	Clearance (n)*
Hr-HPV	6.3% (218/3445)	40.9% (187/457)
HPV 16	2.3% (80/3445)	56.3% (67/119)
HPV 18	1.2% (40/3445)	62.7% (37/59)

n: number
* for clearance, only the women were included who were positive for the specific HPV genotype or group, the results of clearance were compared to women not clearing their HPV infection
Hr-HPV: high-risk HPV

Risk factors for acquiring hr-HPV infections in sexually active women

The risk factors for hr-HPV incidence are presented in Table 3. Factors significantly associated with hr-HPV incidence in univariate analysis were increasing age, smoking, type of current relationship, change in type of relationship, change in having a new relationship, increasing sexual age, total number of sex partners (lifetime), having had two or more partners in the 3 months prior to testing, and a change in number of

Table 3. Odds Ratio's for high-risk HPV, HPV 16 and HPV 18 incidence among sexually active women using univariate logistic regression (n=3445)

		Hr-HPV			HPV 16			HPV 18		
	n	Events* (n)	OR (95% CI)	p	Events* (n)	OR (95% CI)	p	Events* (n)	OR (95% CI)	p
Age (years)**	**3445**	218	1.06 (1.02;1.11)	**<0.01**	80	1.04 (0.97;1.11)	0.33	40	1.04 (0.95;1.15)	0.39
Smoking**	**3427**									
No	2766	161	0.66 (0.48;0.90)	**<0.01**	65	1.04 (0.59;1.83)	0.90	29	0.63 (0.31;1.26)	0.19
Yes	661	57	1.00 (ref)		15	1.00 (ref)		11	1.00 (ref)	
Current OCC use	**3429**									
No	1000	68	1.13 (0.84;1.53)	0.41	23	0.98 (0.60;1.60)	0.93	12	1.08 (0.55;2.14)	0.82
Yes	2429	147	1.00 (ref)		57	1.00 (ref)		27	1.00 (ref)	
Change No	3247	197	0.61 (0.36;1.03)	0.06	77	2.14 (0.52;8.77)	0.29	36	0.65 (0.20;2.15)	0.50
Change Yes	178	17	1.00 (ref)		2	1.00 (ref)		3	1.00 (ref)	
Living with parents**	**3425**									
No	2861	182	1.00 (0.69;1.44)	0.99	69	1.37 (0.70;2.67)	0.36	35	1.39 (0.54;3.55)	0.50
Yes	564	36	1.00 (ref)		10	1.00 (ref)		5	1.00 (ref)	
Relationship	**3439**									
Married	201	9	0.44 (0.22;0.90)	**0.02**	1	0.15 (0.02;1.08)	0.06	1	0.32 (0.04;2.46)	0.27
Living together	998	41	0.41 (0.28;0.60)	**<0.01**	23	0.69 (0.39;1.21)	0.19	10	0.65 (0.28;1.49)	0.31
LAT	1394	87	0.63 (0.46;0.86)	**<0.01**	28	0.60 (0.35;1.02)	0.06	16	0.74 (0.36;1.56)	0.43
Single	846	81	1.00 (ref)		28	1.00 (ref)		13	1.00 (ref)	
Change No	2759	155	0.58 (0.43;0.79)	**<0.01**	59	0.71 (0.42;1.19)	0.19	28	0.56 (0.28;1.11)	0.10
Change Yes	669	62	1.00 (ref)		20	1.00 (ref)		12	1.00 (ref)	
New relationship	**3426**									
No	3247	201	0.63 (0.37;1.06)	0.08	76	1.41 (0.44;4.50)	0.57	38	1.05 (0.25;4.38)	0.95
Yes	179	17	1.00 (ref)		3	1.00 (ref)		2	1.00 (ref)	
Change No	3247	201	0.52 (0.27;1.00)	**0.05**	76	1.15 (0.28;4.75)	0.85	38	1.15 (0.16;8.45)	0.90
Change Yes	98	11	1.00 (ref)		2	1.00 (ref)		1	1.00 (ref)	
Age at first intercourse (years)**	**3402**									
≤ 13	72	4	1.32 (0.41;4.23)	0.64	2	1.31 (0.26;6,65)	0.74	0	1.00 (-;-)	1.00
14-16	1654	118	1.73 (0.94;3.17)	0.08	35	0.99 (0.41;2.39)	0.99	26	2.58 (-;-)	0.99
17-19	1394	84	1.44 (0.78;2.68)	0.25	37	1.25 (0.52;3.00)	0.61	14	1.64 (-;-)	0.99
≥ 20	282	12	1.00 (ref)		6	1.00 (ref)		0	1.00 (ref)	
Sexual Age (years)**, ***	**3400**	218	1.07 (1.03;1.11)	**<0.01**	80	1.03 (0.96;1.10)	0.40	40	1.09 (0.10;1.20)	0.06

Table 3. continued

		Hr-HPV			HPV 16			HPV 18		
	n	**Events*** (n)	**OR (95% CI)**	**p**	**Events*** (n)	**OR (95% CI)**	**p**	**Events*** (n)	**OR (95% CI)**	**p**
Lifetime sex partners (number)**	**3390**									
1	712	25	0.26 (0.16;0.43)	**<0.01**	6	0.20 (0.08;0.52)	**<0.01**	2	0.13 (0.03;0.60)	**<0.01**
2-5	1618	95	0.45 (0.31;0.65)	**<0.01**	39	0.58 (0.32;1.06)	0.08	16	0.45 (0.19;1.05)	0.07
6-10	694	49	0.54 (0.35;0.83)	**<0.01**	19	0.66 (0.33;1.31)	0.24	13	0.85 (0.35;2.08)	0.73
≥ 11	366	45	1.00 (ref)		15	1.00 (ref)		8	1.00 (ref)	
Gender of sex partner(s)	**2965**									
Male, both	2920	188	3.03 (0.42;22.10)	0.28	71	4.03 (-;-)	1.00	34	1.90 (-;-)	1.00
Female	45	1	1.00 (ref)		0	1.00 (ref)		0	1.00 (ref)	
Change No	2912	177	2.07 (0.28;15.24)	0.48	67	3.80 (0.00-;-)	1.00	35	1.97 (0.00;-)	1.00
Change Yes	33	1	1.00 (ref)		0	1.00 (ref)		0	1.00 (ref)	
Sex partners in past 3 months (number)	**3431**									
0	429	22	0.46 (0.26;0.82)	**<0.01**	8	0.37 (0.15;0.90)	**0.03**	4	0.82 (0.18;3.69)	0.80
1	2738	168	0.55 (0.36;0.84)	**<0.01**	59	0.43 (0.23;0.79)	**<0.01**	33	1.06 (0.32;3.48)	0.92
≥ 2	264	28	1.00 (ref)		13	1.00 (ref)		3	1.00 (ref)	
Change No	2686	132	0.39 (0.29;0.52)	**<0.01**	44	0.33 (0.21;0.52)	**<0.01**	25	0.45 (0.23;0.85)	**0.01**
Change Yes	728	85	1.00 (ref)		35	1.00 (ref)		15	1.00 (ref)	
Sexual contact in past 3 months (frequency)	**3426**									
0	431	22	0.88 (0.55;1.40)	0.59	8	0.83 (0.39;1.76)	0.62	4	0.80 (0.28;2.30)	0.68
1-3	338	41	2.26 (1.56;3.28)	**<0.01**	17	2.32 (1.32;4.07)	**<0.01**	7	1.80 (0.78;4.18)	0.17
4-12	417	26	1.09 (0.70;1.68)	0.70	5	0.53 (0.21;1.34)	0.18	3	0.62 (0.19;2.05)	0.43
≥ 13	2240	129	1.00 (ref)		50	1.00 (ref)		26	1.00 (ref)	
Change No	2339	113	0.49 (0.37;0.65)	**<0.01**	51	0.83 (0.52;1.32)	0.43	24	0.66 (0.35;1.24)	0.19
Change Yes	1027	97	1.00 (ref)		27	1.00 (ref)		16	1.00 (ref)	
STD**	**3422**									
No	3397	216	1.63 (0.22;12.10)	0.63	79	0.57 (0.8;4.28)	0.59	39	0.28 (0.04;2.11)	0.22
Yes	25	1	1.00 (ref)		1	1.00 (ref)		1	1.00 (ref)	
Change No	3352	212	1.45 (0.35;6.03)	0.61	74	0.32 (0.10;1.04)	0.06	38	0.51 (0.07;3.76)	0.50
Change Yes	45	2	1.00 (ref)		3	1.00 (ref)		1	1.00 (ref)	

Table 3. continued

		Hr-HPV			HPV 16			HPV 18		
	n	Events* (n)	OR (95% CI)	p	Events* (n)	OR (95% CI)	p	Events* (n)	OR (95% CI)	p
Condom use	**3001**									
Never	1866	101	0.99 (0.57;1.73)	0.98	35	1.03 (0.40;2.66)	0.95	19	1.40 (0.33;6.06)	0.65
Sometimes	511	43	1.59 (0.87;2.92)	0.13	21	2.31 (0.86;6.21)	0.10	5	1.35 (0.26;7.00)	0.72
Most of times	349	36	1.99 (1.07;3.72)	**0.03**	10	1.59 (0.54;4.72)	0.40	10	4.03 (0.88;18.53)	0.07
Always	275	15	1.00 (ref)		5	1.00 (ref)		2	1.00 (ref)	
Change No	1956	104	0.70 (0.51;0.94)	**0.02**	40	0.87 (0.51;1.47)	0.59	17	0.47 (0.24;0.93)	**0.03**
Change Yes	934	70	1.00 (ref)		22	1.00 (ref)		17	1.00 (ref)	

n: number
Sample sizes change because of missing values of the questionnaire
Hr-HPV: high-risk HPV
* number of HPV infections acquired during follow up
OR: Odds Ratio
95% CI: 95% Confidence Interval
p: p-value
** data retrieved at study entry
ref: reference
OCC: oral contraceptives
Change: change in variable in 3 months prior to sample
LAT: living apart together
*** Sexual age in years at baseline with 0 and 1 years combined as well as sexual age higher than 13 years
**** STD, sexually transmitted disease in 3 months prior to sample

partners in 3 months prior to testing. Furthermore, frequency of sexual contact in 3 months prior to testing, change in frequency of sexual contact in 3 months prior to testing, frequency of condom use, and a change in condom use were associated with hr-HPV incidence.

The analysis was concluded by completing a multivariate regression analysis on all factors that showed a significant relationship with hr-HPV incidence in the univariate analysis. The factors independently associated with a risk of acquiring a hr-HPV infection were type of relationship, change in type of relationship, increasing sexual age, and change in number of sex partners in the past 3 months (Table 4).

Risk factors for acquiring an HPV 16 or HPV 18 infection in sexually active women

Risk factors for acquiring an HPV 16 or HPV 18 infection are presented in Table 3 and Table 4.

Univariate analysis for HPV 16 incidence showed that having had a total of 11 or more sex partners (lifetime) compared to having had 1 partner, having had 2 or more sex partners in the past 3 months, change in number of sex partners in past 3 months, as well as frequency of sexual contact in the past 3 months were significantly increasing the risk of acquiring an HPV 16 infection. After multivariate analysis, change of number of sex partners in the past 3 months and frequency sexual contact in the past 3 months remained as independent risk factors for HPV 16 incidence.

In univariate analysis, having had a total of 11 or more sex partners (lifetime), change in number of sex partners in the past 3 months, as well as change in condom use were

Table 4. Adjusted Odds Ratio's for hr-HPV, HPV 16 and HPV 18 incidence among sexually active women using multivariate logistic regression

	Hr-HPV (n=2686)		HPV 16 (n=3346)		HPV 18 (n=2820)	
	Adj. OR (95% CI)	**p**	**Adj. OR (95% CI)**	**p**	**Adj. OR (95% CI)**	**p**
Relationship						
Married	0.29 (0.13;0.69)	**<0.01**	-	-	-	-
Living together	0.35 (0.22;0.56)	**<0.01**	-	-	-	-
LAT	0.58 (0.38;0.86)	**<0.01**	-	-	-	-
Single	1.00 (ref)		-	-	-	-
Change					-	-
No	0.61 (0,43;0.89)	**<0.01**	-	-	-	-
Yes	1.00 (ref)		-	-	-	-
Sexual Age (years)*,**	1.09 (1.04;1.15)	**<0.01**	-	-	-	-
Lifetime sex partners (number)*						
1	-	-	-	-	0.17 (0.04;0.83)	**0.03**
2-5	-	-	-	-	0.42 (0.17;1.05)	0.06
6-10	-	-	-	-	0.79 (0.31;1.99)	0.61
≥ 11	-	-	-	-	1.00 (ref)	
Sex partners in past 3 months (number)						
0	-	-	-	-	-	-
1	-	-	-	-	-	-
≥ 2	-	-	-	-	-	-
Change						
No	0.59 (0.39;0.88)	**<0.01**	0.31 (0.20;0.49)	**<0.01**	0.49 (0.23;1.05)	**0.07**
Yes	1.00 (ref)		1.00 (ref)		1.00 (ref)	
Sexual contact in past 3 months (frequency)						
0	-	-	0.52 (0.24;1.14)	0.10	-	-
1-3	-	-	1.69 (0.94;3.03)	0.08	-	-
4-12	-	-	0.30 (0.09;0.98)	**0.05**	-	-
≥ 13	-	-	1.00 (ref)		-	-

Hr-HPV: high-risk HPV
n: number
Adj. OR: Adjusted Odds Ratio
95% CI: 95% Confidence Interval
p: p-value
ref: reference
LAT: living apart together
Change: change in variable in 3 months prior to sample
* data retrieved at study entry
** Sexual age in years at baseline with 0 and 1 years combined as well as sexual age higher than 13 years

associated with a higher risk of acquiring an HPV 18 infection. After completing the multivariate analysis for HPV 18 incidence, risk factors that were independently related to the risk of acquiring HPV 18 were number of lifetime partners and a change in number of partners in the past 3 months.

Clearance of hr-HPV infections in sexually active women

For analysis of clearance, the results of clearance were compared to women not clearing their HPV infection. Among the transition moments of the sexually active women who were hr-HPV positive (n= 457), the hr-HPV clearance was 40.9% (n=187). For HPV 16 and HPV 18 the clearance rate during follow up was 56.3% (n=67) and 62.7% (n=37), respectively (Table 2).

To compare factors associated with hr-HPV clearance, univariate analysis followed by multivariate analysis was used. Univariate analysis showed that total number of partners (lifetime), number of partners in three months prior to sampling, as well as type of relationship were associated with HPV clearance. Multivariate analysis showed type of relationship and total number of partners (lifetime) to be independently associated with hr-HPV clearance (Table 5).

Factors influencing HPV 16 and HPV 18 clearance in sexually active women

In univariate analyses only the total number of partners (lifetime) showed a significant association with HPV 16 clearance (data not shown).

Univariate analysis for HPV 18 clearance showed type of relationship and never or sometimes using a condom compared to always using condoms as factors positively

Table 5. Hr-HPV clearance, Odds Ratio's and Adjusted Odds Ratio's for hr-HPV clearance among sexually active women using univariate logistic regression (n=457) and multivariate logistic regression (n=446)

	n	Events* (n)	OR (95% CI)	p	Adj. OR (95% CI)	p
Age (years)**	**457**	187	1.00 (0.94;1.06)	0.91	-	-
Smoking**	**457**					
No	321	137	1.28 (0.85;1.94)	0.24	-	-
Yes	136	50	1.00 (ref)		-	-
Current OCC use	**453**					
No	144	59	1.01 (0.67;1.51)	0.97	-	-
Yes	309	126	1.00 (ref)		-	-
Change No	434	179	1.52 (0.57;4.08)	0.41	-	-
Change Yes	19	6	1.00 (ref)		-	-
Living with parents**	**455**					
No	390	157	0.79 (0.46;1.33)	0.37	-	-
Yes	65	30	1.00 (ref)		-	-
Relationship	**455**					
Married	10	8	9.19 (1.88;44.93)	**<0.01**	7.48 (1.47;38.17)	**0.02**
Living together	107	52	2.17 (1.30;3.62)	**<0.01**	1.97 (1.17;3.32)	**0.01**
LAT	183	78	1.70 (1.09;2.68)	**0.02**	1.75 (1.10;2.78)	**0.02**
Single	155	47	1.00 (ref)		1.00 (ref)	
Change No	338	134	0.85 (0.56;1.30)	0.45	-	-
Change Yes	117	51	1.00 (ref)		-	-
New relationship	**452**					
No	426	175	1.12 (0.50;2.52)	0.79	-	-
Yes	26	10	1.00 (ref)		-	-
Change No	426	175	1.05 (0.37;3.00)	0.93	-	-
Change Yes	15	6	1.00 (ref)		-	-
Age at first intercourse (years)**	**455**					
≤ 13	12	4	0.60 (0.15;2.39)	0.47	-	-
14-16	235	101	0.90 (0.44;1.88)	0.79	-	-
17-19	175	67	0.74 (0.35;1.58)	0.44	-	-
≥ 20	33	15	1.00 (ref)		-	-
Sexual Age (years)**, ***	**455**	187	0.99 (0.93;1.05)	0.71	-	-
Lifetime sex partners (number)**	**449**					
1	27	15	1.54 (0.66;3.61)	0.32	1.22 (0.50;2.94)	0.67
2-5	168	76	1.02 (0.62;1.66)	0.94	0.93 (0.56;1.53)	0.78
6-10	149	46	0.55 (0.33;0.93)	**0.02**	0.54 (0.32;0.92)	**0.02**
≥ 11	105	47	1.00 (ref)		1.00 (ref)	
Gender of sex partner(s)	**390**					
Male, both	389	158	1.11 (0.00;-)	1.00	-	-
Female	1	0	1.00 (ref)		-	-
Change No	400	164	-	-	-	-
Change Yes	0	0	-	-	-	-
Sex partners in past 3 months (number)	**455**					
0	59	24	1.83 (0.83;4.02)	0.13	-	-
1	341	146	2.00 (1.06;3.75)	**0.03**	-	-
≥ 2	55	15	1.00 (ref)		-	-
Change No	313	136	1.46 (0.97;2.20)	0.07	-	-
Change Yes	142	49	1.00 (ref)			
Sexual contact in past 3 months (frequency)	**455**					
0	59	24	0.95 (0.54;1.67)	0.85	-	-
1-3	59	18	0.61 (0.33;1.10)	0.10	-	-
4-12	42	20	1.25 (0.66;2.40)	0.49	-	-
≥ 13	295	124	1.00 (ref)			
Change No	308	128	1.03 (0.69;1.54)	0.89	-	-
Change Yes	142	58	1.00 (ref)		-	-
STD**	**455**					
No	446	182	0.86 (0.23;3.25)	0.83	-	-
Yes	9	4	1.00 (ref)		-	-
Change No	440	182	1.41 (0.42;4.76)	0.58	-	-
Change Yes	12	4	1.00 (ref)			
Condom use	**396**					
Never	245	109	1.74 (0.84;3.60)	0.14	-	-

Table 5. continued

	n	Events* (n)	OR (95% CI)	p	Adj. OR (95% CI)	p
Sometimes	67	26	1.37 (0.59;3.19)	0.46	-	-
Most of times	46	15	1.05 (0.42;2.63)	0.92	-	-
Always	38	12	1.00 (ref)			
Change No	244	109	1.50 (0.97;2.31)	0.07	-	-
Change Yes	137	48	1.00 (ref)		-	-

n: number
Sample sizes change because of missing values of the questionnaire
* number of HPV infections cleared during follow up
OR: Odds Ratio
95% CI: 95% Confidence Interval
p: p-value
Adj. OR: Adjusted Odds Ratio
** data retrieved at study entry
ref: reference
-: not applicable
OCC: oral contraceptives
Change: change in variable in 3 months prior to sample
LAT: living apart together
*** Sexual age in years at baseline with 0 and 1 years combined as well as sexual age higher than 13 years
**** STD, sexually transmitted disease in 3 months prior to sample

associated with clearance. However, multivariate analysis showed that condom use was confounded by type of relationship and reversely. Therefore, no independent influencing factors were identified (data not shown).

Discussion

At study entry the point prevalence of hr-HPV was 11.8%. During follow up hr-HPV 6 months incidence was 6.3%. Factors significantly associated with hr-HPV incidence were related to present sexual behaviour like currently being in a relationship and change in number of sexual partners 3 months prior to sampling. These findings are consistent with results from studies describing factors associated with HPV infection.[2;16] However, some studies describe sexual behaviour itself as a risk factor whereas others indicate specific aspects of sexual behaviour.[2;17-19] Several international studies confirm sexual behaviour and a high number of sexual partners as the most prominent risk factors to contract STDs.[7;17;18;20-26]

In this study not only change in the number of sexual partners in the past three months but also number of lifetime sexual partners was associated with a transition from hr-HPV negative status to a hr-HPV positive status. A higher number of lifetime sexual partners increases the risk of getting infected with one or more HPV types in time. Several studies associated past sexual behaviour with HPV incidence.[2;16-18] A possible explanation for the influence of past sexual behaviour on present detection of HPV after a former negative sample could be that the infection was acquired in the past and remained present with levels of shed virus below the threshold of detection as if it were cleared. These infections may be considered as an accidental pick-up.

Detecting an infection is dependent on the sensitivity of the technique used. In this study the highly sensitive SPF_{10} LiPA HPV genotyping assay was used, which could make it difficult to discriminate between truly newly acquired infections and accidental pick-ups because of its low threshold value. For this reason we can only claim to have measured the "presumed" incidence rate of a new type for those negative at the previous test moment.[27]

Change in number of partners in past 3 months was significantly related to acquiring a hr-HPV, an HPV 16 as well as an HPV 18 infection. However, some other specific risk factors differed. For hr-HPV, type of relationship as well as change in current type of relationship, and sexual age were risk factors. For HPV 16, frequency of sexual contact influenced transition, and for HPV 18, lifetime number of partners was associated with HPV incidence. Looking at the transition moments of the separate genotypes, a remark must be made that the numbers are small making the results difficult to interpret. Nevertheless, all factors associated with HPV transition were related to sexual behaviour and therefore influencing the risk of acquiring an HPV infection.

Furthermore, type of relationship was associated with both the risk of acquiring a hr-HPV infection as well as clearance of the previous detected infection. Women being single were most at risk for acquiring an infection, whereas women who were in a relationship, no matter which form, had a better chance of clearing the previously detected infection. This may be explained by the fact that women in a relationship may have more accidental pick-ups which may not be detected during the next measurement. Additionally, singles may have a higher chance of acquiring a true new infection during follow up. These infections may need a longer time to transit to an HPV negative status again than this study provided.

Every HPV infection has its type dependent clearance which takes 8 to 14 months on average.[2;7;9;10;19;27;28] The difference in estimations of time to clearance may be attributed to the interval between test moments. Additionally, the meaning of an intermediate negative test has been inconsistently defined [7;16;29-33], resulting in various definitions of incidence, persistence, and clearance. We did not measure exact time to clearance. Many infections

detected in this study were cleared, i.e. not detected at the next test moment. This is consistent with findings in other natural history studies of cervical HPV infection. [9;10;27;34;35] Several studies have limited their analyses to hr-HPV or grouped specific types together.[10;36;37] Other studies investigated the possible effect of co-infections with multiple HPV types on the natural course of the infection.[7;10;35;38;39] We generally considered the natural course of type specific infections independent of a co-infection with another type, which might be a caveat.

The lack of an observed association with OCC use or smoking is consistent with other studies on risk factors for HPV dynamics.[23;40] Nevertheless, studies on OCC use and smoking show inconsistent results.[2;23;40-43] As the censored women in this study consisted of significantly more smokers this may have biased our results. Additionally, the majority of the participants used OCCs and since OCC use is strongly related to sexual activity, this may have made it difficult to discriminate an effect. In contrast to several other studies, [10;44], age showed no independent relationship with HPV incidence. This may be explained by the fact that our population did not include women above the age of thirty, resulting in no decline in incidence due to higher age.

In conclusion, this study shows that among young unscreened women hr-HPV incidence as well as clearance were related to both past and present sexual behaviour. These results suggest that some infections were newly acquired whereas others were acquired in the past and remained latent below detection level for a long time and may be considered as an accidental pick-up. As HPV infections are very common, it is difficult to discriminate separate risk factors for HPV transition, i.e. acquiring and clearance of infections. Our results indicate that sexual behaviour itself, i.e. being sexually active, is the most important determinant influencing HPV dynamics, and that certain aspects of sexual behaviour may be of particular interest when looking at genotypes separately.

Reference List

(1) Burchell AN, Winer RL, de Sanjose S, Franco EL. Chapter 6: Epidemiology and transmission dynamics of genital HPV infection. Vaccine 2006 Aug 21;24 Suppl 3:S52-S61.

