Dynamics of genital Human papillomavirus in young women

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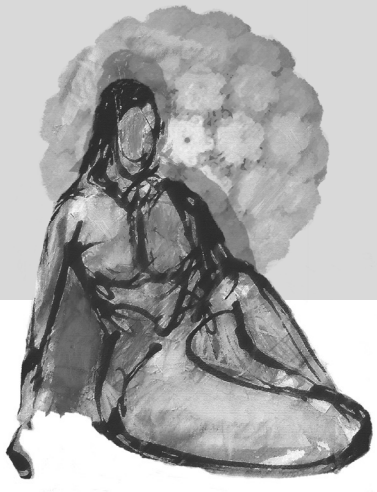


Chapter 1

General introduction and outline of this thesis

Introduction

Cervical cancer is the second most common female cancer in the world, with approximately 490 000 new cases of cancer of the cervix diagnosed yearly, and over 230 000 deaths, of whom 80% occur in developing countries.[1] Since Zur Hausen initiated the concept of viral oncogenesis in the development of cervical cancer, many studies have confirmed that a persistent genital human papillomavirus (HPV) infection is a necessary factor in cervical carcinogenesis.[2-6]

The human papillomavirus

The HPV genome consists of about 8000 basepairs, double stranded, circular DNA. HPV is part of the Papillomaviridae family. Over 120 different HPV genotypes have been identified and more than 40 of them can infect the epithelial and mucosal lining of the anogenital tract.[7;8] According to their carcinogenicity, these anogenital HPV types have been subdivided into low-risk and high-risk HPV genotypes. In condylomata and a subset of cervical intraepithelial neoplasia grade 1 and 2 (CIN1 and CIN2) low-risk types may be present. High-risk types are found in almost all CIN3 lesions and cervical cancers.[9] On the basis of epidemiological criteria HPV types 6, 11, 40, 42, 43, 44, 54, 61, 70, 72 and CP6108 are classified as low-risk. HPV types 6 and 11 are the most frequently detected HPV types in genital warts. There are 15 mucosal HPV types classified as high-risk. These types are HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82. In addition three other HPV types (i.e. HPV 26, 53, and 66) are considered as probably carcinogenic. The high-risk genital HPV types belong to the genus alpha-papillomaviruses.
Phylogenetically related oncogenic types HPV 18, 39, 45, 59 and 68 belong to species 7 and HPV types 16, 31, 33, 35, 52 and 58 to species 9.[9-11] Infections with HPV 16 and 18 account for about 70% of all squamous cervical cancers.[11]

Papillomaviruses are perfectly adapted to their natural host tissue, the differentiating epithelial cells of skin or mucosa. The life cycle of the virus is initiated when infectious particles reach the basal layer of the epithelium, where they bind and enter into the cells. The completion of their life cycle depends on epithelial differentiation. The HPV genome can be subdivided in three regions: a non-coding long control region (LCR), an early encoding region (E), containing 6 early genes and a late encoding region (L), containing 2 late genes (Figure 1). These eight genes are responsible for the translation of a series of functional proteins necessary for the viral life cycle. In the normal viral life cycle the viral proteins E1 and E2 are essential for the basal DNA replication and transcription. Other critical proteins in the process of virus replication are the viral proteins E6 and E7. These proteins enable the virus to replicate by activating DNA synthesis and blocking important apoptotic routes in differentiated non-dividing host cells.[7;10;12-14]

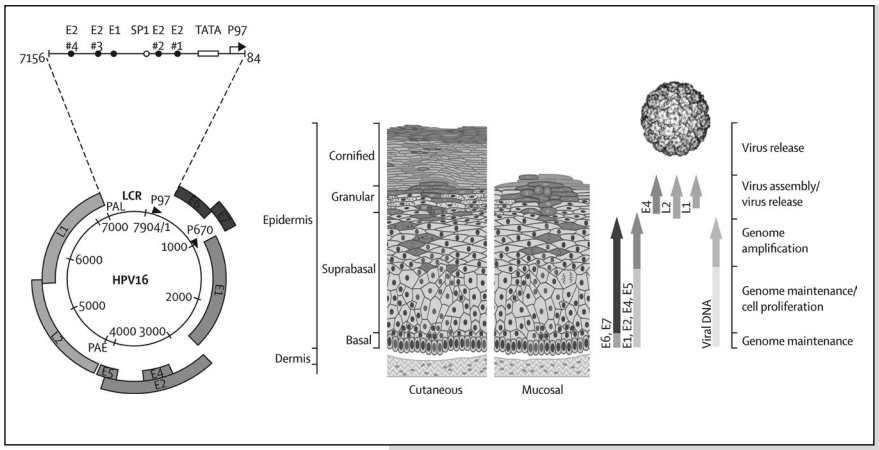


Figure 1: The HPV genome and its expression within the epithelium (adapted from Schiffman et all.[15])

Human papillomavirus detection

Estimates of single point prevalence of genital HPV infection among women worldwide vary from 2% up to 44%.[3;16] In women with normal cervical scrapes, the point prevalence of any-type HPV is influenced by the specific population studied, as they differ in geography and risk factors for development of cervical carcinoma. The prevalence of HPV also differs between age groups, varying from 20% in women aged between 20-25 years, to 6% in women ≥30 years, and some studies show a second peak in HPV prevalence in women above the age of 55 years.[4;17;18] Furthermore the sensitivity of the DNA assay used for the detection of HPV influences the results of these studies.

Several methods for the detection and typing of HPV DNA in cervico-vaginal smears have been developed. The most widely used HPV DNA tests are Hybrid Capture 2 (HC2, Digene Corp., USA) and polymerase chain reaction (PCR) methods: PGMY09/11, GP5+/6+, SPF_{10} consensus primers, and Roche Linear Array HPV genotyping assay.[19-27]

HC2 is a signal amplification method based on hybridization of the target HPV DNA to labeled RNA probes in solution.[28;29] HC2 uses two different probe cocktails one with five low-risk genotypes: 6, 11, 42, 43 and 44, and the other containing 13 high-risk genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. With these probes HC2 distinguishes between the high-risk and low-risk groups, but it does not permit identification of specific HPV genotypes.[25]

The consensus or general PCR primers are based on PCR amplification of the target sequences in the HPV L1 gene, as the L1 region is the most conserved part of the viral genome.[10;21] Reverse hybridization permits detection of type specific infections and detection of multiple HPV types from the PCR product in a single step. The most frequently used reverse hybridization technologies are, line probe assay (LiPA), line blot assay (LBA) or linear array (LA).[25]

The threshold of the viral load that is still detectable determines the sensitivity of the HPV test used. HPV tests using PCR techniques have been generally proven to be more sensitive in detecting high-risk HPV genotypes than hybridization tests like the HC2.[27] Although the GP5+/6+ PCR test has a clinical sensitivity like the HC2. Additionally, the Greiner Bio-One PapilloCheck assay has proven to be clinically compatible to the GP5+/6+ PCR assay.[30] The relative sensitivity of the HC2 assay is equivalent to 1 picogram of HPV DNA, whereas the sensitivity of many PCR assays is at the sub-picogram level.[31] The PCR test SPF_{10}LiPA appeared to have the highest sensitivity to detect HPV DNA.[22;31;32] However, detection of very low viral copy numbers in cervical scrapings may overestimate the risk of high-grade cervical disease.[31;33] Thus, when HPV testing is used to identify women at risk for developing cervical cancer, a highly sensitive HPV detection assay may overestimate the proportion of women who have cervical abnormalities.[25;31;34] On the other hand, accurately studying both the epidemiology of HPV and the global impact of HPV infections, requires a test with the highest analytical sensitivity possible.[31] The choice of an HPV DNA test will therefore depend on its application, whether a high analytical or a high clinical sensitivity is required.

Incidence of the human papillomavirus

Acquisition of genital HPV is very common, particularly among sexually active young adults, and several studies have clearly shown that HPV is predominantly transmitted through sexual intercourse.[35-38] About 50% of the women becoming sexually active contract a genital HPV infection within 2 years, and the lifetime risk of a genital HPV infection is estimated to be 80%.[39] Because HPV is basically sexually transmitted, risk factors associated with acquiring HPV infections are clearly related with the individuals sexual behavior.[40] Most importantly: early age at start of sexual relationships, high number of sexual partners throughout life, and having a recent new sexual partner. The use of condoms is reported to reduce, although not completely prevent, the risk of transmission of HPV between sexual partners.[36;41-47]

Clearance and persistence of the human papillomavirus

In a large prospective study, only 1.73% of the women with a positive HPV test at baseline developed a CIN3 lesion or cervical cancer within 45 months, and 6.92% within 122 months.[48] Therefore it is important to realize that, although a high-risk HPV infection represents the first step of cervical cancer carcinogenesis, most of these infections are transient, while a persistent HPV infection is necessary for the progression to cervical cancer.[6;14] Most infections become undetectable within 1-2 years.[35;49-51] Infections lasting more than 1 year appear to be associated with a lower clearance rate, and therefore pose a greater risk to the infected women.[35;51] Whether infections clear completely, or whether the virus remains latent in the basal cells at undetectable levels, remains unclear.[16] Figure 2 displays the average clearance, persistence, and progression of a high-risk HPV infection in time.

Although there is no consensus on its definition, most investigators define a persistent HPV infection as detection of the same HPV type or group of types on two consecutive visits, but these visits could be from 2 months up to 72 months apart.[52;53] Therefore it is difficult to point out when an HPV infection is truly persistent or may still be transient. Furthermore, within this definition it is also impossible to distinguish a persistent infection from one that represents a cleared infection with subsequent reacquisition of the same HPV type before the next screening round.[16] It would be useful to come to one definition of persistence. This new definition should be based on the duration of infection rather than a positive HPV test on two consecutive screening moments. This definition of persistence should preferably be based on the average duration of an HPV infection predicting oncogenicity, rather than the average duration to clear an infection. Furthermore, consensus should be reached whether this definition should be based on persistence of high-risk HPV as a group or genotype-specific. Finally, this new definition on HPV persistency should also address whether or not there may be a single negative test in between two positive tests, in other words, are two negative test necessary to declare an infection cleared? When there is one definition of persistence, results from epidemiologic studies will be more uniform and stronger conclusions may be drawn in meta analysis on co-factors influencing HPV persistence and cervical carcinogenesis.

Co-factors in the development of cervical intraepithelial neoplasia and cancer

It is not clearly understood why HPV infections resolve in certain individuals and result in CIN lesions in others, but several factors are thought to play a role.[10] Three groups of potential co-factors are; (1) *environmental or exogenous co-factors*; long-term use of hormonal contraceptives, tobacco smoking, parity, condom use, number of sex partners

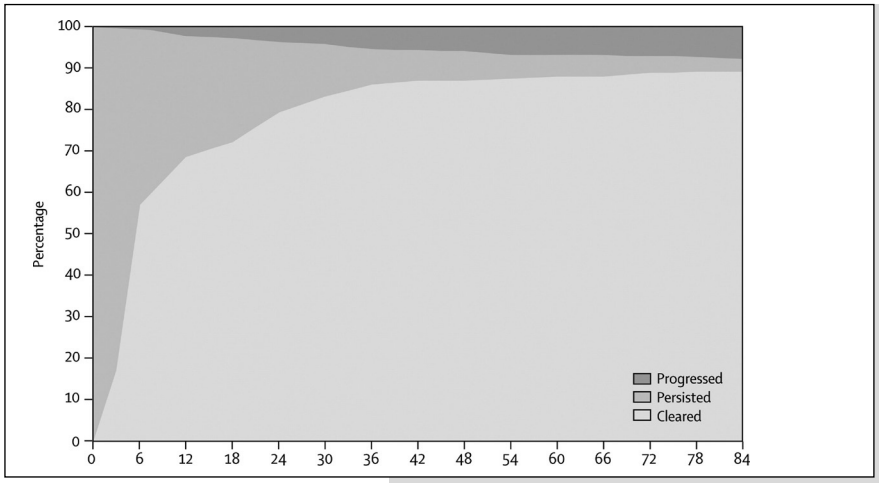


Figure 2: Average clearance, persistence, and progression of carcinogenic HPV infections (adapted from Schiffman et al.[15])

and co-infection with (other than HPV) sexually transmitted agents; [35;54;55] (2) *viral co-factors*; type specific infections, co-infection with another HPV type(s), viral load, and viral integration; [35;56;57] and (3) *host co-factors*; endogenous hormones (fluctuations during menstrual cycle), genetic factors, human histocompatibility leukocyte antigen (HLA) expression and other factors related to immune response.[10;58]
The International Agency for Research on Cancer (IARC) multicentre study found three main factors to have a causal relationship with development of cervical cancer; (1) oral contraceptive pill (OCP), (2) parity, and (3) smoking. [59-61] Among current users of OCPs the risk of cervical cancer increased with increasing duration of use, with a relative risk for 5 years or more versus never use of 1.90 (95% CI: 1.69-2.13). This risk declined after use ceased, and by 10 or more years had returned to that of never users.[62] HPV positive women who reported seven or more full term pregnancies had a fourfold increased risk of cervical cancer compared with similar HPV positive nulliparous women.[60] The effect of parity on cervical cancer risk was less visible in low parity populations.[11] After controlling for other co-factors, the IARC multicentre study found that ever smoking gave a twofold increased risk for cervical carcinoma, with a higher risk for squamous cell carcinoma compared to adenocarcinoma.[61] Generally, these risk factors are only found in studies restricted to HPV DNA positive women and study results about these risk factors and their influence on HPV persistency remain inconsistent.[11]
Despite the increased risk of CIN and cervical cancer ascribed to these co-factors among women with high-risk HPV, the risk of cervical cancer is still mainly a result of a persistent high-risk HPV infection and lack of effective screening.[15]

Cervical cancer prevention
Cervical cancer is a preventable disease. The possibility to detect abnormal cells in cytology and the ability to treat pre-malignancies, prevents progression of lesions towards invasive cervical cancer (especially squamous cell carcinoma).[63;64] The Dutch population based cervical screening aims at women aged 30-60 years, with an interval of 5 years between screening rounds.[65] The sensitivity of conventional cytology for detecting high-grade CIN (CIN2 and 3) is estimated to be about 60%.[66;67] Approximately 70% of the invited women actually participate in the Dutch screening program, this all leads to a mortality reduction of maximal 56%.[68] (Figure 3)

The close association between high-risk HPV and cervical cancer has resulted in the idea to use high-risk HPV testing on cervical scrapes for the detection of ≥CIN2 lesions and cervical cancer. Several studies have clearly shown that application of high-risk HPV testing in cervical screening improves the sensitivity and negative predictive value for ≥CIN2 lesions, compared to cytology alone. This leads to a markedly earlier detection of these lesions.[6;70-73] However, the positive predictive value for ≥CIN2 lesions of even a clinically validated test (i.e. HC2 and GP5+/6+ PCR) is lower than that of cytology because still a substantial number of test positive women do not have high-grade CIN lesions.[71;74;75]

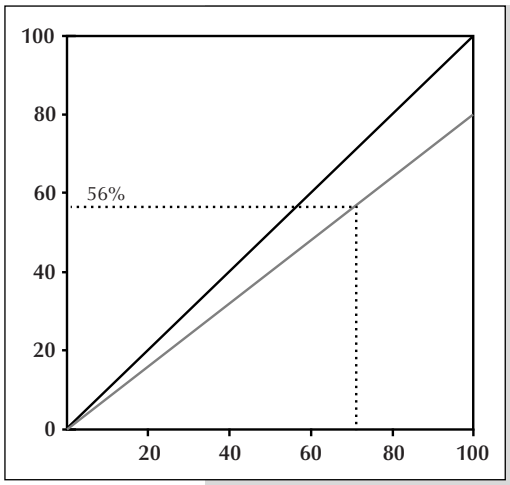


Figure 3: Reduction in cervical cancer mortality in relation to the sensitivity of the screening and the coverage of the target population in the Netherlands. Model based on M. van Ballegooijen.[69] Black line: ideal screening method (100% sensitivity of the combination: screening tool, screening interval, and the cohort to be screened); grey line: ideal line in the Netherlands with 5-yearly screening of women between 30 and 60 years of age; dotted line, actual situation with a 70% coverage rate. (Adapted from Bekkers et al.[68])

In the Dutch national cervical cancer screening program, HPV testing is currently used as an additional test to repeat cytology after a primary borderline or mildly abnormal cytology test. However, in literature it is suggested to implement HPV DNA testing as the sole primary screening test. This is based on the principle that the more sensitive test should be applied first (i.e. HPV DNA testing) and the more specific test (i.e. cytology) should then be used only for HPV-positive women to determine management (i.e. follow-up or treatment).[74] Previous studies show that HPV-positive women with negative cytology can be safely managed by repeating the testing with both cytology and HPV after one year.[72;73;76;77]

Together with preventing the development of precancerous lesions via screening, vaccination has become today's main prevention front against cervical cancer.[67] The two vaccines Gardasil® and Cervarix®, containing immunogens for the HPV genotypes 16 and 18 that cause 70% of all cervical cancers, are highly effective to prevent pre-cancerous disease. Additionally, there is some cross-protection for HPV type 45, 31, 33, 35, 52, 58 infections.[78] Longitudinal studies on the natural behavior of HPV type-specific infections before the implementation of the HPV vaccine provide valuable baseline information for future epidemiologic studies analyzing the impact of HPV vaccination on the dynamics of HPV.

Self-sampling for HPV detection

The introduction of HPV detection in primary screening creates the possibility of the use of self-sampling for HPV detection. However, more studies within general screening populations are necessary before implementation of self-sampling in national cervical cancer screening program is possible. Self-sampling is generally easy to use and is an

inexpensive method, that is highly accepted by women.[79;80] Studies already showed that self-sampling increases participation of non responders in current screening programs.[80-85] In 2005 and 2007, two meta-analyses showed a high level of concordance for HPV DNA detection between self-collected samples and physician-collected samples.[86;87] Because it is easy to use, highly accepted by women, and has a good performance on HPV DNA collection, self-sampling seems to be the ideal method to collect HPV DNA in a prospective epidemiologic study on the dynamics of HPV.

Outline of this thesis

The present thesis is based on the longitudinal data of a large prospective epidemiologic study in young women in the pre-vaccine era. The main objective is to focus on the incidence, clearance and persistence of HPV, and associated risk factors like age, sexual behavior, oral contraceptives and pregnancy. This prospective epidemiologic study on the natural behavior of the human papillomavirus (HPV) was conducted in 2007-2009. The first cross-sectional data on HPV prevalence were described prior to this thesis.[40]
In the first year of follow-up all women were asked to quarterly (i.e. month 0, 3, 6, 9, and month 12) fill in a questionnaire and to self-collect a cervico-vaginal sample for HPV DNA detection in the privacy of their own home. All high-risk HPV positive women at month 12 were asked to participate in a second year of follow-up and to provide two cervico-vaginal self-samples accompanied with a questionnaire at month 18 and 24.
Women who became pregnant during the first year of follow-up were asked to complete follow-up until after delivery. This way we obtained one self-sample at baseline (0-3 months before pregnancy), one in each trimester, and one within the first 6 months after delivery.

First, we reviewed the literature if self-sampling may be widely implemented in cervical cancer screening programs as a tool for HPV detection, and which combination of sample device and HPV test are most suitable. (chapter 2)
In chapter 3 the HPV incidence rates in young females, and whether the relation with the sexual behavior is age-dependent was studied. This will provide baseline information for future studies on the effect of the recently introduced HPV vaccines, targeting high-risk HPV types HPV 16 and 18. Furthermore, the clearance rate of type-specific infections and whether re-infection with the same type takes place during 12 months of follow-up, (i.e. the natural type-specific short-term fluctuations) was studied. Additionally, the potential factors influencing type-specific HPV clearance like age, smoking and sexual behavior were evaluated. In chapter 4, the data of the second year of follow-up are used to determine which factors influence type-specific high-risk HPV persistence, and to relate the results of type-specific HPV detection with available cytology results.
In chapter 5-7, factors potentially influencing the dynamics of HPV, are analyzed and discussed in detail. The potential influence from OCP use and sample timing within the menstrual cycle on HPV detection are described in chapter 5. Chapter 6 covers the poten-

tial influence of pregnancy on HPV detection by evaluating the natural HPV prevalence, incidence and clearance during pregnancy, compared with a matched-control group of non-pregnant women in a low parity population. Finally, the potential use of the FTA elute cartridge to detect premalignancies of cervical cancer is tested with the clinically validated Hybrid Capture (HC2) and GP5+/6+-HPV DNA detection assays. (chapter 7)
This thesis concludes with a general discussion in chapter 8, and a summary in chapter 9.

Reference List

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J.Clin. 2005;55:74-108.
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;87:796-802.
4. Munoz N. Human papillomavirus and cancer: the epidemiological evidence. J.Clin.Virol. 2000;19:1-5.
5. 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;189:12-19.
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;354:20-25.
7. Munger K, Baldwin A, Edwards KM, Hayakawa H, Nguyen CL, Owens M et al. Mechanisms of human papillomavirus-induced oncogenesis. J.Virol. 2004;78:11451-60.
8. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur HH. Classification of papillomaviruses. Virology 2004;324:17-27.
9. 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;348:518-27.
10. Munoz N, Castellsague X, de Gonzalez AB, Gissmann L. Chapter 1: HPV in the etiology of human cancer. Vaccine 2006;24 Suppl 3:S3-1-S310.
11. Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. J.Clin. Pathol. 2002;55:244-65.
12. Doorbar J. The papillomavirus life cycle. J.Clin.Virol. 2005;32 Suppl 1:S7-15.
13. Scheffner M, Romanczuk H, Munger K, Huibregtse JM, Mietz JA, Howley PM. Functions of human papillomavirus proteins. Curr.Top.Microbiol.Immunol. 1994;186:83-99.
14. Snijders PJ, Steenbergen RD, Heideman DA, Meijer CJ. HPV-mediated cervical carcinogenesis: concepts and clinical implications. J.Pathol. 2006;208:152-64.
15. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet 2007;370:890-907.
16. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine 2006;24 Suppl 1:S1-15.
17. 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;81:554-58.
18. Melkert PW, Hopman E, van den Brule AJ, Risse EK, van Diest PJ, Bleker OP et al. Prevalence of HPV in cytomorphologically normal cervical smears, as determined by the polymerase chain reaction, is age-dependent. Int.J.Cancer 1993;53:919-23.
19. Kornegay JR, Roger M, Davies PO, Shepard AP, Guerrero NA, Lloveras B et al.

International proficiency study of a consensus L1 PCR assay for the detection and typing of human papillomavirus DNA: evaluation of accuracy and intralaboratory and interlaboratory agreement. J.Clin.Microbiol. 2003;41:1080-86.

20. Kleter B, van Doorn LJ, Schrauwen L, Molijn A, Sastrowijoto S, ter SJ 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;37:2508-17.

21. Venturoli S, Cricca M, Bonvicini F, Giosa F, Pulvirenti FR, Galli C et al. Human papillomavirus DNA testing by PCR-ELISA and hybrid capture II from a single cytological specimen: concordance and correlation with cytological results. J.Clin.Virol. 2002;25:177-85.

22. Hesselink AT, van Ham MA, Heideman DA, Groothuismink ZM, Rozendaal L, Berkhof J et al. Comparison of GP5+/6+-PCR and SPF10-line blot assays for detection of high-risk human papillomavirus in samples from women with normal cytology results who develop grade 3 cervical intraepithelial neoplasia. J.Clin.Microbiol. 2008;46:3215-21.

23. Kulmala SM, Syrjanen S, Shabalova I, Petrovichev N, Kozachenko V, Podistov J et al. Human papillomavirus testing with the hybrid capture 2 assay and PCR as screening tools. J.Clin.Microbiol. 2004;42:2470-75.

24. Brink AA, Snijders PJ, Meijer CJ. HPV detection methods. Dis.Markers 2007;23:273-81.

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

26. Sabol I, Salakova M, Smahelova J, Pawlita M, Schmitt M, Gasperov NM et al. Evaluation of different techniques for identification of human papillomavirus types of low prevalence. J.Clin.Microbiol. 2008;46:1606-13.

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;155:1473-78.

28. Bozzetti M, Nonnenmacher B, Mielzinska I, I, Villa L, Lorincz A, Breitenbach V, V et al. Comparison between hybrid capture II and polymerase chain reaction results among women at low risk for cervical cancer. Ann. Epidemiol. 2000;10:466.

29. Peyton CL, Schiffman M, Lorincz AT, Hunt WC, Mielzynska I, Bratti C et al. Comparison of PCR- and hybrid capture-based human papillomavirus detection systems using multiple cervical specimen collection strategies. J.Clin.Microbiol. 1998;36:3248-54.

30. Hesselink AT, Heideman DA, Berkhof J, Topal F, Pol RP, Meijer CJ et al. Comparison of the clinical performance of PapilloCheck human papillomavirus detection with that of the GP5+/6+-PCR-enzyme immunoassay in population-based cervical screening. J.Clin. Microbiol. 2010;48:797-801.

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

32. 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;43:2662-67.

33. Snijders PJ, Heideman DA, Meijer CJ. Methods for HPV detection in exfoliated cell and tissue specimens. APMIS 2010;118:520-28.

34. Pagliusi SR, Dillner J, Pawlita M, Quint WG, Wheeler CM, Ferguson M. Chapter 23: International Standard reagents for harmonization of HPV serology and DNA assays--an update. Vaccine 2006;24 Suppl 3:S3-193-S3/200.

35. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N.Engl.J.Med. 1998;338:423-28.

36. Kjaer SK, Chackerian B, van den Brule AJ, Svare EI, Paull G, Walbomers 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;10:101-06.

37. Bosch FX, Castellsague X, Munoz N, de SS, Ghaffari AM, Gonzalez LC et al. Male sexual behavior and human papillomavirus DNA: key risk factors for cervical cancer in Spain. J.Natl.Cancer Inst. 1996;88:1060-67.

38. Burchell AN, Tellier PP, Hanley J, Coutlee F, Franco EL. Human papillomavirus infections among couples in new sexual relationships. Epidemiology 2010;21:31-37.

39. 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;14:95-105.

40. 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:e3743.

41. Hogewoning CJ, Bleeker MC, van den Brule AJ, Voorhorst FJ, Snijders PJ, Berkhof J et al. Condom use promotes regression of cervical intraepithelial neoplasia and clearance of human papillomavirus: a randomized clinical trial. Int.J.Cancer 2003;107:811-16.

42. 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;157:218-26.

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;89:1248-54.

44. Nielson CM, Harris RB, Nyitray AG, Dunne EF, Stone KM, Giuliano AR. Consistent condom use is associated with lower prevalence of human papillomavirus infection in men. J.Infect.Dis. 2010;202:445-51.

45. 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.

46. Castellsague X. Natural history and epidemiology of HPV infection and cervical cancer. Gynecol.Oncol. 2008;110:S4-S7.

47. Rodriguez AC, Burk R, Herrero R, Hildesheim A, Bratti C, Sherman ME et al. The natural history of human papillomavirus infection and cervical intraepithelial neoplasia among young women in the Guanacaste cohort shortly after initiation of sexual life. Sex Transm.Dis. 2007;34:494-502.

48. Sherman ME, Lorincz AT, Scott DR, Wacholder S, Castle PE, Glass AG et al.

Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. J.Natl.Cancer Inst. 2003;95:46-52.

49. Moscicki AB, Shiboski S, Broering J, Powell K, Clayton L, Jay N et al. The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. J.Pediatr. 1998;132:277-84.

50. 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;12:485-90.

51. Plummer M, Schiffman M, Castle PE, Maucort-Boulch D, Wheeler CM. A 2-year prospective study of human papillomavirus persistence among women with a cytological diagnosis of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. J.Infect.Dis. 2007;195:1582-89.

52. Schiffman M, Kjaer SK. Chapter 2: Natural history of anogenital human papillomavirus infection and neoplasia. J.Natl.Cancer Inst. Monogr 2003;14-19.

53. 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;168:123-37.

54. Nielsen A, Kjaer SK, Munk C, Osler M, Iftner T. Persistence of high-risk human papillomavirus infection in a population-based cohort of Danish women. J.Med.Virol. 2010;82:616-23.

55. Louvanto K, Rintala MA, Syrjanen KJ, Grenman SE, Syrjanen SM. Genotype-specific persistence of genital human papillomavirus (HPV) infections in women followed for 6 years in the Finnish Family HPV Study. J.Infect. Dis. 2010;202:436-44.

56. van Duin M., Snijders PJ, Schrijnemakers HF, Voorhorst FJ, Rozendaal L, Nobbenhuis MA et al. Human papillomavirus 16 load in normal and abnormal cervical scrapes: an indicator of CIN II/III and viral clearance. Int.J.Cancer 2002;98:590-95.

57. Munoz N, Hernandez-Suarez G, Mendez F, Molano M, Posso H, Moreno V et al. Persistence of HPV infection and risk of high-grade cervical intraepithelial neoplasia in a cohort of Colombian women. Br.J.Cancer 2009.

58. de Boer MA, Jordanova ES, van Poelgeest MI, van den Akker BE, van der Burg SH, Kenter GG et al. Circulating human papillomavirus type 16 specific T-cells are associated with HLA Class I expression on tumor cells, but not related to the amount of viral oncogene transcripts. Int.J.Cancer 2007;121:2711-15.

59. Moreno V, Bosch FX, Munoz N, Meijer CJ, Shah KV, Walboomers JM et al. Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study. Lancet 2002;359:1085-92.

60. Munoz N, Franceschi S, Bosetti C, Moreno V, Herrero R, Smith JS et al. Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case-control study. Lancet 2002;359:1093-101.

61. Plummer M, Herrero R, Franceschi S, Meijer CJ, Snijders P, Bosch FX et al. Smoking and cervical cancer: pooled analysis of the IARC multi-centric case--control study. Cancer Causes Control 2003;14:805-14.

62. Appleby P, Beral V, Berrington de GA, Colin D, Franceschi S, Goodhill A et al. Cervical cancer and hormonal contraceptives: collaborative reanalysis of individual data

for 16,573 women with cervical cancer and 35,509 women without cervical cancer from 24 epidemiological studies. Lancet 2007;370:1609-21.

63. Bulk S, Visser O, Rozendaal L, Verheijen RH, Meijer CJ. Cervical cancer in the Netherlands 1989-1998: Decrease of squamous cell carcinoma in older women, increase of adenocarcinoma in younger women. Int.J.Cancer 2005;113:1005-09.

64. Bulk S, Visser O, Rozendaal L, Verheijen RH, Meijer CJ. Incidence and survival rate of women with cervical cancer in the Greater Amsterdam area. Br.J.Cancer 2003;89:834-39.

65. van Ballegooijen M., Hermens R. Cervical cancer screening in the Netherlands. Eur.J.Cancer 2000;36:2244-46.

66. Cuzick J, Sasieni P, Davies P, Adams J, Normand C, Frater A et al. A systematic review of the role of human papilloma virus (HPV) testing within a cervical screening programme: summary and conclusions. Br.J.Cancer 2000;83:561-65.

67. Franco EL, Mahmud SM, Tota J, Ferenczy A, Coutlee F. The expected impact of HPV vaccination on the accuracy of cervical cancer screening: the need for a paradigm change. Arch.Med.Res. 2009;40:478-85.

68. 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;100:451-54.

69. van Ballegooijen M., van den Akker-van Marle, Patnick J, Lynge E, Arbyn M, Anttila A et al. Overview of important cervical cancer screening process values in European Union (EU) countries, and tentative predictions of the corresponding effectiveness and cost-effectiveness. Eur.J.Cancer 2000;36:2177-88.

70. 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;119:1095-101.

71. Ronco G, Giorgi-Rossi P, Carozzi F, Confortini M, Palma PD, Del MA et al. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. Lancet Oncol. 2010.

72. Naucler P, Ryd W, Tornberg S, Strand A, Wadell G, Elfgren K et al. Efficacy of HPV DNA testing with cytology triage and/or repeat HPV DNA testing in primary cervical cancer screening. J.Natl.Cancer Inst. 2009;101:88-99.

73. Bulkmans NW, Berkhof J, Rozendaal L, van Kemenade FJ, Boeke AJ, Bulk S et al. Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. Lancet 2007;370:1764-72.

74. Cuzick J, Arbyn M, Sankaranarayanan R, Tsu V, Ronco G, Mayrand MH et al. Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. Vaccine 2008;26 Suppl 10:K29-K41.

75. Clavel C, Masure M, Bory JP, Putaud I, Mangeonjean C, Lorenzato M et al. Human papillomavirus testing in primary screening for the detection of high-grade cervical lesions: a study of 7932 women. Br.J.Cancer 2001;84:1616-23.

76. 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;362:1871-76.

77. Naucler P, Ryd W, Tornberg S, Strand A, Wadell G, Elfgren K et al. Human

papillomavirus and Papanicolaou tests to screen for cervical cancer. N.Engl.J.Med. 2007;357:1589-97.

78. Franco EL, Cuzick J. Cervical cancer screening following prophylactic human papillomavirus vaccination. Vaccine 2008;26 Suppl 1:A16-A23.

79. Anhang R, Nelson JA, Telerant R, Chiasson MA, Wright TC, Jr. Acceptability of self-collection of specimens for HPV DNA testing in an urban population. J.Womens Health (Larchmt.) 2005;14:721-28.

80. Dzuba IG, Diaz EY, Allen B, Leonard YF, Lazcano Ponce EC, Shah KV et al. The acceptability of self-collected samples for HPV testing vs. the pap test as alternatives in cervical cancer screening. J.Womens Health Gend.Based.Med. 2002;11:265-75.

81. 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;186:365-73.

82. Jones HE, Wiegerinck MA, Nieboer TE, Mol BW, Westhoff CL. Women in the Netherlands prefer self-sampling with a novel lavaging device to clinician collection of specimens for cervical cancer screening. Sex Transm.Dis. 2008;35:916-17.

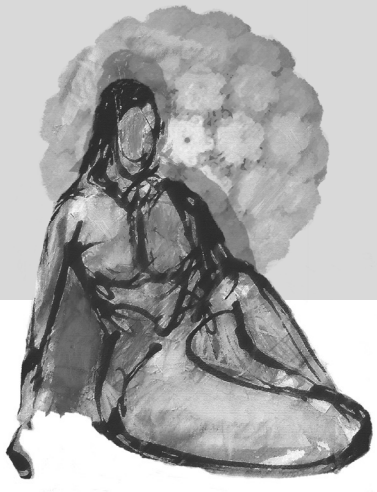
83. Nobbenhuis MA, Helmerhorst TJ, van den Brule AJ, Rozendaal L, Jaspars LH, Voorhorst FJ et al. Primary screening for high risk HPV by home obtained cervicovaginal lavage is an alternative screening tool for unscreened women. J.Clin.Pathol. 2002;55:435-39.

84. 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;13:208-13.

85. Gok M, Heideman DA, van Kemenade FJ, Berkhof J, Rozendaal L, Spruyt JW et al. HPV testing on self-collected cervicovaginal lavage specimens as screening method for women who do not attend cervical screening: cohort study. BMJ 2010;340:c1040.

86. 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;105:530-35.

87. 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;81:207-12.



Chapter 2

The potential role of self-sampling for high-risk Human papillomavirus detection in cervical cancer screening

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Summary

High-risk human papillomavirus (hr-HPV) detection will become an important tool in the screening for cervical cancer. Self-sampling is an inexpensive, well-accepted method for HPV detection that will increase participation of non-responders in current screening programs. Even the more, as self-collected samples are as good as physician-collected samples for HPV detection, self-sampling might be a suitable method for future primary cervical cancer screening.

Introduction

A persistent infection with high-risk human papillomavirus (hr-HPV) is a necessary factor in the multiple-step process of cervical cancer development.[1;2] Based on this etiologic link and the high sensitivity and objectivity of HPV detection, testing for HPV has recently been advocated as a primary cancer screening tool.[3-8]

Currently, physician-collected cervical specimens are considered as the gold standard for HPV detection.[9] However, self-sampling is a specimen collection method that is highly accepted by women, and It may reduce costs as a visit to a physician is omitted. Studies already showed that self-sampling increases participation of non-responders in current screening programs.[10-15] In the period of 2005-2007, one systematic review and two meta-analyses showed a high level of concordance for HPV DNA detection between self-collected samples and physician-collected samples.[16-18] Therefore, it was concluded that HPV DNA testing using self-sampling is promising and might be a suitable alternative method for studies on HPV transmission and vaccine trials.[16;18] Self-sampling might also be an appropriate alternative for cervical screening in low-resource setting or in patients reluctant to undergo pelvic examination.[17;18] After these meta-analyses, 20 new studies were published determining the value of self-sampling compared to physician-sampling for HPV detection. In this review, the studies in the meta-analyses and the recently published reports were analyzed to determine if self-sampling could be implemented in cervical screening as a tool for HPV detection and which combination of sample device and HPV test should be used.

HPV testing in primary cervical cancer screening

The development and implementation of organized and effective cytology-based cervical cancer screening programs has led to significant decreases in the incidence and mortality of cervical cancer.[19] The predicted lifetime risk of cervical cancer in an unscreened population is 2.9% by modeling data from the Netherlands. This is comparable to other European countries with a lifetime risk of 2.5-3.7%. The predicted lifetime risk dropped, however to 0.4% when attending cervical screening.[20] Still, cytology has important limitations. Cytology is based on subjective interpretation of morphological alterations of exfoliated cervical cells and is, therefore, associated with screening errors. The low sensitivity (about 60%) of cytology is its most critical limitation, and therefore, frequent screening is required.[5;21]

HPV testing has recently been advocated as an alternative cancer screening tool, because persistent infection with hr-HPV is associated with an increased risk for cervical cancer development.[1;2] Cytology is subject to sampling errors, processing error, and laboratory interpretation errors. HPV testing accuracy is also subject to sampling errors and laboratory reproducibility. However, HPV DNA detection assays are automated and therefore have a greater reproducibility than cytology. Moreover, the clinical sensitivity of hr-HPV testing for the detection of ≥CIN2 is about 90-95%.[3-5] In primary screening, this sensitivity for the detection of ≥CIN2 is higher compared to conventional cytology.[21-26] This higher

clinical sensitivity may lead to earlier diagnosis of high-grade cervical lesions.[23]
Furthermore, the negative predictive value (NPV) of a double-negative test, that is, normal cytology and no presence of high-risk HPV, is close to 100%,[27;28] and a negative test for HPV provides a greater reassurance against ≥CIN2 than a negative cytology test.[19;27;29-31] Moreover, women with abnormal cytology but negative for HPV have a low risk of CIN3+ lesions.[4;32;33] Therefore, it is suggested that HPV DNA testing could possibly be used to extend screening intervals.[7;8;26;27;30;33-35] In the Netherlands, where standard screening starts at age 30 with an interval of 5 years, studies have already shown that with this screening interval, primary HPV DNA testing or combined testing with cytology leads to higher sensitivity and NPV, with an earlier detection of CIN3+ lesions.[7;8] This suggests that the screening interval may be safely extended.
HPV DNA testing alone or with cytology triage has a higher sensitivity and earlier detection of clinically relevant lesions,[25;28;35] but its specificity for detection of ≥CIN2 is lower (86%) compared with conventional cytology.[5;25;26;29] The lower specificity may be explained by the high prevalence of transient HPV infections, especially in young women. Because a persistent hr-HPV infection is required for the development of severe dysplastic cervical lesions, a single HPV positive test by itself may be a poor predictor of a future intraepithelial lesion;[19;30] therefore, women with an hr-HPV positive test and negative cytology at triage need to be monitored for hr-HPV persistence.[3;24;27;29;35;36] It is suggested that repeating both HPV and cytology after an interval of 1 year allows early detection of newly developed intraepithelial lesions.[6;7;32]
Low specificity of an HPV DNA test may lead to increased numbers of follow-up tests, unnecessary colposcopies, psychosocial distress, and the possibility of over-treatment.[5;23-25;28] Therefore, a clinically validated HPV DNA test is required.[37] This test should be sufficiently sensitive to identify all women at risk for high-grade cervical lesions yet sufficiently specific to avoid unnecessary referral and repeat smears.[36]
HPV testing alone or with cytology triage is more sensitive than cytology alone in cervical screening, and therefore its implementation seems to be inevitable. However, whether HPV DNA testing should be implemented combined with cytology or as a single primary screening tool (with or without cytology triage) is still a subject of study. Cotesting of HPV and cytology is not an issue because it is as sensitive as HPV testing alone. The triage of HPV-positive women with cytology is carried out to decrease the number of colposcopies and is dependent on the settings of screening in different countries.[21;23-25;28;38]
Self-collected cervico-vaginal samples have shown to be suitable for HPV testing.[16;17] The implementation of primary HPV testing in cervical cancer screening leads to the potential use of self-sampling in large scale screening programs.[14] The use of self-sampling requires fewer visits to the physician, and results in less patient inconvenience or noncompliance.[11;15;39;40] The use of self-sampling may therefore lead to a higher acceptability and may potentially lead to a reduction in costs in current screening programs.[39;41] Moreover, provided that HPV detection tests are available, self-collection of cervico-vaginal specimens for HPV DNA testing may help to enhance screening rates in countries that have

been unable to reduce cervical cancer incidence.[11]
Notably, self-sampling will only be a suitable tool if the intended woman indeed does the test and not someone else. In this respect, it is important that she understands the instruction on how to take a cervico-vaginal sample.

Self-sampling for HPV DNA detection

Sampling devices and transport media for HPV DNA detection

Studies with self-sampling used a great variety of collection devices including swabs, cervical brushes, tampons, and cervico-vaginal lavages.
Vaginal Dacron or cotton swabs and cervical brushes are small, easy to manipulate, and can be processed in a similar manner to that of physician-collected swabs.[10;42-44] Additionally, sending and returning swabs and brushes through the mail is feasible, and thus the use of these devices can be home-based.[45-47]
Tampons and lavage mainly collect squamous epithelial cells from the walls of the vagina, together with shed cervical cells.[42] Tampon samples need to be processed more extensively than swabs and brushes before HPV DNA detection tests can be applied.[48] The use of a cervico-vaginal lavage is used as self-sampling method in several studies,[12;13;15;49] and the women in the studies considered the lavage acceptable.[12;13] The most frequently used polymerase chain reaction (PCR) HPV DNA tests, like PGMY09/11, GP5+/6+, SPF_{10}LiPA, and the Roche reverse line blot assay (RBLA), and hybrid capture 2 (HC2) can be used on swabs, brushes, tampons and lavage samples. Nowadays, mRNA tests are developed, and they appear to be more specific for the detection of diseases than DNA testing.[50]
In review, the overall sensitivity for self-collected samples, when Dacron or cotton swabs, or cytobrushes were used, was 74-81% with a specificity of 88-90% using clinician-obtained samples with the same devices as a reference. Tampons performed slightly less well, offering a sensitivity between 67 and 94%.[17]
The majority of the studies assessing self-sampling have used liquid-based storage and transport media.[13;51-53] In our own experience, the use of liquid-based self-samples has the impractical consequence that fluids may leak, and special precautions have to be taken for transport. This potentially hampers the large-scale introduction of cervico-vaginal self-sampling methods.[54;55] The use of dry transport methods may eliminate these disadvantages. Samples may be transported and stored using a dry test tube[56] or the indicating Flinders Technology Associates (FTA) elute cartridge.[54] Previously, the highly sensitive SPF_{10}LiPA HPV DNA test and the less known hpVIR real-time PCR have shown that HPV detection using the FTA elute cartridge is possible.[54;57] The development and improvement of devices and transport methods is still an ongoing process.

Self-collected sampling versus physician-collected sampling

Physician-collected specimens mainly contain cervical cells, whereas self-collected specimens generally represent a mixture of vaginal and cervical cells. However, the

prevalence of carcinogenic HPV types appeared to be similar in vaginal and cervical specimens.[58;59]

Three reviews comparing self-collected samples with physician-collected samples were published.[16-18] In 2005, Ogilvie et al. used the physician-collected samples for HPV DNA as the gold standard, making no distinction between lr-HPV and hr-HPV.[17] They found that self-sampling with Dacron or cotton swabs had the best performance with a sensitivity of 74-81% and specificity of 88-90%.[17] In 2007, Petignat et al. analyzed studies on self-sampling and physician-sampling in which any positive HPV test was considered as reference standard.[16] They found a concordance of 87% between self-sampling and physician-sampling for the detection of any-type HPV. The detection of hr-HPV resulted in a similar level of concordance, but lr-HPV detection was higher in self-sampling. In the same period, Stewart et al. published a systematic review and found a wide range of level of agreement (κ 0.24-0.96) for HPV detection between self-collected samples and physician-collected samples, with little more than half of the studies reporting κ of >0.6.[18] The overall conclusion of these three reviews was that the sensitivity of self-sampling is sufficient to be used as an appropriate alternative for physician-sampling in low resource settings or to increase screening rates, especially in women who are never or seldom screened. Therefore, more evidence is needed before self-sampling might be implemented in national screening.

Twenty new, not previously reviewed, studies comparing self-collected sampling and physician-collected sampling for HPV DNA detection were identified in Pubmed with the key words: self-sampling, human papillomavirus, human papillomavirus infection, and human papillomavirus type and limiting to studies describing research in humans and published since 2000 in English.[39;41;49;53-56;60-72] One study could not be included in our analysis, because a full text version was not available.[68] The characteristics of the remaining 19 studies are shown in Table 1.

Notably, many studies included women in wide age ranges, varying from young sexually active women, likely to have a higher HPV prevalence, up to women 70 years of age. For cervical cancer screening programs, the studies including women from 25 to 30 years and older are most interesting because this will be the age range in which HPV testing will be used.

In Table 1, it is shown that the studies used different sampling procedures. These sampling procedures did not only differ between the studies, but sometimes within studies, different sampling devices or different HPV DNA detection methods for the self-collected sample and physician-collected sample were used. Consequently these results might not represent the true agreement between self-sampling and physician-sampling.[39;49;53;60;63;64;67;70;72]

In most studies both samples are collected on the same day or with a short interval, but one study tested the self-collected samples and physician-collected samples alternatively with 2 months in between.[72] Due to the natural fluctuations of HPV infections in the population, these results may not be directly comparable.