(2) Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. J Clin Virol 2005 Mar;32 Suppl 1:S16-S24.

(3) Bekkers RL, Massuger LF, Bulten J, Melchers WJ. Epidemiological and clinical aspects of human papillomavirus detection in the prevention of cervical cancer. Rev Med Virol 2004 Mar;14(2):95-105.

(4) Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003 Feb 6;348(6):518-27.

(5) van Hamont D, Bekkers RL, Massuger LF, Melchers WJ. Detection, management, and follow-up of pre-malignant cervical lesions and the role for human papillomavirus. Rev Med Virol 2008 Mar;18(2):117-32.

(6) Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999 Sep;189(1):12-9.

(7) Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 1998 Feb 12;338(7):423-8.

(8) Tabora N, Zelaya A, Bakkers J, Melchers WJ, Ferrera A. Chlamydia trachomatis and genital human papillomavirus infections in female university students in Honduras. Am J Trop Med Hyg 2005 Jul;73(1):50-3.

(9) Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine 2006 Mar 30;24 Suppl 1:S1-15.

(10) Goodman MT, Shvetsov YB, McDuffie K, Wilkens LR, Zhu X, Thompson PJ, et al. Prevalence, acquisition, and clearance of cervical human papillomavirus infection among women with normal cytology: Hawaii Human Papillomavirus Cohort Study. Cancer Res 2008 Nov 1;68(21):8813-24.

(11) Hildesheim A, Wang SS. Host and viral genetics and risk of cervical cancer: a review. Virus Res 2002 Nov;89(2):229-40.

(12) Lenselink CH, Melchers WJ, Quint WG, Hoebers AM, Hendriks JC, Massuger LF, et al. Sexual behaviour and HPV infections in 18 to 29 year old women in the pre-vaccine era in the Netherlands. PLoS ONE 2008;3(11):e3743.

(13) Kleter B, van Doorn LJ, Schrauwen L, Molijn A, Sastrowijoto S, ter Schegget J, et al. Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. J Clin Microbiol 1999 Aug;37(8):2508-17.

(14) Melchers WJ, Bakkers JM, Wang J, de Wilde PC, Boonstra H, Quint WG, et al. Short fragment polymerase chain reaction reverse hybridization line probe assay to detect and genotype a broad spectrum of human papillomavirus types. Clinical evaluation and follow-up. Am J Pathol 1999 Nov;155(5):1473-8.

(15) van Hamont D, van Ham MA, Bakkers JM, Massuger LF, Melchers WJ. Evaluation of the SPF10-INNO LiPA human papillomavirus (HPV) genotyping test and the roche linear array HPV genotyping test. J Clin Microbiol 2006 Sep;44(9):3122-9.

(16) Winer RL, Lee SK, Hughes JP, Adam DE, Kiviat NB, Koutsky LA. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. Am J Epidemiol 2003 Feb 1;157(3):218-26.

(17) Kjaer SK, van den Brule AJ, Bock JE, Poll PA, Engholm G, Sherman ME, et al. Determinants for genital human papillomavirus (HPV) infection in 1000 randomly chosen young Danish women with normal Pap smear: are there different risk profiles for oncogenic and nononcogenic HPV types? Cancer Epidemiol Biomarkers Prev 1997 Oct;6(10):799-805.

(18) Lu B, Wu Y, Nielson CM, Flores R, Abrahamsen M, Papenfuss M, et al. Factors associated with acquisition and clearance of human papillomavirus infection in a cohort of US men: a prospective study. J Infect Dis 2009 Feb 1;199(3):362-71.

(19) Oh JK, Ju YH, Franceschi S, Quint W, Shin HR. Acquisition of new infection and clearance of type-specific human papillomavirus infections in female students in Busan, South Korea: a follow-up study. BMC Infect Dis 2008;8:13.

(20) Fenton KA, Hughes G. Sexual behaviour in Britain: why sexually transmitted infections are common. Clin Med 2003 May;3(3):199-202.

(21) Fenton KA, Mercer CH, Johnson AM, Byron CL, McManus S, Erens B, et al. Reported sexually transmitted disease clinic attendance and sexually transmitted infections in britain: prevalence, risk factors, and proportionate population burden. J Infect Dis 2005 Feb 1;191 Suppl 1:S127-S138.

(22) Jonsson M, Karlsson R, Rylander E, Gustavsson A, Wadell G. The associations between risk behaviour and reported history of sexually transmitted diseases, among young women: a population-based study. Int J STD AIDS 1997 Aug;8(8):501-5.

(23) Karlsson R, Jonsson M, Edlund K, Evander M, Gustavsson A, Boden E, et al. Lifetime number of partners as the only independent risk factor for human papillomavirus infection: a population-based study. Sex Transm Dis 1995 Mar;22(2):119-27.

(24) Manhart LE, Holmes KK, Koutsky LA, Wood TR, Kenney DL, Feng Q, et al. Human papillomavirus infection among sexually active young women in the United States: Implications for developing a vaccination strategy. Sex Transm Dis 2006 Aug;33(8):502-8.

(25) Vaccarella S, Franceschi S, Herrero R, Munoz N, Snijders PJ, Clifford GM, et al. Sexual behavior, condom use, and human papillomavirus: pooled analysis of the IARC human papillomavirus prevalence surveys. Cancer Epidemiol Biomarkers Prev 2006 Feb;15(2):326-33.

(26) Sellors JW, Karwalajtys TL, Kaczorowski J, Mahony JB, Lytwyn A, Chong S, et al. Incidence, clearance and predictors of human papillomavirus infection in women. CMAJ 2003 Feb 18;168(4):421-5.

(27) Franco EL, Villa LL, Sobrinho JP, Prado JM, Rousseau MC, Desy M, et al. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. J Infect Dis 1999 Nov;180(5):1415-23.

(28) Giuliano AR, Harris R, Sedjo RL, Baldwin S, Roe D, Papenfuss MR, et al. Incidence, prevalence, and clearance of type-specific human papillomavirus infections: The Young Women's Health Study. J Infect Dis 2002 Aug 15;186(4):462-9.

(29) Koshiol J, Lindsay L, Pimenta JM, Poole C, Jenkins D, Smith JS. Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. Am J Epidemiol 2008 Jul 15;168(2):123-37.

(30) Richardson H, Kelsall G, Tellier P, Voyer H, Abrahamowicz M, Ferenczy A, et al. The natural history of type-specific human papillomavirus infections in female university students. Cancer Epidemiol Biomarkers Prev 2003 Jun;12(6):485-90.

(31) Samoff E, Koumans EH, Markowitz LE, Sternberg M, Sawyer MK, Swan D, et al. Association of Chlamydia trachomatis with persistence of high-risk types of human papillomavirus in a cohort of female adolescents. Am J Epidemiol 2005 Oct 1;162(7):668-75.

(32) Schlecht NF, Kulaga S, Robitaille J, Ferreira S, Santos M, Miyamura RA, et al. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. JAMA 2001 Dec 26;286(24):3106-14.

(33) Sycuro LK, Xi LF, Hughes JP, Feng Q, Winer RL, Lee SK, et al. Persistence of genital human papillomavirus infection in a long-term follow-up study of female university students. J Infect Dis 2008 Oct 1;198(7):971-8.

(34) Moscicki AB, Schiffman M, Kjaer S, Villa LL. Chapter 5: Updating the natural history of HPV and anogenital cancer. Vaccine 2006 Aug 31;24 Suppl 3:S3-42-S3/51.

(35) Trottier H, Mahmud S, Prado JC, Sobrinho JS, Costa MC, Rohan TE, et al. Type-specific duration of human papillomavirus infection: implications for human papillomavirus screening and vaccination. J Infect Dis 2008 May 15;197(10):1436-47.

(36) Cottier O, Sahli R, Mihaescu A, De Grandi P, Boulvain M, Gerber S. Clinical follow-up of women infected with human papillomavirus-16, either alone or with other human papillomavirus types: identification of different risk groups. Am J Obstet Gynecol 2009 Mar;200(3):286.

(37) Insinga RP, Dasbach EJ, Elbasha EH, Liaw KL, Barr E. Incidence and duration of cervical human papillomavirus 6, 11, 16, and 18 infections in young women: an evaluation from multiple analytic perspectives. Cancer Epidemiol Biomarkers Prev 2007 Apr;16(4):709-15.

(38) Bulkmans NW, Berkhof J, Bulk S, Bleeker MC, van Kemenade FJ, Rozendaal L, et al. High-risk HPV type-specific clearance rates in cervical screening. Br J Cancer 2007 May 7;96(9):1419-24.

(39) Woodman CB, Collins S, Winter H, Bailey A, Ellis J, Prior P, et al. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. Lancet 2001 Jun 9;357(9271):1831-6.

(40) Burk RD, Ho GY, Beardsley L, Lempa M, Peters M, Bierman R. Sexual behavior and partner characteristics are the predominant risk factors for genital human papillomavirus infection in young women. J Infect Dis 1996 Oct;174(4):679-89.

(41) Auvinen E, Zilliacus R, Malm C, Karkkulainen T, Fingerroos R, Paavonen J. Repeated human papillomavirus DNA findings among female university students. Int J STD AIDS 2007 Dec;18(12):839-41.

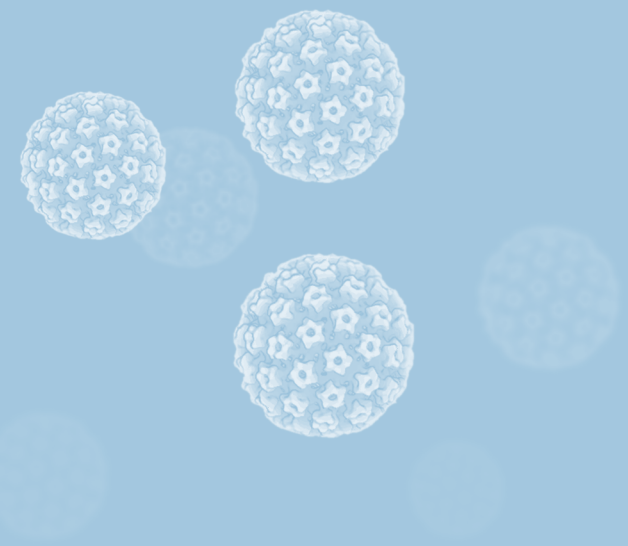
(42) Sarian LO, Hammes LS, Longatto-Filho A, Guarisi R, Derchain SF, Roteli-Martins C, et al. Increased risk of oncogenic human papillomavirus infections and incident high-grade cervical intraepithelial neoplasia among smokers: experience from the Latin American screening study. Sex Transm Dis 2009 Apr;36(4):241-8.

(43) Wang SS, Schiffman M, Shields TS, Herrero R, Hildesheim A, Bratti MC, et al. Seroprevalence of human papillomavirus-16, -18, -31, and -45 in a population-based cohort of 10000 women in Costa Rica. Br J Cancer 2003 Oct 6;89(7):1248-54.

(44) Munoz N, Mendez F, Posso H, Molano M, van den Brule AJ, Ronderos M, et al. Incidence, duration, and determinants of cervical human papillomavirus infection in a cohort of Colombian women with normal cytological results. J Infect Dis 2004 Dec 15;190(12):2077-87.

Chapter 4

Parental acceptance of Human Papillomavirus vaccines



Charlotte H. Lenselink[1]
Marloes M.J.G. Gerrits[1]
Willem J.G. Melchers[2]
Leon F.A.G. Massuger[1]
Dennis van Hamont[1, 2]
Ruud L.M. Bekkers[1]

Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands
[1] Department of Obstetrics & Gynaecology
[2] Department of Medical Microbiology

Eur J Obstet Gynecol Reprod Biol. 2008 Mar;137(1):103-7

Abstract

This study was conducted to determine whether parents would accept Human Papillomavirus (HPV) vaccination for their children and which variables may influence their decision, including knowledge about cervical cancer and HPV. Three-hundred-fifty-six parents of children aged 10 to 12 years were interviewed regarding the acceptance of an HPV vaccine for their children and their knowledge of HPV and cervical cancer. All data were recorded anonymously. Results were compared using the χ^2 - and the Mann-Whitney test. HPV vaccination would be accepted by 88% of the parents, preferably when the child is aged 10 to 12 years. Parents of children who received all the vaccinations of the National Vaccination Programme accepted HPV vaccination significantly more. Less than a third of all parents had heard of HPV, and 14% were aware of the causal relationship of HPV and cervical cancer. Knowledge of HPV and cervical cancer, religion, age, education, and marital status did not show any significant relation with HPV vaccine acceptance. A majority of the parents would accept HPV vaccination. HPV vaccine acceptance seems to be dependent on vaccine acceptance in general, even more than on knowledge of HPV and its causal relation with cervical cancer. However, parents requested more information about cervical cancer, HPV, and HPV vaccination, before the HPV vaccine is introduced.

Introduction

Several studies have shown that a persistent infection with high-risk Human Papillomavirus (hr-HPV) is the most important risk factor for developing cervical cancer and its precursor lesions. Cervical cancer is the second most common cancer in women worldwide and hr-HPV genotypes can be detected in over 99% of all cases.[1-3] In the Netherlands approximately 600 women are diagnosed with invasive cervical cancer annually. The annual mortality rate is approximately 3.0 per 100,000 women.

HPV is primarily transmitted sexually. Sexually active young adults stand a greater risk of being infected with HPV due to the high prevalence of HPV among these age groups.[4] The lifetime risk of acquiring a genital HPV infection is estimated to be at least 80% for sexually active women.[5] In order to prevent HPV infections and reduce the risk of cervical abnormalities and cervical cancer, prophylactic HPV vaccines are being developed.[6-10] In June 2006 the United States Food and Drug Administration (FDA) and in September 2006 the European Medicines Agency (EMEA) announced the approval of a first vaccine developed to prevent cervical cancer and precancerous genital lesions due to hr-HPV genotypes 16 and 18, and genital warts due to low-risk HPV genotypes 6 and 11.

Since 70% of all cervical carcinomas harbour hr-HPV genotypes 16 and 18, the developed vaccines are predominantly targeted against these genotypes. HPV 16 and 18 vaccines seem safe, well tolerated and effective.(7-10) It has been estimated that HPV vaccination will reduce the risk of cervical cancer for 12-year-old girls by 61.8%.[11] To achieve the greatest public health benefit girls and/or boys should be vaccinated prior to the onset of sexual activity. Therefore, the proposed target population for future HPV vaccination consists of pre-teenage children (aged 10-12). Since the pre-teenage children are under age, parental consent will be required. Most studies addressing parental vaccine acceptability have been carried out in the United States and the United Kingdom.[12-14] This study was conducted to assess whether Dutch parents agree to vaccinate their children against HPV infections, which factors influence their decision, and to study their knowledge about HPV, cervical cancer, and HPV vaccination.

Materials and Methods

In order to interview parents of children in the age of 10 to 12 years, the study proposal was discussed with the principals of primary schools in the urban area of Nijmegen, the Netherlands. Forty-five schools were approached, of which 26 reacted and 17 schools participated in this study. Some schools with many pupils from immigrant parents did not participate because of the supposed language barrier of these parents.

All parents of children aged 10 to 12 years received a letter containing a section describing the aims of the study together with some basic information regarding cervical cancer, HPV and HPV vaccination developments, and an informed consent form. Parents who returned a signed informed consent form were interviewed.

Of the 1150 parents approached, 426 parents (37%) responded, and 356 parents (31%) were willing to cooperate of whom, 95.5% were born in the Netherlands.

All interviews lasted 5 to 10 minutes and were conducted by phone by one of the researchers (M.G.) using a survey specifically developed for this study. Most questions were close-ended. Questions were subdivided into socio-demographic items and items regarding knowledge of the risk factors for cervical cancer, the transmission route of HPV, its relationship with cervical cancer, the development of a vaccine against HPV, and the acceptance of this vaccine as prevention of cervical cancer.

In order to avoid influence by the information given in the covering letter of this study, the parents were asked to respond to the knowledge questions disregarding the letter. All data were recorded anonymously. The χ^2 - and Mann-Whitney tests were used to analyse the results. This questionnaire study did not need approval of the Ethics Committee, as it was a fairly non-invasive form of data collection.

Results

All participating parents had sufficient knowledge of the Dutch language and were taking care of at least one child between the age of 10 and 12 years. The parents, of whom 324 (91%) were women and 32 (9%) men, were interviewed by phone. The demography of the population is displayed in Table 1. The mean age of the parents was 42.2 years. Of the 356 parents interviewed 92% were either married or had a partner, 51% had additional education after high school, 16% worked in the medical field, 93% had more than one child, and 60% had both boys and girls.

Of the parents interviewed, 29.5% had ever heard of HPV, and 14.3% of the parents knew HPV is related to cervical cancer (Table 2). Median knowledge scores of HPV, cervical cancer and HPV vaccination were calculated (0-7). The two groups of parents with higher education or working in the medical field had significantly more knowledge about HPV and cervical carcinoma (respectively $p<0.001$; $p<0.001$, Mann-Whitney test). Among the parents working in the medical sector, parents with a university degree had a significantly higher score on the knowledge part than parents who did not have a university degree ($p=0.005$, Mann-Whitney Test). The median knowledge score was also calculated for the sexes and women had a significantly higher score than men (2 versus 1, $p=0.019$,

Table 1. Population characteristics willing or unwilling to vaccinate

	Willingness to vaccinate						
	Total (n=356) (%)		Yes (n=313) (%)		No (n=43) (%)		p
Gender							
Female (324)	324	91.0	282	90.1	42	97.7	n.s.
Male (32)	32	9.0	31	9.9	1	2.3	n.s.
Average age (±SD)*	42.2	(±4.4)	42.1	(±4.4)	43.0	(±3.7)	n.s.
Marital status							
Married/ partner	327	91.9	287	91.7	40	93.0	n.s.
Single/divorced/widow	29	8.1	26	8.3	3	7.0	n.s.
Education							
< High school	174	48.9	156	49.8	18	41.9	n.s.
≥ High school	182	51.1	157	50.2	25	58.1	n.s.
Occupation							
(Para) medic	57	16.0	48	15.3	9	20.9	n.s.
Non (para) medic	299	84.0	244	78.0	31	72.1	n.s.
(Para) medics							
College degree	18	31.6	16	33.3	2	22.2	n.s.
No college degree	39	68.4	32	66.7	7	77.8	n.s.
Country of birth							
The Netherlands	340	95.5	300	95.9	40	93.0	n.s.
Other	16	4.5	13	4.1	3	7.0	n.s.
Religion	202	56.7	180	57.5	22	51.5	n.s.
Practising religion	57	16.0	49	15.7	8	18.6	n.s.
Someone with cervical cancer close to interviewed parent	137	38.5	118	37.7	19	44.2	n.s.
Interviewed parent/ partner has had a Pap smear	342	96.1	300	95.9	42	97.7	n.s.
Interviewed parent/partner treated for abnormal Pap smear	21	5.9	20	6.4	1	2.3	n.s.
Gender children							
Boy(s)	68	19.1	58	18.5	10	23.3	n.s.
Girl(s)	76	21.3	65	20.8	11	25.6	n.s.
Boy(s) and girl(s)	212	59.6	190	60.7	22	51.2	n.s.
Age other children							
<12 years old	177	49.7	156	49.8	21	48.8	n.s.
>12 years old	124	34.8	105	33.6	19	44.2	n.s.
<12 and >12 years old	29	8.2	28	8.9	1	2.3	n.s.
No other children	26	7.3	24	7.7	2	4.7	n.s.

Table 1. continued

	Willingness to vaccinate						
	Total (n=356) (%)		Yes (n=313) (%)		No (n=43) (%)		p
Grade primary school							
6th grade	1	0.3	1	0.3	0	0.0	n.s.
7th grade	183	51.4	158	50.5	25	58.1	n.s.
8th grade	172	48.3	154	49.2	18	41.9	n.s.
Children received all the recommended vaccinations							
Yes	350	98.3	310	99.0	40	93.0	0.004
No	6	1.7	3	1.0	3	7.0	

n: number
p: p-value
n.s.: not significant
* SD: Standard deviation, in years

Table 2. Knowledge of parents regarding HPV, cervical cancer, and the HPV vaccine

Knowledge questions	Total (n=356)	(%)
Had heard of HPV	105	29.5
Knew genital warts can be transmitted sexually	157	44.1
Knew about the causal relation of HPV and cervical cancer	51	14.3
Thinks there is a relationship between smoking and cervical cancer	129	36.2
Thinks there is a relationship between age at first intercourse and cervical cancer	69	19.4
Thinks there is a relationship between number of sexual partners and cervical cancer	226	63.5
Had heard of the HPV vaccine	22	6.2

n: number

Mann-Whitney test). Nevertheless, knowledge did not show a significant relation with acceptance of HPV vaccination of their children. Neither did religion, age, education, and marital status (Multivariate analysis, χ^2 test).

Of the parents interviewed, 313 (87.9%) would accept vaccination of their children against HPV if the Dutch government approves an HPV vaccine. Parents opposed to HPV vaccination thought that the vaccine should first be used for several years before they would agree to vaccinate their children. They were mainly afraid of late side effects. One of the parents was concerned that this vaccination might lead to promiscuity and sexual intercourse at an earlier age. Of all children studied 98.3% received the recommended vaccinations of the National Vaccination Programme. The vaccination level in the group opposed to HPV vaccination was significantly lower, 93.0% versus 99% of the children of parents that accept HPV vaccination (p=0.004, χ^2 test). Many parents, whether they accepted vaccination or not, desired more information regarding HPV, cervical cancer, and HPV vaccination. Based on prior knowledge and the information provided during the interview, 313 parents (87.9%) thought both girls and boys should be vaccinated (Table 3). Parents who accept HPV vaccination were significantly more willing to vaccinate both girls and boys compared to parents opposed to HPV vaccination (91.1% versus 65.1%, respectively, p<0.001, χ^2 test). Almost 19% of the parents indicated that the HPV vaccination should be combined with other vaccinations in the National Vaccination Programme, 13% preferred the age of 9, together with the last vaccinations that are given at that age, like Measles, Rubella, Tetanus, Polio and Diphtheria. The age of 10 to 12 years was preferred by 58.6% of the parents. Of all parents, 23% thought that the child

Table 3. Opinions of parents on HPV vaccination willing or unwilling to vaccinate

	Willingness to vaccinate						
	Total (n=356) (%)		Yes (n=313) (%)		No (n=43) (%)		p
Both boys and girls should be vaccinated	313	87.9	285	91.1	28	65.1	<0.001
Opinion child important	82	23.0	65	20.8	17	39.5	0.006
Age at vaccination (years) *							
0-3	21	5.9	19	6.1	2	4.8	n.s.
9	46	13.0	41	13.1	5	11.9	n.s.
10-12	208	58.6	187	59.7	21	50.0	n.s.
13-18	80	22.5	66	21.1	14	33.3	n.s.

n: number
p: p-value
* one parent, against all vaccinations, did not answer the question
n.s.: not significant

should be involved in deciding whether to be vaccinated against HPV or not. This was significantly higher among the parents who would not accept HPV vaccination for their child (39.5%, p=0.006, χ^2 test).

Discussion

The main target group for vaccination against HPV will consist of pre-teenage children. Therefore, parental consent is needed in order to successfully implement an HPV vaccination programme in the near future. Most studies addressing parental HPV vaccine acceptability have been carried out in the United States and the United Kingdom.[12-14] This study focused on the Dutch parents. In this study the participation rate of 31% was lower than the 35% achieved in a comparable Dutch study of future childhood vaccines.[15] The low response rate can be explained by the fact that parents were approached through their children by letter instead of directly by the researcher. This study by Hak et al. showed 11% of the parents had no intention to accept any new vaccine.[15] This is consistent with findings in our study, if the Dutch authorities approve the HPV vaccine 88% of the parents interviewed accept HPV vaccination of their child. An English study showed that an HPV vaccine uptake rate of 80% would be achievable, yet only 38% were definite in their approval and 15% were opposed to vaccination.[12] In an American study 24% of the parents opposed to vaccination perceived that after HPV vaccination their children would be more likely to initiate sexual intercourse at a younger age.[13] In our study only one parent expressed her concerns. In the American study this question was specifically asked which might have led to the higher outcome, as well as the fact that abstinence until marriage is still a focus in American sexual education.[16;17] The suggestion that widespread vaccination will alter sexual practices is disputed by two other recent American studies.[17;18] Epidemiological studies show that 50% of women contract a genital HPV infection within 2 years after becoming sexually active.[9;19] To be the most effective HPV vaccine should be given before becoming sexually active. According to non-published data from another study in the urban area of Nijmegen among 18 to 25 year-old students, the median age of first sexual intercourse was 16 years. Further analysis showed that at the age of 13.4 years and 11.8 years, less than, respectively 2.0% and 0.5% have had their first sexual encounter. Providing the vaccine to 10 to 12 years olds seems right in order to protect a large majority. Additionally, anti-body-titres in response to vaccination are higher at a younger age. In this study parents opposed to HPV vaccination were significantly more likely to think that it is of great value that their children should express their opinion about the vaccination. Therefore, they selected more often adolescents in the age 13 to 18 as the target group for vaccination. However, the majority of all parents would agree with vaccination in early puberty (9 to 12 years of age). If the HPV vaccination were to be implemented in the National Vaccination Programme for children under 13 years of age, it would be advisable to offer the HPV vaccine to unvaccinated children later on in their lives as well, as the mean titre for vaccine induced antibodies to HPV were over 80 times higher than those seen in natural infections.[7]

Despite the fact that the participants were predominantly female and had experience with the cervical screening programme, only about one third of the parents had ever heard of HPV, and less than 15% knew about the causal relation of HPV and cervical cancer. Similar results and even lower awareness are shown in British studies.[14;20;21] An American study showed that a brief educational intervention of the parents opposed to or undecided about the HPV vaccine significantly improved the acceptance of an HPV vaccine from 55 to 75%.[13] This was contradicted by a study by Dempsey et al. In this study a random half of the study participants received an "information sheet" together with the survey. There was no significant difference between the 2 groups with respect to the mean parental vaccine acceptability scale score, suggesting that receipt of the HPV information sheet did not substantially alter parental acceptability of HPV vaccines.[22] An already high level of acceptance at the onset of this study could explain the difference. However, it may have been due to the different methodologies employed, and the fact that they did not specifically report on undecided or opposed parents.