When studying the agreement between self-collected samples and physician-collected

samples, the order of sampling could potentially influence the amount of cells collected by the sampling method. However, previously, Harper et al. randomized the order of sampling for self-sampling with a tampon and physician-sampling with a swab, and they concluded that the order of sampling does not seem to influence the outcome.[48]

All studies, previously reviewed and more recently published, reporting results on the concordance and/or kappa (*κ*), were used to determine the level of agreement of HPV detection between self-sampling and physician-sampling. Additionally, all the used combinations of the sample device and HPV test were compared. Studies reporting the concordance of self-sampling and physician-sampling for hr-HPV detection using the same sample device and the same HPV DNA detection method are shown in Table 2. Because of the potential influence of the sample device on the performance, studies using different devices for the self-sample and physician-sample were analyzed separately (Table 3). One study used different sampling devices and different HPV DNA detection methods for self-sampling and physician-sampling, and therefore was not included.[56]

The HC2 is used by many studies,[39-41;43;48;63;63;64;69;73;74] and is compared with the group of PCR-based methods in Tables 2 and 3. The PCR methods used were mainly PGMY09/11 and RLBA,[41;51;51;52;60;63;63;69;71;75-81] other PCR based methods like SPF_{10}LiPA,[54] GP5+/6+,[13;49] and less known tests like HPVDNAChip^{tm} [67] and QIAamp DNA test [82] were also used. The most frequently used sample devices were swabs and brushes, only 2 studies used the lavage for self-sampling compared with physician-sampling.[13;49]

Table 1.
Characteristics of studies comparing self-sampling with physician-collected samples

	Reference	Country	N	Inclusion Criteria	
1	Belinson	China	2625	Age 16-54	
2	Bhatla et al. [60]	Developing country	546	Age ≥ 30, sexually active, gynecologic symptoms	
3	Brink et al. [49]	the Netherlands	96	Age 18-59, referred to gynecologist for colposcopy-directed biopsy or healthy volunteer	
4	Daponte et al. [82]	Greece	98	HIV negative, referred with abnormal cervical cytology, with proven abnormal histology	
5	De Alba et al. [62]	California, USA	1213	Age ≥ 18, hispanic	
6	Holanda et al. [39]	Brazil	878	Age 15-70, sexually active	
7	Jones et al. [63]	South Africa	450	Age ≥ 18, sexually active, attending community health centre	
8	Karwalajtys et al. [64]	NA	307	Age 15-49, HPV+ a year earlier Age ≥ 50; randomly	
9	Khanna et al. [65]	Cananda	398* 399#	Age 18-69, presenting for routine gynecology care	
10	Lack et al. [53]	Gambia	210	RMS survey 83	
11	Lenselink et al. [54]	the Netherlands	45	Visiting gynecologist for follow-up diethylstilbestrol exposition in utero, cervical dysplasia or cytology abnormalities	
12	Longatto-Filho et al. [66]	Brazil, Argentina	311* 770#	Latin female population with different risks of cervical abnormalities 84	
13	Moscicki et al. [72]		537	Age 13-21, < 5 years of sexual experience 85	
14	Safaeian et al. [41]	Uganda	606~	Age 15-49, participants RCCS 86	
15	Seo et al. [67]	South Korea	118	Abnormal cytology	
16	Sowjanya et al. [69]	India	432	Age ≥ 25, intact uterus, mentally competent	
17	Stenval et al. [56]	Sweden	43	Age 23-58, previous positive cytology	
18	Szarewski et al. [70]	Great Britain	920	Attending routine cervical smear	
19	Winer et al. 71	Washington, USA	374	Age 18-25, newly recruited in longitudinal study of genital HPV infection, who never had vaginal intercourse or first intercourse within past 3 months Age 23-32, recruited 5-10 years earlier to longitudinal study of genital HPV infection	

	Exclusion Criteria	Self-sample device	Physician-sample device	HPV test
	Pregnancy, pelvic radiation, hysterectomy, treatment cervical cancer or HIV+	Brush	Brush	HC2; typing: Roche RLBA
	NA	Brush	Ayre Spatula Brush	HC2, PGMY09/11
	NA	Lavage	Brush	GP5+/6+
	NA	Cytobrush	Cytobrush	PCR
	Papsmear last year, pregnancy, hysterectomy, cervical cancer	Cotton swab	NA	HC2
	Menstruation, sexual intercourse < 24 h, using vaginal cream	Brush	Ayre spatula	HC2
	Pregnant	Half swab and half tampons	Brush	Roche RLBA, HC2
	NA	Dacron swab	Brush (after papsmear)	HC2
	Presenting symptoms Abnormal Pap test, genital cancer, cervical surgery, or immune treatment of the cervix within one year	Christmass tree cervical sampling brush (Digene)	Christmass tree cervical sampling brush (Digene)	HC2
	Pregnant	Dacron swab, tampons	Cytobrush	GP5+/6+
	NA	Viba-Brush (Rovers Medical devices)	Cervex-Brush (Rovers Medical devices)	SPF10LiPA
	NA	Brush, 1 sample	Brush, 2 samples	HC2
	immunosuppressd, pregnant, surgery cervix 85	Dacron swab	Lavage	PGMY09/11
	NA	Swab	Swab	HC2, RBLA, PGMY09/11
	NA	Dacron swab	Cervical brush (cytology), Dacron swab (HPV)	HPVDNACHipTM (PCR based)
	NA	Swab	Ayre spatual & Swab (cytology) Swab Digene cervical sampler (HPV)	HC2, Roche prototype RLBA
	NA	Qvintip	Cytobrush	Self-sample HC2 Physician-sample GP5+/6+
	No previous cervical treatment	Cotton swab	Spatual and brush (cytology) Brush (HPV)	HC2
	NA	Dacron swab	Dacron swab	Roche RLBA, PGMY09/11

~ Number of samples instead of number of patients
* Number of self-samples
\# Number of physician-collected samples
^ Study also including immunocompromised (HIV-positive) women
NA Not available or not applicable; HC2, hybrid capture 2; RLBA, reverse line blot assay; HPV, human papillomavirus
RMS, reproductive morbidity survey; RCCS, Rakai community cohort study

When using the same device for self-sampling and physician-sampling, the overall mean concordance is 87.1% (SD 12.3) with a nearly excellent agreement of κ=0.73 (SD 0.15). Studies using different devices for self-sampling and physician-sampling showed a concordance of 85.2% but with a lower, still fairly good, agreement of κ=0.60 (SD 0.12).
In Table 2, studies that did show an excellent agreement (κ> 0.75) between self-sampling and physician-sampling are all PCR-based. When a swab for sampling and a PCR-based HPV DNA test was used, the mean κ was excellent (κ=0.81 [SD 0.09]). The use of a brush combined with PCR results in a nearly excellent mean κ (κ=0.71 [SD 0.18]), with three of the five studies with an excellent agreement (κ=0.76-0.88). In Table 3, only studies using tampons for self-sampling and a PCR HPV detection method reported an excellent concordance (κ=0.76). The higher sensitivity to detect hr-HPV is probably the reason why PCR-based studies show better results than studies performed with HC2. The SPF$_{10}$LiPA, the PCR test with the highest analytical sensitivity,[87] was only used in one study. This study showed a concordance of 93.3% with an excellent agreement (κ=0.86).[54]
Three studies used both HC2 and PCR-based methods for HPV detection. Bhatla et al. showed a higher diagnostic accuracy for both physician- and self-samples with PGMY09/11. The agreement between HPV testing with HC2 and PGMY09/11 was 90.9% (κ=0.64) for self-samples and 95.3% (κ=0.80) for physician-samples.[60] Sowjanaya et al. showed the concordance between HC2 and RLBA for physician-sampling and self-sampling for HPV detection of 94.4% (κ=0.8), with a better performance for RLBA.[69] Jones et al. also showed a better performance for RLBA, the κ with RLBA compared to the κ with HC2 between self-sampling and physician-sampling using swabs was κ=0.71 versus κ=0.61, and when using tampons it was κ=0.75 versus κ=0.55.[63] The strongest predictor of self-sampling performance in this study was the HPV viral load in the physician-collected specimens, estimated by HC2 relative light units.
Based on these data, self-sampling with PCR-based HPV DNA testing is more promising than with HC2. Before implementation of self-sampling in cervical screening, further research is needed to determine if the difference in performance of HC2 and PCR on self-sampling is clinically relevant.

Table 2.
Concordance of hr-HPV detection using the same sample device for self- and physician-sampling

Sample device	HPV test	N	Concordance Mean % [range] (SD)	Kappa Mean % [range] (SD)
Swab	HC2 [40;41;64;69]	2539	91.3 [85.7-99.1] (4.9)	0.59 [0.37-0.75] (0.17)
	PCR [41;51;67;69;75-77, 71–,*]	2255	84.9 [51.0-98.8] (17.2)	0.81 [0.65-0.96] (0.09)
Brush	HC2 [39;43;73]	1177	87.5 [83.0-92.0] (6.4)	0.71 [0.70-0.71] (0.01)
	PCR [54;79, 60;78*, 82^]	1362	89.5 [77.0-93.9] (8.33)	0.71 [0.51-0.88] (0.18)
Lavage	PCR [13]	71	75	0.47
Total		7404	87.1 [51.0-99.1] (12.3)	0.73 [0.37-0.96] (0.15)

* Any-type HPV
^ Only HPV 16 detection
~ Study used two different groups of participants and reported two different rates of concordance and kappa.
κ=0.0, no agreement at all; κ<0.4, poor, κ=0.4-0.75, fairly good; κ>0.75, excellent; κ=1.0 = complete agreement between the test results.

Table 3.
Concordance of hr-HPV detection using different sample devices for self- and physician-sampling

Self-sample device	HPV test	N	Concordance Mean % [range] (SD)	Kappa Mean % [range] (SD)
Swab	HC2 [63;74]	1637	81.8 [81.5-82.0] (0.35)	0.53 [0.45-0.61] (0.11)
	PCR [63;81]	475	85.6	0.66 [0.60-0.71] (0.08)
Brush	PCR [52]	285	NA	0.45
Tampon	HC2 [63, 48–]	321	84.2 [80.5-87.8] (5.2)	<0.5-0.55
	PCR [63, 80*]	502	88.5 [87.8-89.2] 1.0	0.76 [0.75-0.76] (0.01)
Lavage	PCR [49]	96	87	0.71
Total		3316	85.2 [80.5-89.2] (3.4)	0.60 [0.45-0.76] (0.13)

* Any-type HPV
~ HPV detection with HC
κ=0.0, no agreement at all; κ<0.4, poor, κ=0.4-0.75, fairly good; κ>0.75, excellent; κ=1.0 = complete agreement between the test results.
SD, standard deviation; HPV, Human papillomavirus; HC2, hybrid caputre 2; PCR, polymerase chain reaction

Detection of cervical abnormalities by self-sampling and physician-sampling

Studies reporting data on sensitivity and specificity of HPV testing for detection of cervical intraepithelial neoplasia (CIN) lesions were included to obtain insight in the performance of self-sampling and physician-sampling. Table 4 presents the results of studies performed in screening populations, mainly including HPV testing and cytology prior to histology, and Table 5 shows data of studies performed in colposcopy clinics on populations considered to be at high risk of CIN lesions.

The available results on the accuracy of hr-HPV detection with self-sampling compared with physician-sampling for detection of CIN lesions in screening populations are limited. From the results shown in Table 4, there was no statistically significant difference in ≥CIN1 detection between self-sampling and physician-sampling in screening populations. For the detection of ≥CIN2, only studies using HC2 were available. The mean sensitivity (94.3%) of physician-sampling with a brush was higher than the mean sensitivity of self-sampling (82.7%). Self-sampling with a swab had a lower mean sensitivity to detect ≥CIN2 (73.6%), but could not be compared with physician-sampling as results were not available. Although the sensitivity of HPV testing for ≥CIN2 detection for both physician-sampling and self-sampling is high, it will not reach 100% sensitivity. This may be explained by the vaccine trials that showed that HPV prevalence is not necessarily related to HPV attribution of the ≥CIN2 lesion that developed.

Because of the limited number of data available more research is necessary to evaluate the clinical performance of self-sampling for hr-HPV DNA in screening populations, especially since data on ≥CIN2 detection with PCR-based HPV DNA detection methods are lacking and HPV detection with self-sampling and PCR have a better agreement with physician-sampling compared to testing with HC2.

In high-risk populations for cervical abnormalities (Table 5), physician-samples for HPV DNA testing showed a higher sensitivity for detection of ≥CIN2 than self-sampling. However, the specificity did not differ between both groups. The highest sensitivity of 98.3% for detection of ≥CIN2 was obtained when a physician-sample with a swab and HC2 were used. The best results on sensitivity of self-sampling for detection of ≥CIN2 were obtained by using a swab or a brush with HC2; both had a sensitivity of 86%. Although the number of studies are limited, these results suggest that physician-sampling is therefore preferred for detecting cervical abnormalities in high-risk populations, and self-sampling seems to be a good alternative for women otherwise not reached.

Table 4.
Accuracy of hr-HPV detection with self-sampling compared with physician-sampling for detection of CIN lesion general screening population

CIN lesion	HPV DNA test	Sample Device	Self-sample		Physician-sample	
			Sensitivity %	Specificity %	Sensitivity %	Specificity %
			Mean% [range] (SD)	Mean% [range] (SD)	Mean% [range] (SD)	Mean% [range] (SD)
≥CIN1	HC2	Brush [43]	89.5	48.5	NA	NA
		Swab [40]	96.3	91.8	NS	NS
	PGMY09/11	Brush [78]	75.0*	95.7*	75*	97.1*
≥CIN2	HC2	Brush[43;55;66;70;88]	82,7 [62.5-100] (15.6)	64.6 [45.2-77.2] (17.1)	94.3 [83.9-100] (6.4)	84.9 [79.7-90.2] (4.3)
		Swab [70;74]	73.6 [66.1-81.0] (10.5)	82.5 [82.0-82.9] (0.64)	NA	NA

* Any-type HPV.
NA Not available; NS, not statistically significant different from HPV testing with self-sampling; CIN1, cervical intraepithelial neoplasia grade 1; CIN2, cervical intraepithelial neoplasia grade 2; SD, standard deviation; HPV, human papillomavirus; HC2, hybrid capture 2.

Table 5.
Accuracy of hr-HPV detection with self-sampling compared with physician-sampling for detection of CIN lesion in a high-risk population

CIN lesion	HPV DNA test	Sample Device	Self-sample		Physician-sample	
			Sensitivity %	Specificity %	Sensitivity %	Specificity %
			Mean% [range] (SD)	Mean% [range] (SD)	Mean% [range] (SD)	Mean% [range] (SD)
≥ CIN1	HC2	Brush [73]	86.0	NA	80	NA
≥ CIN2	HC2	Brush [60*, 73]	86.0 [80.0*-92] (8.5)	88.1*	91.0 [90.0*-92] (1.4)	91.7*
		Swab [44]	86.2	53.5	98.3	52.1
	PGMY09/11	Brush [60*,79]	65.8 [49.0-82.5*] (23.7)	93.6* + NS	84.9 [82.2-87.5*] (3.7)	93.2* + NS
	GP5+/6+	Lavage [13]	81	68	91	43
CIN3	HPVDNA Chip[tm] (PCR)	Swab [67]	90.5	29.0	88.1	32.9

* Any-type HPV.
NA Not available; NS, specificity not statistically significant different from HPV testing with self-sampling and physician-sampling; CIN1, cervical intraepithelial neoplasia grade 1; CIN2, cervical intraepithelial neoplasia grade 2; CIN3, cervical intraepithelial neoplasia grade 3; HPV, human papillomavirus; HC2, hybrid capture 2; PCR, polymerase chain reaction.

Acceptance of self-sampling

Cervico-vaginal self-sampling is a user-friendly method because self-sampling eliminates the need of a speculum examination.[77] In studies reporting acceptability of self-sampling, the majority of women found self-sampling devices easy to use,[12;43;62] time saving, less embarrassing, more comfortable, and women report that they were more relaxed doing it.[11;14;78] This potentially improves participation rates for cervical cancer screening.

Overall, moderate to high rates of acceptance, 75-93% have been reported for self-sampling.[11-13;41;78] These studies included women in wide age ranges, from 15 to 63 years old.[12;13;41;78] The majority of women, however, was of the ages 35-44 years [12;13;78] Most of the studies showed that women preferred self-sampling above a physician-collected sample, suggesting that self-sampling is likely to provide better population coverage.[11;12;41] However, these data are based on study populations, and therefore acceptability of self-sampling needs to be further explored in a national cervical screening program. Furthermore, to be representative, the acceptability of self-sampling should only be examined among women ≥30 years because HPV testing in screening will be offered to this group of women.

Although the responses are mainly positive, one study reported an acceptance rate of only 46% for self-sampling. This study was performed in women aged 15-49 years, who were invited on their return visit for annual cervical cytology, representing a group of women at low risk who already participated in screening.[64] Another study reported that, despite women reported that self-sampling was easy to use (69%) and less painful (62%), a majority of 68% of the women still preferred the physician-collected sample.[89] Although women respond positively to self-sampling, they also report uncertainty about their performance of the procedure.[11;13;90;91] Even women who reported to be fairly to very confident, were less confident compared to when the physician-collected test was used. This is the main reason for the preference of physician-sampling. Although this concern does not stop them to use the self-sample again if offered in the future.[14]

Cultural and religious beliefs do not appear to influence women's choice for self-sampling,[90;92;93] although in a group of Muslim women attending for cervical screening, limited enthusiasm for self-sampling was shown. They did show a clear preference for a swab rather than a cervico-vaginal lavage.[94] In a low-resource setting where women are not used to tampons, the use of swabs was preferred above the use of tampons for self-sampling, and the use of tampons was preferred above physician-collected sampling.[53]

Because it is known that about 50% of the cases with invasive cervical cancer arise in women who are not adequately screened,[95-97] non-responding women represent a high risk category for developing cervical cancer and are therefore important to reach.[98] Self-sampling is regarded as a possible alternative to facilitate the screening of women who refuse to participate in current cervical cancer screening programs.[11;13;42] Recently, it has been shown that the participation in screening of non-responders improved with the use of self-sampling methods.[15;75;99;100] Because the majority of all women included in these studies were ≥30 years of age, the high rates of acceptance in these studies

were promising for self-sampling as a public health option. For example, in one study, a group of non-responders was offered a cervico-vaginal lavage self-sample and the control group consisted of non-responders who received the normal recall for cytology. The compliance rate was significantly higher in the group offered the self-sample, 26.6% versus 16.4%.[15] Another study invited non-responders of the national cervical screening program to perform a self-sample with the Qvintip; 58% of these women responded.[100] Both studies proved that self-sampling is an attractive cervical screening method for non-responders and increases their participation.

Conclusion

Self-sampling showed a good overall agreement with physician-sampling when a swab or a brush was used in combination with a PCR-based test for HPV DNA detection. The agreement was only moderate when using other sampling devices or HC2 for HPV DNA detection. However, the clinical sensitivity of self-sampling with PCR based tests needs to be validated. A high sensitivity of HPV detection should result in a high detection of ≥CIN2 lesions, but a highly sensitive HPV detection assay may also overestimate the proportion of women who have cervical abnormalities.[87;101;102] Future studies should focus especially on general screening populations to determine if self-sampling combined with PCR based HPV testing is a valid tool for national screening.

In high-risk populations, although based on only a few studies, physician-sampling had a higher sensitivity to detect ≥CIN2 lesions than self-sampling, and HC2 seemed to be better than self-sampling with PCR-based HPV DNA detection. This was not expected because PCR-based tests performed better than HC2 in studies comparing self-sampling and physician-sampling for HPV detection. Moreover, in high-risk populations, there is a higher prevalence of lesions and a sensitive test like PCR is expected to detect those lesions. Therefore, future studies on self-sampling in national screening should also address the performance of HC2 and PCR-based tests for the detection of ≥CIN2 lesions in high-risk populations.

The majority of women accept a sampling method performed by the woman herself, in the privacy of her own home. Furthermore, self-sampling lowers the threshold and increases the uptake of screening in non-responders. Therefore, addition of a self-sampling option to a national screening program may increase screening coverage, and thus overall efficacy.

Today self-sampling for HPV detection has proven that its sensitivity is sufficient to screen women otherwise not screened, and may be implemented in national screening to reach non-responders. Further research is necessary before a wide implementation in an already successful national screening program is possible.

Reference List

1. 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;87:796-802.
2. Schiffman MH, Bauer HM, Hoover RN, Glass AG, Cadell DM, Rush BB et al. Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. J.Natl. Cancer Inst. 1993;85:958-64.
3. Bulk S, Bulkmans NW, Berkhof J, Rozendaal L, Boeke AJ, Verheijen RH et al. Risk of high-grade cervical intra-epithelial neoplasia based on cytology and high-risk HPV testing at baseline and at 6-months. Int.J.Cancer 2007;121:361-67.
4. Ronco G, Giorgi-Rossi P, Carozzi F, Dalla PP, Del MA, De ML et al. Human papillomavirus testing and liquid-based cytology in primary screening of women younger than 35 years: results at recruitment for a randomised controlled trial. Lancet Oncol. 2006;7:547-55.
5. 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;119:1095-101.
6. Naucler P, Ryd W, Tornberg S, Strand A, Wadell G, Elfgren K et al. Human papillomavirus and Papanicolaou tests to screen for cervical cancer. N.Engl.J.Med. 2007;357:1589-97.
7. Bulkmans NW, Berkhof J, Rozendaal L, van Kemenade FJ, Boeke AJ, Bulk S et al. Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. Lancet 2007;370:1764-72.
8. Bulkmans NW, Rozendaal L, Voorhorst FJ, Snijders PJ, Meijer CJ. Long-term protective effect of high-risk human papillomavirus testing in population-based cervical screening. Br.J.Cancer 2005;92:1800-02.
9. Daponte A, Tsezou A, Oikonomou P, Hadjichristodoulou C, Maniatis AN, Pournaras S et al. Use of real-time PCR to detect human papillomavirus-16 viral loads in vaginal and urine self-sampled specimens. Clin.Microbiol.Infect. 2008;14:619-21.
10. 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;186:365-73.
11. Dzuba IG, Diaz EY, Allen B, Leonard YF, Lazcano Ponce EC, Shah KV et al. The acceptability of self-collected samples for HPV testing vs. the pap test as alternatives in cervical cancer screening. J.Womens Health Gend.Based.Med. 2002;11:265-75.
12. Jones HE, Wiegerinck MA, Nieboer TE, Mol BW, Westhoff CL. Women in the Netherlands prefer self-sampling with a novel lavaging device to clinician collection of specimens for cervical cancer screening. Sex Transm. Dis. 2008;35:916-17.
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;55:435-39.

14. 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;13:208-13.

15. Gok M, Heideman DA, van Kemenade FJ, Berkhof J, Rozendaal L, Spruyt JW et al. HPV testing on self-collected cervicovaginal lavage specimens as screening method for women who do not attend cervical screening: cohort study. BMJ 2010;340:c1040.

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;105:530-35.

17. 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;81:207-12.

18. 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;29:817-28.

19. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet 2007;370:890-907.

20. Berkhof J, de Bruijne MC, Zielinski GD, Meijer CJ. Natural history and screening model for high-risk human papillomavirus infection, neoplasia and cervical cancer in the Netherlands. Int.J.Cancer 2005;115:268-75.

21. Franco EL, Mahmud SM, Tota J, Ferenczy A, Coutlee F. The expected impact of HPV vaccination on the accuracy of cervical cancer screening: the need for a paradigm change. Arch.Med.Res. 2009;40:478-85.

22. Bulkmans NW, Rozendaal L, Snijders PJ, Voorhorst FJ, Boeke AJ, Zandwijken GR et al. POBASCAM, a population-based randomized controlled trial for implementation of high-risk HPV testing in cervical screening: design, methods and baseline data of 44,102 women. Int.J.Cancer 2004;110:94-101.

23. Leinonen M, Nieminen P, Kotaniemi-Talonen L, Malila N, Tarkkanen J, Laurila P et al. Age-specific evaluation of primary human papillomavirus screening vs conventional cytology in a randomized setting. J.Natl. Cancer Inst. 2009;101:1612-23.

24. Cuzick J, Sasieni P, Davies P, Adams J, Normand C, Frater A et al. A systematic review of the role of human papilloma virus (HPV) testing within a cervical screening programme: summary and conclusions. Br.J.Cancer 2000;83:561-65.

25. Ronco G, Giorgi-Rossi P, Carozzi F, Confortini M, Palma PD, Del MA et al. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. Lancet Oncol. 2010.