Most parents in our study indicated that they would like to receive more information on HPV, cervical cancer, and HPV vaccination. However, as in the Dempsey study, knowledge was not a significant predicting factor for HPV vaccination acceptance. This indicates that an educational campaign should cover not only knowledge of HPV and cervical carcinoma, but also beliefs and behaviours associated with the acceptance of vaccination.

In conclusion, despite the fact that our study had several limitations like less participation of immigrant parents, a participation rate of 31%, 91% female participants, and a relatively high educational level, it has shown a remarkable correspondence with results of other studies.[12;14;22;23] The only population characteristic that significantly influenced parental HPV vaccination acceptance was whether parents had given their children all the recommended vaccinations or not. HPV vaccine acceptance seems to be dependent on vaccine acceptance in general, i.e. trust in the recommending body. In this study population vaccine acceptance in general was already high. Despite a lack of knowledge of HPV infections, cervical cancer, and HPV vaccination, most parents accept vaccination of their 10 to 12 year-old children against HPV infections once recommended by the government.

Reference List

(1) Bosch FX, de Sanjose S. Chapter 1: Human papillomavirus and cervical cancer--burden and assessment of causality. J Natl Cancer Inst Monogr 2003;(31):3-13.

(2) Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999 Sep;189(1):12-9.

(3) Cogliano V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F. Carcinogenicity of human papillomaviruses. Lancet Oncol 2005 Apr;6(4):204.

(4) Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. J Clin Virol 2005 Mar;32 Suppl 1:S16-S24.

(5) Bekkers RL, Massuger LF, Bulten J, Melchers WJ. Epidemiological and clinical aspects of human papillomavirus detection in the prevention of cervical cancer. Rev Med Virol 2004 Mar;14(2):95-105.

(6) Garnett GP, Waddell HC. Public health paradoxes and the epidemiological impact of an HPV vaccine. J Clin Virol 2000 Oct;19(1-2):101-11.

(7) Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. Lancet 2004 Nov 13;364(9447):1757-65.

(8) Harper DM, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, Roteli-Martins CM, et al. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. Lancet 2006 Apr 15;367(9518):1247-55.

(9) Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, et al. A controlled trial of a human papillomavirus type 16 vaccine. N Engl J Med 2002 Nov 21;347(21):1645-51.

(10) Villa LL, Costa RL, Petta CA, Andrade RP, Ault KA, Giuliano AR, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. Lancet Oncol 2005 May;6(5):271-8.

(11) Taira AV, Neukermans CP, Sanders GD. Evaluating human papillomavirus vaccination programs. Emerg Infect Dis 2004 Nov;10(11):1915-23.

(12) Brabin L, Roberts SA, Farzaneh F, Kitchener HC. Future acceptance of adolescent human papillomavirus vaccination: a survey of parental attitudes. Vaccine 2006 Apr 12;24(16):3087-94.

(13) Davis K, Dickman ED, Ferris D, Dias JK. Human papillomavirus vaccine acceptability among parents of 10- to 15-year-old adolescents. J Low Genit Tract Dis 2004 Jul;8(3):188-94.

(14) Noakes K, Yarwood J, Salisbury D. Parental response to the introduction of a vaccine against human papilloma virus. Hum Vaccin 2006 Nov;2(6):243-8.

(15) Hak E, Schonbeck Y, De Melker H, Van Essen GA, Sanders EA. Negative attitude of highly educated parents and health care workers towards future vaccinations in the Dutch childhood vaccination program. Vaccine 2005 May 2;23(24):3103-7.

(16) Darroch JE, Landry DJ, Singh S. Changing emphases in sexuality education in U.S. public secondary schools, 1988-1999. Fam Plann Perspect 2000 Sep;32(5):204-11, 265.

(17) Nicoletti A. Cervical cancer vaccine. J Pediatr Adolesc Gynecol 2006 Dec;19(6):423-4.

(18) Monk BJ, Wiley DJ. Will widespread human papillomavirus prophylactic vaccination change sexual practices of adolescent and young adult women in America? Obstet Gynecol 2006 Aug;108(2):420-4.

(19) Kjaer SK, Chackerian B, van den Brule AJ, Svare EI, Paull G, Walboomers JM, et al. High-risk human papillomavirus is sexually transmitted: evidence from a follow-up study of virgins starting sexual activity (intercourse). Cancer Epidemiol Biomarkers Prev 2001 Feb;10(2):101-6.

(20) Waller J, McCaffery K, Forrest S, Szarewski A, Cadman L, Wardle J. Awareness of human papillomavirus among women attending a well woman clinic. Sex Transm Infect 2003 Aug;79(4):320-2.

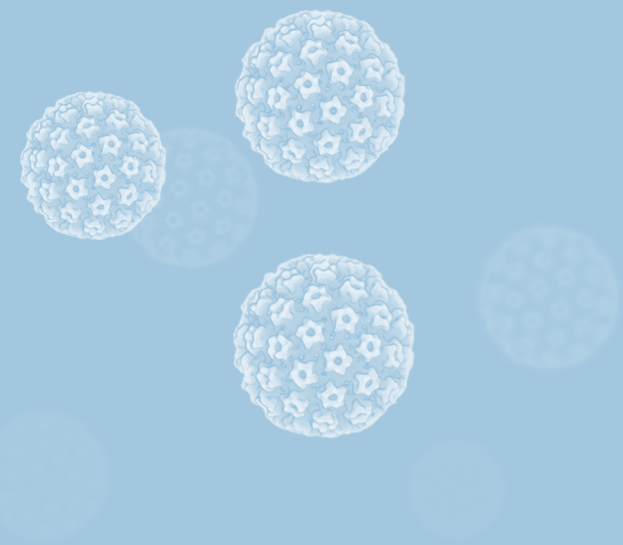
(21) Waller J, McCaffery K, Wardle J. Beliefs about the risk factors for cervical cancer in a British population sample. Prev Med 2004 Jun;38(6):745-53.

(22) Dempsey AF, Zimet GD, Davis RL, Koutsky L. Factors that are associated with parental acceptance of human papillomavirus vaccines: a randomized intervention study of written information about HPV. Pediatrics 2006 May;117(5):1486-93.

(23) Zimet GD. Improving adolescent health: focus on HPV vaccine acceptance. J Adolesc Health 2005 Dec;37(6 Suppl):S17-S23.

Chapter 5

Young adults and acceptance of the Human Papillomavirus vaccine



Charlotte H. Lenselink[1]
Channa E. Schmeink[1]
Willem J.G. Melchers[2]
Leon F.A.G. Massuger[1]
Jan C.M. Hendriks[3]
Dennis van Hamont[1, 2]
Ruud L.M. Bekkers[1]

Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands
[1] Department of Obstetrics & Gynaecology
[2] Department of Medical Microbiology
[3] Department of Epidemiology, Biostatistics and HTA

Public Health. 2008 Dec;122(12):1295-301

Abstract

To determine whether young Dutch adults had ever heard of human papillomavirus (HPV) and whether they would accept vaccination, a cross-sectional survey was performed. Additionally, factors influencing their decision were assessed. Six hundred participants aged 18 to 25 years were recruited from two university departments and one non-university technical college. One hundred and six (17.7%) participants had heard of HPV and 536 (94%) had heard of cervical carcinoma. Women had significantly more knowledge of cervical carcinoma than men. A medical education, knowledge of HPV, knowledge of cervical cancer and knowledge of the cervical screening programme were not significantly associated with acceptance of HPV vaccination, whereas gender and age did show a significant relationship. In total, 61% of the female participants and 48% of the male participants were willing to accept a catch-up HPV vaccination. This study found that average knowledge levels of HPV and cervical cancer were low. Despite this lack of knowledge, a small majority of the study population would accept a catch-up HPV vaccination. Women and younger participants were significantly more willing to accept HPV vaccination. However, in these subgroups, acceptance of HPV vaccination seems to be affected by other, still unidentified, factors. These factors could be evaluated in a more qualitative orientated study. An educational campaign is needed to cover knowledge about HPV and cervical carcinoma, and beliefs and behaviours associated with the acceptance of vaccination.

Introduction

Infection with Human Papillomavirus (HPV) is a necessary event in the multi-step process of cervical carcinogenesis. HPV has been detected in almost 100% of cervical cancers.[1] More than 35 HPV genotypes can infect the genital tract, of which at least 13 are considered high-risk (hr). HPV genotypes 16 and 18 account for almost 70% of all cervical carcinomas.[2] Prophylactic vaccines have been developed to prevent HPV 16 and 18 infections specifically. Randomised double-blind placebo-controlled studies show a decrease of > 90% in the incidence and up to a complete decrease in the persistence of HPV 16 and 18 infections and associated cytological abnormalities and lesions.[3-6] It has been estimated that the best results of a prophylactic vaccine will be achieved by vaccinating women before they become sexually active.[6] This means that the main target group for vaccination consists of pre-teenagers. In order to decrease cervical cancer without a 15 to 20 year lag time, catch-up vaccination is necessary. The main target group for catch-up vaccination consists of women aged 15 to 26 years.

Before introduction of an HPV vaccine, it is important to consider whether the public is aware of the causal relationship between HPV infection and cervical cancer.[7] Studies in the United States (US) and the United Kingdom (UK) have found low awareness among women in health care and university settings.[8-11]

In June and September 2006, respectively, the US Food and Drug Administration and the European Medicines Agency announced their approval of a vaccine developed to prevent cervical cancer and precancerous genital lesions due to hr-HPV genotypes 16 and 18, and genital warts due to low-risk HPV genotypes 6 and 11.

It is also very important to consider whether society accepts the vaccination of adolescents/young adults against a sexually transmitted disease (STD).[7] A British study investigated attitudes and behaviour regarding vaccination against (sexually transmitted) hepatitis B. The study showed that there was low awareness, but that nearly all participants were in favour of vaccination.[12] Most studies exploring HPV vaccine acceptability among young adults and students have been performed in the US and the UK.[13-19] In order to enhance vaccine acceptability, factors influencing individual decision-making need to be studied. In earlier reports knowledge, number of sexual partners, educational level, and effectiveness of the vaccine have been associated with vaccine acceptability.[13-15;17] In this Dutch study, information on attitudes about HPV vaccination and predictors of intention to receive a vaccine were assessed, as well as knowledge of HPV and other risk factors of cervical cancer.

Materials and Methods

For this cross-sectional survey, 659 young adults in Nijmegen, the Netherlands were recruited during August and September 2005, before reports about the HPV vaccine appeared in newspapers in early October 2005. Participants were approached at random at two university departments and one non-university technical college during their lunch break. The inclusion criteria were: age between 18 and 25 years; sufficient knowledge of the Dutch language to answer the questionnaire; and studying at one of the three institutions at which the study was conducted. Six hundred (91.0%) students took part in the study; 150 were medical students, 250 were attending the language department of the university, and 200 students were attending the non-university technical college. The most common reason for refusing to participate was time related. A self-administered questionnaire was used for this study, which students were asked to fill out individually under the supervision of the researcher (CS). The questionnaire data were processed anonymously. The questionnaire contained questions about demography, sexual activity, knowledge of HPV in general, cervical carcinoma, Pap smears, and acceptance of HPV 16 and 18 vaccination. The questions about knowledge were multiple-choice questions and were not preceded by any extra information besides the questionnaire itself. If the participants had heard of cervical carcinoma, they were given a list of potential risk factors and then asked to indicate if they thought each was a risk factor; the question contained a "don't know" option. For the statistical analysis of knowledge, a score was computed which was corrected for guessing. The multiple-choice question on HPV vaccine acceptability also contained a 'don't know' option. Participants who had never heard of HPV were not excluded from the statistical analysis on vaccine acceptance, as a recent study showed that HPV vaccine acceptance seems to be dependent on vaccine acceptance in general, more than on knowledge of HPV and its causal relationship with cervical cancer.[12;20]

Fisher's exact test was used to test differences between men and women for statistical significance for two proportions, and the t-test was used for continuous variables. Chi-square test was used to test differences between the education groups for statistical significance for categorical variables, and one-way analysis of variance was used for continuous variables.

Univariate logistic regression was used to study the ability of the variables to discriminate between participants who accept HPV vaccination and those who do not, separately for each variable. The dependent variable was acceptance of HPV vaccination (yes, no), as present in the questionnaire. Due to the small numbers, sexual activity, sexarche, and number of partners were grouped. The crude odds ratios with 95% confidence interval (CI) are presented.

Multivariate logistic regression with forward selection procedures was used to identify variables that contributed independently to probability of acceptance of HPV. Again, the dependent variable was acceptance of HPV vaccination. In the selection procedure, all the variables with a p-value < 0.10 in the univariate regression were used, as these were potentially related to acceptance of HPV vaccination. P-values for entry into the model were considered in the forward selection procedures in order to identify other potentially important variables. The adjusted odds ratios with 95% CI of the final model are presented.

Results

Of the 600 participants, 377 (62.8%) were women and 223 (37.2%) were men. This participation rate reflects the male-female distribution of students in this region. The average age was 19.8 years, and 91% (n=547) were born in the Netherlands. At the time of study, 440 participants (73.3%) considered themselves to be sexually active in the present or past. The average age of first sexual intercourse was 16.6 years (standard deviation (SD) 1.6). The mean number of sexual partners was 2.1 (SD 3.6), and 0.8% (n=5) had ever had an STD.

Knowledge of HPV and cervical carcinoma

Table 1 and 2 present the knowledge of HPV, risk factors for cervical carcinoma, and Pap smears. Of the 600 participants, 106 (17.7%) had heard of HPV, of whom 84% (n=89) knew that HPV was transmitted sexually. Of these 106 participants, 16% (n=17) knew that using a condom is not fully protective, and 29 participants (27.4%) knew that the lifetime risk of acquiring a genital HPV infection was >50%. The causal relationship with cervical carcinoma was known by 86 participants (81%).

Of the 600 participants, 565 (94.2%) had heard of cervical carcinoma. The mean knowledge of risk factors was low (3.1 out of 8). The risk factors 'promiscuity ' and 'smoking' were mentioned by 22.3% (n=126) and 42.8% (n=242) of the students, respectively. The misconception that a hereditary cause was a risk factor for developing cervical carcinoma was reported by 72% (n=407) of the students, and urinary tract infection and age were mentioned as risk factors by 27.3% (n=154) and 49.7% (n=281) of the students, respectively.

Table 1. Knowledge of HPV, risk factors for cervical carcinoma, and Pap smears by gender

	Total n=600		Men n=223		Women n=377		p
Had heard of HPV	106	(17.7%)	27	(12.1%)	79	(21%)	<0.01
Had heard of cervical carcinoma	565	(94.2%)	192	(86.1%)	373	(98.9%)	<0.01
If heard of cervical carcinoma, named risk factors*							
Early sexarche	61	(10.8%)	17	(8.8%)	44	(11.9%)	0.32
Promiscuity	126	(22.3%)	42	(21.9%)	84	(22.5%)	0.91
No condom use	110	(19.5%)	39	(20.3%)	71	(19.0%)	0.74
Urinary tract infections	154	(27.3%)	60	(31.3%)	94	(25.2%)	0.14
Oral contraconceptive	93	(16.5%)	25	(13.0%)	67	(18.0%)	0.15
Smoking	242	(42.8%)	87	(45.3%)	155	(41.6%)	0.42
Heredity	407	(72.0%)	134	(69.8%)	273	(73.2%)	0.43
Age	281	(49.7%)	79	(41.1%)	202	(54.2%)	<0.01
Knowledge of cervical carcinoma (score 0-8) (mean) (SD)	3.1	(1.3)	2.8	(1.6)	3.2	(1.2)	<0.01**
Knew that from the age of 30 years, Dutch women get a Pap smear	195	(32.5%)	67	(30.2%)	128	(34.0%)	0.78
Knew Pap smears diagnose cervical carcinoma and pre-malignancies	352	(62.3%)	114	(51.4%)	238	(63.1%)	<0.01
Knew an abnormal Pap smear is not always due to cervical carcinoma	326	(54.3%)	87	(39.2%)	239	(63.4%)	<0.01
Will get a Pap smear in the future***	-	-	-	-	314	(83.3%)	-

n: number
p: p-value for difference between men and women using the Fisher exact test
* more answers possible
SD: Standard Deviation
** using t-test
-: not applicable
*** women only

Table 2. Knowledge of HPV, risk factors for cervical carcinoma, and Pap smears by education

	Medical faculty n=150		Non-medical faculty n=250		Technical college n=200		p
Had heard of HPV	93	(62.0%)	3	(1.2%)	10	(5.0%)	**<0.01**
Had heard of cervical carcinoma	148	(98.7%)	240	(96%)	177	(88.5%)	**<0.01**
If heard of cervical carcinoma, named risk factors*							
Early sexarche	43	(29.1%)	9	(3.8%)	9	(5.1%)	**<0.01**
Promiscuity	71	(48.0%)	30	(12.5%)	25	(14.1%)	**<0.01**
No condom use	53	(35.8%)	38	(15.8%)	19	(10.7%)	**<0.01**
Urinary tract infections	38	(25.7%)	67	(27.9%)	49	(27.7%)	0.86
Oral contra conceptive	24	(16.2%)	42	(17.5%)	26	(14.7%)	0.78
Smoking	60	(40.5%)	101	(42.1%)	81	(45.8%)	0.42
Heredity	109	(73.6%)	186	(77.5%)	112	(63.3%)	**<0.01**
Age	102	(68.9%)	118	(49.2%)	61	(34.5%)	**<0.01**
Knowledge of cervical carcinoma (score 0-8) (mean) (S.D.)	3.3	(1.3)	2.9	(1.2)	3.0	(1.5)	0.07**
Knew that from the age of 30 years, Dutch women get a Pap smear	52	(34.7%)	73	(29.2%)	59	(29.5%)	0.47
Knew Pap smears diagnose a cervical carcinoma and pre-malignancies	98	(65.3%)	145	(58.0%)	108	(54.0%)	**<0.01**
Knew an abnormal Pap smear is not always due to cervical carcinoma	121	(80.7%)	126	(50.4%)	78	(39.0%)	**<0.01**
Will get a Pap smear in the future***	96	(85.0%)	141	(89.8%)	78	(75.0%)	**<0.01**

n: number
p: p-value for differences between groups of education using the Chi-Square test
* more answers possible
** one-way analysis of variance (ANOVA)
*** women only

Men versus women

Table 1 presents the knowledge of HPV, risk factors for cervical carcinoma, and Pap smears by gender. Significantly more women had heard of HPV than men. Women were also significantly more likely to have heard of cervical carcinoma than men (98.9% (n=373) versus 86.1% (n=192)).

Of the 600 participants, 58.9% (n=352) knew that a Pap smear could detect cervical carcinoma and pre-malignant lesions. Significantly more women were aware of this fact than men. Additionally, 326 (54.5%) students were aware that an abnormal Pap smear is not always due to cervical carcinoma. Significantly more women knew about this. Of all 377 women, 314 (83.3%) intended to have a Pap smear in the future.

Type of education

Table 2 presents knowledge of HPV, risk factors for cervical carcinoma, and Pap smears by education. Of the 106 participants who had heard of HPV, 93 (87.7%) were medical students. Students at the technical college had heard of cervical cancer significantly less often. As expected, the risk factors 'promiscuity' and 'early sexarche' were mentioned significantly more often by medical students than by non-medical students. Medical students were significantly more aware of the fact that an abnormal Pap smear is not always due to cervical carcinoma. Women at the technical college were significantly less likely to report their intention to have a Pap smear in the future.

Acceptance of HPV vaccination

Of the 600 participants, 336 (56.0%) were willing to accept HPV vaccination. A medical education, knowledge of HPV, knowledge of cervical cancer and knowledge of the cervical screening programme were not significantly associated with acceptance of HPV vaccination (Table 3). In addition, no association was found with sexual activity, sexarche and number of sexual partners. Men and older participants were less likely to accept HPV vaccination in both univariate and multivariate logistic regression analysis (Table 3).

Discussion

In general, knowledge of HPV and cervical cancer in this study population of young adults aged 18 to 25 years was low. However, multivariate analysis showed that acceptance of HPV vaccination is not influenced by knowledge or a medical education.

Table 3. Odds Ratios and adjusted Odds Ratios for the acceptance of HPV vaccination using logistic regression

	n	OR (95% C.I.)	p	Adj. OR (95% C.I.) (n=346)	p
Gender Male Female	373	0.31 (0.16;0.63) 1.00 (ref)	**<0.01**	0.32 (0.16;0.63)	**<0.01**
Education Medical Non-medical-university Non-university technical	373	0.65 (0.28;1.49) 1.01 (0.44;2.33) 1.00 (ref)	0.31 0.99	-	-
Age (year)	371	0.84 (0.71;0.99)	**0.04**	0.84 (0.71;0.99)	**0.04**
Had heard of HPV Yes No	373	1.93 (0.92;4.03) 1.00 (ref)	0.08	-	-
Knowledge of HPV	373	0.82 (0.64;1.06)	0.10	-	-
Knowledge of cervical cancer	373	0.79 (0.59;1.06)	0.12	-	-
Knowledge of national cervical screening programme	348	1.28 (0.87;1.89)	0.21	-	-
Sexual activity Yes No	373	2.00 (0.99;4.10) 1.00 (ref)	0.05	-	-
Sexarche	373	1.21 (0.96;1.52)	0.11	-	-
Number of sexual partners	352	1.21 (0.82;1.78)	0.34	-	-

n: number
OR: Odds Ratio
95% C.I.: 95% Confidence Interval
Adj. OR: Adjusted Odds Ratio, adjusted for the other variable in the model
ref: reference
-: not selected using multivariable logistic regression model with selection procedures

Only a lower age and female gender were associated with vaccine acceptance. This is in accordance with the results of Gudmundsdottir et al.[21] As expected, only 48% of the men in this study would accept HPV vaccination. If male participants were told explicitly about genital warts and the minor risk of penile carcinoma, their acceptance of

vaccination may increase. This indicates that in order to increase herd immunity by including males in the "catch-up" vaccination campaign, strategies to improve their motivation to accept vaccination are needed. Otherwise, their vaccine acceptability is of less value.

An American study investigated the predictors of intention to receive a vaccine among 52 women aged between 18 and 30 years. Knowledge and higher number of sexual partners were associated with acceptance of an HPV vaccine.[15] These associations were not confirmed in this present much larger study.

Only a minority of the study participants had heard of HPV and even fewer were aware of its causal relationship with cervical carcinoma. This low level of knowledge of HPV is comparable with results from a study performed in Canada in 2000, where 13% of high school students (aged 15 to 20 years) had heard of HPV.[8] The awareness of HPV was also limited among students attending a public university in Florida; HPV was the STD about which they knew the least.[11] This resemblance is notable, as it was expected that the awareness of HPV would have changed substantially since 2000 due to new developments. However, the present study was conducted before the front-page news about the HPV vaccine was reported in the newspapers.

Although most students in this study (93.8%) had heard of cervical carcinoma, only a minority could identify the risk factors correctly. These findings are in good agreement with earlier reports. Waller et al. also concluded that the level of knowledge of risk factors was low. Only 14% of males and females aged 16 to 75 years were aware that there was a link between sexual activity and cervical carcinoma. It also appeared that young adolescents (16 to 24 years) had the least knowledge of risk factors. Educational level was an important predictor of the level of knowledge.[22] In the present study, educational level did not reach significance; this may be explained by the fact that all participants were relatively highly educated. As expected, medical students had the best knowledge, and non-university technical college students had the poorest knowledge, but this difference was not reflected in vaccine acceptance.