26. Davies P, Arbyn M, Dillner J, Kitchener HC, Meijer CJ, Ronco G et al. A report on the current status of European research on the use of human papillomavirus testing for primary cervical cancer screening. Int.J.Cancer 2006;118:791-96.

27. Clavel C, Cucherousset J, Lorenzato M, Caudroy S, Nou JM, Nazeyrollas P et al. Negative human papillomavirus testing in

normal smears selects a population at low risk for developing high-grade cervical lesions. Br.J.Cancer 2004;90:1803-08.

28. Cuzick J, Arbyn M, Sankaranarayanan R, Tsu V, Ronco G, Mayrand MH et al. Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. Vaccine 2008;26 Suppl 10:K29-K41.

29. Shi JF, Belinson JL, Zhao FH, Pretorius RG, Li J, Ma JF et al. Human papillomavirus testing for cervical cancer screening: results from a 6-year prospective study in rural China. Am.J.Epidemiol. 2009;170:708-16.

30. Sherman ME, Lorincz AT, Scott DR, Wacholder S, Castle PE, Glass AG et al. Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. J.Natl.Cancer Inst. 2003;95:46-52.

31. Cuschieri KS, Cubie HA, Whitley MW, Gilkison G, Arends MJ, Graham C et al. Persistent high risk HPV infection associated with development of cervical neoplasia in a prospective population study. J.Clin.Pathol. 2005;58:946-50.

32. 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;362:1871-76.

33. Dillner J, Rebolj M, Birembaut P, Petry KU, Szarewski A, Munk C et al. Long term predictive values of cytology and human papillomavirus testing in cervical cancer screening: joint European cohort study. BMJ 2008;337:a1754.

34. Ronco G, Segnan N. HPV testing for primary cervical cancer screening. Lancet 2007;370:1740-42.

35. Naucler P, Ryd W, Tornberg S, Strand A, Wadell G, Elfgren K et al. Efficacy of HPV DNA testing with cytology triage and/or repeat HPV DNA testing in primary cervical cancer screening. J.Natl.Cancer Inst. 2009;101:88-99.

36. Brink AA, Snijders PJ, Meijer CJ, Berkhof J, Verheijen RH. HPV testing in cervical screening. Best.Pract.Res.Clin.Obstet. Gynaecol. 2006;20:253-66.

37. 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;124:516-20.

38. Zhao C, Florea A, Austin RM. Clinical utility of adjunctive high-risk human papillomavirus DNA testing in women with Papanicolaou test findings of atypical glandular cells. Arch. Pathol.Lab Med. 2010;134:103-08.

39. Holanda F, Jr., Castelo A, Veras TM, de Almeida FM, Lins MZ, Dores GB. Primary screening for cervical cancer through self-sampling. Int.J.Gynaecol.Obstet. 2006;95:179-84.

40. Chang CC, Tseng CJ, Liu WW, Jain S, Horng SG, Soong YK et al. Clinical evaluation of a new model of self-obtained method for the assessment of genital human papilloma virus infection in an underserved population. Chang Gung.Med.J. 2002;25:664-71.

41. Safaeian M, Kiddugavu M, Gravitt PE, Ssekasanvu J, Murokora D, Sklar M et al. Comparability of self-collected vaginal swabs and physician-collected cervical swabs for detection of human papillomavirus infections in Rakai, Uganda. Sex Transm.Dis. 2007;34:429-36.

42. Morris BJ, Rose BR. Cervical screening in the 21st century: the case for human papillomavirus testing of self-collected

specimens. Clin.Chem.Lab Med. 2007;45:577-91.

43. Dannecker C, Siebert U, Thaler CJ, Kiermeir D, Hepp H, Hillemanns P. Primary cervical cancer screening by self-sampling of human papillomavirus DNA in internal medicine outpatient clinics. Ann.Oncol. 2004;15:863-69.

44. 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;163:513-18.

45. 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:e3743.

46. Baay MF, Verhoeven V, Lambrechts HA, Pattyn GG, Lardon F, Van RP et al. Feasibility of collecting self-sampled vaginal swabs by mail: quantity and quality of genomic DNA. Eur.J.Clin.Microbiol.Infect.Dis. 2009;28:1285-89.

47. Harper DM, Raymond M, Noll WW, Belloni DR, Duncan LT, Cole BF. Tampon samplings with longer cervicovaginal cell exposures are equivalent to two consecutive swabs for the detection of high-risk human papillomavirus. Sex Transm.Dis. 2002;29:628-36.

48. Harper DM, Hildesheim A, Cobb JL, Greenberg M, Vaught J, Lorincz AT. Collection devices for human papillomavirus. J.Fam.Pract. 1999;48:531-35.

49. Brink AA, Meijer CJ, Wiegerinck MA, Nieboer TE, Kruitwagen RF, van KF 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;44:2518-23.

50. Cuschieri K, Wentzensen N. Human papillomavirus mRNA and p16 detection as biomarkers for the improved diagnosis of cervical neoplasia. Cancer Epidemiol. Biomarkers Prev. 2008;17:2536-45.

51. Gravitt PE, Lacey JV, Jr., Brinton LA, Barnes WA, Kornegay JR, Greenberg MD et al. Evaluation of self-collected cervicovaginal cell samples for human papillomavirus testing by polymerase chain reaction. Cancer Epidemiol.Biomarkers Prev. 2001;10:95-100.

52. Baldwin S, Santos C, Mendez BE, Nuno T, Giuliano A, Davis J et al. Comparison of type-specific human papillomavirus data from self- and clinician directed sampling. Gynecol.Oncol. 2005;97:612-17.

53. Lack N, West B, Jeffries D, Ekpo G, Morison L, Soutter WP et al. Comparison of non-invasive sampling methods for detection of HPV in rural African women. Sex Transm. Infect. 2005;81:239-41.

54. Lenselink CH, de Bie RP, van HD, 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;47:2564-70.

55. Belinson JL, Hu S, Niyazi M, Pretorius RG, Wang H, Wen C et al. Prevalence of type-specific human papillomavirus in endocervical, upper and lower vaginal, perineal and vaginal self-collected specimens: Implications for vaginal self-collection. Int.J.Cancer 2009.

56. Stenvall H, Wikstrom I, Backlund I, Wilander E. Accuracy of HPV testing of vaginal smear obtained with a novel self-sampling device. Acta Obstet.Gynecol.Scand. 2007;86:16-21.

57. Gustavsson I, Lindell M, Wilander E, Strand A, Gyllensten U. Use of FTA card for dry collection, transportation and storage of cervical cell specimen to detect high-risk HPV. J.Clin.Virol. 2009;46:112-16.

58. Castle PE, Rodriguez AC, Porras C, Herrero R, Schiffman M, Gonzalez P et al. A comparison of cervical and vaginal human papillomavirus. Sex Transm.Dis. 2007;34:849-55.

59. Castle PE, Schiffman M, Glass AG, Rush BB, Scott DR, Wacholder S et al. Human papillomavirus prevalence in women who have and have not undergone hysterectomies. J.Infect.Dis. 2006;194:1702-05.

60. Bhatla N, Dar L, Patro AR, Kumar P, Kriplani A, Gulati A et al. Can human papillomavirus DNA testing of self-collected vaginal samples compare with physician-collected cervical samples and cytology for cervical cancer screening in developing countries? Cancer Epidemiol. 2009;33:446-50.

61. Daponte A, Pournaras S, Mademtzis I, Hadjichristodoulou C, Kostopoulou E, Maniatis AN et al. Evaluation of high-risk human papillomavirus types PCR detection in paired urine and cervical samples of women with abnormal cytology. J.Clin.Virol. 2006;36:189-93.

62. De A, I, nton-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;17:2163-68.

63. Jones HE, Allan BR, van de Wijgert JH, Altini L, Taylor SM, de KA et al. Agreement between self- and clinician-collected specimen results for detection and typing of high-risk human papillomavirus in specimens from women in Gugulethu, South Africa. J.Clin.Microbiol. 2007;45:1679-83.

64. Karwalajtys T, Howard M, Sellors JW, Kaczorowski J. Vaginal self-sampling versus physician cervical sampling for HPV among younger and older women. Sex Transm. Infect. 2006;82:337-39.

65. Khanna N, Mishra SI, Tian G, Tan MT, Arnold S, Lee C et al. Human papillomavirus detection in self-collected vaginal specimens and matched clinician-collected cervical specimens. Int.J.Gynecol.Cancer 2007;17:615-22.

66. Longatto-Filho A, Roteli-Martins C, Hammes L, Etlinger D, Pereira SM, Erzen M et al. Self-sampling for human papillomavirus (HPV) testing as cervical cancer screening option. Experience from the LAMS study. Eur.J.Gynaecol.Oncol. 2008;29:327-32.

67. Seo SS, Song YS, Kim JW, Park NH, Kang SB, Lee HP. Good correlation of HPV DNA test between self-collected vaginal and clinician-collected cervical samples by the oligonucleotide microarray. Gynecol.Oncol. 2006;102:67-73.

68. Soisson AP, Reed E, Brown P, Ducatman B, Armistead J, Kennedy S et al. Self-test device for cytology and HPV testing in rural Appalachian women: an evaluation. J.Reprod.Med. 2008;53:441-48.

69. Sowjanya AP, Paul P, Vedantham H, Ramakrishna G, Vidyadhari D, Vijayaraghavan K et al. Suitability of self-collected vaginal samples for cervical cancer screening in periurban villages in Andhra Pradesh, India. Cancer Epidemiol.Biomarkers Prev. 2009;18:1373-78.

70. 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;14:34-42.

71. 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;34:371-77.

72. Moscicki AB, Widdice L, Ma Y, Farhat S, Miller-Benningfield S, Jonte J et al. Comparison of natural histories of human papillomavirus detected by clinician- and self-sampling. Int.J.Cancer 2010;127:1882-92.

73. Hillemanns P, Kimmig R, Huttemann U, Dannecker C, Thaler CJ. Screening for cervical neoplasia by self-assessment for human papillomavirus DNA. Lancet 1999;354:1970.

74. Wright TC, Jr., Denny L, Kuhn L, Pollack A, Lorincz A. HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer. JAMA 2000;283:81-86.

75. Petignat P, Hankins C, Walmsley S, Money D, Provencher D, Pourreaux K et al. Self-sampling is associated with increased detection of human papillomavirus DNA in the genital tract of HIV-seropositive women. Clin.Infect.Dis. 2005;41:527-34.

76. Palmisano ME, Gaffga AM, Daigle J, Brinkman J, Mire K, Lenczyk K et al. Detection of human papillomavirus DNA in self-administered vaginal swabs as compared to cervical swabs. Int.J.STD AIDS 2003;14:560-67.

77. Kahn JA, Slap GB, Huang B, Rosenthal SL, Wanchick AM, Kollar LM et al. Comparison of adolescent and young adult self-collected and clinician-collected samples for human papillomavirus. Obstet.Gynecol. 2004;103:952-59.

78. Agorastos T, Dinas K, Lloveras B, Font R, Kornegay JR, Bontis J et al. Self-sampling versus physician-sampling for human papillomavirus testing. Int.J.STD AIDS 2005;16:727-29.

79. Garcia F, Barker B, Santos C, Brown EM, Nuno T, Giuliano A et al. Cross-sectional study of patient- and physician-collected cervical cytology and human papillomavirus. Obstet.Gynecol. 2003;102:266-72.

80. Coutlee F, Hankins C, Lapointe N. Comparison between vaginal tampon and cervicovaginal lavage specimen collection for detection of human papillomavirus DNA by the polymerase chain reaction. The Canadian Women's HIV Study Group. J.Med. Virol. 1997;51:42-47.

81. Lorenzato FR, Singer A, Ho L, Santos LC, Batista RL, Lubambo TM et al. Human papillomavirus detection for cervical cancer prevention with polymerase chain reaction in self-collected samples. Am.J.Obstet. Gynecol. 2002;186:962-68.

82. Daponte A, Pournaras S, Mademtzis I, Hadjichristodoulou C, Kostopoulou E, Maniatis AN et al. Evaluation of HPV 16 PCR detection in self- compared with clinician-collected samples in women referred for colposcopy. Gynecol.Oncol. 2006;103:463-66.

83. Walraven G, Scherf C, West B, Ekpo G, Paine K, Coleman R et al. The burden of reproductive-organ disease in rural women in The Gambia, West Africa. Lancet 2001;357:1161-67.

84. Syrjanen K, Naud P, Derchain S, Roteli-

Martins C, Longatto-Filho A, Tatti S et al. Comparing PAP smear cytology, aided visual inspection, screening colposcopy, cervicography and HPV testing as optional screening tools in Latin America. Study design and baseline data of the LAMS study. Anticancer Res. 2005;25:3469-80.

85. Scott ME, Ma Y, Farhat S, Shiboski S, Moscicki AB. Covariates of cervical cytokine mRNA expression by real-time PCR in adolescents and young women: effects of Chlamydia trachomatis infection, hormonal contraception, and smoking. J.Clin.Immunol. 2006;26:222-32.

86. Wawer MJ, Gray RH, Sewankambo NK, Serwadda D, Paxton L, Berkley S et al. A randomized, community trial of intensive sexually transmitted disease control for AIDS prevention, Rakai, Uganda. AIDS 1998;12:1211-25.

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

88. Belinson JL, Qiao YL, Pretorius RG, Zhang WH, Rong SD, Huang MN et al. Shanxi Province cervical cancer screening study II: self-sampling for high-risk human papillomavirus compared to direct sampling for human papillomavirus and liquid based cervical cytology. Int.J.Gynecol.Cancer 2003;13:819-26.

89. Anhang R, Nelson JA, Telerant R, Chiasson MA, Wright TC, Jr. Acceptability of self-collection of specimens for HPV DNA testing in an urban population. J.Womens Health (Larchmt.) 2005;14:721-28.

90. Forrest S, McCaffery K, Waller J, Desai M, Szarewski A, Cadman L et al. Attitudes to self-sampling for HPV among Indian, Pakistani, African-Caribbean and white British women in Manchester, UK. J.Med. Screen. 2004;11:85-88.

91. Barata PC, Mai V, Howlett R, Gagliardi AR, Stewart DE. Discussions about self-obtained samples for HPV testing as an alternative for cervical cancer prevention. J.Psychosom. Obstet.Gynaecol. 2008;29:251-57.

92. Howard M, Lytwyn A, Lohfeld L, Redwood-Campbell L, Fowler N, Karwalajtys T. Barriers to acceptance of self-sampling for human papillomavirus across ethnolinguistic groups of women. Can.J.Public Health 2009;100:365-69.

93. Barbee L, Kobetz E, Menard J, Cook N, Blanco J, Barton B et al. Assessing the acceptability of self-sampling for HPV among Haitian immigrant women: CBPR in action. Cancer Causes Control 2009.

94. Szarewski A, Cadman L, shdown-Barr L, Waller J. Exploring the acceptability of two self-sampling devices for human papillomavirus testing in the cervical screening context: a qualitative study of Muslim women in London. J.Med.Screen. 2009;16:193-98.

95. 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;73:1001-05.

96. 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;14:95-105.

97. 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;100:451-54.

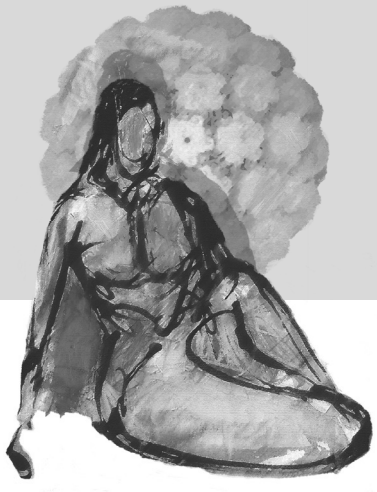
98. Stenvall H, Wikstrom I, Wilander E. High prevalence of oncogenic human papilloma virus in women not attending organized cytological screening. Acta Derm.Venereol. 2007;87:243-45.

99. Sanner K, Wikstrom I, Strand A, Lindell M, Wilander E. Self-sampling of the vaginal fluid at home combined with high-risk HPV testing. Br.J.Cancer 2009;101:871-74.

100. Wikstrom I, Stenvall H, Wilander E. Attitudes to self-sampling of vaginal smear for human papilloma virus analysis among women not attending organized cytological screening. Acta Obstet.Gynecol.Scand. 2007;86:720-25.

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

102. Pagliusi SR, Dillner J, Pawlita M, Quint WG, Wheeler CM, Ferguson M. Chapter 23: International Standard reagents for harmonization of HPV serology and DNA assays--an update. Vaccine 2006;24 Suppl 3:S3-193-S3/200.



Chapter 3

Prospective follow-up of 2065 young unscreened women to study Human papillomavirus incidence and clearance

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Submitted

Abstract

Background: Human papillomavirus (HPV) is a necessary factor in the development of cervical intraepithelial neoplasia and cervical cancer. However, HPV is also a very common sexually transmitted virus and many women clear their infection.

Objective: To analyze HPV type-specific incidence and clearance and its relation with age and sexual behavior in a group of young, unvaccinated, unscreened women.

Methods and Materials: 2065 women, aged 18-29 years, were followed for 12 months and were asked to provide a self-collected cervico-vaginal sample and fill-out a questionnaire every three months. For HPV DNA detection the SPF_{10}-DEIA $LiPA_{25}$ system was used.

Results: Incidence rates of any-type high-risk HPV and low-risk HPV were 17.0 per 1000-person months, and 14.3 per 1000-person months, respectively. HPV types 16, 52, 51, and 31 had the highest type specific incidence rates. Co-factors independently influencing HPV incidence were type of relationship, having a new relationship, number of lifetime sex partners, frequency of sexual contacts in the past 3 months, and condom use. The overall clearance of the newly detected type-specific high-risk HPV infections and low-risk HPV infections was 61.2% and 69.0%, respectively. Having a sexual relationship and sexual age independently influenced the clearance of any-type high-risk HPV. Women with HPV 16 and co-infection with other high-risk HPV types had a lower proportion of clearance than women only infected with HPV 16.

Conclusion: HPV incidence rates in this young Dutch study population are comparable to incidence rates in young women in other western countries and not related to age. Independent factors significantly influencing incidence and clearance were all related to past or current sexual behavior.

Introduction

It is generally accepted that a persistent human papillomavirus (HPV) infection is the necessary cause of cervical cancer. Acquisition of an HPV infection is therefore the main precursor of the series of events that eventually lead to cervical cancer.[1-4] Fortunately, most of the acquired HPV infections are transient and clear spontaneously within 12 months after first detection, and about 90% of the women will have cleared their HPV infection within 24 months.[5-13]

HPV is a very common sexually transmitted virus, mainly acquired early in sexual life.[14;15] Rates of acquiring an HPV infection are high following first sexual intercourse, and with each new sexual partner.[7;16;17] The risk factors associated with acquiring an HPV infection are therefore mainly related to the individuals sexual behavior.[18]

HPV prevalence, however, is not only influenced by sexual activity but also varies by age and geography, as shown by the International Agency for Research on Cancer (IARC).[19] Young women have high rates of HPV acquisition, and incidence tends to decline with age.[10;17;20;21] Whether age is also related to the duration of the infection remains controversial. Some studies report that there is no influence of age on the clearance rate of the HPV infection.[10;22;23] Whereas other studies report a lower clearance rate with increasing age,[24;25] or in contrary a faster clearance rate in older women (> 45 year vs. < 25 year old women).[8] These different study results are probably the result of variation in study populations with respect to age, geography and other risk factors related to cervical carcinoma development, as well as the sensitivity of the HPV DNA test used.

Although the majority of type-specific HPV infections clear within 24 months, some women may become HPV positive again for the same HPV type after one or more negative test results. These women are either newly infected by a new sexual partner with the same HPV genotype, re-infected by their own partner or have a reactivation of their initial infection.[9]

The objective of this 12-month prospective cohort study of young, unscreened, and unvaccinated women is to analyze HPV type-specific incidence and clearance and its relation to age and sexual behavior.

Methods and Materials

This study covers 12 months follow-up of a large prospective cohort study on HPV prevalence, incidence and clearance, performed in the Netherlands from 2007–2010. At study entry, 2065 unscreened women 18-29 years of age were included, as described previously.[26] In the 12-month follow-up period, women were asked to provide five self-samples with a 3-month interval (study month 0, 3, 6, 9, and 12). All women received a self-sample kit and the additional questionnaires by mail and performed the cervico-vaginal self-sample in the privacy of their own home. Written informed consent was obtained from all participants. This study was approved by the Local Medical Ethics Committee.

The self-sample kit contained an explanatory letter, a questionnaire, an illustrated instruction form on how to perform the cervico-vaginal self-sample, a small brush in a

sterile cover (Rovers Vibabrush®, Rovers Medical Devices, Oss, the Netherlands), and a collection tube containing medium (SurePath™, Tripath Imaging®, Inc., Burlington NC, U.S.A.).[26;27]

Broad-spectrum HPV DNA amplification was performed using a short-PCR-fragment assay. Extracted DNA was used for PCR amplification with the SPF_{10}primer sets.[28;29] The samples were run through an HPV DNA enzyme immunoassay (DEIA) to obtain an OD reading, and categorized as HPV DNA negative, positive, or borderline. In the DEIA essay a cocktail of 9 general probes are used to identify at least 54 different HPV types in the anogenital region. Amplimers from SPF_{10}-DEIA-positive samples were used to identify the HPV genotype by reverse hybridization on a line probe assay ($LiPA_{25}$) ($SPF_{10}HPVLiPA_{25}$, version 1; Labo Bio-Medical Products B.V., Rijswijk, the Netherlands), which detects 25 HPV genotypes: high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68/73 and low-risk HPV types: type 6, 11, 34, 40, 42, 43, 44, 53, 54, 66, 70, and 74. Furthermore an HPV group "X" is identified (DEIA positive and LiPA negative samples) and contains mucosal and cutaneous HPV types not included in the type specific list, and considered to be low-risk HPV.

Statistical analysis

Incidence was defined by the cumulative number of newly detected HPV per 1000-person months. When analyzing incidence for a specific HPV type, women had to be negative for that type-specific HPV type at baseline to be included in the analysis. The type-specific positive women could still be included in the analysis of the other HPV types, therefore baseline HPV positive women were not excluded from the study.

HPV types were considered cleared during follow-up when a positive test was followed by two consecutive negative tests for the same HPV type. The duration of infection was defined as the time from first detection of the HPV type to the first negative test for the same HPV type. When an HPV type (either prevalently or newly detected) was considered cleared, but again detected during follow-up it was defined as a re-infection or reactivation of that certain HPV type. The re-infection/reactivation rates per 1000-person months of any-type hr-HPV and lr-HPV were lower when clearance was defined as having two consecutive HPV negative samples compared to when clearance was defined as having an infection followed by one negative sample, 30.8 [95% CI: 19.1-49.5] and 39.5 [95% CI: 27.1-57.7] per 1000-person months, compared to 60.0 [95% CI: 49.0-73.4] and 51.7 [95% CI: 41.6-64.2] per 1000-person months. The definition of clearance, a positive test followed by two consecutive negative tests, gives therefore more certainty of clearance. This supports the use of the definition of clearance, a positive test followed by two consecutive negative, in an epidemiological study like ours.

To identify any potential factors influencing incidence and clearance the following factors were analyzed; age (baseline), smoking (baseline), oral contraceptive pill (OCP) use, age at first sexual intercourse, sexual age (number of sexually active years) (baseline), type of sexual relationship, having a new sexual partner, lifetime number of sexual

partners, gender of sexual partner(s), number of sexual partners in the past 3 months, number of sexual contacts in the past 3 months, ever diagnosed with an STI, condom use, and having multiple HPV infections.
The categorical variables were individually analyzed in univariate analysis using log-rank testing, for the continuous variables age and sexual age univariate cox-regression testing was used. Multivariate analysis was performed using cox-regression analysis with forward stepwise selection. A variable entered the model when its p-value was <0.15. All statistical analysis was performed using Stata/IC (version 11.2 for Windows; StataCorp LP, College Station, TX, USA).
Due to small numbers of re-infection/reactivation, potential factors of influence could not be analyzed.