This study found that there are many misconceptions about cervical cancer. A majority of students identified heredity as a cause of cervical cancer. It may well be that misconceptions have a negative influence on vaccine acceptance. This shows that it is important to provide information about the true risk factors of HPV infection and the possible clinical sequelae, as well as ensuring that misconceptions are corrected.

A study conducted in Michigan with men and women aged 20 to 46 years showed that the preferred time to receive information about HPV was before becoming sexually active. In this group the mean age of first sexual intercourse was 18 years.[9] In the present study, the mean age of first sexual intercourse was 16.6 years (SD 1.6).

The results of this study cannot be generalised to the whole Dutch population as it was based on students aged 18 to 25 years with a relatively high education. Additionally, participants had to have sufficient knowledge of the Dutch language to answer the questionnaire. Only 73 % considered themselves to be sexually active, and less than 1% ever had an STD. This indicates that knowledge levels could be even lower in the general population. No bias was expected between the participants and the non-participants, as the students were approached ad random and the major reason for refusal was time related.

In conclusion, this study found that almost all participants had heard of cervical carcinoma, but knowledge of risk factors was low. Only a few students had heard of HPV and its relationship with cervical carcinoma. Despite this general lack of knowledge, a small majority of young adult women aged 18 to 25 years would accept HPV vaccination. Multivariate analysis showed that acceptance of HPV vaccination is influenced by younger age and female gender but not by knowledge or educational level. This study found that the exact factors which influence vaccine acceptance in this age group, in both men and women, remain to be elucidated. These factors could be evaluated in a future, more qualitative orientated, explorative study. The fact that "knowledge" did not reach significance shows that an intervention is needed that covers knowledge of HPV and cervical cancer, and beliefs and behaviours associated with the acceptance of HPV vaccination.

Ethical Approval

The local Medical Ethics Committee advised that formal approval was not required for this non-invasive anonymised study.

Reference List

(1) Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999 Sep;189(1):12-9.

(2) Bosch FX, de Sanjose S. Chapter 1: Human papillomavirus and cervical cancer--burden and assessment of causality. J Natl Cancer Inst Monogr 2003;(31):3-13.

(3) Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. Lancet 2004 Nov 13;364(9447):1757-65.

(4) Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, et al. A controlled trial of a human papillomavirus type 16 vaccine. N Engl J Med 2002 Nov 21;347(21):1645-51.

(5) Harper DM, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, Roteli-Martins CM, et al. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. Lancet 2006 Apr 15;367(9518):1247-55.

(6) Villa LL, Costa RL, Petta CA, Andrade RP, Ault KA, Giuliano AR, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. Lancet Oncol 2005 May;6(5):271-8.

(7) Tjalma WA, Van Damme P. Is the public enough aware to accept a vaccination program against human papillomavirus? Vaccine 2005 May 9;23(25):3231.

(8) Dell DL, Chen H, Ahmad F, Stewart DE. Knowledge about human papillomavirus among adolescents. Obstet Gynecol 2000 Nov;96(5 Pt 1):653-6.

(9) Holcomb B, Bailey JM, Crawford K, Ruffin MT. Adults' knowledge and behaviors related to human papillomavirus infection. J Am Board Fam Pract 2004 Jan;17(1):26-31.

(10) Waller J, McCaffery K, Forrest S, Szarewski A, Cadman L, Wardle J. Awareness of human papillomavirus among women attending a well woman clinic. Sex Transm Infect 2003 Aug;79(4):320-2.

(11) Yacobi E, Tennant C, Ferrante J, Pal N, Roetzheim R. University students' knowledge and awareness of HPV. Prev Med 1999 Jun;28(6):535-41.

(12) Hinds A, Cameron JC. Acceptability of universal hepatitis B vaccination among school pupils and parents. Commun Dis Public Health 2004 Dec;7(4):278-82.

(13) Boehner CW, Howe SR, Bernstein DI, Rosenthal SL. Viral sexually transmitted disease vaccine acceptability among college students. Sex Transm Dis 2003 Oct;30(10):774-8.

(14) Hoover DR, Carfioli B, Moench EA. Attitudes of adolescent/young adult women toward human papillomavirus vaccination and clinical trials. Health Care Women Int 2000 Jul;21(5):375-91.

(15) Kahn JA, Rosenthal SL, Hamann T, Bernstein DI. Attitudes about human papillomavirus vaccine in young women. Int J STD AIDS 2003 May;14(5):300-6.

(16) McClelland A, Liamputtong P. Knowledge and acceptance of human papillomavirus vaccination: perspectives of young Australians living in Melbourne, Australia. Sex Health 2006 May;3(2):95-101.

(17) Zimet GD, Mays RM, Winston Y, Kee R, Dickes J, Su L. Acceptability of human papillomavirus immunization. J Womens Health Gend Based Med 2000 Jan;9(1):47-50.

(18) Zimet GD. Improving adolescent health: focus on HPV vaccine acceptance. J Adolesc Health 2005 Dec;37(6 Suppl):S17-S23.

(19) Zimet GD, Perkins SM, Sturm LA, Bair RM, Juliar BE, Mays RM. Predictors of STI vaccine acceptability among parents and their adolescent children. J Adolesc Health 2005 Sep;37(3):179-86.

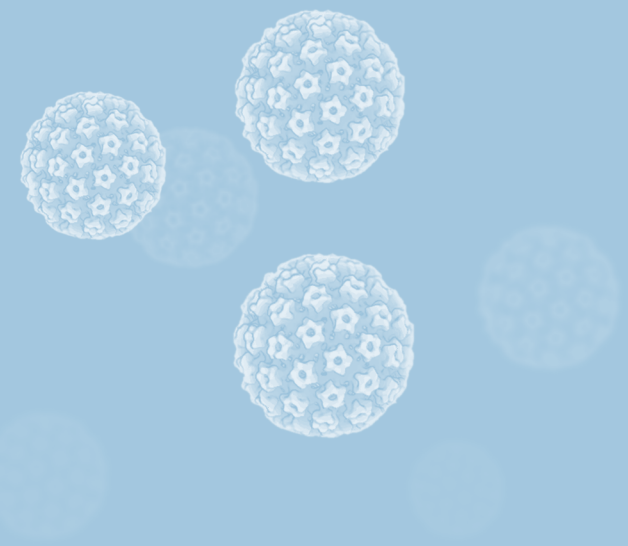
(20) Lenselink CH, Gerrits MM, Melchers WJ, Massuger LF, van Hamont D, Bekkers RL. Parental acceptance of Human Papillomavirus vaccines. Eur J Obstet Gynecol Reprod Biol 2008 Mar;137(1):103-7.

(21) Gudmundsdottir T, Tryggvadottir L, Allende M, Mast TC, Briem H, Sigurdsson K. Eligibility and willingness of young Icelandic women to participate in a HPV vaccination trial. Acta Obstet Gynecol Scand 2003 Apr;82(4):345-50.

(22) Waller J, McCaffery K, Wardle J. Beliefs about the risk factors for cervical cancer in a British population sample. Prev Med 2004 Jun;38(6):745-53.

Chapter 6

Individual risk factor assessment of sexually active women prior to HPV vaccination; how effective is your HPV vaccination?



Charlotte H. Lenselink[1]
Channa E. Schmeink[1]
Jan C.M. Hendriks[2]
Willem J.G. Melchers[3]
Wim G.V. Quint[4]
Leon F. A.G. Massuger[1]
Ruud L.M. Bekkers[1]

[1, 2, 3] Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands
[1] Department of Obstetrics & Gynaecology
[2] Department of Epidemiology, Biostatistics and HTA
[3] Department of Medical Microbiology
[4] DDL Diagnostic Laboratory, Voorburg, the Netherlands

Submitted

Abstract

Efficacy of Human Papillomavirus (HPV) vaccines has been proven in women who are HPV 16 and/or 18 negative at time of vaccination. The benefit of HPV vaccination of sexually naïve women is likely to be higher than that of older already sexually active women. The individual decision of these women to get vaccinated will be balanced between costs and personal benefit. A risk assessment may determine ones personal benefit from vaccination and may be helpful in counselling individual women in outpatient settings by providing insight in one's personal situation. This study is based on the results of a large prospective epidemiologic study among 2065 unscreened women aged 18 to 29 years, and includes mathematic modelling to estimate the probability of an HPV 16 and/or HPV 18 infection. Finally, data were used from 1322 women aged 18-25 years who reported to be sexually active in past or present time. Women returned a self-collected cervico-vaginal specimen and filled out a questionnaire. The model predicting the optimal probability of being HPV 16 and/or 18 positive was based on the combination of age, number and gender of sexual partners, condom use, and frequency of sexual contact in past 6 months. A nomogram including significant predictors is provided to calculate the probability of being HPV 16 and/or 18 infected. In total 16% of the variance (R-square) in this model could be explained by the selected variables, the discriminatory power (AUC) was 79%. After the internal validation using bootstrap methods the corrected R-square and AUC were 10.2% and 75%, respectively. The basal risk of being HPV 16 and/or 18 positive was 4%. The cut-off value to estimate an HPV 16 and/or 18 infection was 8%. This was based on the point where the sum of the sensitivity and twice the specificity was maximal. The sensitivity and specificity at this point were 51% and 88%, respectively. This risk assessment tool may be helpful in individual counselling. Additionally, women with a high probability of HPV 16 and/or 18 positivity may benefit from HPV testing prior to vaccination.

Introduction

Widespread application of the HPV vaccine is expected to have a significant impact on the incidence of cervical pre-malignant lesions and cervical cancer. Presently, vaccination programmes have started in many countries around the world, targeting 11 to 16 year old girls.[1;2] Additionally, catch-up vaccination of already sexually active women is under consideration in many countries in order to get a faster decrease in cervical cancer incidence. Some studies question whether comprehensive universal strategies for nationwide catch-up vaccination are superior to a "targeted" approach.[3-5] An attempt to target a nationwide subpopulation based on risk factors does not seem to be a reasonable option as many women eligible for vaccination would be excluded based on the high prevalence of risk factors. However, as some of these women could potentially benefit from vaccination, identifying women individually with a higher probability of being HPV vaccine-type positive may be helpful in counselling.

In the Netherlands, the vaccine is assimilated into the National Vaccination Programme (NVP) and will be free of charge for the target group which consists of 12 year old girls. Single catch-up vaccination of girls aged 13 to 16 years will be implemented in the NVP as well. HPV vaccination has started recently.

Older adolescent women and young adult women (i.e. 17 through 25 years) who cannot take part in the NVP based on their age, may therefore need to pay for the vaccine. As women age, they are more likely to have engaged in sexual activity resulting in exposure to HPV in general as well as vaccine specific types. It must be emphasized that HPV vaccines are prophylactic, not therapeutic, and have no efficacy against existing HPV infection or disease.[6;7] Therefore, the clinical benefit afforded to older sexually active women is likely to be less than that of younger sexually naïve women. Thereby, the decision to get vaccinated will be balanced between personal benefit and costs. It is likely that these women will ask physicians and gynaecologists about the health benefit of this vaccine and the level of benefit for their individual situation.

In order to decide whether vaccination on an individual basis may be beneficial, assessment of a risk profile may be important. Additionally, it provides the opportunity to test women at risk on an individual basis before excluding them from vaccination. To estimate the risk of currently being infected with high-risk (hr) HPV vaccine-type 16 and/or 18, knowledge of epidemiology of type specific HPV infections in relation to socio-demographic characteristics like ethnicity and education, and sexual behaviour is important. However, little is known about the association between behavioural risk factors of young women and past or prevalent HPV infection.[2;3;5;8] To optimize the individual risk estimation of HPV 16 and/or 18 positivity at time of vaccination, we explored the possibility of composing a risk factor assessment tool, i.e. a prediction model, that may be helpful in counselling individual women in outpatient settings by providing insight in one's personal situation.

Materials and Methods

Study population and study design

This study is based on the results of a large prospective epidemiologic study performed among 2065 unscreened women aged 18 to 29 years. Women were recruited between June and September 2007, using different advertisements, as well as active recruitment sites, and posters at general practices in the city regions of Arnhem, Nijmegen, and Den Bosch, the Netherlands. Furthermore, advertisements on the internet were used, which were accessible in the whole of the Netherlands. Of the 2297 women who responded to the advertisements, 2065 (89.9%) consented with the study. Of the 2065 women, 1430 were aged 18 to 25 years, i.e. a target group for vaccination as the prophylactic HPV vaccines are registered through the age of 25 years. Of these 1430 women 1428 provided information on sexual activity of whom 7.4% (n=106) reported not be sexually active. Data of these sexually naïve women were not used to compose the prediction model as this study assesses the risk of sexually active women of being HPV positive. Therefore, we only used data from the 1322 (92.6%) women aged 18 to 25 years who reported to be sexually active in past or present time.
Written informed consent was obtained from all participants. This study was approved by the Local Medical Ethics Committee.

Specimen Collection and Processing

All women were asked to fill out a questionnaire and to self-collect a cervico-vaginal sample in the privacy of their own home. Women received an explanatory letter, an informed consent form, a questionnaire, and a self-sample kit by mail. The self sample kit contained a collection device (a small brush packaged in an individual sterile cover, Rovers® Viba-brush, Rovers Medical Devices B.V., Oss, the Netherlands), a collection tube containing medium (SurePath™, Tripath Imaging®, Inc., Burlington, NC, USA), instructions how to perform the cervico-vaginal self-sample (written and in cartoon), and a return package consisting of a leak-proof seal bag, absorption sheet, and a reclosable plastic return envelope (Easyslider, Transposafe Systems Holland BV, Sassenheim, the Netherlands). The self-sample was taken and processed as described earlier.[8]
All HPV DNA-positive samples were genotyped using the SPF_{10}-LiPA HPV genotyping assay. The HPV genotyping assay was performed as described previously.[9;10]
Samples that tested positive using the DNA enzyme immunoassay but that showed no results on the LiPA strip were considered to be HPV X type, i.e. genotypes not available on the LiPA strip. Low-risk HPV (lr-HPV) types were defined as HPV type 6,11, 34, 40, 42, 43, 44, 53, 54, 55, 58, 66, 70, 74, and "X"; and hr-HPV types as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59, 68, 73, and 82.

Questionnaire

We used a questionnaire consisting of two parts. The first part was composed of questions regarding smoking, medication use, contraceptive use, and socio-demographic variables like educational level, religion, and ethnicity. Race and ethnicity were self-reported into different categories. The second part consisted of questions regarding sexual behaviour to gain insight in risk factors for acquiring genital HPV. Sex was defined as vaginal, oral, and/or anal sex. Questions were asked on age at first sexual contact, age of first sex partner, number of sex partners before the age of sixteen, number of sex partners lifetime, number of sex partners in the past 6 months, gender of sex partners, frequency of sexual contact, condom use, and history of sexually transmitted diseases (STD).

Statistical analyses

In this study we aimed at identifying sexually active women at risk for hr-HPV vaccine types 16 and/or 18 from those not at risk. The risk of a present HPV 16 and/or 18 infection was chosen over the single risk of a present infection with both HPV 16 and 18 simultaneously, as the presence of a single infection with HPV 16 or a single infection with HPV 18 already influences the protective effect of the vaccine and therefore may influence a woman's decision to get vaccinated.
Categories of specific variables were grouped in case of small numbers or in case of a similar risk of being infected. Age higher than 24 years was grouped into one category, as a plateau phase was reached in HPV prevalence after the age of 24.[8] Lifetime number of partners was divided into 3 categories and number of partners in the past six months was divided into four categories. Gender of sex partners was divided into 2 categories; category 1 consisting of male gender, category 2 consisting of female and both female and male gender. Frequency of sexual contact was grouped into five categories. Years of being sexually active (i.e. sexual age) ranged from 0 to 23 years, the category "0" years consisted of women who became sexually active in the past year. Because of the small numbers, 0 and 1 year were combined as well as 13 to 23 years. Previous infection with at least one of the following was defined as having had a previous STD: Chlamydia, genital warts, Syphilis, Gonorrhoea, Genital Herpes, or HIV.
Univariate logistic regression was used to study the ability of socio-demographic and sexual behaviour variables to discriminate between women with an HPV 16 and/or 18 infection from those without an HPV 16 and/or 18 infection. This was performed for each variable

separately. The dependent variable was HPV 16 and/or 18 infection. The crude odds ratios (OR) with 95% confidence interval (CI) are presented.

Multivariate logistic regression with backward variable deletion was used to estimate the probability of HPV 16 and/or 18 infection. The variables used were selected based on previous study.[8] Again, the dependent variable was HPV 16 and/or 18 infection. The following possible HPV 16 and/or 18 infection related variables were used in the selection procedure: age, current smoking, living with parents, type of relationship, sexual age i.e. years of being sexually active, number of partners during lifetime, gender of sexual partner(s), number of partners in last six months, frequency of sexual intercourse in last six months, ever diagnosed with a STD, and condom use. The adjusted odds ratios with 95% confidence interval (CI) of the final model are presented. Participants with missing data on variables included in the multivariate analysis were excluded. The R-square is presented to indicate the total percentage explained variance in the outcome. The area under the curve (AUC) of the receiver operating characteristic curve is presented as a measure of predictive discrimination. In general, these measures will be to high because the model is developed solely using the study sample and this model may perform less on a different random sample. Therefore, to evaluate the reliability of the created prediction model an internal validation was performed using bootstrap methods.[11] The corrected R-square and the corrected AUC are presented.

Using the multivariable prognostic model, a boundary value of the risk of HPV 16 and/or 18 infection, given the values of the prognostic variables only, was constructed under the condition of higher 'costs' of misclassification of non-cases compared to cases. This resulted in a high-specific test. In other words, the cut-off value for the probability to be infected was selected so that the sum of the sensitivity and two times the specificity discriminating infected subjects from non-infected subjects was maximal.

Finally, a nomogram was constructed using this multivariable prognostic model.

In all tests, p values < 0.05 were regarded statistically significant. Statistical analyses were performed using SAS 8.2 (SAS Institute Inc., Cary, NC, USA) and SPSS 16.0 (Chicago, Illinois, USA), and R 2.1 (r-project.org).

Results

HPV Prevalence

Of the 1322 sexually active women 17.6% (n=233) tested positive for one or more HPV genotypes. A single HPV type was detected in 13.6% (n=180) of all sexually active women, while multiple types were found in 4.0% (n=53) of these women. The prevalence of hr-HPV types was 11.5% (n=152) and of lr-HPV types 7.7% (n=102), including co-infections. Simultaneous infection with HPV 16 (2.8%) and 18 (1.3%) occurred in only 2 women (0.2%). Although HPV DNA was detected in 4 of the 106 sexually naïve women, these data were not used for further analyses. It concerned three single infections with a hr-HPV type of which two times HPV type 16, and a co-infection with a lr- and a hr-HPV type. Simultaneous infection with HPV 16 (n=2, 1.9%) and 18 (n=0) did not occur.

Prediction Model

Table 1 shows the ORs (OR's) of the probability of being infected with HPV 16 and/or 18. Except for increasing age, all factors significantly associated with HPV 16 and/or 18 prevalence were related to sexual behaviour.

Multivariate logistic regression with backward variable deletion showed five factors contributing independently to the risk of being HPV 16 and/or 18 positive. These factors were age, the number of lifetime sexual partners, gender of sexual partner(s), condom use, and frequency of sexual intercourse in the last 6 months (Table 2).

To calculate the probability of being infected with HPV 16 and/or 18 a prediction model was constructed. This model was constructed using this multivariate analysis. In total 16% of the variance (R-square) in this model could be explained by the selected variables, the discriminatory power (AUC) was 79%. After the internal validation using bootstrap methods the corrected R-square was 10.2% and the corrected AUC was 75%. In this study the basal risk of being HPV 16 and/or 18 positive was 4%. The cut-off value to predict the likelihood of having an HPV 16 and/or 18 infection was 8%. This was based on the point where the sum of the sensitivity and twice the specificity was maximal. The sensitivity and specificity at this point were 51% and 88%, respectively. When having a probability of >8% the subject could be defined as having high risk for being infected with HPV 16 and/or 18. Note this test has higher specificity than a test that is based on equal "costs" of misclassification of cases and non-cases.

Figure 1 shows a nomogram to calculate the probability of being infected with HPV 16 and/or 18 based on the prediction model. This procedure allocated the weight to each variable in the model. To read the probability from the prediction model the corresponding number of points of each of the 5 variables can be read from the scale above. Subsequently, all points are added up into a total score. With use of the total score, the corresponding probability of being HPV 16 and/or 18 infected can be read from the scale below. This procedure is illustrated using two examples below.

Example 1:

For instance, a 22 year old women (age: 34 points) visits the outpatient gynaecological clinic asking for advise on HPV vaccination. She has had a total number of 3 sexual

Table 1. Odds Ratio's for the risk of an infection with HPV 16 and/or 18 among sexually active women using logistic regression

	n	OR	(95% C.I.)
Age* (years)	**1322**	1,24	(1.06;1.44)
Current smoking	**1316**		
No	1083	0.90	(0.45;1.82)
Yes	233	1.00	(ref)
Using OCC	**1321**		
No	245	1.49	(0.78;2.84)
Yes	1076	1.00	(ref)
Living with parents	**1313**		
No	1019	0.94	(0.48;1.81)
Yes	294	1.00	(ref)
Relationship	**1316**		
Single	370	1.90	(1.05;3.43)
Married, LT	265	0.66	(0.27;1.65)
LAT	681	1.00	(ref)
Age at first intercourse** (years)	**1319**		
≤ 13	29	3.15	(0.42;23.43)
14-16	662	1.95	(0.46;8.31)
17-19	541	1.55	(0.35;6.76)
≥ 20	87	1.00	(ref)
Sexual Age*** (years)	**1318**	1.56	(1.04;1.28)
Lifetime sex partners (number)	**1317**		
1	329	0.07	(0.02;0.28)
2-5	657	0.38	(0.21;0.67)
>5	331	1.00	(ref)
Gender of sex partner(s)	**1319**		
Male	1258	0.24	(0.11;0.54)
Female, both	61	1.00	(ref)
Sex partners in past 6 months (number)	**1316**		
0	124	0.13	(0.03;0.66)
1	997	0.31	(0.13;0.72)
2	131	0.46	(0.15;1.37)
>2	64	1.00	(ref)
Sexual intercourse in past 6 months (frequency)	**1287**		
0	110	0.28	(0.08;1.02)
1-6	132	1.00	(ref)
7-24	138	0.30	(0.09;0.95)
25-54	492	0.21	(0.09;0.49)
>54	415	0.56	(0.27;1.16)
Ever diagnosed an STD?	**1317**		
No	1231	0.62	(0.24;1.59)
Yes	86	1.00	(ref)
Condom use	**1316**		
Never (0%)	630	0.90	(0.25;3.20)
Sometimes (1-99%)	555	2.96	(0.90;9.76)
Always (100%)	131	1.00	(ref)

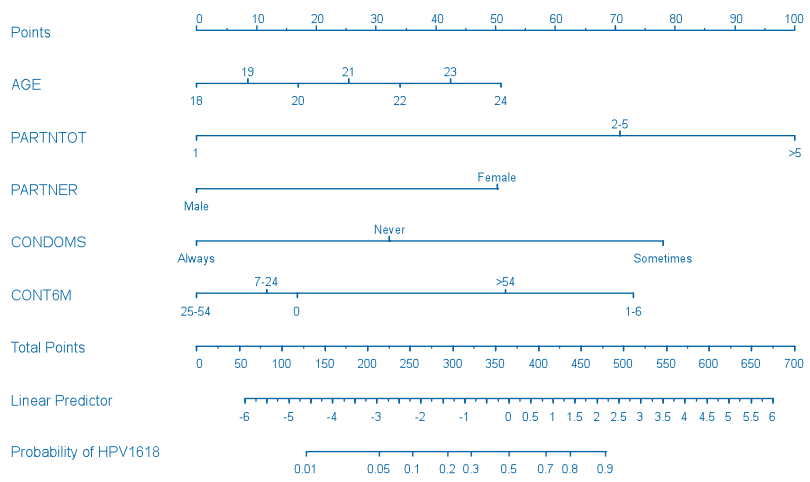
n: number
OR: Odds Ratio
95% C.I.: 95% Confidence Interval
* Age, grouped in one category when 24 or higher
ref: reference
OCC: oral contraceptives
LT: Living together
LAT: Living apart together
** below the age of 10 years several cases of sexual abuse were reported
*** Sexual age in years with 0 and 1 combined as well as sexual age higher than 13
STD: Sexually Transmitted Disease

partners (lifetime, 71 points), the gender of her partners was male (0 points), she never uses condoms (32 points), and has had sexual intercourse once a week i.e. 26 times in the past six months (0 points). If we sum up the points 34+71+0+32+0 this results in 137 points. Her corresponding probability of being HPV 16 and/or 18 positive is 1.3%. This is below the basal risk of 4% and below the cut-off value of 8%. Therefore, according to this "risk assessment tool", this woman has a low likelihood of vaccine-type hr-HPV infection and she may decide for direct HPV vaccination.