Results

Of the 2065 women initially included, 1871 (90.6%) completed 12 months of follow-up. The median follow-up time was 12.3 months [0.0-17.0], with a median period of 3.0 months [1.4-15.0] between samples. A total of 1798 women provided all 5 samples, and 267 women had one or more missing samples (total 541 missing samples).
The mean age of the women was 23.5 years [18-29]. At baseline almost all women (94.3%) were sexually active and the mean sexual age (i.e. years of being sexually active) was 6.8 years [0-23]. Previously, the baseline demographic characteristics have been described extensively.[26]

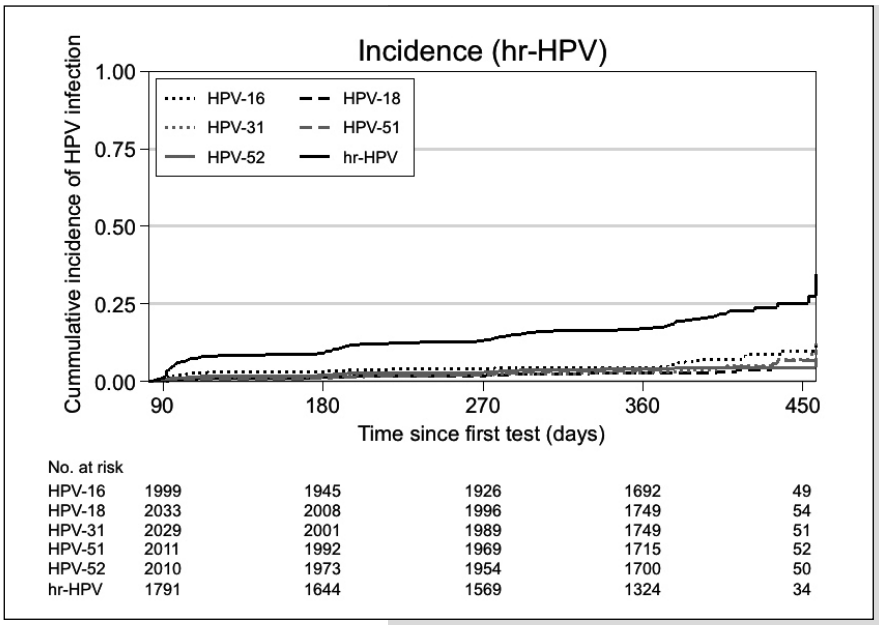
Human papillomavirus incidence

HPV type-specific incidence is shown in Table 1. There were 687 type-specific hr-HPV incident infections in total and 482 type-specific lr-HPV infections. The incidence rate of any-type hr-HPV was 17.0 infections per 1000-person months. Hr-HPV types 16, 52 51, 31 and 18 have the highest incidence rates, 4.8, 3.5, 3.4, 3.0, and 2.4 per 1000-person months, respectively. Figure 1 shows the Kaplan-Meier curves of the cumulative incidence rates of any-type hr-HPV and the five most prevalent hr-HPV types (HPV 16, 51, 52, 31, and 18). This figure shows that during the 12 months of follow-up the cumulative incidence of the five most prevalent hr-HPV types is almost as high as the incidence rate of any-type hr-HPV. Indicating that the remaining hr-HPV types only have a small contribution to hr-HPV incidence.
The incidence rate of any-type lr-HPV was 14.3 infections per 1000-person months. Generally, hr-HPV infections have a higher incidence rate than lr-HPV infections. The 95% confidence interval for the difference between the incidence rates of any-type hr-HPV and any type lr-HPV does not contain the value zero [95% CI: 0.37-5.15] and is therefore significant. The lr-HPV types 53, 66, 6, 54, and 74 have the highest incidence rates, 2.9, 2.4, 1.9, 1.7, and 1.3 per 1000-person months, respectively. The Kaplan-Meier curves of incidence rate of any-type lr-HPV and the five most prevalent lr-HPV types, 66, 53, 54, 6, and 42 are displayed in Figure 2.

Table 1.
HPV type-specific prevalence at baseline and incidence

HPV-type	Prevalent infections N (%)	Total person months at risk	Incident infections N	Incidence rate per 1000-person months (95% CI)
Hr-HPV*	254 (12.3)	20445.0	348	17.0 (15.3-18.9)
16	57 (2.8)	24356.6	118	4.8 (4.0-5.8)
18	30 (1.5)	25072.8	59	2.4 (1.8-3.0)
31	31 (1.5)	25008.7	74	3.0 (2.4-3.7)
33	18 (0.9)	25473.6	23	0.9 (0.6-1.4)
35	6 (0.3)	25625.6	17	0.7 (0.4-1.1)
39	20 (1.0)	25259.1	54	2.1 (1.6-2.8)
45	3 (0.1)	25636.9	25	1.0 (0.7-1.4)
51	51 (2.5)	24763.6	85	3.4 (2.8-4.2)
52	52 (2.5)	24599.2	86	3.5 (2.8-4.3)
56	15 (0.7)	25391.6	50	2.0 (1.5-2.6)
58	6 (0.3)	25541.6	31	1.2 (0.9-1.7)
59	9 (0.4)	25578.1	22	0.9 (0.6-1.3)
68/73	12 (0.6)	25428.4	43	1.7 (1.3-2.3)
Lr-HPV#	179 (8.7)	21800.2	311	14.3 (12.8-15.9)
6	12 (0.6)	25352.6	47	1.9 (1.4-2.5)
11	4 (0.2)	25697.3	8	0.3 (0.2-0.6)
34	0 (0.0)	25755.3	9	0.3 (0.2-0.7)
40	3 (0.1)	25701.0	12	0.5 (0.3-0.8)
42	5 (0.2)	25627.9	25	1.0 (0.7-1.4)
43	1 (0.0)	25698.8	19	0.7 (0.5-1.2)
44	2 (0.1)	25648.8	23	0.9 (0.6-1.3)
53	35 (1.7)	24982.3	73	2.9 (2.3-3.7)
54	14 (0.7)	25398.6	44	1.7 (1.3-2.3)
66	39 (1.9)	24996.4	61	2.4 (1.9-3.1)
70	4 (0.2)	25687.2	14	0.5 (0.3-0.9)
74	3 (0.1)	25613.1	34	1.3 (0.9-1.9)
X	72 (3.5)	24374.1	113	4.6 (3.9-5.6)

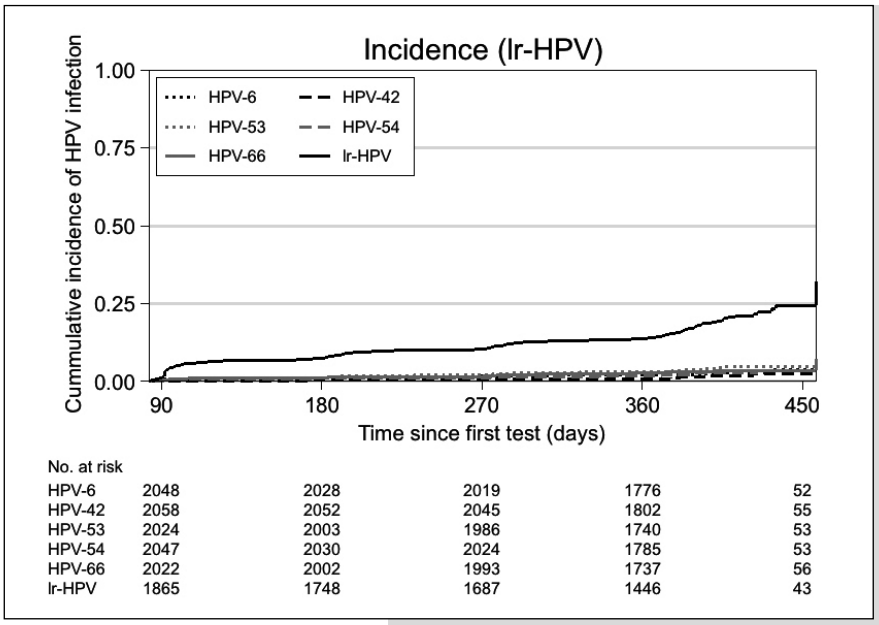
n number;
* Any-type hr-HPV;
\# Any-type lr-HPV
Total type-specific incident hr-HPV infections: 687
Total type-specific incident lr-HPV infections: 482



No. at risk

HPV-16	1999	1945	1926	1692	49
HPV-18	2033	2008	1996	1749	54
HPV-31	2029	2001	1989	1749	51
HPV-51	2011	1992	1969	1715	52
HPV-52	2010	1973	1954	1700	50
hr-HPV	1791	1644	1569	1324	34

Figure 1



No. at risk

HPV-6	2048	2028	2019	1776	52
HPV-42	2058	2052	2045	1802	55
HPV-53	2024	2003	1986	1740	53
HPV-54	2047	2030	2024	1785	53
HPV-66	2022	2002	1993	1737	56
lr-HPV	1865	1748	1687	1446	43

Figure 2

Only risk factors associated with sexual behavior; the type of sexual relationship, having a new sexual partner in the past 3 months, the lifetime number of sexual partners, the number of sexual contacts in the past 3 months and condom use, independently influenced HPV incidence, as shown in Table 2.

Couples living apart (HR: 0.3 [95% CI: 0.1-1.0]), living together (HR: 0.4 [95% CI: 0.1-1.0]), or who are married (HR: 0.3 [95% CI: 0.1-0.9]) have a lower risk of acquiring any-type hr-HPV, compared to being single. Married women also have a lower risk (HR: 0.2 [95% CI: 0.0-0.7]) of acquiring any-type lr-HPV infection compared to single women. Having a new sexual relationship in the past 3 months increases the risk of acquiring any-type hr-HPV (HR: 2.6 [95% CI: 1.7-3.9]), HPV 18 (HR: 4.5 [95% CI: 2.1-9.5]) or any-type lr-HPV (HR: 2.2 [95% CI: 1.4-3.6]).

Higher numbers of lifetime sexual partners increase the risk of having a newly detected HPV type. Compared to having 1 lifetime sexual partner at baseline, women with 2-5, 6-10, and >10 lifetime sexual partners have an increased risk for acquiring any-type hr-HPV (HR: 1.5 [95% CI:1.0-2.3], HR: 1.8 [95% CI: 1.1-2.8], and HR: 3.6 [95% CI: 2.1-6.2], respectively), for acquiring any-type lr-HPV (HR: 1.5 [95% CI: 0.9-2.2], HR: 2.4 [95% CI:1.5-3.9], HR: 2.4 [95% CI:1.4-4.2], respectively), and for acquiring HPV 16 (HR: 2.1 [95% CI: 1.0-4.3], HR: 3.0 [95% CI:1.4-6.3], and HR: 4.3 [95% CI: 1.9-9.4], respectively). Additionally, compared to women with 1 lifetime sexual partner at baseline, women with 6-10 partners had an increased risk (HR: 3.7 [95% CI:1.0-13.3]) for acquiring HPV 18. This risk was not significantly different compared to women with 2-5 and >10 lifetime sexual partners.

Compared to women with 1-6 sexual contacts in the past 3 months, frequent sexual contacts (>54 times) had a higher risk of having a newly detected any-type hr-HPV (HR: 2.8 [95% CI: 1.5-5.2]). Condom use only influenced a woman's risk of acquiring any-type lr-HPV, but not the acquisition of hr-HPV. The risk of having a newly detected any-type lr-HPV infection was lower (HR: 0.6 [95% CI: 0.3-1.0]) in women using condoms most of the times (>50%) compared to women who never used condoms.

Figure 3 displays the relation of age with the detection of a newly acquired hr-HPV type, in this young group of women. Within the Cox-regression model, where age was analyzed as a continuous variable and not categorical, the relation between age and acquiring a new hr-HPV type was not significant. However, women aged 24-29 years (except for women 26 years) have a higher hr-HPV incidence rate compared to women 18-23 years of age (p-value < 0.0001).

Table 2.
Factors independently associated with acquiring HPV

	Any-type Hr-HPV		HPV 16		HPV 18		Any-type Lr-HPV	
	HR [95% CI]	P-value	HR [95% CI]	P-value	HR [95% CI]	P-value	HR [95% CI]	P-value
Relationship								
Single	1.0		NS	NS	NS	NS	1.0	
Married	0.3 [0.1-1.0]	0.047	NS	NS	NS	NS	0.2 [0.0-0.7]	0.012
Living together	0.4 [0.1-1.0]	0.044	NS	NS	NS	NS	0.4 [0.1-1.4]	0.151
Couple living apart	0.3 [0.1-0.9]	0.025	NS	NS	NS	NS	0.6 [0.2-1.9]	0.351
New sexual relationship								
No	1.0		NS	NS	1.0		1.0	
Yes	2.6 [1.7-3.9]	<0.001	NS	NS	4.5 [2.1-9.5]	<0.001	2.2 [1.4-3.6]	0.001
Lifetime sex partners (N)*								
1	1.0		1.0		1.0		1.0	
2-5	1.5 [1.0-2.3]	0.042	2.1 [1.0-4.3]	0.038	1.9 [0.5-6.5]	0.332	1.5 [0.9-2.2]	0.089
6-10	1.8 [1.1-2.8]	0.021	3.0 [1.4-6.3]	0.005	3.7 [1.0-13.3]	0.043	2.4 [1.5-3.9]	<0.001
>10	3.6 [2.1-6.2]	<0.001	4.3 [1.9-9.4]	<0.001	3.8 [1.0-15.1]	0.055	2.4 [1.4-4.2]	0.001
N sex contacts < 3 months								
1-6	1.0		NS	NS	1.0		NS	NS
7-24	1.0 [0.6-1.8]	0.870	NS	NS	0.2 [0.1-0.9]	0.029	NS	NS
25-54	1.2 [0.7-2.1]	0.402	NS	NS	0.8 [0.3-1.9]	0.538	NS	NS
>54	2.8 [1.5-5.2]	<0.001	NS	NS	0.9 [0.3-3.1]	0.902	NS	NS
Condom use								
Never	NS	NS	NS	NS	NS	NS	1.0	
Always	NS	NS	NS	NS	NS	NS	0.7 [0.4-1.3]	0.302
Sometimes (<50%)	NS	NS	NS	NS	NS	NS	0.9 [0.6-1.3]	0.423
Most times (>50%)	NS	NS	NS	NS	NS	NS	0.6 [0.3-1.0]	0.044

Cox regression multivariate analysis; HR: Hazard ratio's; CI: confidence interval
Factors not included in the table do not significantly influence acquiring a newly detected HPV infections: age, smoking, oral contraceptive pill (OCP) use, age at first sexual intercourse, sexual age (number of sexually active years), gender of sexual partner(s), number of sexual partners in the past 3 months, ever diagnosed with an STI, and having multiple HPV infections.
NS=not significantly associated with HPV incidence
* baseline data

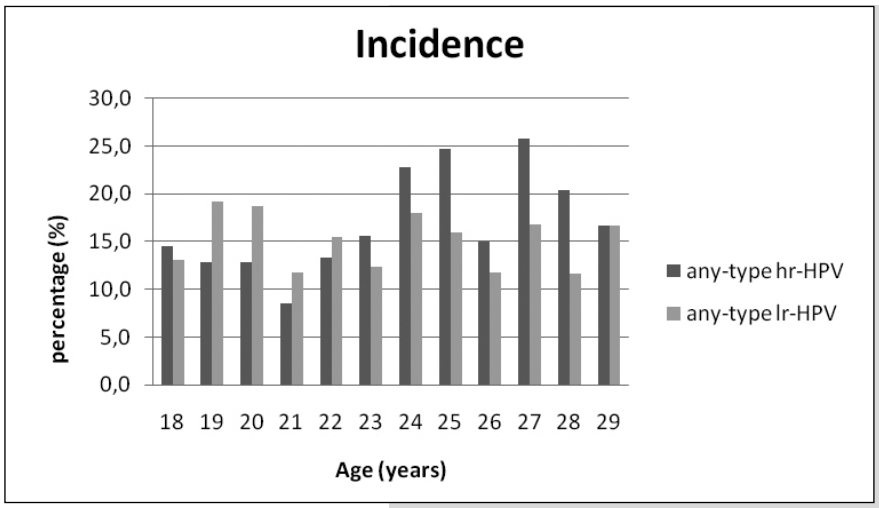


Figure 3: Hr- en lr-HPV incidence and age.
Incidence: proportion of subjects with an incident infection

Human papillomavirus clearance

In the 12-month follow-up of this young group of women an overall clearance proportion of 61.2% [95% CI: 56.3-65.9] for type specific hr-HPV infections and 69.0% [95% CI: 63.1-74.5] for lr-HPV infections was found. Table 3 shows the type-specific time to clearance of incident infections. Overall, the clearance proportion between hr-HPV types and lr-HPV types was not significantly different.

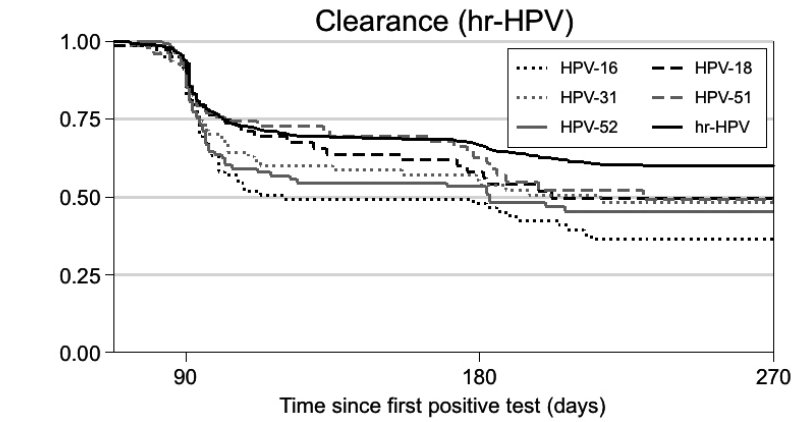
Fifty three percent of the incident any-type hr-HPV infections cleared with a median time to clearance of 6.4 months. This clearance was significantly higher than the clearance of prevalently detected (i.e. already present at the first sample) any-type hr-HPV infections, who had a median clearance time of 9.0 months (data not shown). The clearance proportions of incident hr-HPV types 16, 51, 52, 31, and 18 were 62.0%, 44.7%, 63.8%, 61.4%, and 59.5%, respectively, with a median time to clearance of 3.9, not estimable (NE), 3.2, 3.7, and 5.8 months, respectively. Figure 4 displays the Kaplan-Meier curves of the clearance of any-type hr-HPV and HPV 16, 51, 52, 31, and 18. The figure shows that HPV 16 had the highest clearance and that the clearance of HPV 18, 31, and 51 are almost identical after six months of follow-up.

Sixty percent of the incident any-type lr-HPV infections cleared after a median time of 3.5 months, which was faster, however not significant, than the median time to clearance (6.0 months) of prevalently detected any-type lr-HPV (data not shown). The clearance rates of incident lr-HPV types, 66, 53, 54, 6, and 42 were 61.3%, 56.1%, 54.2%, 81.3%, and 64.3% respectively, with a median time to clearance of 3.5, 3.4, 5.9, 3.2, and 3.0 months, respectively. Figure 5 displays the Kaplan-Meier curves of the clearance of any-type hr-HPV and HPV 66, 53, 54, 6, and 42. The figure shows that HPV 6 clears twice as fast as any-type lr-HPV.

Table 3.
HPV type-specific clearance

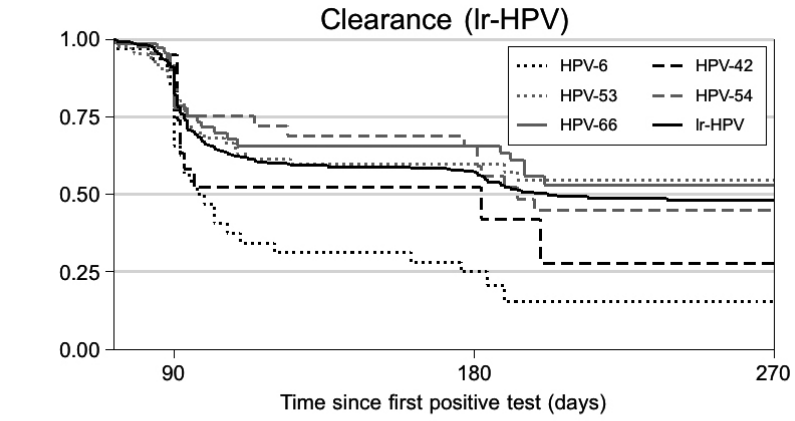
HPV-type	Incident infections N	Clearance portion N (%) [95% CI]	Time (months) Median [95% CI]
Hr-HPV*	233	123 (52.8) [46.2-59.3]	6.4 [3.9-NE]
16	79	49 (62.0) [50.4-72.7]	3.9 [3.3-7.0]
18	37	22 (59.5) [42.1-75.2]	5.8 [3.4-NE]
31	44	27 (61.4) [45.5-75.9]	3.7 [3.1-NE]
33	9	4 (44.4) [13.7-78.8]	NE [2.7-NE]
35	11	6 (54.5) [23.4-83.3]	6.1 [3.0-NE]
39	31	16 (51.6) [33.1-69.8]	7.5 [3.0-NE]
45	16	8 (50.0) [24.7-75.3]	3.9 [3.0-NE]
51	38	17 (44.7) [28.6-61.7]	NE [3.1-NE]
52	58	37 (63.8) [50.1-76.0]	3.2 [3.1-5.6]
56	26	18 (69.2) [48.2-85.7]	3.1 [3.0-6.7]
58	22	16 (72.7) [49.8-89.3]	3.0 [3.0-4.4]
59	15	12 (80.0) [51.9-95.7]	3.0 [3.0-3.8]
68/73	26	20 (76.9) [56.4-91.0]	3.2 [3.0-3.9]
Lr-HPV#	190	113 (59.5) [52.1-66.5]	3.5 [3.2-6.2]
6	32	26 (81.3) [63.6-92.8]	3.2 [3.0-3.9]
11	6	2 (33.3) [4.3-77.7]	NE [3.0-NE]
34	7	5 (71.4) [29.0-96.3]	3.7 [3.0-NE]
40	8	8 (100.0) [63.1-100]	3.0 [2.8-3.2]
42	14	9 (64.3) [35.1-87.2]	3.1 [3.0-NE]
43	11	11 (100.0) [71.5-100]	3.1 [2.8-3.6]
44	16	11 (68.8) [41.3-89.0]	3.0 [2.8-NE]
53	41	23 (56.1) [39.7-71.5]	3.4 [3.0-NE]
54	24	13 (54.2) [32.8-74.4]	5.9 [3.0-NE]
66	31	19 (61.3) [42.2-78.2]	3.5 [3.0-NE]
70	8	5 (62.5) [24.5-91.5]	3.2 [2.8-NE]
74	17	11 (64.7) [38.3-85.8]	3.1 [3.0-NE]
X	56	44 (78.6) [65.6-88.4]	3.0 [3.0-3.2]

n: number; NE: not estimable; *: Any-type hr-HPV; #: Any-type lr-HPV
Mean clearance type-specific hr-HPV: 61.2% [56.3-65.9]
Mean clearance type-specific lr-HPV: 69.0% [63.1-74.5]