Example 2:
However, if she was 22 years (34 points), has had a total number of 6 sexual partners (lifetime, 100 points), the gender of her partners were both male and female (50 points), she sometimes used condoms (78 points), and has had sexual intercourse 2 times in the past six months (72 points), her total of points would raise to 34+100+50+78+72=334 points, resulting in a corresponding probability of 35%. In this situation she would be considered to have a high risk of being infected with HPV 16 and/or 18. This provides the woman with insight in her personal situation, and as this does not concern population based circumstances, she may choose to test for HPV prior to vaccination.

Figure 1.

Nomogram to read the probability of HPV 16 and/or 18 infection.



To read the probability from the prediction model, the corresponding number of points of each of the 5 variables can be read from the scale above. All points are added up, and using the total score the corresponding probability of being HPV 16 and/or 18 infected can be read from the scale below.
The maximal probability of HPV 16 and/or 18 is 45%, i.e. 351 points.
Age 24: ≥ 24, i.e. 24+25 (in years)
Partntot: total number of partners (lifetime)
Partner: gender of partner; male=male, female= female or both male and female
Cont6m: frequency of sexual intercourse in past 6 months

Table 2. Adjusted Odds Ratio's for the risk of an infection with HPV 16 and/or 18 among sexually active women using multivariate logistic regression (n=1274)

	Adj. OR	(95% C.I.)
Age* (years)	1.18	(1.01;1.38)
Lifetime sex partners (number)		
1	0.14	(0.03;0.64)
2-5	0.57	(0.30;1.07)
>5	1.00	(ref)
Gender of sex partner(s)		
Male	0.38	(0.16;0.91)
Female, both	1.00	(ref)
Condom use		
Never (0%)	1.87	(0.38;9.27)
Sometimes (1-99%)	4.54	(1.01;20.32)
Always (100%)	1.00	(ref)
Sexual intercourse in past 6 months (frequency)		
0	0.34	(0.09;1.27)
1-6	1.00	(ref)
7-24	0.30	(0.09;0.99)
25-54	0.24	(0.10;0.61)
>54	0.66	(0.30;1.47)

n: number
Adj. OR: Adjusted Odds Ratio
95% C.I.: 95% Confidence Interval
*Age, grouped in one category when 24 or higher
ref: reference

Discussion

We explored the possibility of composing a risk factor assessment tool, i.e. a prediction model, regarding individual HPV vaccine benefit, which may be helpful in counselling individual women in outpatient settings. The information needed is easy to obtain during an outpatient clinic visit and does not include expensive diagnostic tools or invasive pelvic examinations. The prediction model has been constructed using data from 1322 sexually active women aged 18 through 25 years who may form the group for additional (catch-up) vaccination. This risk factor assessment tool provides a simple method to read the probability of currently being infected with hr-HPV vaccine-types 16 and/or 18. It may be used as a decision-aid in outpatient clinics to provide women with insight into their own individual situation. Furthermore, it provides an individual based strategy to identify women with a higher probability of being positive, providing the opportunity to perform an HPV test before vaccination, as part of these women may still benefit from vaccination.

Population based strategies may consist only of selectively vaccinating women without risk factors or with a low risk profile, or vaccinating women with a high risk of future acquisition of HPV.[3;12] This study shows an opportunity for the use of risk profiling besides

the questionable population-based vaccination eligibility approach. Since vaccination is costly, sexually active women may need more insight in their personal situation before deciding to get vaccinated. Individually-focused risk profiling may be used to answer questions on an individual basis. Moreover, it may be used to predict whether the vaccine may be administrated immediately or if HPV-testing before vaccination may be indicated. This may be translated into a personal "benefit" i.e. cost savings. The vaccination of sexually active women may considerably increase the speed with which results are obtained in the fight against cervical cancer.[5] Women with previous exposure to HPV or a current HPV infection may still benefit to some extent from vaccination, provided that they have not been infected with both vaccine hr-HPV types. In this study only 2 women (0.2%) were positive for both hr-HPV vaccine types 16 and 18 simultaneously, resulting in no benefit of vaccination. Looking at population based level most women will have the potential to benefit from vaccination since the prevalence of HPV vaccine type 16 and/or 18 is low.[5;13] Therefore, a vaccination strategy consisting of testing all sexually active women prior to vaccination will be senseless as it will be expensive and excessive. However, looking at counselling individual sexually active women with a higher probability of being hr-HPV vaccine-type positive, a strategy with HPV testing may be useful. Therefore, this model provides insight in personal "risk factors" and therefore may contribute to a woman's conscious decision regarding direct vaccination or prior HPV testing.

A limitation of using cross-sectional data is the identification of current HPV infection and partial retrospective analyses of potential risk factors. An additional disadvantage of using cross-sectional data for the prediction model may be that information on previous infection is lacking. However, this problem is contradicted by a study performed by Schwarz et al. which concluded that vaccination of women who are serologically negative at time of vaccination, without knowledge of previous infections, is effective.[14] Therefore, transient infections, that play a very small role in the overall development of clinical disease, are of importance at the moment when a woman is considering vaccination.

In conclusion, we composed a risk assessment tool that may be helpful in counselling individual women in outpatient settings. It provides an estimation of the probability of being infected with HPV 16 and/or 18 and therefore provides insight into the personal situation. Furthermore, it may provide a guideline to discriminate between sexually active individuals eligible for direct vaccination and sexually active individuals who may benefit from prior HPV testing. Furthermore, future research is of specific interest as risk profiling may be used to predict hr-HPV persistence and future infections, both affecting vaccine efficacy.

What is already known on this topic

Infection with HPV is a necessary event in the multi-step process of cervical carcinogenesis. Prophylactic vaccines are developed to prevent specifically HPV 16 and 18 infections. Efficacy of HPV vaccines has been proven in women who are HPV 16 and/or 18 negative at time of vaccination. The benefit of HPV vaccination of sexually naïve women is likely to be higher than that of older already sexually active women.

What this study adds

The individual decision of older sexually active women to get vaccinated will be balanced between costs and personal benefit. This risk assessment tool may be used in outpatient settings to estimate the probability of HPV 16 and/or 18 infection in sexually active individuals requesting counselling before HPV vaccination. It provides insight in one's personal situation. Furthermore, it may provide a guideline to discriminate between sexually active individuals eligible for direct vaccination and sexually active individuals who may benefit from prior HPV testing.

Reference List

(1) Brabin L, Roberts SA, Stretch R, Baxter D, Chambers G, Kitchener H, et al. Uptake of first two doses of human papillomavirus vaccine by adolescent schoolgirls in Manchester: prospective cohort study. BMJ 2008 May 10;336(7652):1056-8.

(2) Manhart LE, Holmes KK, Koutsky LA, Wood TR, Kenney DL, Feng Q, et al. Human papillomavirus infection among sexually active young women in the United States: Implications for developing a vaccination strategy. Sex Transm Dis 2006 Aug;33(8):502-8.

(3) Dempsey AF, Gebremariam A, Koutsky LA, Manhart L. Using risk factors to predict human papillomavirus infection: implications for targeted vaccination strategies in young adult women. Vaccine 2008 Feb 20;26(8):1111-7.

(4) Dempsey AF, Gebremariam A, Koutsky L, Manhart L. Behavior in early adolescence and risk of human papillomavirus infection as a young adult: results from a population-based study. Pediatrics 2008 Jul;122(1):1-7.

(5) Torne A, Alonso I, Puig-Tintore LM, Pahisa J. Clinical role of cervical cancer vaccination: when and whom to vaccinate? Gynecol Oncol 2008 Sep;110(3 Suppl 2):S15-S16.

(6) Stanley M. Human papillomavirus vaccines versus cervical cancer screening. Clin Oncol (R Coll Radiol) 2008 Aug;20(6):388-94.

(7) Hildesheim A, Herrero R, Wacholder S, Rodriguez AC, Solomon D, Bratti MC, et al. Effect of human papillomavirus 16/18 L1 viruslike particle vaccine among young women with preexisting infection: a randomized trial. JAMA 2007 Aug 15;298(7):743-53.

(8) Lenselink CH, Melchers WJ, Quint WG, Hoebers AM, Hendriks JC, Massuger LF, et al. Sexual behaviour and HPV infections in 18 to 29 year old women in the pre-vaccine era in the Netherlands. PLoS ONE 2008;3(11):e3743.

(9) Kleter B, van Doorn LJ, Schrauwen L, Molijn A, Sastrowijoto S, ter Schegget J, et al. Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. J Clin Microbiol 1999 Aug;37(8):2508-17.

(10) Melchers WJ, Bakkers JM, Wang J, de Wilde PC, Boonstra H, Quint WG, et al. Short fragment polymerase chain reaction reverse hybridization line probe assay to detect and genotype a broad spectrum of human papillomavirus types. Clinical evaluation and follow-up. Am J Pathol 1999 Nov;155(5):1473-8.

(11) Harrell FE. Regression modeling strategieswith applications to linear models, logistic regression, and survival analysis. New York: Springer; 2001.

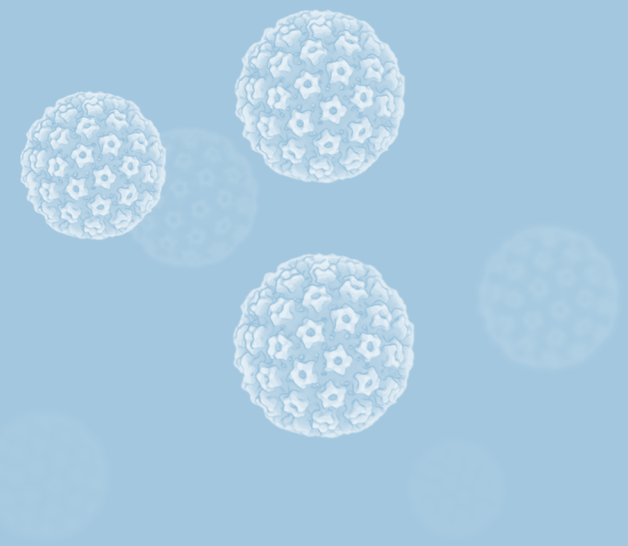
(12) Dempsey AF, Zimet GD. Human papillomavirus vaccine and adolescents. Curr Opin Obstet Gynecol 2008 Oct;20(5):447-54.

(13) Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine 2006 Mar 30;24 Suppl 1:S1-15.

(14) Schwarz TF, Spaczynski M, Schneider A, Wysocki J, Galaj A, Perona P, et al. Immunogenicity and tolerability of an HPV-16/18 AS04-adjuvanted prophylactic cervical cancer vaccine in women aged 15-55 years. Vaccine 2009 Jan 22;27(4):581-7.

Chapter 7

Detection and genotyping of Human Papillomavirus in self-obtained cervico-vaginal samples by using the FTA cartridge: New possibilities for cervical cancer screening



Charlotte H. Lenselink[1]
Roosmarie P. de Bie[1]
Dennis van Hamont[1]
Judith M.J.E. Bakkers[2]
Wim G.V. Quint[3]
Leon F.A.G. Massuger[1]
Ruud L.M. Bekkers[1]
Willem J.G. Melchers[2]

[1,2] Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands
[1] Department of Obstetrics & Gynaecology,
[2] Department of Medical Microbiology
[3] DDL Diagnostic Laboratory, Voorburg, the Netherlands

J Clin Microbiol 2009 Aug;47(8):2564-70

Abstract

This study assesses Human Papillomavirus (HPV) detection and genotyping in self-sampled genital smears applied to an indicating FTA elute cartridge (FTA cartridge). The study group consisted of 96 women, divided into two sample sets. All samples were analysed by the HPV-SPF$_{10}$ Line Blot 25. Set 1 consisted of 45 women attending the gynaecologist; all obtained a self-sampled cervico-vaginal smear, which was applied to an FTA cartridge. HPV results were compared to a cervical smear (liquid-based) taken by a trained physician. Set 2 consisted of 51 women who obtained a self-sampled cervico-vaginal smear at home, which was applied to an FTA cartridge and to a liquid-based medium. DNA was obtained from the FTA cartridges by simple elution as well as extraction. Of all self-obtained samples of set 1, 62.2% tested HPV-positive. The overall agreement between self- and physician-obtained samples was 93.3%, in favour of the self-samples. In sample set 2, 25.5% tested HPV-positive. The overall agreement for high-risk HPV presence between the FTA cartridge and liquid-based medium and between DNA elution and extraction was 100%.

This study shows that HPV detection and genotyping in self-obtained cervico-vaginal samples applied to an FTA cartridge is highly reliable. It shows a high level of overall agreement with HPV detection and genotyping in physician-obtained cervical smears and liquid-based self-samples. DNA can be obtained by simple elution and is therefore easy, cheap, and fast. Furthermore, the FTA cartridge is a convenient medium for collection and safe transport at ambient temperatures. Therefore, this method may contribute to a new way of cervical cancer screening.

Introduction

Infection with Human Papillomavirus (HPV) is a necessary event in the multi-step process of cervical carcinogenesis.[1;2] As a result, the clinical value of HPV testing has been well-established.[3-8] In the United States, the Food and Drug Administration (FDA) has authorized a high-risk HPV (hr-HPV) assessment for primary screening in women aged 30 and older. This is in addition to regular cytological screening as well as for the triage of smears of atypical cells of undetermined significance. In the Netherlands, additional hr-HPV testing has been approved and recommended in all follow-up smears after the detection of a first-time borderline or mild dysplasia (BMD) smear. The beneficial effect of HPV testing will most likely increase in case hr-HPV assessment replaces cytology as primary screening tool.[3-5;7]

Regarding (hr-)HPV testing, material from vaginal lavages or self-sampling brushes has proven to be highly representative for the cervical (hr-)HPV status.[9-14] In addition, cervico-vaginal self-samples have repetitively been proven to be as reliable as physician-taken samples.[15;16] Subsequently, several studies have shown that self-sampling for HPV testing was highly acceptable to women, although some women were concerned about performing the test properly.[14;17] Hr-HPV testing on self-sampled materials might be a promising opportunity to increase the efficacy of population-based screening programmes worldwide.[9;10;13;18] Cervico-vaginal self-sampling may be an easy, accessible, user-friendly, and timesaving alternative for the physician-based collection of cervico-vaginal material.[14;17]

In the Dutch cervical screening programme approximately 70% of the women who are invited actually take part. Tragically, half of the cervical carcinomas are diagnosed in the remaining group of non-responders.[13;19;20] Cervical cancer incidence would decrease significantly if these non-responders could be reached.[5;8] Several studies have shown that non-responders actually do take part in self-sampling studies.[9;13;21] Self-sampling is a less costly and a less invasive collection method.[16] Self-sampled material could be more easily obtained in populations that are difficult to reach and in settings with limited resources, facilitating the introduction of organised HPV-based cervical screening programmes in developing countries as well.

However, the vast majority of studies assessing self-sampling have used liquid-based storage and transport media.[9;12;13;21] Since these solutions can be inflammable, hazardous, and potentially infectious, careful handling is required and regular mailing may even not be allowed. This severely hampers the introduction of cervico-vaginal self-sampling methods. Dried fluid spots or solid carriers have already been used for decades in the postnatal screening of certain congenital disorders and diseases. Solid carriers have also been successfully used in studies detecting and genetically characterizing measles virus

Figure 1.

Whatman® indicating FTA® elute cartridge.

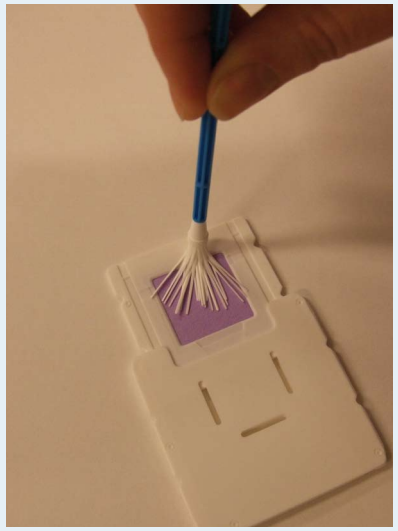


Figure 2.

Whatman® indicating FTA® elute cartridge upon application.

strains, as well as in studies assessing viral load and genotypic-resistance for human immunodeficiency virus (HIV).[22-24] As dried material on a solid carrier is neither hazardous nor inflammable, applying genital self- samples on these solid carriers (like FTA cartridges) can solve storage and transportation problems.

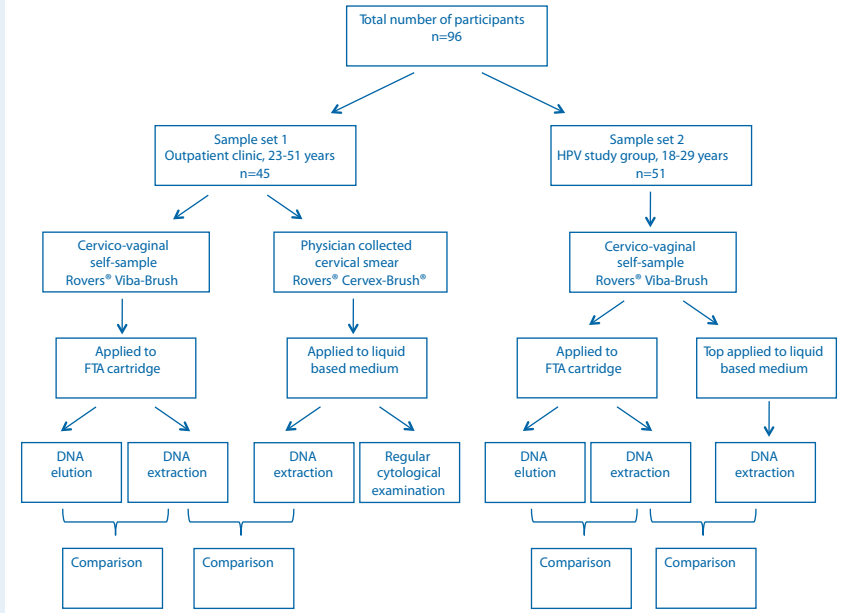
In this study, we have assessed the use of self-sampled cervico-vaginal smears applied to a new FTA cartridge, i.e. the Whatman® indicating FTA® elute cartridge (Figure 1 and 2) which allows easy storage and transport as the virus is denaturised upon application. Additionally, the cartridge overcomes the uncertainty of women about performing the procedure properly, as it has an indicating dye which changes from purple to white when a (genital) sample is applied. Furthermore, we assessed the novel method of direct HPV DNA elution without requiring further purification.

Material and Methods

The study group consisted of 96 women, divided into two sample sets (Figure 3).

Figure 3.

Study design.



Sample set 1

Between September and October 2008, 45 women were recruited at the Department of Obstetrics and Gynaecology of the Radboud University Nijmegen Medical Centre, the Netherlands. These participants visited the gynaecologist for follow-up after diethyl-stilbestrol-exposition in utero, treatment of cervical dysplasia, or follow up after two BMD smears. The median age was 38 years (standard deviation 6.85 years, range 23 to 51 years).

All women were asked to self-collect a cervico-vaginal sample after having received instructions on how to perform the self-sample (verbally, written, and in cartoon).

In brief, participants were instructed to wash their hands before opening the brush cover (Rovers® Viba-Brush, Rovers Medical Devices B.V., Oss, the Netherlands), to hold the brush by the end of the handle, to insert the brush approximately 7 cm into the vagina (similar to inserting a tampon), and to gently turn the brush 5 times. Subsequently, the brush was applied to the FTA cartridge (Whatman® indicating FTA® elute cartridge, catalogue number WB 659223, GE Healthcare, United Kingdom) (Figure 1 and 2). The FTA cartridge was dried to air. After self-sampling, a vaginal speculum was inserted and a physician obtained a regular cervical smear using a Rovers® Cervex-brush® (Rovers Medical Devices B.V., Oss, the Netherlands) that was rinsed in a Thinprep® vial (Cytyc corp. Boxborough, MA, USA). Regular liquid-based cytological (LBC) examination was performed and 0.5mL of LBC homogenised medium was used for HPV assessment.
In order to assess the samples anonymously, all self-obtained samples and cervical LBC samples were provided with an unique patient code before they were sent to the laboratory.

Sample set 2

Sample set 2 consisted of 51 healthy participants who were randomly recruited from a prospective self-sampling study of HPV prevalence, incidence and clearance among 2065 unscreened women between 18 and 29 years of age.[25] All women were asked to self-collect a cervico-vaginal sample in the privacy of their own home. Women received an explanatory letter, an informed consent form, and a self-sample kit by mail. The self-sample kit was provided with an anonymous code to ensure privacy. The self-sample kit contained a collection device (a small brush packaged in an individual sterile cover, Rovers® Viba-Brush, Rovers Medical Devices B.V., Oss, the Netherlands), an FTA cartridge (Whatman® indicating FTA® elute cartridge, catalogue number WB 659223, GE Healthcare, United Kingdom) (Figure 1 and 2), a collection tube containing medium (SurePath™, Tripath Imaging®, Inc., Burlington, NC, USA), instructions how to perform the cervico-vaginal self-sample (written and in cartoon), and a return package consisting of a leak-proof seal bag, absorption sheet, and a reclosable plastic return envelope (Easyslider, Transposafe Systems Holland BV, Sassenheim, the Netherlands). Except for the fact that they used an additional liquid based medium, the instructions of how to perform the self-sample were similar to the instructions described above. In brief, participants were instructed to first apply the self-sample on the FTA cartridge and to subsequently place the top of the brush in the collection tube. The collection tube was closed and enclosed in the seal bag. Finally, the collection tube was placed in the return envelope together with the dried FTA cartridge and sent to the Department of Obstetrics and Gynaecology for further processing and HPV assessment at the Department of Medical Microbiology. The samples were stored at room temperature. In the original study of the 2065 women, a control for sample sufficiency, i.e. detection of human beta-globin, was performed and showed less than 1% false negative samples.[25]
The self-sampling material on the FTA cartridge was compared to the self-sampling material stored in the liquid-based medium (Figure 3).

Specimen preparation LBC

For isolation of DNA from cervical scrapes in liquid-based cytology medium, the MagNAPure LC Isolation station (Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany) was used; 500µL of material was isolated using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics GmbH, Roche Molecular Biochemicals, Mannheim, Germany), as described by the manufacturer. With each set of 28 cervical scrape samples four negative controls were included. Nucleic acid was resuspended in a final volume of 50µL; 10µL was used for PCR analysis.[26]

Specimen preparation of the indicating FTA elute cartridge

The indicating FTA elute matrix contains an indicating dye that changes from purple to white upon application of a colourless sample such as cervico-vaginal swab. The FTA cartridges were punched using a sterilised perforator specifically designed for the FTA cartridges (3-mm Harris Uni-core device, Whatman). The sample amount varied between samples, and to optimize the number of punches to cover this variation, pilots were performed using a different number of punches. For this study, only three punches were considered to compare DNA elution and extraction, as well as individual genotypes.
The FTA elute matrix is chemically treated with proprietary reagents that lyses cells upon contact, causing the release of nucleic acids. DNA was recovered from the FTA elute matrix through a simplified elution process using heat and water. Inhibitory components, such as haemoglobin, are retained on the FTA elute matrix.

Elution

The three punches were transferred into a 1.5-mL microfuge tube, 1500µL of sterile water was added to the punches and immediately pulse vortexed 3 times, for a total of 5 seconds. The water was removed with a sterile fine-tip pipette. Fifty microliters of sterile water was added to the punches, and the tube was transferred to a heating block at 95°C for 30 minutes. At the end of the incubation period the sample was removed from the heating block and pulse vortexed approximately 60 times. It was additionally

centrifuged for 30 seconds, and the eluted DNA was placed into a new microcetrifuge tube with a pipette. The eluted DNA was stored at -80°C.
Finally, 10µL of the eluate was used for PCR analysis.

Isolation

For additional comparison, DNA was extracted from three other punches using the QIAGEN® DNeasy Tissue Kit (QIAGEN Inc, Valencia, CA, USA), as described by the manufacturer.
Subsequently, HPV DNA assessment was performed identically as for the LBC specimens, as described below. All HPV tests were performed by laboratory assistants unaware of the cytological status and the results from the comparative HPV detection tests.