No. incident infections			
HPV-16	72	37	20
HPV-18	64	30	17
HPV-31	68	34	22
HPV-51	87	35	13
HPV-52	99	43	24
hr-HPV	454	274	160

Figure 4



No. incident infections			
HPV-6	27	7	2
HPV-42	18	5	2
HPV-53	68	29	13
HPV-54	37	21	10
HPV-66	62	30	17
lr-HPV	314	144	73

Figure 5

Table 4.
Factors independently associated with HPV clearance of incident infections

	Any-type Hr-HPV		HPV 16		HPV 18		Any-type Lr-HPV	
	HR [95% CI]	P-value	HR [95% CI]	P-value	HR [95% CI]	P-value	HR [95% CI]	P-value
Relationship								
Single	1.0		NS	NS	NS	NS	1.0	
Married	7.3 [3.3-15.7]	<0.001	NS	NS	NS	NS	1.6 [0.6-4.8]	0.361
Living together	4.0 [2.2-7.3]	<0.001	NS	NS	NS	NS	1.8 [1.1-3.1]	0.028
Couple living apart	2.9 [1.6-5.0]	<0.001	NS	NS	NS	NS	2.1 [1.3-3.4]	0.003
Sexual age#	0.9 [0.9-1.0]	0.031	NS	NS	NS	NS	NS	NS
Lifetime sex partners (N)								
1	NS	NS	NS	NS	1.0		NS	NS
2-5	NS	NS	NS	NS	0.1 [0.0-0.8]	0.029	NS	NS
6-10	NS	NS	NS	NS	0.3 [0.1-1.6]	0.169	NS	NS
>10	NS	NS	NS	NS	0.2 [0.0-1.3]	0.100	NS	NS
Multiple hr-HPV infections								
No	NE	NE	1.0		NS	NS	NE	NE
Yes	NE	NE	0.1 [0.0-0.6]	0.007	NS	NS	NE	NE

Cox regression multivariate analysis; HR: Hazard ratio's; CI: confidence interval; NS=not significantly associated with HPV clearance; NE= not estimable; none of the women infected with more than 1 infection were cleared from all of their infections Factors not included in the table do not significantly influence acquiring a newly detected HPV infections: age, smoking, OCP use, age at first sexual intercourse, having a new sexual partner, gender of sexual partner(s), number of sexual contacts in the past 3 months, ever diagnosed with an STI, and condom use.
* baseline data
# number of years being sexually active

The clearance of both any-type hr-HPV and any-type lr-HPV is not affected by age in this group of young women (see Figure 6). Factors that did independently influence clearance of newly detected HPV types are shown in Table 4. This table shows that women with increasing sexual age (i.e, the number of sexual active years) are less likely to clear their infection (HR: 0.9 [95% CI: 0.9-1.0]). Additionally, women with a sexual relationship, married, living together or a couple living apart are more likely to clear their any-type hr-HPV infection compared to single women (HR: 7.3 [95% CI: 3.3-15.7], HR: 4.0 [95% CI:2.2-7.3], and HR: 2.9 [95% CI: 1.6-5.0], respectively). Women with a sexual relationship, either living together or a couple living apart were also more likely to clear their any-type lr-HPV infection compared to single women (HR: 1.8 [95% CI: 1.1-3.1] and HR: 2.1 [95% CI: 1.3-3.4], respectively).
The only factor influencing the clearance of HPV 16 was whether a woman had multiple hr-HPV infections or not. If a woman had multiple hr-HPV infections she was not likely

Figure 6: Hr- en lr-HPV clearance and age

to clear her HPV 16 infection (HR: 0.1 [95% CI: 0.0-0.6]). The clearance of HPV 18, however, was only influenced by the number of lifetime sexual partners. Women with 2-5 lifetime sexual partners at baseline compared to women with 1 lifetime sexual partner were less likely to clear their HPV 18 infection (HR: 0.1 [95% CI: 0.0-0.8). This lower clearance rate ceased when a woman had more lifetime sexual partners.
For the prevalently detected HPV types (i.e. already present at the first sample), clearance of any-type hr-HPV infections, HPV 16 and HPV 18 was not related to a woman's sexual behavior. However, the clearance of any-type lr-HPV was associated with the type of sexual relationship. Women living together with their sexual partner had a higher clearance rate compared to single women (HR 1.9 [95% CI 1.1-3.2]). (Data not shown in table)

Human papillomavirus re-infection/reactivation
The re-infection/reactivation rates of any-type hr-HPV and any-type lr-HPV were 30.8 and 39.5 per 1000-person months, respectively. These re-infection/reactivation rates of the type-specific hr-HPV infections and lr-HPV infections are based on small numbers and vary from 0.0 to 41.1 and 0.0 to 87.5 infections per 1000-person months, respectively. Notable, no re-infection/reactivation is detected in this one year follow-up study for the hr-HPV types 35, 45, 56, and 59, and the lr-HPV types 6, 11, 34, 40, 42, 70.
Due to the small rates of re-infection/reactivation we were not able to identify any risk factors associated to re-infection or reactivation of HPV.

Discussion

This is the first Dutch prospective epidemiological study analyzing the incidence and clearance of HPV among unscreened young women in the pre-vaccine era. This study therefore provides important baseline information on the natural behavior of HPV for future studies analyzing the impact of HPV vaccination on HPV incidence and clearance and whether type-replacement and cross-protection takes place.

Incidence

The incidence of any-type hr-HPV in this prospective 12-month follow-up study of 2065 young women was 17.0 per 1000-person months. This was significantly higher than the incidence of any-type lr-HPV, which was 14.3 per 1000-person months. Coinciding incidence rates of hr-HPV, 14.0 per 1000-person months, and lr-HPV, 12.4 per 1000-person months were reported in a follow-up study of female university students.[9] In the past, several other studies also found a higher incidence of hr-HPV types compared to the incidence of lr-HPV types.[13;30] Whereas Goodman et al. reported the same incidence rate for hr-HPV and lr-HPV.[8] In this study population, HPV types 16, 51, 52, and 31 had the highest type specific incidence rates, which has also been reported in other studies.[7-9;30;31]

We found that sexual behavior related co-factors were the only factors independently influencing HPV incidence, this supports the conclusion of previous studies that HPV is basically a sexually transmitted disease.[18;32] Interestingly, incidence was related to both recent as well as past sexual behavior factors. The recent sexual behavior factors were, type of relationship, having a new relationship, frequency of sexual contacts in the past 3 months, and condom use. These factors have been described before in other studies and fit in with the concept that with new sexual partners, or risk-full sexual behavior the chances of acquiring a new HPV type are increased.[16;18] Additionally, HPV incidence was related to the number of lifetime sex partners, which in fact is past sexual behavior. The number of lifetime sex partners was an independent factor related to the incidence of any-type hr-HPV, any-type lr-HPV, as well as to type specific HPV 16 and HPV 18 incidence. Several other studies also associated past sexual behavior with HPV incidence.[14;16;33-35] Generally, one would expect past sexual behavior to be related to HPV prevalence (as shown before by our group) but not to HPV incidence.[26] A possible explanation could be that women with a high number of sexual partners in the past may potentially date men who also have a high number of sexual partners in the past, a group likely to have a higher HPV prevalence, and therefore increase the risk of acquiring HPV. However, another possible explanation for the influence of past sexual behavior on current HPV incidence may be that the infection was acquired in the past and has remained present in levels of low copy numbers, normally not detectable by current HPV DNA detection methods (i.e. viral latency). Accidental detection of this latent virus will thus be regarded as an incident infection, while in fact it is a non-productive virus residing in cells, or a reactivated previous infection. Past sexual behavior is related to past HPV incidence, and

thus related to the risk of having one or multiple latent HPV infections. So, the probability to detect a latent HPV accidentally, or detect a re-activated HPV infection, mimicking an incident HPV infection, is increased with increasing number of lifetime sexual partners. Therefore, we can only measure presumed incidence because it is presently impossible to distinguish a new infection from an accidental pick-up or reactivation of a latent infection.[13;36]

Although women aged 24-29 years had a higher incidence rate compared to 18-23 years old women, age was not an independent risk factor for HPV incidence. When analyzing the age groups compared to the variable 'having a new sexual partner during follow-up', women in the age group 24-29 years have more new sexual partners compared to the younger women (p-value = 0.145). This may potentially explain why age as continuous factor is not an independent factor influencing HPV incidence in this young group of women.

Some studies, however, do report a decline of incidence with increasing age in women <30 years, but they found the lowest risk for women 30 years and older.[21;36]

Clearance

In this 12-month follow-up study the overall clearance rate of the newly detected (i.e. incident) hr-HPV and lr-HPV infections was 61.2% and 69.0%, respectively. Confirming the finding that most incident HPV types are not detected 6-12 months later.[14]

The overall clearance rate of hr-HPV types and lr-HPV types was not significantly different. Other studies, however, often reported a higher clearance rate for lr-HPV.[9;13;21] This may possibly be explained by the duration of follow-up, because it was found that the difference between the clearance rate of lr-HPV and hr-HPV types became significant after a follow-up of 12 months.[13]

The time to clearance was not significantly different between incident hr-HPV and lr-HPV infections in this study, varying from 3.0-7.5 months for hr-HPV types and 3.0-5.9 months for lr-HPV types. Several studies, on the other hand, have reported that infections with hr-HPV types tend to have a longer duration compared to lr-HPV types.[9;13;21;37] Especially HPV 16 and associated types like HPV 31, tend to persist longer than other genotypes.[9;15;21;25;38]

Factors independently related to HPV clearance were type of sexual relationship, sexual age, and number of lifetime sexual partners. Single women had a lower clearance of hr-HPV and lr-HPV infections compared to women with a partner, or living together/ married. Women, living together with their sexual partner or married, may probably have more accidental pick-up of latent HPV. An accidental pick-up is much more likely to be detected only once and considered cleared during follow-up, whereas single women may have more new (true incident) infections, likely to remain detected for a longer duration before the immune system clears the infection, or before it is maintained in a latent state. Furthermore, the fact that an increasing sexual age decreases the clearance rate of any-type hr-HPV, and that an increasing number of lifetime sexual partners decreases the clearance of HPV 18, underlines the influence of past sexual behavior on current HPV

dynamics.
Interestingly, the clearance rate of HPV 16 is not related to sexual behavior in this study. Women with multiple HPV infections were less likely to clear their HPV 16 infection. The fact that multiple HPV types are detected may indicate that the host immune system is failing in some respect, which may be responsible for a slower clearance of HPV 16 in these patients. This relation may also exist for other type-specific HPV infections, but the numbers were too small in this study to analyze this for all individual HPV types. The presence of multiple infections in a woman may potentially be an indicator of a (partially) failing immune system.
No relation between age and HPV clearance was found in this young study population. This was probably due to the young age of the women and the small age range. In literature some studies report that women, older than 45 years clear their HPV infections faster than women <25 years,[8] and vice versa.[25] However, many other studies did not find a difference in clearance among different age groups.[10;22]
Factors like OCP use, condom use and smoking, reported by some authors to be associated with HPV clearance and persistence were not identified as independent risk factors within this study population.
Considering that we only found either past sexual behavior to influence clearance or having multiple infections, we suggest that HPV clearance is likely to be mainly related to the host immune response, or other intrinsic host factors and not to present behavior factors.
Previous studies showed that detection or reactivation of latent HPV exists, even in the absence of sexual activity. For example, Trottier et al. found that in women >40 years of age, 39.2% with no new sexual partners and 71,4% with new sexual partners became HPV-positive during follow-up.[35;39] The risk of reactivation was found highest in immune compromised women.[40;41] This supports our theory that newly detected HPV types must be distinguished in true incident infections, accidental pick-up of latent virus, and re-activation of latent virus. Only when we are able to identify these subgroups, true HPV incidence and clearance data can be studied in relation to behavioral factors. This will be the challenge for future epidemiological HPV studies.
In conclusion, HPV incidence rates in this young Dutch study population are comparable to incidence rates in young women from other western countries. HPV types 16, 51, 52, 31, and 18 had the highest type specific incidence rates. The high clearance rates and short duration of infections in this study confirm that most HPV infections are cleared within 6-12 months. The fact that incidence and clearance were not only related to current sexual behavior, but also to past sexual behavior indicates that accidental pick-up or re-activation of a latent HPV may be common. HPV clearance is probably more related to host immune factors, as clearance did show a relation with multiple infections. Future studies should therefore try to distinguish accidental pick-up, or reactivation, from true HPV incidence in order to be able to investigate the influence of intrinsic host factors on HPV dynamics.

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;348:518-27.
2. Schiffman MH, Bauer HM, Hoover RN, Glass AG, Cadell DM, Rush BB et al. Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. J.Natl.Cancer Inst. 1993;85:958-64.
3. Schiffman MH, Castle P. Epidemiologic studies of a necessary causal risk factor: human papillomavirus infection and cervical neoplasia. J.Natl.Cancer Inst. 2003;95:E2.
4. 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;14:95-105.
5. Rodriguez AC, Burk R, Herrero R, Hildesheim A, Bratti C, Sherman ME et al. The natural history of human papillomavirus infection and cervical intraepithelial neoplasia among young women in the Guanacaste cohort shortly after initiation of sexual life. Sex Transm.Dis. 2007;34:494-502.
6. Plummer M, Schiffman M, Castle PE, Maucort-Boulch D, Wheeler CM. A 2-year prospective study of human papillomavirus persistence among women with a cytological diagnosis of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. J.Infect.Dis. 2007;195:1582-89.
7. 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.
8. 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;68:8813-24.
9. 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;12:485-90.
10. Dalstein V, Riethmuller D, Pretet JL, Le Bail CK, Sautiere JL, Carbillet JP et al. Persistence and load of high-risk HPV are predictors for development of high-grade cervical lesions: a longitudinal French cohort study. Int.J.Cancer 2003;106:396-403.
11. Molano M, van den BA, Plummer M, Weiderpass E, Posso H, Arslan A et al. Determinants of clearance of human papillomavirus infections in Colombian women with normal cytology: a population-based, 5-year follow-up study. Am.J.Epidemiol. 2003;158:486-94.
12. Castle PE, Rodriguez AC, Burk RD, Herrero R, Wacholder S, Alfaro M et al. Short term persistence of human papillomavirus and risk of cervical precancer and cancer: population based cohort study. BMJ 2009;339:b2569.
13. 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;180:1415-23.
14. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. J.Clin.Virol.

2005;32 Suppl 1:S16-S24.

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

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;157:218-26.

17. Velicer C, Zhu X, Vuocolo S, Liaw KL, Saah A. Prevalence and incidence of HPV genital infection in women. Sex Transm.Dis. 2009;36:696-703.

18. Castellsague X. Natural history and epidemiology of HPV infection and cervical cancer. Gynecol.Oncol. 2008;110:S4-S7.

19. Clifford GM, Gallus S, Herrero R, Munoz N, Snijders PJ, Vaccarella S et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. Lancet 2005;366:991-98.

20. Fukuchi E, Sawaya GF, Chirenje M, Magure T, Tuveson J, Ma Y et al. Cervical Human Papillomavirus Incidence and Persistence in a Cohort of HIV-Negative Women in Zimbabwe. Sex Transm.Dis. 2009.

21. 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;190:2077-87.

22. Bae J, Seo SS, Park YS, Dong SM, Kang S, Myung SK et al. Natural history of persistent high-risk human papillomavirus infections in Korean women. Gynecol.Oncol. 2009.

23. Nobbenhuis MA, Helmerhorst TJ, van den Brule AJ, Rozendaal L, Voorhorst FJ, Bezemer PD et al. Cytological regression and clearance of high-risk human papillomavirus in women with an abnormal cervical smear. Lancet 2001;358:1782-83.

24. Lai CH, Chao A, Chang CJ, Chao FY, Huang HJ, Hsueh S et al. Host and viral factors in relation to clearance of human papillomavirus infection: A cohort study in Taiwan. Int.J.Cancer 2008;123:1685-92.

25. Kulmala SM, Shabalova IP, Petrovitchev N, Syrjanen KJ, Gyllensten UB, Johansson BC et al. Type-specific persistence of high-risk human papillomavirus infections in the New Independent States of the former Soviet Union Cohort Study. Cancer Epidemiol.Biomarkers Prev. 2007;16:17-22.

26. 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:e3743.

27. Schmeink CE, Massuger LF, Lenselink CH, Quint WG, Melchers WJ, Bekkers RL. Effect of the menstrual cycle and hormonal contraceptives on human papillomavirus detection in young, unscreened women. Obstet.Gynecol. 2010;116:67-75.

28. 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;155:1473-78.

29. 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;44:3122-29.

30. 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;186:462-69.

31. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine 2006;24 Suppl 1:S1-15.

32. Bosch FX, de SS. The epidemiology of human papillomavirus infection and cervical cancer. Dis.Markers 2007;23:213-27.

33. 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;6:799-805.

34. 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;199:362-71.

35. Gonzalez P, Hildesheim A, Rodriguez AC, Schiffman M, Porras C, Wacholder S et al. Behavioral/lifestyle and immunologic factors associated with HPV infection among women older than 45 years. Cancer Epidemiol. Biomarkers Prev. 2010;19:3044-54.

36. Rodriguez AC, Schiffman M, Herrero R, Hildesheim A, Bratti C, Sherman ME et al. Longitudinal study of human papillomavirus persistence and cervical intraepithelial neoplasia grade 2/3: critical role of duration of infection. J.Natl.Cancer Inst. 2010;102:315-24.

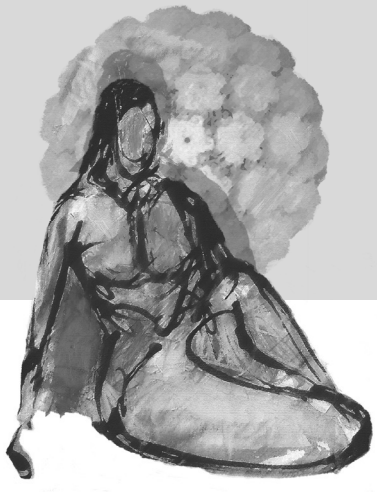
37. Insinga RP, Dasbach EJ, Elbasha EH, Liaw KL, Barr E. Incidence and duration of cervical human papillomavirus 6, 11, 16, and 18 infections in young women: an evaluation from multiple analytic perspectives. Cancer Epidemiol.Biomarkers Prev. 2007;16:709-15.

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;357:1831-36.

39. Trottier H, Ferreira S, Thomann P, Costa MC, Sobrinho JS, Prado JC et al. Human papillomavirus infection and reinfection in adult women: the role of sexual activity and natural immunity. Cancer Res. 2010;70:8569-77.

40. Theiler RN, Farr SL, Karon JM, Paramsothy P, Viscidi R, Duerr A et al. High-risk human papillomavirus reactivation in human immunodeficiency virus-infected women: risk factors for cervical viral shedding. Obstet. Gynecol. 2010;115:1150-58.

41. Taylor S, Wang C, Wright TC, Denny L, Tsai WY, Kuhn L. Reduced acquisition and reactivation of human papillomavirus infections among older women treated with cryotherapy: results from a randomized trial in South Africa. BMC.Med. 2010;8:40.



Chapter 4

Human papillomavirus persistence in young unscreened women, a prospective cohort study

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Abstract

Objective: To evaluate hr-HPV persistence and associated risk factors in a prospective cohort of young unscreened women. Additionally, the relation between hr-HPV status and cytology/histology results is examined.

Methods and Principal Findings: Two year follow-up of 235 out of 2065 young women (18–29 years), participating in a large, one year epidemiological study, with questionnaires, self-collected cervico-vaginal samples (Vibabrush), and SPF_{10}LiPA for HPV detection. Only women hr-HPV positive at sample month 12 were invited for a second year of follow-up. After study follow-up, available cytology/histology data were requested from PALGA (the national network and registry of histo- and cytopathology in the Netherlands). These data were compared with available cytology/histology data of the month 12 hr-HPV negative women from the same cohort. 44.1% of the hr-HPV types detected at study month 12, persisted during follow-up. HPV types 45, 31, 16 and 18 were most likely to persist with percentages of 60.0%, 56.8%, 54.4%,and 50.0%, respectively. Compared to newly detected infections at month 12, infections present since 6 months or baseline had an increased risk to persist (OR 3.09 [95% CI: 1.74-5.51] and OR 4.99 [95% CI: 2.67-9.32], respectively). Other co-factors influencing persistence were, multiple HPV infections, smoking and multiple lifetime sexual partners. The percentage of women with a HSIL/CIN2+ (12.1%) in the persistent HPV group, was not significantly different (p=0.107) from the 5.3% of the women who cleared the hr-HPV infection, but was significantly (p 0.000) higher than to the 1.6% of women in the hr-HPV negative control group.

Conclusion: We showed that HPV genotype, multiple infections, smoking, and multiple lifetime sexual partners are co-factors that increase the risk of hr-HPV persistency. Most importantly, we showed that hr-HPV infections are more likely to persist the longer they have been present and that women with a persistent hr-HPV infection have a high risk of HSIL/CIN2+ development.

Introduction

It has been well established that a human papillomavirus (HPV) infection is a necessary cause in the development of cervical intraepithelial neoplasia (CIN) and cervical cancer.[1-3] HPV appears to be the most common sexually transmitted infection and about 80% of all sexually active women will acquire an HPV infection during their lifetime.[2] Fortunately, only a small proportion of these infections lead to CIN and cervical cancer. A persistent high risk (hr) HPV infection is necessary for the development of cervical carcinoma. Therefore the detection of a persistent hr-HPV infection represents an important marker of an increased risk of CIN and cervical carcinoma.[4-6] However, there is no consensus on the definition of persistence.

Most investigators define a persistent HPV infection as detection of the same HPV type, or group of types, on two consecutive visits, but these could be from 2 months up to 72 months apart.[7,8] Several studies have shown that most infections become undetectable within 1-2 years.[5,9-11] Additionally, infections lasting more than 1 year appear to be associated with a lower clearance rate.[5,9] Therefore it is more informative to monitor the duration of the infection rather than the number of positive tests.[12]

Several risk factors have been identified that are associated with HPV persistence. Especially viral characteristics like viral load, and HPV genotype are linked to persistence.[13,14] Given that HPV16 and 18 are the most carcinogenic HPV genotypes, it would be useful to know whether their risk of persistence differs from other hr-HPV genotypes.[15] Additionally, some authors have shown that multiple HPV infections are associated with an increased duration of high-risk HPV infections,[9,16] whereas others have failed to show such an increase.[11,17] Other co-factors like age, condom use, smoking, long-term use of oral contraceptives, high parity, number of sex partners, and exposure to other sexually transmitted diseases have also been associated with HPV persistence.[9,18,19]

In this study, a prospective cohort of young unscreened women (18-29 years) in the pre-vaccine era was followed for 2 years, to examine the influence of viral factors (i.e. duration of infection, HPV-type and co-infection), and co-factors (i.e. sexual behavior and smoking) on hr-HPV persistence. Additionally we examined the relation between hr-HPV status and follow-up cytology/histology results.

Methods and Materials

A large prospective cohort study on HPV prevalence, incidence and clearance was performed in the Netherlands, in 2007–2010. At study entry, 2065 unscreened women 18 to 29 years of age were included.[20] Exclusion criteria were, being pregnant, or not fluent in Dutch. Of the 2065 women initially included, 1871 (90.6%) completed the first year of follow-up. Women, who were hr-HPV positive at month 12 (n=257 (13.7%)), were invited to participate in a second year of follow-up in order to study hr-HPV persistence. In total 235 (91.4%) of the invited women, completed the second year of follow-up and were included in this analysis of persistence. The study was closed after the sample at month 24, therefore all the hr-HPV positive women were referred to their general

practitioner or gynaecologist for additional follow-up or treatment. Written informed consent was obtained from all participants. This study was approved by the Local Medical Ethics Committee.