HPV detection and genotyping

Broad-spectrum HPV DNA amplification was performed using a short-PCR-fragment assay (HPV SPF_{10} Line Blot 25, Labo Biomedical Products BV, Rijswijk, the Netherlands). This assay amplifies a 65-bp fragment of the L1 open reading frame, and allows detection of a broad range of hr-, low-risk (lr) and possible hr-HPV genotypes.[27]
Twenty-eight oligonucleotide probes which recognize 25 different types were tailed with poly(dT) and immobilised as parallel lines to membrane strips (Labo Biomedical Products BV, Rijswijk, the Netherlands). The HPV genotypes detectable are hr-HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59, and 68/73 and two probable hr-HPV types (53 and 66). Samples that tested positive using the DNA enzyme immunoassay but that showed no results on the LiPA strip were considered to be HPV "X" type, i.e. genotypes not available on the LiPA strip. Lr-HPV types were defined as HPV type 6, 11, 34, 40, 42, 43, 44, 54, 55, 58, 70, 74, and X. The HPV genotyping assay was performed as described previously.[28] The LiPA strips were visually inspected, and interpreted using the provided reference guide.

Study design

All samples were assessed for HPV genotyping using the HPV SPF_{10} Line Blot 25 assay. For the first sample set, the self-sampled material on the FTA cartridge was compared to a liquid-based cervical smear obtained for diagnostic purposes by a trained physician in the outpatient clinic. Additionally, as HPV DNA elution is a novel method to obtain HPV DNA from an FTA Elute cartridge, results of DNA elution were compared to results from HPV DNA extraction (Figure 3).

In the second sample set, the self-sampled material stored in liquid based solution was compared to self-sampled material on the FTA cartridge. Again, results of DNA elution and extraction from the FTA cartridge were compared (Figure 3). In the original study population of sample set 2 (n=2065), detection of beta-globin was used as a control for sample sufficiency and showed less than 1% false negatives.
Comparing the presence of hr-HPV between the two samples, results were termed concordant or discordant based on the following definitions. If analyses showed identical genotypes in both samples, the results were termed concordant. Genotyping results were termed discordant when no similarities in the genotypes existed.
This study was approved by the local Medical Ethics Committee. All participants were informed and provided an informed consent.

Statistics

All data were analysed using SPSS version 16.0 for Windows (Chicago, Illinois, USA). Agreement was measured by absolute agreement and Cohen's kappa statistics, a measure of the agreement between two methods that is in excess of that due to chance.

Results

The study group consisted of 96 women between 18 and 51 years of age. The results of the two sample sets are described separately, since sample set 2 consisted of healthy unscreened women and sample set 1 consisted of women with a higher risk of an HPV infection than in the general population as they had initially been referred to the gynaecologist for cervical follow-up for several reasons.

Sample set 1

The median age of the 45 women in sample set 1 was 38 years (standard deviation 6.85 years, range 23 to 51 years).

Cervico-vaginal self-obtained sample versus physician-obtained cervical smear

Of the 45 self-collected cervico-vaginal samples on the FTA cartridges, 62.2% (n=28) tested positive for one or more HPV genotypes. This high prevalence was expected due to the nature of the follow-up. Of these 28 samples, 25 also tested positive for HPV in the cervical smear sample obtained by the physician.

Of the 28 HPV positive samples, 19 samples showed similar types, 5 samples showed an additional genotype (sample no. 2,5,9,16, and 25) (Table 1), and 4 samples showed a different genotype (sample no. 8,10, 18, and 20) (Table 1). The overall agreement for HPV positivity between self-sampling and the cervical smear taken by the physician was 93.3% (kappa value 0.86, 95% confidence interval (C.I.) 0.713;1.013).

Concordance and discordance of hr-HPV

Table 1 shows a summary of the genotypes per sample set as well as the concordance and discordance for hr-HPV. Taking the samples of all 45 women into account, 42 samples (93.3%) were concordant and 3 samples (6.7%) were discordant for hr-HPV presence. In these 3 samples, the physician-obtained smear did not contain a hr-HPV type in contrast to the self-obtained sample (sample no. 8, 16, and 18) (Table 1).

Of the 42 concordant samples, 25 showed no hr-HPV DNA in both self- and physician-obtained samples. In 20 of the 45 (44.4%) self-obtained samples, one or more hr-HPV types were detected; 17 patients also tested hr-HPV positive in the cervical smears obtained by the physician. The overall agreement for hr-HPV positivity was 93.3% (kappa value 0.86, 95% C.I. 0.713;1.013).

Concordance and discordance of lr-HPV

In 13 of the 45 (28.9%) self-obtained samples, one or more lr-HPV types were detected, 10 cases also tested lr-HPV positive in the cervical smears obtained by the physician. The overall agreement for lr-HPV positivity was 93.3% (kappa value 0.83, 95% C.I. 0.635;1.016). Table 1 shows a summary of the genotypes per sample set as well as the concordance and discordance for lr-HPV. Taking the samples of all 45 women into account, 42 samples (93.3%) were concordant and 3 samples (6.7%) were discordant for lr-HPV presence. In these 3 samples the physician-obtained smear did not contain a lr-HPV type in contrast to the self-obtained sample (sample no. 2, 5, and 10) (Table 1). Of the 42 concordant samples, 32 showed no lr-HPV DNA in both self- and physician-obtained samples.

DNA elution versus DNA extraction

As DNA elution is a novel method of obtaining DNA from an FTA cartridge, HPV DNA from the self-sampled material on the FTA cartridge yielded by DNA elution was compared to HPV DNA yielded from the cartridge by DNA extraction. The results showed a perfect overall agreement of 100% (kappa value 1.0, 95% C.I. 1.0;1.0) indicating the reliability of this procedure (Table 1).

Table 1. HPV detection by SPF_{10} Line Blot 25 for corresponding genital self-obtained smears and physician-obtained cervical smears (sample set 1)

Sample	HPV detection using physician-obtained samples (LBC)			HPV detection using self-obtained samples (FTA cartridge)				HPV Accordance	
	DNA extraction	hr	lr	DNA extraction	DNA elution	hr	lr	hr	lr
1	52	+	-	52	52	+	-	c	c
2	51	+	-	11, 31, 51	11, 31, 51	+	+	c	d
3	18, 31	+	-	18, 31	18, 31	+	-	c	c
4	16	+	-	16	16	+	-	c	c
5	53	+	-	6, 53	6, 53	+	+	c	d
6	16	+	-	16	16	+	-	c	c
7	6	-	+	6	6	-	+	c	c
8	N	-	-	52	52	+	-	d	c
9	16, 66	+	-	66	66	+	-	c	c
10	N	-	-	11	11	-	+	c	d
11	16	+	-	16	16	+	-	c	c
12	66	+	-	66	66	+	-	c	c
13	X	-	+	X	X	-	+	c	c
14	59	+	-	59	59	+	-	c	c
15	39	+	-	39	39	+	-	c	c
16	42	-	+	18, 42	18, 42	+	+	d	c
17	16	+	-	16	16	+	-	c	c
18	N	-	-	16	16	+	-	d	c
19	51	+	-	51	51	+	-	c	c
20	68, 70	+	+	52, 70	52, 70	+	+	c	c
21	51	+	-	51	51	+	-	c	c
22	X	-	+	X	X	-	+	c	c
23	6, 51, 58	+	+	6, 51, 58	6, 51, 58	+	+	c	c
24	58	-	+	58	58	-	+	c	c
25	31	+	-	31, 51	31, 51	+	-	c	c
26	70	-	+	70	70	-	+	c	c
27	6	-	+	6	6	-	+	c	c
28	X	-	+	X	X	-	+	c	c
29-45	N	N	N	N	N	N	N	c	c

LBC: liquid based cytology
hr: hr-HPV types were 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59, and 68/73, probable hr-HPV: 53 and 66
lr: lr-HPV types were 6, 11, 34, 40, 42, 43, 44, 54, 55, 58, 70, 74, and X
c: concordant
d: discordant
N: HPV negative
-: negative
+: positive

Sample set 2

The median age of the women in sample set 2 was 21 years (range 18 to 29 years). Of the 51 self-collected cervico-vaginal samples applied to a liquid-based medium, 13 (25.5%) tested positive for one or more HPV genotypes. All of these HPV positive samples also tested positive for HPV on the FTA cartridge (Table 2). Moreover, the overall agreement for HPV positivity between the FTA cartridge and liquid-based medium was 100% (kappa value 1.0, 95% C.I. 1.0;1.0).
Of the 13 HPV positive samples, 10 samples showed similar types, 2 samples showed an additional genotype (sample no. 9 and 12) (Table 2), and 1 sample showed a different genotype (sample no. 13) (Table 2).

Concordance and discordance of hr-HPV

Table 2 shows a summary of the genotypes per sample set as well as the concordance of hr-HPV between the liquid-based and filter-based samples. Taking all 51 samples into account, all samples were concordant for hr-HPV detection, of which 9 (17.6%) were hr-HPV positive. In these 9 liquid-based stored self-samples, one or more hr-HPV types were detected; all samples (100%) also tested hr-HPV positive on the FTA cartridges.
Additionally, the overall agreement for hr-HPV positivity between FTA cartridge and liquid-based medium was 100% (kappa value 1.0, 95% C.I. 1.0;1.0).

Concordance and discordance of lr-HPV

One or more lr-HPV types were detected in 7 of the liquid-based stored self-obtained samples (13.7%) versus 7 on the FTA cartridges (Table 2). However, not all samples showed similar types, resulting in only 5 concordant lr-HPV types. The overall agreement for lr-HPV positivity between FTA cartridge and liquid-based medium was 96.1% (kappa value 0.83, 95% C.I. 0.609;1.059).

DNA elution versus DNA extraction

In sample set 2, HPV DNA yielded from the self-obtained material on the FTA cartridge by DNA elution was again compared to DNA yielded from the FTA cartridge by extraction. The results showed an overall agreement of 100% (kappa value 1.0, 95% C.I. 1.0;1.0).

Table 2. HPV detection by SPF_{10} Line Blot 25 for corresponding genital self-obtained smears with liquid-based medium versus FTA cartridge (sample set 2)

Sample	HPV detection using self-obtained samples (LBC)			HPV detection using self-obtained samples (FTA cartridge)				HPV Accordance	
	DNA extraction	hr	lr	DNA extraction	DNA elution	hr	lr	hr	lr
1	18, 51, 54, 68	+	+	18, 51, 54, 68	18, 51, 54, 68	+	+	c	c
2	56	+	-	56	56	+	-	c	c
3	X	-	+	X	X	-	+	c	c
4	X	-	+	X	X	-	+	c	c
5	42, 51, 54	+	+	42, 51, 54	42, 51, 54	+	+	c	c
6	52	+	-	52	52	+	-	c	c
7	X	-	+	X	X	-	+	c	c
8	16	+	-	16	16	+	-	c	c
9	16, 45, 51	+	-	11, 16, 45, 51	11, 16, 45, 51	+	+	c	d
10	68	+	-	68	68	+	-	c	c
11	31	+	-	31	31	+	-	c	c
12	39, 54	+	+	39	39	+	-	c	d
13	54	-	+	X	X	-	+	c	c
14-51	N	-	-	N	N	-	-	c	c

LBC: liquid based cytology
hr: hr-HPV types were 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59, and 68/73, probable hr-HPV: 53 and 66
lr: lr-HPV types were 6, 11, 34, 40, 42, 43, 44, 54, 55, 58, 70, 74, and X
c: concordant
d: discordant
N: HPV negative
-: negative
+: positive

Discussion

HPV testing in cervical cancer screening has a beneficial effect in patient management and can increase the success rate of population-based screening programmes in reducing cervical cancer incidence.[3-5;7] Regarding hr-HPV testing, cervico-vaginal self-obtained samples have repetitively been proven to be as reliable as physician-obtained samples.[15;16] This present study underlines the reliability of using cervico-vaginal self-samples for hr-HPV testing.

However, despite differences in self-sampling methods, many previous studies have used liquid-based sample storage and transport media. Use of these solutions may result in a delay or inability to implement at-home self-sampling of population-based screening non-responders because of a number of reasons. For example, one reason is the high cost due to legislations for these potentially hazardous liquid-based techniques, which require difficult and therefore expensive logistics. An alternative for the transport of potentially hazardous solutions could be storage on filter papers, i.e., FTA cartridges. These FTA cartridges, for example, are less prone to contamination and are therefore easy to handle. For instance, filters have been used for decades in the postnatal screening of certain congenital disorders and diseases. The air-dried samples showed stability at room temperature for months, up to years.[29] Furthermore, at-home collection for HIV testing on filter papers has been considered feasible and acceptable in a high-risk cohort. Additionally, also viral load and genotypic-resistance assessments in applied whole blood and plasma of HIV-positive patients appear to be possible.[22;23]

To compare the transport media used in this study, it would be ideal if all conditions across the groups were equal. However, as we are particularly interested in whether results of HPV detection in cervico-vaginal self-obtained samples are comparable to the results of HPV detection in physician-taken cervical smears, the "golden standard", despite or precisely because the conditions differ, we think it is important to compare the self-sampling method with the "regular" physician-taken smear as a proof of principle. This study shows a high level of overall agreement of HPV detection and genotyping between physician-obtained cervical smears which are applied to a liquid-based medium and self-obtained cervico-vaginal samples that are subsequently applied to an FTA cartridge. Additionally, all hr-HPV positive physician-obtained smears were hr-HPV positive in the cervico-vaginal self-samples as well. Besides the reliability of the FTA cartridge regarding hr-HPV testing, its unique properties make it easy to handle. For instance, the air dried FTA cartridges showed stability at room temperature for months. Furthermore, the uncertainty about performing the self-sampling procedure properly will be overcome since the indicating FTA cartridge has an indicating dye which changes upon application of the sample. Furthermore, the contamination risk is reduced as the virus is denaturised upon application, making it biohazard free and safe for transport by mail. This allows cervical or self-obtained genital samples to be added to this FTA cartridge and sent to designated central laboratories for analysis. Even more important, by using the FTA cartridge, processing costs will be low as DNA is eluted by a simple method using only water and heat, without requiring expensive DNA extraction. Besides the use in existing screening programmes, usage of the FTA cartridge could simplify the introduction of organised HPV-based cervical screening programmes in developing countries as well.

It has been shown that self-sampling methods are unsuitable for cytological analysis.[9;13] To complete the diagnosis for the individual hr-HPV positive patient, a subsequent physician-obtained smear ought to be performed. Preferably, this is solely done in women who are persistently hr-HPV positive. Whether the self-sampling women are willing to have an additional cytology smear taken in case of hr-HPV persistence has not yet been studied.
Since cervico-vaginal self-sampling could be an easily accessible and user-friendly method, women not participating in the screening programme due to fear or other reasons might be interested to actually participate since this technique could be applicable to at-home self-sampling. Therefore, the introduction of cervico-vaginal self-sampling will probably increase the participation rate.[9;10;13;18] Recently, Bais et al. showed that the active response to self-sampling in populations-based screening non-responders was significantly higher than the active response to an extra recall for conventional cytology.[21]

For HPV detection and genotyping, we used the HPV SPF_{10} Line Blot 25. This assay has previously shown high concordance with various other systems.[26;28] This indicates the suitability of the FTA cartridge for various other HPV detection and genotyping systems like the Roche Amplicor and Linear Array assays. Preliminary studies indeed showed an excellent concordance (data not shown). However, further study may be needed to assess genital self-sampled FTA cartridges using other commercially available HPV detection tests with lower analytical sensitivity (e.g. Hybrid Capture II). Additionally, since this was a pilot study and sample sizes were small, further research should be conducted. Furthermore, since not all samples were checked for specimen sufficiency (e.g. beta globin), future research should include a measure for sample sufficiency.

In conclusion, the results of HPV detection and genotyping on self-sampled cervico-vaginal samples using a Rovers® Viba-brush and the Whatman® indicating FTA® elute cartridge are highly representative for the cervical HPV status.
Furthermore and equally important, this study shows that elution of DNA from the Whatman® indicating FTA® elute cartridge, without the necessity of DNA extraction procedures, is a fast, cheap, and reliable method. The Whatman® Indicating FTA® Elute cartridge technique is a convenient medium for collection, as the colour of the FTA cartridge changes after application of the self-sampled material, confirming proper use. Additionally, the FTA cartridges can be stored at ambient temperatures for months, and since the method is non-hazardous, the samples are allowed to be sent by regular mail. This suggests that this method might be applicable to at-home self-sampling in population-based screening non-responders, as well as for the introduction of primary

HPV-based cervical cancer prevention and for establishing cervical cancer screening programmes in developing countries.

Acknowledgements

This study was partially supported by GlaxoSmithKline. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Concerning this manuscript no conflict of interest exists.

We would like to thank Nel H.T.H. Struijk- van der Zanden for the logistical support critical to this study. Furthermore, Delft Diagnostic Laboratory kindly provided the HPV SPF_{10} Line Blot 25 genotyping test and detection reagents, Rovers Medical Devices B.V. supplied the Rovers Cervex®-brushes and Rovers® Viba-brushes, and GE Healthcare provided the Whatman® indicating FTA® elute cartridges.

Reference List

(1) Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003 Feb 6;348(6):518-27.

(2) Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999 Sep;189(1):12-9.

(3) Arbyn M, Paraskevaidis E, Martin-Hirsch P, Prendiville W, Dillner J. Clinical utility of HPV-DNA detection: triage of minor cervical lesions, follow-up of women treated for high-grade CIN: an update of pooled evidence. Gynecol Oncol 2005 Dec;99(3 Suppl 1):S7-11.

(4) Bais AG, Rebolj M, Snijders PJ, de Schipper FA, van der Meulen DA, Verheijen RH, et al. Triage using HPV-testing in persistent borderline and mildly dyskaryotic smears: proposal for new guidelines. Int J Cancer 2005 Aug 10;116(1):122-9.

(5) Bekkers RL, Meijer CJ, Massuger LF, Snijders PJ, Melchers WJ. Effects of HPV detection in population-based screening programmes for cervical cancer; a Dutch moment. Gynecol Oncol 2006 Mar;100(3):451-4.

(6) Cuzick J, Beverley E, Ho L, Terry G, Sapper H, Mielzynska I, et al. HPV testing in primary screening of older women. Br J Cancer 1999 Oct;81(3):554-8.

(7) Cuzick J, Szarewski A, Cubie H, Hulman G, Kitchener H, Luesley D, et al. Management of women who test positive for high-risk types of human papillomavirus: the HART study. Lancet 2003 Dec 6;362(9399):1871-6.

(8) Bekkers RL, Massuger LF, Bulten J, Melchers WJ. Epidemiological and clinical aspects of human papillomavirus detection in the prevention of cervical cancer. Rev Med Virol 2004 Mar;14(2):95-105.

(9) Brink AA, Meijer CJ, Wiegerinck MA, Nieboer TE, Kruitwagen RF, van Kemenade F, et al. High concordance of results of testing for human papillomavirus in cervicovaginal samples collected by two methods, with comparison of a novel self-sampling device to a conventional endocervical brush. J Clin Microbiol 2006 Jul;44(7):2518-23.

(10) De Alba I, Anton-Culver H, Hubbell FA, Ziogas A, Hess JR, Bracho A, et al. Self-sampling for human papillomavirus in a community setting: feasibility in Hispanic women. Cancer Epidemiol Biomarkers Prev 2008 Aug;17(8):2163-8.

(11) Winer RL, Feng Q, Hughes JP, Yu M, Kiviat NB, O'Reilly S, et al. Concordance of self-collected and clinician-collected swab samples for detecting human papillomavirus DNA in women 18 to 32 years of age. Sex Transm Dis 2007 Jun;34(6):371-7.

(12) Sellors JW, Lorincz AT, Mahony JB, Mielzynska I, Lytwyn A, Roth P, et al. Comparison of self-collected vaginal, vulvar and urine samples with physician-collected cervical samples for human papillomavirus testing to detect high-grade squamous intraepithelial lesions. CMAJ 2000 Sep 5;163(5):513-8.

(13) Nobbenhuis MA, Helmerhorst TJ, van den Brule AJ, Rozendaal L, Jaspars LH, Voorhorst FJ, et al. Primary screening for high risk HPV by home obtained cervicovaginal lavage is an alternative screening tool for unscreened women. J Clin Pathol 2002 Jun;55(6):435-9.

(14) Harper DM, Noll WW, Belloni DR, Cole BF. Randomized clinical trial of PCR-determined human papillomavirus detection methods: self-sampling versus clinician-directed--biologic concordance and women's preferences. Am J Obstet Gynecol 2002 Mar;186(3):365-73.

(15) Ogilvie GS, Patrick DM, Schulzer M, Sellors JW, Petric M, Chambers K, et al. Diagnostic accuracy of self collected vaginal specimens for human papillomavirus compared to clinician collected human papillomavirus specimens: a meta-analysis. Sex Transm Infect 2005 Jun;81(3):207-12.

(16) Petignat P, Faltin DL, Bruchim I, Tramer MR, Franco EL, Coutlee F. Are self-collected samples comparable to physician-collected cervical specimens for human papillomavirus DNA testing? A systematic review and meta-analysis. Gynecol Oncol 2007 May;105(2):530-5.

(17) Waller J, McCaffery K, Forrest S, Szarewski A, Cadman L, Austin J, et al. Acceptability of unsupervised HPV self-sampling using written instructions. J Med Screen 2006;13(4):208-13.

(18) Szarewski A, Cadman L, Mallett S, Austin J, Londesborough P, Waller J, et al. Human papillomavirus testing by self-sampling: assessment of accuracy in an unsupervised clinical setting. J Med Screen 2007;14(1):34-42.

(19) Sasieni PD, Cuzick J, Lynch-Farmery E. Estimating the efficacy of screening by auditing smear histories of women with and without cervical cancer. The National Co-ordinating Network for Cervical Screening Working Group. Br J Cancer 1996 Apr;73(8):1001-5.

(20) Bos AB, Rebolj M, Habbema JD, van Ballegooijen M. Nonattendance is still the main limitation for the effectiveness of screening for cervical cancer in the Netherlands. Int J Cancer 2006 Nov 15;119(10):2372-5.

(21) Bais AG, van Kemenade FJ, Berkhof J, Verheijen RH, Snijders PJ, Voorhorst F, et al. Human papillomavirus testing on self-sampled cervicovaginal brushes: an effective alternative to protect nonresponders in cervical screening programs. Int J Cancer 2007 Apr 1;120(7):1505-10.

(22) Ayele W, Schuurman R, Messele T, Dorigo-Zetsma W, Mengistu Y, Goudsmit J, et al. Use of dried spots of whole blood, plasma, and mother's milk collected on filter paper for measurement of human immunodeficiency virus type 1 burden. J Clin Microbiol 2007 Mar;45(3):891-6.

(23) Spielberg F, Critchlow C, Vittinghoff E, Coletti AS, Sheppard H, Mayer KH, et al. Home collection for frequent HIV testing: acceptability of oral fluids, dried blood spots and telephone results. HIV Early Detection Study Group. AIDS 2000 Aug 18;14(12):1819-28.

(24) Kerr RJ, Player G, Fiscus SA, Nelson JA. Qualitative human immunodeficiency virus RNA analysis of dried blood spots for diagnosis of infections in infants. J Clin Microbiol 2009 Jan;47(1):220-2.

(25) Lenselink CH, Melchers WJ, Quint WG, Hoebers AM, Hendriks JC, Massuger LF, et al. Sexual behaviour and HPV infections in 18 to 29 year old women in the pre-vaccine era in the Netherlands. PLoS ONE 2008;3(11):e3743.

(26) van Ham MA, Bakkers JM, Harbers GK, Quint WG, Massuger LF, Melchers WJ. comparison of two commercial assays for detection of human papillomavirus (HPV) in cervical scrape specimens: validation of the Roche AMPLICOR HPV test as a means to screen for HPV genotypes associated with a higher risk of cervical disorders. J Clin Microbiol 2005 Jun;43(6):2662-7.

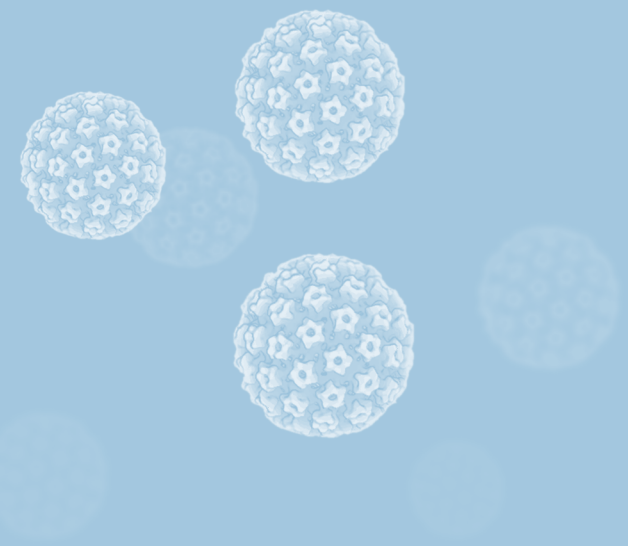
(27) Melchers WJ, Bakkers JM, Wang J, de Wilde PC, Boonstra H, Quint WG, et al. Short fragment polymerase chain reaction reverse hybridization line probe assay to detect and genotype a broad spectrum of human papillomavirus types. Clinical evaluation and follow-up. Am J Pathol 1999 Nov;155(5):1473-8.