Specimen collection and HPV DNA detection and genotyping

The women provided five self-samples with a 3-month interval (month 0, 3, 6, 9, and 12) during the first year of the study. In the second year of follow-up, women provided two additional self-samples with a 6 month interval (month 18 and 24). All women received the self-sample kit and the additional questionnaires by mail.

The self-sample kit contained an explanatory letter, a questionnaire, an illustrated instruction form on how to perform the cervico-vaginal self-sample, a small brush in a sterile cover (Rovers Vibabrush®, Rovers Medical Devices, Oss, the Netherlands), and a collection tube containing medium (SurePath™, Tripath Imaging®, Inc., Burlington NC, U.S.A.), as described previously.[20,21]

Broad-spectrum HPV DNA amplification was performed using a short-PCR-fragment assay. Extracted DNA was used for PCR amplification with the SPF_{10}primer sets.[22,23] The samples were run through an HPV DNA enzyme immunoassay (DEIA) to obtain an OD reading, and categorized as HPV DNA negative, positive, or borderline. The same SPF_{10} amplimers were used on SPF_{10}-DEIA-positive samples to identify HPV genotype by reverse hybridization on a line probe assay (LiPA) (SPF_{10}HPVLiPA$_{25}$, version 1; Labo Bio-Medical Products B.V., Rijswijk, the Netherlands), which detects the following HPV genotypes: low-risk HPV types: type 6, 11, 34, 40, 42, 43, 44, 53, 54, 55, 66, 70, 74, and "X" (DEIA positive and LiPA negative samples); and high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82.

Type-specific hr-HPV; clearance, persistence, and history

An HPV infection was considered cleared when a woman had two consecutive HPV negative samples.[11,24,25] Therefore, hr-HPV positive women with one intermittent type-specific hr-HPV negative sample were considered persistent for that hr-HPV type. All women included in the second year of follow-up were hr-HPV positive at sample month 12, thus only women who were negative for hr-HPV at sample month 18 and 24 were considered to have cleared their hr-HPV infection. (see Table 1)

The sampling interval in the 2nd study year was 6 months and persistence is generally defined as two consecutive HPV positive samples. Thus, a type–specific hr-HPV infection was considered persistent when it was detected for at least 6 months. Women who were positive at sample month 18 and/or month 24 were therefore considered to have a persistent type-specific hr-HPV infection in the 2nd study year (see Table 1). Four of the 235 women were treated for abnormal cytology (and one cervical carcinoma) and cleared hr-HPV before sample month 18, therefore the natural course of clearance or persistence was disturbed. The data of these women were excluded in the analysis of persistence.

To analyze whether the duration of the type-specific hr-HPV infection (the history) prior

to sample month 12 influences persistence in the 2nd study year, we determined at which sample moment the hr-HPV infection was first detected. In order to calculate with the same time interval between the samples in the 1st and the 2nd study year, the cut-off point for first detection of the type-specific hr-HPV were made at baseline (month 0), sample month 6 and sample month 12 (see Table 1). This analysis was performed for the total number of type-specific hr-HPV infections.

Hr-HPV status related to cytology and histology

The study protocol was based on self-sampling for HPV detection. Gynecologic histo- and cytopathology data were thus not available at the end of the study. To be able to evaluate the relation between HPV status and cervical lesions, we requested information from PALGA (the national network and registry of histo- and cytopathology in the Netherlands).[26] To compare data of the hr-HPV positive women included in the 2nd study year, a control group was selected from the women who were hr-HPV negative at sample month 12. These women were 27-29 years at study baseline, and had received an invitation of the Dutch national cervical cancer screening program. In order to relate cytology/histology results to the hr-HPV status, information of all sample points in the 1st and 2nd study year had to be available. The four women who were treated for abnormal cytology before sample month 18, were also excluded in the analysis of persistence related to cytology. This resulted in 224 women hr-HPV positive at sample month 12 and 336 women hr-HPV negative at sample month 12 (control group). PALGA data on

Table 1.
Definitions of duration of type-specific hr-HPV detection

	Study sample month					
Type-specific hr-HPV history	Month 0	Month 6	Month 12*	Month 18	Month 24	Type-specifc hr-HPV during 2nd study year
Detected since baseline	+	+	+			
	+	-	+			
Detected since study month 6	-	+	+			
Newly detected at study month 12	-	-	+			
				-	-	Clearance
				+	-	Persistence
				+	+	
				-	+	

* only women positive for hr-HPV at sample month 12 are included in the 2nd study year and in the analysis of HPV persistency

gynecologic histo- and cytopathology, test date, and age of the woman at time of the test, were available for 166 (74.1%) hr-HPV positive women and 246 (73.2%) women of the control group. Due to privacy legislation, identification data were made anonymous by a Third Trusted Party (ZorgTTP, Houten, the Netherlands). To relate these anonymous gynecologic histo- and cytopathology test results with the hr-HPV status, women were grouped.

1) Month 12 hr-HPV positive women:
   a. type-specific hr-HPV type persistent in the 2nd study year
   b. cleared type-specific hr-HPV type in the 2nd study year
2) Control group: Month 12 hr-HPV negative women.

Statistical analysis

The baseline characteristics of the study population were analyzed and presented in frequencies, mean and standard deviation (SD). The percentage and 95% confidence interval (95% CI) of the hr-HPV prevalence at sample month 12 and persistence in the second year of follow-up were calculated using the number of infections instead of the number of women. Data from the questionnaires were used to determine, with simple logistic regression analysis, whether co-factors like OCP use, smoking and sexual behavior influenced the persistence in the 2nd study year. To analyze the relation between hr-HPV status and histo- and cytopathology test results significance was calculated with the Fischer's exact test. All statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL).

Results

The 235 hr-HPV positive women included in this analysis had a mean follow-up time of 25.3 months (SD 1.5) and were all born in the Netherlands. At study baseline these women were 18-29 years of age with a mean age of 24 years (SD 3.2), and 233 (99.1%) women reported to ever have had sexual intercourse. Sixty six (28.1%) women reported that they were current smokers, and there were 165 (70.2%) current oral contraceptive pill (OCP) users. These baseline data are shown in Table 2.

In order to be included in the 2nd study year, women had to be hr-HPV positive at sample month 12. Of the 235 women, 163 had one hr-HPV type, 54 had two hr-HPV types, 15 had 3 hr-HPV types, two had 4 hr-HPV types and one woman had 6 hr-HPV types at sample month 12. This resulted in a total of 330 type-specific hr-HPV infections detected at sample month 12. HPV 16 had the highest prevalence of 30.2%, followed by HPV 51 (19.1%), HPV 31 (16.6%), and HPV 52 (14.9%). HPV 18 was prevalent in 11.1% of the women. (see Table 3)

Table 2.
Baseline demographics

	Sample size N (%)
Total	235
Age (years), mean (SD)	24 (3.2)
18	8 (3.4)
19	16 (6.8)
20	19 (8.1)
21	16 (6.8)
22	25 (10.6)
23	15 (6.4)
24	25 (10.6)
25	24 (10.2)
26	18 (7.7)
27	33 (14.0)
28	23 (9.8)
29	13 (5.5)
Ethnicity	
Dutch	235 (100)
Other	0 (0.0)
Education	
Lower secondary/Lower vocational training	5 (2.1)
Higher secondary/Vocational training	46 (19.6)
Higher vocational training/University	184 (78.3)
Current Smoking	
Yes	66 (28.1)
No	169 (71.9)
Using OCP	
Yes	165 (70.2)
No	70 (29.8)
Ever had sexual intercourse	
Yes	233 (99.1)
No	2 (0.9)

The percentage of the type-specific hr-HPV infections, present at sample month 12, persisting in the 2nd study year are shown in Table 3. For 315 of the 330 type-specific hr-HPV infections data were available on persistence. Of these 315 type-specific infections, 139 (44.1%) infections were persistent in the 2nd study year. HPV 45 was the most persistent type, with a persistence rate of 60.0%, followed by HPV 31 (56.8%), HPV 16 (54.4%), and HPV 18 (50.0%). Notably, HPV 45 was only number 10 in prevalence with 11 (4.7%) type-specific infections at month 12, whereas HPV 51 (second in prevalence with 45 infections) was one of the least persistent infections with a persistence rate of 25.0%. Not only the hr-HPV type, but also the duration of the infection prior to sample month

Table 3.
Ranking of type-specific hr-HPV prevalence at month 12 and type-specific hr-HPV persistence in 2nd study year

Rank	HPV type	Prevalence at month 12		HPV type	Persistence in 2nd study year *	
		N	% (95% CI)		N	% (95% CI)
1	16	71	30.2 (24.4-36.5)	45	6/10	60.0 (26.2-87.8)
2	51	45	19.1 (14.3-24.8)	31	21/37	56.8 (39.5-72.9)
3	31	39	16.6 (12.1-22.0)	16	37/68	54.4 (41.9-66.5)
4	52	35	14.9 (10.6-20.1)	18	13/26	50.0 (29.9-70.1)
5	56	28	11.9 (8.1-16.8)	33	5/10	50.0 (18.7-81.3)
6	18	26	11.1 (7.4-15.8)	56	12/26	46.6 (26.6-66.6)
7	39	25	10.6 (7.0-15.3)	39	10/25	40.0 (21.1-61.3)
8	68	21	8.9 (5.6-13.3)	68	7/19	36.8 (16.3-61.6)
9	33	12	5.1 (2.7-8.7)	52	12/34	35.3 (19.7-53.5)
10	45	11	4.7 (2.4-8.2)	35	2/7	28.6 (3.7-71.0)
11	59	9	3.8 (1.8-7.1)	51	11/44	25.0 (13.2-40.3)
12	35	8	3.4 (1.5-6.6)	59	2/9	22.2 (2.8-60.0)
	Total	330	140.4 %	Total	139/315	44.1 (38.6-49.8)

* Four of the 235 women were treated for abnormal cytology before sample month 18, therefore the natural fluctuation of clearance and persistence was disturbed. The data of these women were excluded in the analysis of persistence in the 2nd study year

12, seemed to influence persistence in the 2nd study year. Overall, a type-specific infection detected since baseline had an odds ratio (OR) of 4.99 (95% CI: 2.67-9.32) to persist in the 2nd study year, compared to infections newly detected at month 12. Infections first detected at sample month 6 also had an increased risk, OR of 3.09 (95% CI: 1.74-5.51), to persist in the 2nd study year, compared to newly detected infections (see Table 4).

Table 4.
Influence of the duration of type-specific hr-HPV detection on persistence in 2nd study year

Type-specific hr-HPV history	N	persistent N (%)	OR (95% CI)	P-value
Detected since baseline	63	43 (68.3)	**4.99 (2.67-9.32)**	**0.000**
Detected since study month 6	70	40 (57.1)	**3.09 (1.74-5.51)**	**0.000**
Newly detected at month 12	166	50 (30.1)	**1 (ref)**	

Having multiple HPV infections at sample month 12, irrespective whether these infections were only hr-HPV or also lr-HPV types, increased a woman's risk to have a type-specific hr-HPV persistent infection in the 2nd study year. This was almost a twofold increased risk, OR 1.93 (95% CI: 1.14-3.26). (See Table 5)

Furthermore, Table 5 shows that smoking and number of lifetime sexual partners are co-factors influencing hr-HPV persistence. Smokers have an almost twofold increased risk of persistence than non-smokers (OR: 1.87 [95% CI: 1.03-3.39]) and women with multiple lifetime sexual partners at baseline have an increased risk of persistence compared to women with 1 lifetime sexual partner. The OR's for 2-5, 6-10 and more than 10 lifetime sexual partners were 4.29 [95% CI: 1.13-16.26], 6.82 [95% CI: 1.76-26.33] and 4.15 [95% CI: 1.05-16.34], respectively. Of the 15 women with 1 lifetime sexual partner at baseline, only three had a type-specific persistent infection during follow-up. Of the three persistent infections in women with 1 lifetime sexual partner, one (HPV 16) was newly detected at month 12, and two (HPV 16 and HPV 56) were detected since month 6.

Twelve of the 15 women with 1 lifetime sexual partner at baseline reported to have a new sexual partner during the study follow-up. Together they had 16 type-specific hr-HPV infections detected at sample month 12, of whom 10 were newly detected, four were first detected at month 6, and two at baseline. Because most hr-HPV types were newly detected at month 12 and newly detected hr-HPV infections are less likely to persist, this might be a confounding factor. However, confounding could not be ruled out with multiple logistic regression analysis because 'duration of hr-HPV detection' and 'number of lifetime partners' are based on different dependent variables, 'number of infections' and 'number of women', respectively.

The following variables; age, OCP use, age of first sexual intercourse, sexual age, having a new sexual partner during study follow-up, current type of relationship, current number of sexual partners, current frequency of sexual intercourse, current condom use and having a STI during study follow-up, did not influence persistence of a type-specific hr-HPV infection in the 2nd study year (see Table 5).

Table 5.
Woman's risk of having a persistent type-specific hr-HPV type in the 2nd study year

	N* (n=231)	Persistent N (%)	OR (95% CI)	P-value
Age Mean: 24.0 years SD: 3.16	231	122 (52.1)	1.07 (0.99-1.16)	0.106
Education				
Lower secondary / Lower vocational training	5	3 (60.0)	1.31 (0.21-8.04)	0.769
Higher secondary / Vocational training	45	23 (51.1)	0.92 (0.48-1.76)	0.898
Higher vocational training/University	180	96 (53.3)	1 (ref)	
Current Smoking				
No	166	81 (48.8)	1 (ref)	
Yes	64	41 (64.1)	1.87 (1.03-3.39)	0.039
OAC at T12				
No	75	36 (48.0)	0.72 (0.41-1.25)	0.240
Yes	151	85 (56.3)	1 (ref)	
Age at first sexual intercourse				
≤ 13	8	6 (75.0)	2.57 (0.37-17.83)	0.339
14-16	119	61 (51.3)	0.90 (0.29-2.84)	0.859
17-19	90	48 (53.3)	0.98 (0.31-3.15)	0.972
≥ 20	13	7 (53.8)	1 (ref)	
Sexual Age^				
Mean: 7.5	228	122 (52.6)	1.07 (0.99-1.16)	0.197
SD: 3.37				
Lifetime sexual partners				
1	15	3 (20.0)	1 (ref)	
2-5	87	45 (51.7)	4.29 (1.13-16.26)	0.032
6-10	73	46 (63.0)	6.82 (1.76-26.33)	0.005
>10	55	28 (50.9)	4.15 (1.05-16.34)	0.042
New sexual partner during study period?				
No	55	35 (63.6)	1.75 (0.90-3.43)	0.103
Yes	102	51 (50.0)	1 (ref)	
Type of relationship at study month 12				
Married or Living together	47	28 (59.6)	1.47 (0.73-2.98)	0.281
Couple, living apart	83	44 (53.0)	1.13 (0.63-2.03)	0.686
Single	98	49 (50.0)	1 (ref)	
Number of sexual partners past 3 months at study month 12				
0	32	14 (43.8)	1.36 (0.33-5.59)	0.669
1	161	86 (53.4)	2.01 (0.57-7.12)	0.281
2	26	18 (69.2)	3.94 (0.89-17.37)	0.070
≥3	11	4 (36.4)	1 (ref)	

Table 5. Continued

	N* (n=231)	Persistent N (%)	OR (95% CI)	P-value
Sexual frequency past 3 months at T12				
1-3	42	26 (61.9)	1.49 (0.68-3.26)	0.318
4-12	25	10 (40.0)	0.61 (0.24-1.55)	0.299
13-27	61	35 (57.4)	1.23 (0.62-2.47)	0.552
>27	69	36 (52.2)	1 (ref)	
Condom use at study month 12				
Never	101	60 (59.4)	1.13 (0.45-2.81)	0.800
Sometimes	39	18 (46.2)	0.66 (0.23-1.86)	0.431
Most of times	34	16 (47.1)	0.68 (0.24-1.98)	0.484
Always	23	13 (56.5)	1 (ref)	
Having multiple infections at study month 12? #				
Yes	107	66 (61.7)	1.93 (1.14-3.26)	0.015
No	123	56 (45.5)	1 (ref)	
Other STI during study period?				
No	209	112 (53.6)	1.27 (0.52-3.12)	0.602
Yes	21	10 (47.6)	1 (ref)	

SD: standard deviation
* Number = based on available data, as not all 231 women completely filled-in the questionnaire.
^ Sexual age = years of sexual activity (current age minus age of first sexual intercourse)
# Multiple infections = irrespective whether these infections were only hr-HPV or also lr-HPV

Hr-HPV persistence related to cytology and histology

The results from PALGA on cytology and histology related to the hr-HPV status of the women are presented in Table 6. The mean age of the women with available cytology results was 28,4 (SD: 2.8). It was not possible to measure the exact time interval between sample month 12 and first cytology because the cases were made anonymous.

However, all dates of self-sampling and cytology were available. We calculated the time interval between the date when 50% of the women had taken their self-sample en when 50% of the women had their cytology performed. For the study group women (1a and 1b) this interval was 13 months and for the control group women (2a and 2b) the interval was 12 months.

In the study group 1a; 11 (12.1%) of the 91 women with a persistent hr-HPV infection were identified with HSIL/CIN2+. Four of the 75 women (5.3%) in group 1b, who cleared their type-specific hr-HPV infection in the 2nd study year had HSIL/CIN2+. This difference between both groups was, not significant (*p 0.107*). In the control group, 4 of the 246 women (1.6%) developed HSIL/CIN2+. Women with a persistent hr-HPV type were significantly more often diagnosed with HSIL/CIN2+ compared to women in the control group (*p 0.000*).

Table 6.
Histo- and cytopathology follow-up results

	Cytology		Histology				
	classification	n (%)	Normal	CIN 1	CIN 2	CIN 3+	No histology
1. Study group*							
a. Persisting type specific hr-HPV (n=91)	No cytology	1 (1.1)	1				
	Normal	55 (60.4)					55
	ASCUS	16 (17.6)	4	2	1		9
	LSIL	10 (11.0)	1	6	1		6
	HSIL	9 (9.9)			3	5	1
b. Cleared type-specific hr-HPV (n=75)	Normal	52 (69.3)					52
	ASCUS	15 (20.0)	3	1			11
	LSIL	5 (6.7)	1	1	1	1	1
	HSIL	3 (4.0)		1	1	1	0
2. Control group# (n=246)							
	Normal	227 (92.3)					227
	ASCUS	14 (5.7)	1				13
	LSIL	2 (0.8)			1		1
	HSIL	3 (1.2)				2	1

* Study group: study month 12 hr-HPV positive women. Mean interval between study month 12 and cytology=13 months
# Control group: study month 12 hr-HPV negative women. Mean interval between study month 12 and cytology=12 months

Discussion

In this 2-year prospective cohort study among young unscreened women, we showed that an already persistent hr-HPV infection has an increased risk to persist during follow-up compared to newly detected infections. Hr-HPV types persistent for 6 and 12 months or longer had a respective, threefold or fivefold, increased risk of persistence. Other studies also reported that HPV infections are more likely to persist the longer they have been present.[4,5,11,14,27] Of the newly detected infections 70% were cleared before the next sample (6 month interval). This is a slightly higher clearance rate than reported in some other studies, who found a mean time to clearance of 6-8 months for newly acquired HPV infections.[9,28]

Persistence was also influenced by the genotype. The overall persistence of type-specific hr-HPV in this study is 44.1%, HPV types 45, 31, 16, and 18, had the highest rate of persistence and the highly prevalent HPV type 51 had a low tendency to persist. In agreement with our findings, other studies also showed that HPV 16, 18, 31, and 45 have an

increased risk to persist compared to other HPV types.[11,17,29-32] Additionally, the IARC multicentre case-control study showed that HPV 16, 18, 45 and 31 were the most common HPV types in cervical carcinoma.[33]

Having multiple HPV infections is a risk factor for hr-HPV persistence. The risk to have a persistent type-specific hr-HPV infection during follow-up increased almost twofold in women with multiple (hr- and/or lr-HPV) HPV types at month 12. These results are in agreement with other studies showing that women with multiple infections have a higher risk to have a persistent type-specific HPV infection during follow-up.[9,16,18] A possible explanation may be that the multiple infections increase the overall viral load of the infection which by sheer volume overcomes immune control. Whether this only happens in women with a lower immunity or also in the general population is not well known. Another explanation may be that there are specific interactions between different HPV genotypes. The impact of having multiple infections on persistence, however, probably ceases in time. Cuschieri et al., showed that over a period of two to three years multiple hr-HPV infections did not constitute a higher risk factor for the development of cervical neoplasia compared with single hr-HPV infections.[34]

Only two co-factors, smoking and number of lifetime sexual partners, were found to correlate with the likelihood that a hr-HPV type would persist. Smokers had a two-fold increased risk of hr-HPV persistence. Smoking is a known immune suppressor and a risk factor for cervical carcinoma.[35] However, some studies did not find a difference in HPV persistence between current and non-current smokers.[27.36] In our study only smoking at baseline was registered, therefore we could not identify differences in the risks between current or past smokers, nor for the duration of smoking, or number of cigarettes a day.

Women with 1 lifetime sexual partner at baseline were at lower risk for a persistent infection than women with multiple lifetime sexual partners. The highest risk was found for women with 6-10 lifetime sexual partners at baseline. It should be noted, however, that the majority of the women with 1 lifetime sexual partner at baseline, had a new sexual partner during study follow-up and newly identified hr-HPV types. Newly detected hr-HPV types are less likely to persist, so this might be a confounding factor. In literature, the number of lifetime sexual partners is not a consistent risk factor for hr-HPV persistency.[36,37] Therefore, the potential influence of the number of lifetime sexual partners on hr-HPV persistency still needs to be elucidated.

The use of OCP's is also a potential risk factor for the development of cervical carcinoma.[38] However, results from our study and others did not find a relation between OCP use and persistence.[21,27,36] This increased risk is reported to be strongly related to the duration of use and the effect proved reversible after cessation. This influence of OCP use on HPV persistence, however, is still controversial.[35]

Previously it has been shown that women with a persistent type-specific hr-HPV infection are significantly more likely to have or develop CIN than those who were sequentially infected by different hr-HPV types or who cleared their infection.[34,39,40] We also found that having a persistent type-specific hr-HPV infection is associated with a higher (not

significant) rate of HSIL/CIN2+ development (12.1%) compared to women who cleared the type-specific hr-HPV infection (5.3%), and significantly higher (*p 0.000*) compared to women who were hr-HPV negative at study month 12 (1.6%).

Due to the anonymous histo- and cytopathology data, we were not able to correlate the histo- and cytopathology results to the type-specific hr-HPV types, nor if the woman had multiple HPV types. Therefore we could not confirm whether the infections with HPV types 45, 31, 16, and 18 were more likely to cause HSIL/CIN2+ and whether the number of infections was correlated to the severity of the lesion as described previously.[41]

There is considerable interest in the possibility of using HPV testing as a primary cervical cancer screening tool.[42,43] Our data support that following prevalently detected hr-HPV types for persistence is useful to identify women with an increased risk of CIN in the following years.[44] In order to do so, a clear definition of a clinically relevant persistent HPV infection should be determined. This definition should be based on the duration of the type-specific hr-HPV presence that is predictive of CIN development rather than based on two consecutive hr-HPV positive visits.

Based on our results in this population of young women, we suggest that this interval should be a minimal of 6 months, because a 6 month persistent hr-HPV type had a threefold increased risk to persist during follow-up compared to a newly detected hr-HPV infection. However, this interval may be extended to 12 months, because 68.3% of the hr-HPV types already persistent for 12 months or longer continued to persist during follow-up. Therefore a 12 month interval for detection of type-specific hr-HPV persistency will select a group of women that needs close surveillance for HSIL/CIN2+ development in the following year(s).