(28) van Hamont D, van Ham MA, Bakkers JM, Massuger LF, Melchers WJ. Evaluation of the SPF10-INNO LiPA human papillomavirus (HPV) genotyping test and the roche linear array HPV genotyping test. J Clin Microbiol 2006 Sep;44(9):3122-9.

(29) Chibo D, Riddell MA, Catton MG, Birch CJ. Applicability of oral fluid collected onto filter paper for detection and genetic characterization of measles virus strains. J Clin Microbiol 2005 Jul;43(7):3145-9.

Chapter 8

Final Considerations



The identification of the central aetiological role of high-risk (hr) HPV in cervical carcinogenesis has led to the development of prophylactic vaccines against the two most prevalent hr-HPV genotypes, HPV 16 and HPV 18.[1-5] As HPV transmission mainly occurs sexually, the best results of prophylactic vaccination will be achieved by vaccinating girls before they become genitally infected i.e. sexually active. Given the lag time between age of vaccination and age at development of cervical cancer, the effect of vaccination on cervical cancer rates will take several decades after the introduction of the vaccine. In order to decrease cervical cancer incidence without a 15 to 20 years lag time, vaccination of older already sexually active women is under consideration in many countries. In the group up to 30 years of age, little is known about epidemiology of HPV infections. The studies in this thesis mainly focus on the HPV epidemiology among unscreened women aged 18 to 29 years in the pre-vaccine era. Additionally, the studies provide insight in factors influencing HPV infection as well as vaccine acceptance.

HPV and sexual behaviour

Being sexually active itself has been described as the main risk for exposure to HPV. Besides being sexually active, we also explored several aspects of sexual behaviour like number of sexual partners and condom use. Several studies questioned whether condom use reduces transmission of HPV.[6;7] Unfortunately, results are too inconsistent to produce actual estimates. This may partly be due to different populations studied, as well as differences in study design. In chapter two and three we show that the use of contraceptive methods like condoms was influenced by type of relationship and therefore was not independently related to HPV prevalence or incidence. Furthermore, studies raised young age at first intercourse and corresponding cervical immaturity as an influencing factor of susceptibility of infection and consequent development of abnormalities.[8;9] In our population, age at first intercourse was not associated with HPV infection, as it was influenced by time interval between age at first intercourse and current age as well as by lifetime number of partners. Both lifetime number of partners and type of relationship were related to prevalence, incidence as well as clearance of infection. It was striking that aspects of present as well as past sexual behaviour were independent factors influencing current presence of HPV. These results suggest that some infections detected were newly acquired whereas others were acquired in the past and remained latent below detection level for a long time and may be considered as randomly detected latent infections.

HPV epidemiology and vaccination

Equally important, besides providing insight into HPV dynamics in the pre-vaccine era, the studies in this thesis provide baseline data to allow future research on shifts in HPV epidemiology due to HPV vaccination. With HPV mass vaccination, HPV epidemiology may change gradually by progressive reduction in HPV 16 and HPV 18 infections, as well as a possible decrease in non-vaccine types HPV 45 and HPV 31, induced by cross-protection.[4;10] Theoretically, it is possible that these vacated niches may be filled by other genotypes, leading to (as yet unproven) type replacement.[11] This may raise concerns about the potential of other oncogenic HPV types to replace the position of HPV 16 and HPV 18 as the main initiator of cervical cancer development. As a result of extensive vaccination, it may be possible that new HPV 16 or 18 subtypes or intra-type variants develop, influencing vaccination efficacy. Monitoring these changes on a population level may prove crucial in assessing the effect of mass vaccination on HPV epidemiology as well as the success of vaccination as primary prevention strategy.

For HPV vaccination to be successfully incorporated into the fight against cervical cancer, widespread vaccine coverage is crucial. Presently, vaccination programmes have started in many countries around the world, primarily targeting 9 to 16 year old girls.[12-14] At the verge of European vaccine licensure in 2006, we conducted a study among parents of 10 to 12 year old children to determine their acceptance of HPV vaccination.[15] This study showed an 88% acceptance among parents, with the remark that they requested additional information about HPV and HPV vaccination. When the vaccine was implemented into the funded Dutch national vaccination programme early 2009, the coverage of catch-up vaccination among girls aged 13 to 16 years reached only 50%. This relatively low coverage was attributed to the age of the catch-up group and even more important to negative media attention and a lack of appropriate information. In the United Kingdom vaccine coverage for the first dose among girls aged 12-13 years was 70.6%.[12] Again, the main reason for parents' refusal of vaccination was insufficient information about the vaccine and its long term safety. These findings support the need of educational campaigns to reach a high vaccine coverage.

Although the vaccines have shown cross-protection for other genotypes than originally invented for, protection does not include all hr-HPV genotypes. As the current vaccines only protect against up to 70 to 80% of cervical cancer, the need for additional protection remains a challenge. Therefore, future HPV vaccination needs to focus on the development of a new generation of preventive vaccines that are capable of protecting against additional types and subsequently protect against most cervical cancers. Given the lag

in time between HPV infection and the development of cervical cancer, the impact of (universal) vaccination on cervical cancer rates will take several decades after vaccine introduction. Taken the above into account, the Cervical Screening Programme (CSP) must be continued after the introduction of vaccination. However, along with routine vaccination, screening guidelines may need adaptation in order to retain efficient and cost-effective prevention measures.

HPV and cervical screening in the (post-) vaccination era

In the (post-) vaccination era, HPV 16 and HPV 18 infection will be prevented which will lead to a subsequent decrease in incidence of cervical cancer and its precursors. In the present screening setting 2-5% of the smears contain abnormalities, with an overall sensitivity for CIN2+ of 53.0% (48.6-57.4%).[16] However, sensitivity varies enormously between studies reviewing screening.[16-18] The reduction in prevalence of abnormal cytology may lead to smears not being read as attentively and thoroughly as before given the expectation that abnormalities will be rare. This would result in more false-negative diagnoses with consequent loss in sensitivity.[19] Additionally, the decrease in prevalence of abnormalities would also lead to a decrease in positive predictive value of cervical cytology, the parameter that indicates how correct a positive result would be in triggering management.[19] This may lead to unnecessary medical interventions and patient stress. Therefore, the CSP will need transformation and new screening tools will be necessary to meet the new post-vaccination screening requirements.

Given the strong etiologic link between hr-HPV infection and cervical cancer an alternative for cytology-based cervical cancer screening may be hr-HPV testing.[20] Unlike cytology, HPV tests are objective and highly reproducible. HPV testing is based on highly standardized and validated systems which do not suffer from the pitfalls that typically affect the performance of cervical cytology. For detecting high-grade lesions or cancer, HPV testing has a 20 to 40% greater sensitivity, but about 5 to 10% lower specificity than cervical cytology.[16;21] This lower specificity may be explained by the detection of transient HPV infections instead of detecting exclusively persistent infections. Studies have shown that this somewhat lower specificity may be compensated by using cervical cytology for triage testing. Other potential triage tests, like methylation detection assays, are being studied. HPV genotyping can also be used as a triage test in HPV positive women, as it allows detection of HPV type specific persistence and is therefore important in individual patient management. Because the risk of cervical abnormalities in the five year period following a HPV negative test is much lower than the risk predicted by normal cytology, the screening interval may be extended.

Self-sampling

Throughout the Netherlands, the participation rate of the CSP is approximately 70%. Unfortunately, half of the cervical cancers are diagnosed in the remaining 30% non-participating women. Several studies have shown that non-responders do actually take part in self-sampling studies and that self-sampling for HPV testing was highly acceptable to the majority of women.[22-25] Besides, it will probably be a less costly and less invasive collection procedure than a cervical smear. With regard to hr-HPV detection, self sampling has repetitively been proven to be as reliable as physician-taken samples.[24;26] However, standardization of self-sampling procedures may lead to better comparability across studies. HPV testing on self-sampled materials might be a promising opportunity for future screening. Using HPV genotyping assays instead of HPV detection assays will enable the possibility of monitoring shifts in the natural history of HPV.

HPV vaccination in combination with continued cervical screening will eventually lead to a major reduction in cervical cancer deaths and cervical precancerous lesions. Studies performed in the pre-vaccine era contribute to the understanding of risk factors associated with HPV infections and provide a basis for research on possible future shifts in HPV epidemiology due to mass vaccination. In time, CSPs will need modification to maximize synergy with primary prevention. The challenge remains to link data from screening to data from immunisation allowing epidemiological surveillance of vaccinated populations.

Reference List

(1) Future II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. N Engl J Med 2007 May 10;356(19):1915-27.

(2) Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. Lancet 2004 Nov 13;364(9447):1757-65.

(3) Harper DM. Impact of vaccination with Cervarix (trade mark) on subsequent HPV-16/18 infection and cervical disease in women 15-25 years of age. Gynecol Oncol 2008 Sep;110(3 Suppl 1):S11-S17.

(4) Paavonen J, Naud P, Salmeron J, Wheeler CM, Chow SN, Apter D, et al. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet 2009 Jul 25;374(9686):301-14.

(5) Villa LL, Costa RL, Petta CA, Andrade RP, Ault KA, Giuliano AR, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. Lancet Oncol 2005 May;6(5):271-8.

(6) Manhart LE, Koutsky LA. Do condoms prevent genital HPV infection, external genital warts, or cervical neoplasia? A meta-analysis. Sex Transm Dis 2002 Nov;29(11):725-35.

(7) Winer RL, Hughes JP, Feng Q, O'Reilly S, Kiviat NB, Holmes KK, et al. Condom use and the risk of genital human papillomavirus infection in young women. N Engl J Med 2006 Jun 22;354(25):2645-54.

(8) International Collaboration of Epidemiological Studies of Cervical Cancer. Cervical carcinoma and sexual behavior: collaborative reanalysis of individual data on 15,461 women with cervical carcinoma and 29,164 women without cervical carcinoma from 21 epidemiological studies. Cancer Epidemiol Biomarkers Prev 2009 Apr;18(4):1060-9.

(9) Edelstein ZR, Madeleine MM, Hughes JP, Johnson LG, Schwartz SM, Galloway DA, et al. Age of diagnosis of squamous cell cervical carcinoma and early sexual experience. Cancer Epidemiol Biomarkers Prev 2009 Apr;18(4):1070-6.

(10) Harper DM, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, Roteli-Martins CM, et al. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. Lancet 2006 Apr 15;367(9518):1247-55.

(11) Franco EL, Trottier H. Reassessing the epidemiology of human papillomavirus infection: back to basics. Sex Transm Dis 2008 Mar;35(3):283-5.

(12) Brabin L, Roberts SA, Stretch R, Baxter D, Chambers G, Kitchener H, et al. Uptake of first two doses of human papillomavirus vaccine by adolescent schoolgirls in Manchester: prospective cohort study. BMJ 2008 May 10;336(7652):1056-8.

(13) Brotherton JM, Deeks SL, Campbell-Lloyd S, Misrachi A, Passaris I, Peterson K, et al. Interim estimates of human papillomavirus vaccination coverage in the school-based program in Australia. Commun Dis Intell 2008 Dec;32(4):457-61.

(14) Shefer A, Markowitz L, Deeks S, Tam T, Irwin K, Garland SM, et al. Early experience with human papillomavirus vaccine introduction in the United States, Canada and Australia. Vaccine 2008 Aug 19;26 Suppl 10:K68-K75.

(15) Lenselink CH, Gerrits MM, Melchers WJ, Massuger LF, van Hamont D, Bekkers RL. Parental acceptance of Human Papillomavirus vaccines. Eur J Obstet Gynecol Reprod Biol 2008 Mar;137(1):103-7.

(16) Cuzick J, Clavel C, Petry KU, Meijer CJ, Hoyer H, Ratnam S, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. Int J Cancer 2006 Sep 1;119(5):1095-101.

(17) Nanda K, McCrory DC, Myers ER, Bastian LA, Hasselblad V, Hickey JD, et al. Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. Ann Intern Med 2000 May 16;132(10):810-9.

(18) Wright TC, Jr. Cervical cancer screening in the 21st century: is it time to retire the PAP smear? Clin Obstet Gynecol 2007 Jun;50(2):313-23.

(19) Franco EL, Cuzick J, Hildesheim A, de Sanjose S. Chapter 20: Issues in planning cervical cancer screening in the era of HPV vaccination. Vaccine 2006 Aug 31;24 Suppl 3:S3-171-S3/177.

(20) Meijer CJ, Berkhof J, Castle PE, Hesselink AT, Franco EL, Ronco G, et al. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. Int J Cancer 2009 Feb 1;124(3):516-20.

(21) Franco EL. Chapter 13: Primary screening of cervical cancer with human papillomavirus tests. J Natl Cancer Inst Monogr 2003;(31):89-96.

(22) Brink AA, Meijer CJ, Wiegerinck MA, Nieboer TE, Kruitwagen RF, van Kemenade F, et al. High concordance of results of testing for human papillomavirus in cervicovaginal samples collected by two methods, with comparison of a novel self-sampling device to a conventional endocervical brush. J Clin Microbiol 2006 Jul;44(7):2518-23.

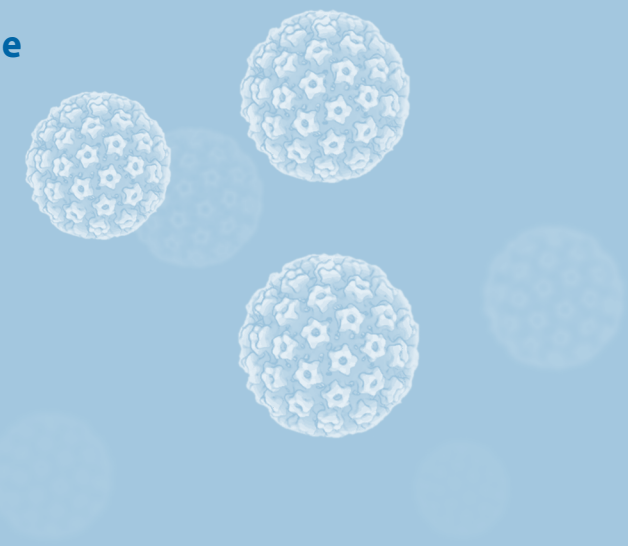
(23) Nobbenhuis MA, Helmerhorst TJ, van den Brule AJ, Rozendaal L, Jaspars LH, Voorhorst FJ, et al. Primary screening for high risk HPV by home obtained cervicovaginal lavage is an alternative screening tool for unscreened women. J Clin Pathol 2002 Jun;55(6):435-9.

(24) Ogilvie GS, Patrick DM, Schulzer M, Sellors JW, Petric M, Chambers K, et al. Diagnostic accuracy of self collected vaginal specimens for human papillomavirus compared to clinician collected human papillomavirus specimens: a meta-analysis. Sex Transm Infect 2005 Jun;81(3):207-12.

(25) Waller J, McCaffery K, Forrest S, Szarewski A, Cadman L, Austin J, et al. Acceptability of unsupervised HPV self-sampling using written instructions. J Med Screen 2006;13(4):208-13.

(26) Petignat P, Faltin DL, Bruchim I, Tramer MR, Franco EL, Coutlee F. Are self-collected samples comparable to physician-collected cervical specimens for human papillomavirus DNA testing? A systematic review and meta-analysis. Gynecol Oncol 2007 May;105(2):530-5.

Chapter 9



Summary

Chapter 1

Genital infection with the Human Papillomavirus (HPV) is one of the most common sexually transmitted diseases among young sexually active women. Studying aspects of sexual behaviour may inform us about the risk of exposure to HPV and the effect on the course of infection. Fortunately, most HPV infections are transient.

The HPV genotypes that are able to infect the genital epithelium have been classified low-risk or high-risk (hr) according to their oncogenic potential. A persistent infection with a hr-HPV type is a risk factor for the development of cervical abnormalities. As hr-HPV genotypes 16 and 18 together account for the majority of cervical carcinomas and its precursors, prophylactic vaccines against these two hr-HPV types have been developed. Worldwide mass vaccination with HPV vaccines will most certainly change HPV epidemiology. To provide a basis for understanding possible future shifts in genotypes, as well as to provide insight in the HPV epidemiology of a target group for vaccination, baseline data should be gathered before vaccination takes place. Additionally, in order to correlate risk factors associated with HPV infection in the pre- and the post-vaccine era, data regarding sexual behaviour are needed. In chapter 1, aspects of HPV mediated carcinogenesis, HPV detection methods, and sampling methods are addressed as well.

Chapter 2

Infection with HPV is a necessary event in the multi-step process of cervical carcinogenesis. Little is known about the natural history of HPV infection among young unscreened adults. As prophylactic vaccines have been developed to prevent specifically HPV 16 and 18 infections, shifts in prevalence in the post-vaccination era may be expected. Chapter 2 describes a cross-sectional study among 2065 unscreened women aged 18 to 29 years. Women returned a self-collected cervico-vaginal specimen and filled out a questionnaire. All HPV DNA-positive samples were genotyped using the SPF_{10} LiPA HPV genotyping assay. HPV point prevalence was 19% and hr-HPV point prevalence was 11.8%. In the majority of the infections it concerned an infection with a single HPV type. HPV vaccine types 16 (2.8%) and 18 (1.4%) were found concomitantly in only 3 (0.1%) women. HPV prevalence increased till 22 years of age, afterwards a plateau phase was reached. Factors independently associated with HPV prevalence were mainly related to sexual behaviour. Number of lifetime sexual partners was the most powerful predictor of HPV positivity followed by type of relationship. Combination of these results with the relative low prevalence of HPV 16 and/or 18 may be promising for expanding the future

target group for catch-up vaccination. These results provide a basis for research on possible future shifts in HPV genotype prevalence.

Chapter 3

The natural course of an HPV infection in healthy unscreened young women may be influenced by viral, host, and environmental factors. In addition to Chapter 2, this part of the prospective epidemiologic study analyses the results of HPV incidence and clearance in 1812 women aged 18-29 years, of whom 1729 were sexually active during follow-up. Women provided three consecutive cervico-vaginal self-samples with a 6 month interval and filled out accompanying questionnaires. During the 12 month follow up hr-HPV incidence in sexually active women was 6.3%. HPV 16 was the most commonly acquired hr-HPV type. The risk of hr-HPV acquisition increased with being single, change in current type of relationship, as well as change in number of sexual partners 3 months prior to sampling, and sexual age at study entry. Hr-HPV clearance was significantly associated with currently being in a relationship as well as total number of sexual partners (lifetime). Hr-HPV incidence as well as clearance were related to past and present sexual behaviour. These results suggest that some infections were newly acquired whereas others were acquired in the past and remained latent below detection level for some time and may be considered as randomly detected latent infections. As HPV infections are very common, it is difficult to discriminate separate risk factors for HPV dynamics. Our results indicate that sexual behaviour itself, i.e. being sexually active, is the most important determinant.

Chapter 4

Before introduction of an HPV vaccine, it is important to consider whether the public is aware of the causal relationship between HPV infections and cervical cancer and whether they would be willing to accept vaccination. This chapter describes the results of the cross-sectional survey performed before the front-page news about the HPV vaccine appeared in the newspapers. To determine whether parents would accept HPV vaccination for their children and which variables may influence their decision, 356 parents of children aged 10 to 12 years were interviewed. HPV vaccination would be accepted by 88% of the parents, preferably at the age of 10 to12 years. Parents of children who received all the vaccinations of the National Vaccination Programme were significantly more likely to accept HPV vaccination. Parents who were not willing to vaccinate their children were more likely to think that the child should be involved in deciding whether to be vaccinated against HPV or not. Less than a third of all parents had heard of HPV and 14% was aware of the causal relationship of HPV and cervical cancer. Knowledge of HPV and cervical cancer, religion, age, educational level, and marital status did not show any significant relation with HPV vaccine acceptance. Among these parents HPV vaccine acceptance seems to be dependent on vaccine acceptance in general, even more than on knowledge of HPV and its causal relationship with cervical cancer. However, parents requested more information about cervical cancer, HPV, and HPV vaccination, before the HPV vaccine is introduced into the vaccination programme.

Chapter 5

In order to decrease cervical cancer without a 15 to 20 years lag time, catch-up vaccination is necessary. The main target group for catch-up vaccination consists of women aged 15 to 25 years. In addition to chapter 4, this chapter describes knowledge of HPV, vaccine acceptability as well as influencing factors among 600 male and female participants aged 18 to 25 years recruited and surveyed at two university departments and one non-university technical college. The majority of the participants had heard of cervical cancer but only a minority could correctly identify risk factors. Female participants had significantly more knowledge of cervical cancer. Only a minority of the participants had ever heard of HPV. Despite this lack of knowledge, a small majority would accept catch-up HPV vaccination. Women and younger participants had a significantly higher acceptance rate. Educational level and knowledge of HPV and cervical cancer were not significantly related to vaccine acceptance. However, this may be influenced by the relatively high educational level of the participants as well as the fact that the general knowledge level of HPV was very low. The exact factors that do influence vaccine acceptance in this age group, in both men and women, remain to be eluded. To reach a high vaccine coverage in this group, an educational campaign is needed that not only covers knowledge of HPV and cervical cancer, but also beliefs and behaviours associated with vaccine acceptance.

Chapter 6

Efficacy of HPV vaccines has been proven in women who are HPV 16 and/or 18 negative at time of vaccination. The benefit of HPV vaccination of sexually naïve women is likely to be higher than that of older already sexually active women. The individual decision of these women to get vaccinated will be balanced between personal benefit and costs. This study is based on the results of a large prospective epidemiologic study performed among 2065 unscreened women aged 18-29 years. Finally, data were used from 1322 women aged 18-25 years who reported to be sexually active in past or present time. HPV

detection was performed on self-collected cervico-vaginal specimens and a questionnaire regarding sexual activity was completed. Multivariate logistic regression with backward variable deletion was used to estimate the probability of HPV infection. The model predicting the optimal probability of being HPV 16 and/or 18 positive was based on the combination of age, number and gender of sexual partners, condom use, and frequency of sexual contact in past 6 months. A nomogram including significant predictors calculates the probability of being HPV 16 and/or 18 infected. This risk assessment tool may be helpful in counselling individual women in outpatient settings. It provides an estimation of the probability of being infected with HPV 16 and/or 18 and therefore provides insight into the personal situation. Furthermore, it may provide a guideline to discriminate between sexually active individuals eligible for direct vaccination and sexually active individuals who may benefit from prior HPV-testing.

Chapter 7

HPV testing in cervical cancer screening has a beneficial effect in patient management and may increase the success rate of population-based screening programmes. Regarding HPV testing, cervico-vaginal self-samples have been proven to be as reliable as physician-taken samples. The introduction of cervico-vaginal self-sampling might increase the participation rate of screening programmes and may therefore potentially reduce cancer incidence. In chapter 7 we assess the reliability of HPV detection and genotyping in self-sampled genital smears applied to an indicating FTA elute cartridge (FTA cartridge). All samples were analysed by the SPF_{10} LiPA HPV genotyping assay. The study group consisted of 96 women, divided in two sample sets. In set 1, women obtained a cervico-vaginal self-sample which was applied to an FTA cartridge. In addition, a cervical smear (liquid-based) was taken by a trained physician. In set 2, women obtained a cervico-vaginal self-sample at home which was applied to an FTA cartridge and to a liquid-based medium. DNA was obtained from the FTA cartridges by simple elution as well as extraction. In sample set 1 overall agreement between self- and physician-obtained samples was 93.3%, in favour of the self-samples. In sample set 2, overall agreement for hr-HPV presence between FTA cartridge and liquid-based medium was 100%. In both sample sets overall agreement for hr-HPV presence between DNA elution and DNA extraction was 100%. This shows that HPV detection and genotyping in self-obtained cervico-vaginal samples applied to an FTA cartridge is highly reliable. It shows a high level of overall agreement with HPV detection and genotyping in physician-obtained cervical smears and liquid-based self-samples. DNA can be obtained by simple elution and is therefore easy, cheap, and fast. Furthermore, as the FTA cartridge is non-hazardous it is a convenient medium for collection and safe transport at ambient temperatures. Therefore, this method may contribute to a new way of cervical cancer screening.

Chapter 8

In this section we consider our results and speculate about vaccine uptake and the effects of HPV vaccination on HPV epidemiology and the current screening programmes.

Samenvatting

Hoofdstuk 1

Een genitale infectie met het Humaan Papillomavirus (HPV) is een van de meest voorkomende seksueel overdraagbare aandoeningen onder seksueel actieve jonge vrouwen. Het in kaart brengen van seksueel gedrag kan inzicht bieden in het risico van blootstelling aan HPV en het effect op het verloop van de infectie. Gelukkig zijn de meeste HPV infecties van voorbijgaande aard.
De HPV genotypen die het genitale gebied kunnen infecteren zijn op basis van hun oncogene potentie ingedeeld in hoog en laag risico typen. Een persistente infectie met een hoog risico HPV type (hr-HPV) is een risicofactor voor de ontwikkeling van afwijkingen aan de cervix (baarmoederhals). Hr-HPV type 16 en 18 zijn samen verantwoordelijk voor de meerderheid van de gevallen van baarmoederhalskanker en de voorstadia ervan. Daarom zijn er tegen deze typen profylactische vaccins ontwikkeld. Wereldwijde vaccinatie met het HPV vaccin zal een verandering tot gevolg hebben in het voorkomen van HPV. Om een basis te vormen voor toekomstig onderzoek naar verschuivingen in het vóórkomen van HPV genotypen zullen er data verzameld moeten worden voordat vaccinatie plaats gaat vinden. Deze gegevens zullen ook inzicht bieden in het voorkomen van HPV in een mogelijke doelgroep voor vaccinatie en de mogelijkheid bieden om in het pre- en postvaccinatie tijdperk risicofactoren met HPV te correleren. In hoofdstuk 1 komen tevens aspecten van de rol van HPV in de carcinogenese alsmede HPV detectie-methoden en manier van monsterafname aan de orde.

Hoofdstuk 2

Infectie met HPV is een voorwaarde voor het ontstaan van baarmoederhalskanker. Er is weinig bekend over het natuurlijk verloop van HPV infecties bij ongescreende jongvolwassenen. Aangezien er profylactische vaccins ontwikkeld zijn om specifiek infectie met HPV 16 en 18 te voorkomen, kan er in het postvaccinatie tijdperk een verschuiving in het voorkomen van HPV worden verwacht. Hoofdstuk 2 beschrijft een cross-sectionele studie onder 2065 ongescreende vrouwen van18 tot en met 29 jaar. De vrouwen vulden een vragenlijst in en namen zelf een cervico-vaginaal monster (zelfsample) af en stuurden dit tezamen terug. Alle HPV DNA-positieve monsters werden gegenotypeerd met behulp van de SPF_{10} LiPA HPV genotyperingstest. De HPV prevalentie bedroeg 19%. De hr-HPV prevalentie was 11.8% en HPV 16 en HPV 18 werden bij respectievelijk 2.8% en 1.4% van de vrouwen aangetoond. In de meerderheid van de infecties betrof het een infectie met een enkel HPV type. Bij slechts 3 vrouwen (0.1%) werden de HPV vaccin types

16 en 18 tegelijkertijd aangetroffen. De HPV prevalentie nam toe tot de leeftijd van 22 jaar waarna een plateaufase werd bereikt. De factoren die onafhankelijk van invloed waren op de HPV prevalentie waren met name gerelateerd aan seksueel gedrag. Het totale aantal seksuele partners hing het sterkst samen met het aanwezig zijn van HPV; dit werd gevolgd door het type relatie. Deze resultaten, samen met de relatief lage prevalentie van HPV 16 en 18 zou veelbelovend kunnen zijn voor het uitbreiden van de toekomstige doelgroep voor catch-up vaccinatie. Verder vormen deze resultaten een basis voor onderzoek naar mogelijke toekomstige verschuivingen in HPV epidemiologie.

Hoofdstuk 3

Het natuurlijk verloop van een HPV infectie in gezonde ongescreende jonge vrouwen kan beïnvloed worden door virale, gastheer / vrouw en omgevingsfactoren. In aanvulling op hoofdstuk 2, worden in dit hoofdstuk de resultaten van HPV incidentie en klaring geanalyseerd van de 1812 vrouwen die deelnemen aan het prospectieve deel van de studie. Van deze vrouwen waren er 1729 seksueel actief tijdens de follow-up van de studie. De deelneemsters vulden een vragenlijst in en namen bij het begin van de studie een zelfsample af gevolgd door twee opeenvolgende zelfsamples met een interval van zes maanden. Tijdens de 12 maanden follow-up bedroeg de hr-HPV incidentie onder de seksueel actieve vrouwen 6.3%. Het hr-HPV type dat het meest werd opgelopen was HPV 16. Het risico om hr-HPV op te lopen werd groter wanneer iemand “single” was, wanneer er sprake was van een verandering in het type relatie of in het aantal seksuele partners in de 3 maanden voor het afnemen van het monster, alsmede een toename in het aantal jaar van seksuele activiteit bij het starten van de studie. Het klaren van hr-HPV werd geassocieerd met het hebben van een relatie alsmede met het totale aantal seksuele partners. Zowel hr-HPV incidentie als hr-HPV klaring waren gerelateerd aan zowel huidig seksueel gedrag als seksueel gedrag in het verleden. Deze resultaten suggereren dat sommige aangetoonde infecties nieuw opgelopen infecties waren terwijl andere infecties reeds in het verleden waren opgelopen en enige tijd onder de detectiegrens aanwezig zijn gebleven en nu toevallig zijn opgepikt door de test. Aangezien HPV infecties veel voorkomen, is het moeilijk om specifieke risicofactoren aan te wijzen. Onze resultaten laten zien dat het seksueel actief zijn zelf de meest belangrijke factor is.

Hoofdstuk 4

Voordat een HPV vaccin geïntroduceerd wordt, is het van belang te weten of de populatie zich bewust is van de relatie tussen HPV en het ontstaan van afwijkingen aan de

baarmoederhals. Het is ook van belang te weten of men bereid is zich te laten vaccineren dan wel of ouders bereid zouden zijn hun kinderen te laten vaccineren en welke factoren hun keuze zouden beïnvloeden. Dit hoofdstuk beschrijft de resultaten van een cross-sectioneel onderzoek dat verricht is voordat het nieuws over het HPV vaccin in oktober 2005 de voorpagina’s van de kranten haalde. Telefonisch werden 356 ouders van kinderen tussen de 10 en de 12 jaar geïnterviewd. Van de ouders zou 88% de HPV vaccinatie accepteren, met een voorkeur voor vaccinatie op de leeftijd van 10 tot 12 jaar. Ouders van kinderen die reeds alle aanbevolen vaccinaties van het nationale vaccinatie programma hadden gekregen, waren significant vaker bereid hun kinderen tegen HPV te laten vaccineren. Ouders die niet bereid waren om hun kinderen te laten vaccineren waren significant vaker van mening dat het kind betrokken moest worden bij deze keuze. Minder dan één derde van alle ouders had ooit gehoord van HPV en slechts 14% was zich bewust van de relatie met baarmoederhalskanker. Kennis van HPV en baarmoeder-halskanker, religie, leeftijd, opleidingsniveau, en burgerlijke staat waren niet significant van invloed op de vaccinatiebereidheid. Onder de geïnterviewde ouders bleek de vaccinatiebereidheid afhankelijk te zijn van vaccinatiebereidheid in het algemeen, en niet zo zeer van kennis van HPV en baarmoederhalskanker. De ouders gaven evenwel aan, dat zij graag meer informatie zouden ontvangen over baarmoederhalskanker, HPV en HPV vaccinatie voordat het vaccin geïntroduceerd zou worden in het vaccinatie programma.

Hoofdstuk 5

Om de incidentie van baarmoederhalskanker te verlagen zonder een vertraging van 15 tot 20 jaar zal catch-up vaccinatie noodzakelijk zijn. De doelgroep van catch-up vaccinatie zal voornamelijk bestaan uit vrouwen van 17 tot en met 25 jaar. In aanvulling op hoofdstuk 4, beschrijft dit hoofdstuk de kennis van HPV, vaccinatiebereidheid en factoren die hiervan op invloed zijn in een groep van 600 mannen en vrouwen van 18 tot en met 25 jaar. De deelnemers werden geïnterviewd op twee universitaire faculteiten en op één technische beroepsopleiding. De meerderheid van de deelnemers had ooit gehoord van baarmoederhalskanker, maar slechts een minderheid kon correct de risicofactoren benoemen. Vrouwelijke deelnemers hadden significant meer kennis van baarmoeder-halskanker. Zeer weinig deelnemers hadden ooit gehoord van HPV. Ondanks dit gebrek aan kennis zou een kleine meerderheid bereid zijn zich te laten vaccineren tegen HPV. Vrouwelijke en jongere deelnemers waren significant vaker bereid zich te laten vaccineren. Opleidingsniveau en kennis van HPV en baarmoederhalskanker waren niet significant geassocieerd met vaccinatiebereidheid. Deze uitkomst zou echter beïnvloed kunnen zijn door het relatief hoge opleidingsniveau alsmede de geringe kennis over

HPV. De exacte factoren die in deze groep mannen en vrouwen van invloed zijn op de vaccinatiebereidheid blijven onduidelijk. Om met de catch-up vaccinatie een hoge dekkingsgraad te bereiken, zal er een campagne nodig zijn die niet alleen voorlichting geeft over HPV en baarmoederhalskanker maar die ook in gaat op gedrag en overtuigingen met betrekking tot vaccinatiebereidheid.

Hoofdstuk 6

De effectiviteit van de HPV vaccins is aangetoond bij vrouwen die op het moment van vaccineren HPV 16 en/of 18 negatief waren. Het voordeel van vaccinatie zal groter zijn voor vrouwen die nog niet seksueel actief zijn, dan voor (oudere) vrouwen die al wel seksueel actief zijn en dus blootgesteld zijn aan HPV. De individuele beslissing van deze seksueel actieve vrouwen om zich te laten vaccineren, zal een afweging zijn tussen kosten verbonden aan vaccinatie en baat bij vaccinatie. Dit hoofdstuk beschrijft een model dat de schatting weergeeft van de kans om HPV 16 en/of 18 positief te zijn. Deze studie is gebaseerd op de uitkomsten van de prospectieve studie onder 2065 ongescreende vrouwen van 18 tot 29 jaar zoals beschreven in hoofdstuk 2 en 3. Uiteindelijk zijn de resultaten gebruikt van 1322 seksueel actieve vrouwen die tussen de 18 en de 25 jaar oud waren. De deelneemsters vulden een vragenlijst in en namen een zelfsample af. De monsters werden op HPV getest. Door middel van "multivariate logistic regression analysis with backward variable deletion" werd de waarschijnlijkheid van het aanwezig zijn van een HPV infectie geschat. Het model dat de optimale schatting van de aanwezigheid van HPV 16 en/of 18 weergeeft, werd gebaseerd op de combinatie van leeftijd, aantal en geslacht van seksuele partners, condoomgebruik en coïtusfrequentie in de afgelopen 6 maanden. Een nomogram gebaseerd op bovengenoemde factoren berekent de kans op de aanwezigheid van HPV 16 en/of 18. Dit model kan van pas komen in het begeleiden van individuele vrouwen bij hun keuze om zich wel of niet te laten vaccineren. Het model geeft een schatting van de waarschijnlijkheid om geïnfecteerd te zijn met HPV 16 en/of 18 en geeft op deze manier inzicht in de persoonlijke situatie. Verder biedt het de mogelijkheid om een verschil te kunnen maken tussen seksueel actieve vrouwen die direct gevaccineerd kunnen worden en seksueel actieve vrouwen die baat zouden hebben bij testen op HPV voorafgaand aan vaccinatie.

Hoofdstuk 7

Het testen op hr-HPV in het bevolkingsonderzoek is van voordeel in de patiënt-beleidsvoering en kan zo het succespercentage van het bevolkingsonderzoek vergroten. Ten aanzien van het testen op HPV is bewezen dat cervico-vaginale zelfsamples net zo betrouwbaar zijn als cervicale monsters die zijn afgenomen door een arts. De introductie van cervico-vaginale zelfsampling zou de opkomst van het bevolkingsonderzoek kunnen verhogen en op deze manier kunnen bijdragen aan het voorkómen van baarmoederhalskanker. In hoofdstuk 7 wordt de betrouwbaarheid in kaart gebracht van HPV detectie en genotypering in cervico-vaginale zelfsamples die aangebracht zijn op een "indicating FTA elute cartridge" (FTA cartridge). Alle monsters werden op HPV getest met de SPF_{10} LiPA HPV genotyperingstest. De onderzoeksgroep bestond uit 96 vrouwen die konden worden opgedeeld in twee groepen. In groep 1 namen vrouwen een zelfsample die zij op een FTA cartridge aanbrachten. Verder werd er door een arts een cervicale uitstrijk (opgeslagen in een vloeistof medium) afgenomen. In groep 2 namen vrouwen een zelfsample die zij op een FTA cartridge aanbrachten alsmede in een vloeistof medium uitschudden. Het DNA werd van de FTA cartridge gehaald door middel van eenvoudige elutie alsmede extractie. In groep 1 was de overeenkomst tussen het zelf- en arts-verkregen monster 93.3%, in het voordeel van de zelfsamples. In groep 2 was de overeenkomst tussen de FTA cartridge en het vloeistof medium ten aanzien van de aanwezigheid van hr-HPV 100%. In beide groepen was de overeenkomst tussen DNA elutie en DNA extractie ten aanzien van de aanwezigheid van hr-HPV 100%. Deze resultaten laten zien dat HPV detectie en genotypering van cervico-vaginale zelfsamples die aangebracht zijn op een FTA cartridge zeer betrouwbaar is. Het toont een hoge overeenkomst met HPV detectie en genotypering in arts-verkregen monsters en in vloeistof medium opgeslagen zelfsamples. Het DNA kan verkregen worden door middel van eenvoudige elutie, wat makkelijk, goedkoop en snel is. De FTA cartridge is een praktisch medium om materiaal op te verzamelen en te versturen omdat het geen gevaarlijke stoffen bevat en het aangebrachte materiaal niet meer infectieus is en stabiel blijft bij wisselende temperaturen. Daarom zou deze methode onderdeel uit kunnen maken van een nieuwe manier van baarmoederhalskankerscreening.

Hoofdstuk 8

In dit deel worden de resultaten besproken en in perspectief geplaatst van de huidige ontwikkelingen. Verder wordt er gediscussieerd over de mogelijke effecten van HPV vaccinatie op HPV epidemiologie en de huidige screeningsprogramma's.

Dankwoord

Bij deze wil ik iedereen bedanken die heeft bijgedragen aan het tot stand komen van dit proefschrift.

Beste Leon, dank voor de begeleiding van de afgelopen tijd. Je wist altijd net even dat beetje extra te motiveren en nieuwe ideeën te lanceren. Dank voor de leerzame jaren.

Beste Willem, als ik dr. Melchers bleef zeggen, moest ik in Praag aan een tafeltje apart zitten, gelukkig was dat niet nodig. Dank voor het razendsnel stukken van commentaar voorzien en al je ideeën, maar vooral ook de gezellige congressen, je verhalen en je interesse.

Beste Ruud, naast copromotor zijn, was je ook erg betrokken. Vele besprekingen over uiteraard de studie, maar met name over "het leven" maakten het promoveren een mooie tijd! Samen namen we uiteindelijk de "midden-bocht", dank voor je persoonlijke begeleiding!

Beste Petra, als ik jou toch niet had gehad…dan was ik hier niet eens aan begonnen! Dank!!

Mede auteurs: Jan Hendriks, Nel Roeleveld en Marloes Gerrits, hartelijk dank voor jullie bijdragen.

MMB-lab, beste Judith, Annelies en Onno, dank voor jullie eindeloze geduld en tomeloze inzet bij de duizenden monsters die altijd gisteren klaar moesten zijn.

Beste Wim Quint, dank voor het bepalen van alle monsters die o.a. in zakjes werden aangeleverd, ik heb ervan geleerd! Ook dank voor het gezellige congres in Praag, ik heb de kunst toen goed af kunnen kijken.

Beste Wim Abma, "access", je legde het me uit, we dachten na over de database/queries/etc, en het werkte! 10.000 monsters en vragenlijsten gingen (bijna) automatisch de deur uit, daar kan geen agenda tegen op!!

Beste student-stagiaires, Annette, Fanny, Marieke, Daphne-Sophie, Dion, Neeltje, Marloes en Moniek, dank voor al jullie enthousiasme en jullie inzet. Annette, zonder jouw inzet van het hele eerste jaar was het nooit zo gestroomlijnd gelopen, dank hiervoor!

Beste Nel, even brainstormen met jou en de monsters van de poli stroomden binnen. Dank voor je hulp en de gezellige borrelmomenten met o.a.Tommy.

Kantoortuin: teveel om op te noemen… dank voor de leuke onderzoekersweekenden, de etentjes en alle gezellige koffie momenten met "publicatie-taart".

Dennis, mijn helpdesk, één wanhopige blik vanachter mijn compu-scherm vandaan was genoeg… Dat ik later deze taak over kon nemen, blijft een wonder!

Irene, als overbuurvrouw erg gezellig en jij weet gewoon altijd alles, als nieuwkomer in de tuin en in Nijmegen was dat erg praktisch ;-)

Angèle, BP zat toch op t Valkhof? Jij wist me op zaterdag naar de Houtstraat te leiden, met succes! Dank voor de fijne start die je me bood in de tuin.

Channa, inclusiekanon van het eerste uur. Het was best moeilijk de studie uit handen te geven, maar de follow-up van de studie is je toevertrouwd. Dank voor de gezellige congressen in Nice en Malmö en uiteraard alle "overleg" momenten.

Eva en Anne, de nieuwe overburen, een knipoog en een terrasje op zijn tijd maakte de afgelopen jaren erg geslaagd!

Anneke, door jou leerde ik mijn nieuwe Nijmeegse collegae snel en goed kennen. Dank voor je gezelligheid!

Lieve AIOS collegae, dank voor jullie gezelligheid, de hulp bij de eerste stapjes in Nijmegen, onderzoeksland en die in de kliniek. Met jullie is het altijd gezellig.

Lieve Ralph, Marieke en Jolieneke, de eerste opvang in het Nijmeegse verliep soepel, waarvoor veel dank!

Lieve ladies van de Jc en uit Amersfoort, ondanks dat jullie in het begin geen idee hadden van wat ik nou eigenlijk deed de hele dag, waren jullie altijd erg geïnteresseerd en behulpzaam! Dank voor jullie gezelligheid en sorry voor het tijdelijk afhaken, we halen het zeker in ☺!!

Lieve zussen & co, dank voor jullie plezier en gezelligheid van alle jaren, voor jullie interesse in het onderzoek en het feit dat ik op de meest rare momenten op mocht bellen om een of ander woord te checken… Lieve Lies en Peter, dank voor jullie hulp bij het reviseren!

Lieve papa en mama, wat zijn jullie toch altijd trots op ons en wat is dat fijn! Jullie hebben altijd met alles achter me gestaan en me gestimuleerd door te gaan, ook toen ik 't Stichtse heb verlaten. Julie zijn echt geweldig en ik ben dankbaar dat ik zulke lieve ouders heb.

Lieve Stefke, al jaren supervriendinnen. Jij naar Venlo en ik naar Nijmegen, dat kwam mooi uit! Dank voor je "zen-momenten" in de Limburgse lucht en je nuchtere commentaar op zaken... Geweldig dat jij mijn paranimf bent!

Lieve Wiet, wat hebben we een lol samen, gelukkig vinden "wij" het zelf altijd erg geslaagd! Dat jij mijn paranimf zou zijn, was vanzelfprekend maar is toch ook heel bijzonder.

Bibliography

Termote JU, Donders AR, Schalij-Delfos NE, **Lenselink CH**, Derkzen van Angeren CS, Lissone SC, Cats BP. Can screening for retinopathy of prematurity be reduced? Biol Neonate. 2005;88(2):92-7.

Lenselink CH, Massuger LFAG, Melchers WGJ, Hendriks JCM, van Hamont D, Bekkers RLM. Vaccinatie tegen het Humaan Papillomavirus; hoe staat de Nederlandse bevolking er tegenover, NTOG. 2007;120:16-24.

Lenselink CH, Gerrits MM, Melchers WJ, Massuger LF, van Hamont D, Bekkers RL. Parental acceptance of Human Papillomavirus vaccines. Eur J Obstet Gynecol Reprod Biol. 2008 Mar;137(1):103-7.

Lenselink CH, Schmeink CE, Melchers WJ, Massuger LF, Hendriks JC, van Hamont D, Bekkers RL. Young adults and acceptance of the human papillomavirus vaccine. Public Health. 2008 Dec;122(12):1295-301.

Schmeink CE, **Lenselink CH**, Bekkers RLM. Pilgebruik en een verhoogde kans op cervixcarcinoom. Ned Tijdschr Geneeskd. 2008 Aug 2;152(31):1717-8.

Lenselink CH, Massuger LFAG, Melchers WGJ, Hendriks JCM, van Hamont D, Bekkers RLM. Vaccinatie tegen het Humaan Papillomavirus; hoe staat de Nederlandse bevolking er tegenover. NTOG, huisartseneditie, september 2008:13-18.

Lenselink CH, Melchers WGJ, Quint WGV, Hoebers AMJ, Hendriks JCM, Massuger LFAG, Bekkers RLM. Sexual behaviour and HPV infections in 18 to 29 year old women in the pre-vaccine era in the Netherlands. PLoS ONE. 2008;3(11):e3743.

Lenselink CH, de Bie RP, van Hamont D, Bakkers JM, Quint WG, Massuger LF, et al. Detection and genotyping of human papillomavirus in self-obtained cervicovaginal samples by using the FTA cartridge: new possibilities for cervical cancer screening. J Clin Microbiol 2009 Aug;47(8):2564-70.

Lenselink CH, Schmeink CE, Hendriks JC, Melchers WJG, Quint WGV, Massuger LFAG, Bekkers RLM. Individual risk-factor assessment of sexually active women prior to HPV vaccination; how effective is your HPV vaccination? Submitted

Lenselink CH, Schmeink CE, Quint WGV, Roeleveld N, Melchers WJG, Massuger LFAG, Bekkers RLM. Factors influencing HPV incidence and clearance in young women in the pre-vaccine era; a prospective study. In preparation

Lenselink CH, Massuger LFAG, Bekkers RLM. HPV-vaccinatiebereidheid, de rol van ouders en voorlichting. Tijdschrift voor Gezondheidswetenschappen. In press

Schmeink CE, **Lenselink CH**, Quint WGV, Massuger LFAG, Melchers WJG, Bekkers RLM. Effect of the menstrual cycle and hormonal contraceptives on HPV detection in young unscreened women. Submitted

Curriculum Vitae

De levensloop van Charlotte Lenselink tussen 1978 en 2009

Als tienjarig meisje in 1988, wist Charlotte al wat ze wilde worden: arts. Geen speld tussen te krijgen. Ze kwam op het idee nadat ze een tv-programma had gezien over Artsen zonder Grenzen. Dit werk leek haar fantastisch. Dat ze het echt meende, is wel gebleken.

Het stedelijk gymnasium in Amersfoort doorliep de jongste van het gezin Lenselink glansrijk, terwijl ze ondertussen aan steile wand klimmen deed, roeide, paardreed en veelvuldig de stad inging met vriendinnen. In één keer werd ze in 1996 ingeloot voor Geneeskunde in Utrecht. Voortvarend als altijd, stortte ze zich in het studenten- en verenigingsleven. Maar ook de studie werd serieus aangepakt, wat resulteerde in een propedeuse een jaar later en een doctoraal in 2001.

Welke richting ze vervolgens op zou gaan wist ze toen nog niet. Alhoewel haar wetenschappelijke stage in 2001 met betrekking tot retinopathie van de prematuur in het Wilhelmina Ziekenhuis in Utrecht, zeer goed bevallen was. Toen ze in 2003 in Tilburg het keuze co-schap Gynaecologie & Obstetrie volgde, viel de zaak op zijn plaats. Dit was het vakgebied waarin deze inmiddels arts, verder wilde.

Na een korte stop in Tilburg ging ze in 2004 in het Rijnstate Ziekenhuis te Arnhem als ANIOS aan de slag. Dit werk maakte Charlotte gelukkig. Om serieus door te kunnen in de gynaecologie besloot zij onderzoek te gaan doen. Het UMC St Radboud in Nijmegen bood hier de mogelijkheid voor. Professor Massuger, dr. Bekkers en dr. Melchers begeleidden haar tussen 2006 en 2009, wat resulteerde in dit proefschrift.

Sinds oktober 2009 is Charlotte werkzaam als AIOS gynaecologie in het Catharina Ziekenhuis te Eindhoven. Ondertussen geniet ze van het leven in Nijmegen, haar vrienden en familie en haar weg naar de volgende mijlpaal.