In conclusion, we showed that co-factors increasing the risk of hr-HPV persistency are, genotype specific (45, 31,16, and 18, are most likely to persist), multiple infections, smoking, and multiple sexual lifetime partners. Most importantly, we showed that hr-HPV infections are more likely to persist the longer they have been detected and that women with a persistent hr-HPV infection have a higher rate of HSIL/CIN2+ detection in the following year. Thus, women with a persistent hr-HPV infection should be monitored for HSIL/CIN2+ development.

Reference List

1 Schiffman MH, Bauer HM, Hoover RN, Glass AG, Cadell DM, et al. (1993) Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. J Natl Cancer Inst 85: 958-964.

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

3 Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, et al. (1999) Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 189: 12-19.

4 Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S (2007) Human papillomavirus and cervical cancer. Lancet 370: 890-907.

5 Plummer M, Schiffman M, Castle PE, Maucort-Boulch D, Wheeler CM (2007) A 2-year prospective study of human papillomavirus persistence among women with a cytological diagnosis of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. J Infect Dis 195: 1582-1589.

6 Syrjanen K, Kulmala SM, Shabalova I, Petrovichev N, Kozachenko V, et al. (2008) Epidemiological, clinical and viral determinants of the increased prevalence of high-risk human papillomavirus (HPV) infections in elderly women. Eur J Gynaecol Oncol 29: 114-122.

7 Schiffman M, Kjaer SK (2003) Chapter 2: Natural history of anogenital human papillomavirus infection and neoplasia. J Natl Cancer Inst Monogr 14-19.

8 Koshiol J, Lindsay L, Pimenta JM, Poole C, Jenkins D, et al. (2008) Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. Am J Epidemiol 168: 123-137.

9 Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD (1998) Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 338: 423-428.

10 Moscicki AB, Shiboski S, Broering J, Powell K, Clayton L, et al. (1998) The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. J Pediatr 132: 277-284.

11 Richardson H, Kelsall G, Tellier P, Voyer H, Abrahamowicz M, et al. (2003) The natural history of type-specific human papillomavirus infections in female university students. Cancer Epidemiol Biomarkers Prev 12: 485-490.

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

13 van Duin M, Snijders PJ, Schrijnemakers HF, Voorhorst FJ, Rozendaal L, et al. (2002) Human papillomavirus 16 load in normal and abnormal cervical scrapes: an indicator of CIN II/III and viral clearance. Int J Cancer 98: 590-595.

14 Munoz N, Hernandez-Suarez G, Mendez F, Molano M, Posso H, et al. (2009) Persistence of HPV infection and risk of high-grade cervical intraepithelial neoplasia in a cohort of Colombian women. Br J Cancer.

15 Castle PE (2008) Invited commentary: is monitoring of human papillomavirus infection for viral persistence ready for use in cervical cancer screening? Am J Epidemiol 168: 138-144.

16 Trottier H, Mahmud S, Prado JC, Sobrinho JS, Costa MC, et al. (2008) Type-specific duration of human papillomavirus infection: implications for human papillomavirus screening and vaccination. J Infect Dis 197: 1436-1447.

17 Kulmala SM, Shabalova IP, Petrovitchev N, Syrjanen KJ, Gyllensten UB, et al. (2007) Type-specific persistence of high-risk human papillomavirus infections in the New Independent States of the former Soviet Union Cohort Study. Cancer Epidemiol Biomarkers Prev 16: 17-22.

18 Nielsen A, Kjaer SK, Munk C, Osler M, Iftner T (2010) Persistence of high-risk human papillomavirus infection in a population-based cohort of Danish women. J Med Virol 82: 616-623.

19 Louvanto K, Rintala MA, Syrjanen KJ, Grenman SE, Syrjanen SM (2010) Genotype-specific persistence of genital human papillomavirus (HPV) infections in women followed for 6 years in the Finnish Family HPV Study. J Infect Dis 202: 436-444.

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

21 Schmeink CE, Massuger LF, Lenselink CH, Quint WG, Melchers WJ, et al. (2010) Effect of the menstrual cycle and hormonal contraceptives on human papillomavirus detection in young, unscreened women. Obstet Gynecol 116: 67-75.

22 Melchers WJ, Bakkers JM, Wang J, de Wilde PC, Boonstra H, et al. (1999) 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 155: 1473-1478.

23 van Hamont D., van Ham MA, Bakkers JM, Massuger LF, Melchers WJ (2006) Evaluation of the SPF10-INNO LiPA human papillomavirus (HPV) genotyping test and the roche linear array HPV genotyping test. J Clin Microbiol 44: 3122-3129.

24 Winer RL, Lee SK, Hughes JP, Adam DE, Kiviat NB, et al. (2003) Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. Am J Epidemiol 157: 218-226.

25 Giuliano AR, Harris R, Sedjo RL, Baldwin S, Roe D, et al. (2002) Incidence, prevalence, and clearance of type-specific human papillomavirus infections: The Young Women's Health Study. J Infect Dis 186: 462-469.

26 Casparie M, Tiebosch AT, Burger G, Blauwgeers H, van Krieken JH, et al. (2007) Pathology databanking and biobanking in the Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. Cell Oncol 19-24.

27 Maucort-Boulch D, Plummer M, Castle PE, Demuth F, Safaeian M, et al. (2009) Predictors of human papillomavirus persistence among women with equivocal or mildly abnormal cytology. Int J Cancer.

28 Nobbenhuis MA, Walboomers JM, Helmerhorst TJ, Rozendaal L, Remmink AJ, et al. (1999) Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study. Lancet 354: 20-25.

29 Schiffman M, Herrero R, Desalle R, Hildesheim A, Wacholder S, et al. (2005) The carcinogenicity of human papillomavirus types reflects viral evolution. Virology 337: 76-84.

30 Schiffman M, Rodriguez AC, Chen Z, Wacholder S, Herrero R, et al. (2010) A

population-based prospective study of carcinogenic human papillomavirus variant lineages, viral persistence, and cervical neoplasia. Cancer Res 70: 3159-3169.

31 Syrjanen K, Shabalova I, Naud P, Kozachenko V, Derchain S, et al. (2009) Persistent High-Risk Human Papillomavirus Infections and Other End-Point Markers of Progressive Cervical Disease Among Women Prospectively Followed up in the New Independent States of the Former Soviet Union and the Latin American Screening Study Cohorts. Int J Gynecol Cancer 19:934-942.

32 Iftner T, Eberle S, Iftner A, Holz B, Banik N, et al. (2010) Prevalence of low-risk and high-risk types of human papillomavirus and other risk factors for HPV infection in Germany within different age groups in women up to 30 years of age: an epidemiological observational study. J Med Virol 82: 1928-1939.

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

34 Cuschieri KS, Cubie HA, Whitley MW, Gilkison G, Arends MJ, et al. (2005) Persistent high risk HPV infection associated with development of cervical neoplasia in a prospective population study. J Clin Pathol 58:946-950.

35 Castellsague X, Munoz N (2003) Chapter 3: Co-factors in human papillomavirus carcinogenesis--role of parity, oral contraceptives, and tobacco smoking. J Natl Cancer Inst Monogr 20-28.

36 Bae J, Seo SS, Park YS, Dong SM, Kang S, et al. (2009) Natural history of persistent high-risk human papillomavirus infections in Korean women. Gynecol Oncol.

37 Fukuchi E, Sawaya GF, Chirenje M, Magure T, Tuveson J, et al. (2009) Cervical Human Papillomavirus Incidence and Persistence in a Cohort of HIV-Negative Women in Zimbabwe. Sex Transm Dis.

38 Appleby P, Beral V, Berrington de GA, Colin D, Franceschi S, et al. (2007) Cervical cancer and hormonal contraceptives: collaborative reanalysis of individual data for 16,573 women with cervical cancer and 35,509 women without cervical cancer from 24 epidemiological studies. Lancet 370: 1609-1621.

39 Sundstrom K, Eloranta S, Sparen P, Arnheim DL, Gunnell A, et al. (2010) Prospective study of HPV types, HPV persistence and risk of squamous cell carcinoma of the cervix. Cancer Epidemiol Biomarkers Prev.

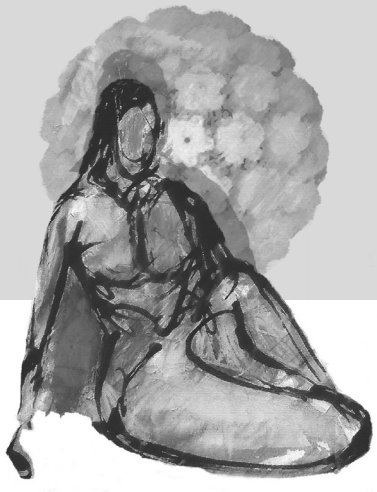
40 Lai CH, Chao A, Chang CJ, Chao FY, Huang HJ, et al. (2008) Host and viral factors in relation to clearance of human papillomavirus infection: A cohort study in Taiwan. Int J Cancer 123: 1685-1692.

41 Bello BD, Spinillo A, Alberizzi P, Cesari S, Gardella B, et al. (2009) Cervical infections by multiple human papillomavirus (HPV) genotypes: Prevalence and impact on the risk of precancerous epithelial lesions. J Med Virol 81: 703-712.

42 Brink AA, Snijders PJ, Meijer CJ, Berkhof J, Verheijen RH (2006) HPV testing in cervical screening. Best Pract Res Clin Obstet Gynaecol 20: 253-266.

43 Franco EL, Mahmud SM, Tota J, Ferenczy A, Coutlee F (2009) The expected impact of HPV vaccination on the accuracy of cervical cancer screening: the need for a paradigm change. Arch Med Res 40: 478-485.

44 Castle PE, Rodriguez AC, Burk RD, Herrero R, Wacholder S, et al (2009) Short term persistence of human papillomavirus and risk of cervical precancer and cancer: population based cohort study. BMJ 339: b2569.



Chapter 5

Effect of the menstrual cycle and hormonal contraceptives on Human papillomavirus detection in young unscreened women

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Abstract

Objective: To estimate the effect of the menstrual cycle and oral contraceptive pill (OCP) use on the prevalence, incidence, and persistence of human papillomavirus (HPV).

Methods and Materials: A longitudinal study was conducted among 2065 women aged 18-29 years. The women returned a self-collected cervicovaginal sample and filled out a questionnaire. A total of 1812 women participated at all three time points, month 0, month 6 and month 12.

Results: Low- and high-risk Human papillomavirus (lr- and hr-HPV) prevalence at study entry was 8.9% and 11.8%, respectively. The annual incidence of lr-HPV infections was 12.5% and the persistence was 2.0%. For hr-HPV, the incidence and persistence was 12.1% and 4.5%, respectively. These results did not differ between OCP users and non-users. A significant relation between hr-HPV detection and the timing of sampling was found when OCP users and nonusers were analyzed separately. In the second half of the menstrual cycle hr-HPV detection decreased in nonusers (P=.007) and increased in OCP users (P=.021). When women used OCPs continuously, hr-HPV detection returned to level of the first half of the menstrual cycle.

Conclusion: Hr-HPV detection was significantly influenced by sample timing in the menstrual cycle when analyzed separately for OCP users and women with a natural menstrual cycle. This may have implications in the future, when hr-HPV detection may become a primary screening tool in cervical cancer prevention.

Introduction

Human papillomavirus (HPV) infection is a necessary event in the carcinogenesis of cervical cancer. However, only a small fraction of women infected with high-risk HPV types will eventually progress to high-grade intraepithelial lesions and cervical cancer. High-parity, long-term oral contraceptive pill (OCP) use, smoking, and coinfection with other sexually transmitted diseases, are the most consistently identified co-factors in cervical carcinogenesis.[1-3]

Oral contraceptive use is associated with invasive cervical cancer. The international collaboration of epidemiological studies of cervical cancer (International Agency for Research on Cancer) showed that for current users of OCP, the risk of invasive cervical cancer increased with increasing duration of use with a relative risk of 1.90 (95% confidence interval [CI] 1.69-2.13) for 5 years or more. This increased relative risk returned to normal after stopping OCP use for 10 years or more.[4] Although OCPs are defined as a risk factor for cervical cancer, there is no evidence for an association with HPV positivity.[5]

Despite the lack of proof for a relation between OCP use and HPV positivity, there are indications that hormonal factors might influence HPV detection. The detection of HPV related to a woman's last menstrual period (LMP) has been examined in a number of studies. Some studies found a relation between the LMP and HPV detection. However, their results were not concordant because they found different phases of the menstrual cycle to be optimal for HPV detection.[6-9] On the other hand, studies that found no relation between HPV detection and the menstrual cycle suggest that only the sampling technique or the materials used may affect HPV detection.[10-12] These studies on HPV prevalence and LMP are summarized in Table 1.

The mucosal immunity of the female genital tract, which is influenced by immunoglobin, cytokines and reproductive hormones, may explain this possible effect of the menstrual cycle on HPV detection. Exogenous and endogenous hormones increase the production of immunoglobin-producing cells in cervical secretions.[13] Total immunoglobulin (Ig) A and IgG levels were higher in OCP users than in woman with a natural menstrual cycle (nonusers).[13;14] In OCP users, the immunoglobin titer in the cervical mucus lowest during the week OCP use is stopped and increases with the start of use, whereas in nonusers, the IgA and IgG levels were highest during the follicular phase and lowest around ovulation.[13-15] These patterns in Ig levels during the menstrual cycle suggest an important role for reproductive hormones in the regulation of mucosal immunity.[14]

Because the use of OCP is associated with an increased relative risk of cervical cancer, we expected that the prevalence and persistence of high-risk HPV is higher in women using OCPs compared with nonusers. According to a previous study from our group,[8] we would expect to find a higher high-risk (hr) HPV detection rate in the second week of the menstrual cycle in nonusers. Whether fluctuating Ig levels truly influence HPV detection and the mode of this potential influence needs to be explored. When OCP use and sample timing within the menstrual cycle do affect HPV detection, this may

have implications in the future as HPV testing using self-samples becomes an important tool in cervical cancer screening programs. We analyzed cervicovaginal self-samples and questionnaires of a large population of young, unscreened women to estimate any potential influence from OCP use and sample timing within the menstrual cycle on HPV detection. Additionally, we investigated the HPV prevalence, incidence, and persistence in this group of young, unscreened women. Furthermore, we investigated the potential difference in the HPV prevalence, incidence, and persistence between OCP users and nonusers.

Methods and Materials

Study population & study design

This study is part of a 1-year prospective longitudinal study on HPV prevalence, clearance and persistence, performed in the Netherlands in 2007–2008 among 2065 unscreened women 18-29 years of age.[16] Participants were recruited through advertisements, posters, flyers, the Internet, and active recruitment sites. Exclusion criteria were: not being in the age range of 18-29 years, being pregnant, or not being fluent in Dutch. Of the 2297 women who responded, 2065 (90%) were eligible for participation and provided an informed consent. A total of 253 (12.3%) women were excluded from further analyses

Table 1.
Studies on Human Papillomavirus prevalence and menstrual cycle

Author	Sampling Technique	HPV Detection Test used	
Fairly, C.K., et al.	Single tampon specimen (in/out specimen)	PCR amplification	
Harper, D.M., et al.	5 HPV screening test: clinician-directed ectocervical swab, clinician-directed endocervical swab, a self-sampled synthetic polyester fiber swab (Dracon), a second self-sampled Dracon swap, and a self-sample tampon, in random order	PCR amplification	
Schneider, A., et al.	Single point cervical swabs cumulative every 5 weeks in 1 year by clinician	PCR mixture	
Sherman, M.E., J.D. Carreon, and M. Schiffman	2 samples: Papette tm broom (Wallach Surgical, Orange, CT, USA) and a Dracon swab by clinician	PCR-based assay	
Van Ham, M.A., et al.	4 samples in one mentrual period by clinician with the Cervix brush (Rovers, Oss, the Netherlands)	SPF10-PCR LiPA	
Wheeler, C.M., et al.	12 weekly vulvar and cervical swabs were taken by clinician as well as cervicovaginal saline lavage s. 10 were used for evaluation.	PCR and the ViraPap (Digene Diagnostics, Silver Spring, MD) DNA dot-blot assay	

HPV, human papillomavirus; PCR, polymerase chain reaction.

from the 2065 participants at study entry. These women became pregnant (n=63, [3.0%]), were 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 (94.0%) 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 the sexually active women were used for further analyses.

Written informed consent was obtained from all participants. This study was approved by the local medical ethics committee.

All women received a questionnaire and a self-sample kit by mail. The self-sample kit contained an explanatory letter, an illustrated instruction form on how to perform the cervicovaginal self-sample, a small brush in a sterile cover (Rovers Vibabrush®, Rovers Medical Devices, Oss, the Netherlands), and a collection tube containing medium (SurePath™, Tripath Imaging®, Inc., Burlington NC, U.S.A.). To return the self-sample, a leakproof sealed bag, absorption sheet, and a recloseable plastic return envelope (easyslider, Transposafe Systems Holland BV, Sassenheim, the Netherlands) were added, as described before.[16] The accuracy of cervico-vaginal self-samples is highly comparable to physician-taken samples and it is inexpensive, feasible, and viewed by women as a convenient and acceptable method.[17;18]

The questionnaire consisted of two parts. One part concerned demographic information and the other part included questions on sexual behavior as well as questions about current OCP use and the LMP. To ensure privacy, the questionnaires were provided with

	HPV Types	Number subjects	Result
	High-risk: 16, 18 Low-risk: 6,11	298	Pellet volume of tampon specimen, but not HPV detection, vary during the menstrual cycle
	High-risk: 16,18, 26, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 68, NM4, NM7, NM9) Low-risk: 6, 11, 40, 42, 53, 54, 57, 66, MM8)	103	Percentage of positive samples for HPV did not differ by week of the menstrual cycle
	High-risk: 16	21	HPV 16 detection significantly (P= 0.019) higher in luteal phase
	High-risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68	5060	Small effect of mid-cycle specimens on highest HPV load
	25 different HPV types	20	Significantly (P=0.02) higher rate of HPV positive samples in the follicular phase (7th to 11th day)
	High-risk; 16, 18, 31, 33, 35, 39, 45, 51, 52, 59 Low-risk: 6, 11, 53, 54	72	No correlation between HPV DNA detection and phase of menstrual cycle was observed

an anonymous code and all the data were entered in a computer program. The self-samples were stored at room temperature and were sent to the Department of Medical Microbiology for processing and HPV detection.

Broad-spectrum HPV DNA amplification was performed using a short-PCR-fragment assay (SPF_{10} HPV PCR, Laboratory Biomedical Products B.V., Rijswijk, the Netherlands) as described before.[19;20] HPV types 6, 11, 34, 40, 42, 43, 44, 53, 54, 55, 58, 66, 70, 74, and "X" are defined as low-risk types and HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59, 68, 73, and 82 as high-risk types.

Data at study entry, month 0, were used for the analysis of HPV prevalence. The annual incidence of HPV was a result of the women acquiring a new HPV type at month 6 or month 12. Women positive for any HPV type or group (lr- or hr-HPV) at all three measuring points were defined as persistent.

To investigate whether there were any potential confounding factors influencing hr-HPV, the data on demographics and sexual behavior at study entry were compared between the OCP users and nonusers. At study entry 72.7% (n=1315) of the 1812 women were using OCPs.

During the follow-up period of 1 year, 61.1% (n=1108) of the women were using OCPs during the whole year of follow-up and 20.9% (n=378) never used OCPs. This results in 18.0% (n=326) women changeably using OCPs. These changeable users were excluded from the analysis when comparing the incidence and persistence between OCP users and nonusers. Women who reported using the Nuvaring® were registered as OCP users, because this creates local exogenous hormonal fluctuations comparable to OCP use.

The menstrual cycle was divided into the first half (days 1-14) and the second half (days 15-28) of the cycle to examine if the timing of sampling related to the LMP or oral contraceptive withdrawal bleeding influences the HPV detection. The cycle was divided into four weeks (days 1-7, 8-14, 15-21 and 22-28) for further analysis of these results. The high-risk HPV detection rate in women on continuous OCP use, with a cycle of more than 28 days, was also analyzed.

Of the 5436 samples (at three time points, 1812 samples) taken during the follow-up period, 3904 samples (71.8%) had data on the LMP and oral contraceptive withdrawal bleeding and were taken within 28 days or less of the menstrual cycle. Data on OCP use also needed to be available for the analysis of the combined effect of sample timing related to the LMP and oral contraceptive withdrawal bleeding and OCP use on hr-HPV detection. This resulted in 3893 (99.7%) self-samples taken within 28 days or less of the menstrual cycle. Specified for OCP users and nonusers, there were 2893 and 1000 samples, respectively. From women on continuous OCP use, samples taken more than 28 days after the oral contraceptive withdrawal bleeding, 684 samples were available for analysis.

Analysis on demographic data and HPV prevalence, incidence and persistence were performed using frequencies, cross tables, and chi-square. Data on LMP and OCP use were calculated separately and combined. The binary logistic regression model was used

as univariable test to see if there was a potential confounder between OCP users and nonusers. Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL).

Results

Of the 1812 participants, 1750 (96.6%) were of Dutch ethnicity with a mean age of 23.2 years (standard deviation 3.3), 335 (18.5%) women smoked, and 1315 (72.7%) women used OCPs. In total, 1703 (94.0%) women were sexually active at study entry. From these sexually active women 356 (20.9%) had one lifetime partner, 809 (47.5%) had two to five, 347 (20.4%) had six to 10, 183 (10.7%) had more than 10 lifetime partners and for eight (0.5%) women no data were available on the number of lifetime partners. As shown in Table 2, there are no confounding factors within the demographic characteristics and data on sexual behavior that could have significantly influenced the odds ratio for hr-HPV prevalence between OCP users and nonusers. Interestingly, this table shows that condom use did not differ significantly between OCP users and nonusers. Thus, condom use was not considered a confounding factor for hr-HPV detection between these two groups.

No effect of the sampling period on hr-HPV detection was found when analyzing the total group of women and all three sampling times together. However, a significant difference was found in hr-HPV detection with regard to the sampling time when analyzing OCP users (3577 samples) and nonusers (1000 samples) separately. Hr-HPV detection increased significantly in the second half of the cycle for the OCP users (*P*=.021), whereas a significant (*P*=.007) decrease in the hr-HPV detection was observed in the second half of the menstrual cycle for the nonusers (data shown in Table 3).

After dividing the menstrual cycle into four sampling weeks, the decrease of hr-HPV detection in nonusers remained significant (*P*=.049), although the increase of hr-HPV detection during the contraceptive cycle became a non significant linear trend (*P*=.073), as shown in Figure 1.

At study entry, HPV DNA was detected in 18.9% (95% CI: 17.15-20.81) of all 1812 women. For lr-HPV and hr-HPV, the prevalence was 8.9% (95% CI: 7.61-10.29) and 11.8% (95% CI: 10.31-13.33), respectively. No significant difference in HPV prevalence between OCP users and nonusers was found. Furthermore, the prevalence of lr-HPV and hr-HPV was also equally divided within the OCP users and nonusers, 9.1 % (95% CI: 7.62-10.81) compared with 8.1% (95% CI: 5.84-10.84) and 11.7 % (95% CI: 10.02-13.57) compared with 11.9% (95% CI: 9.20-15.10), respectively.

Of all women, 67.2% (95% CI: 65.00-69.38) never had any type of HPV at time months 0, 6, and 12. This was 80.0% (98% CI: 78.11-81.84) for lr-HPV and 77.3% (95% CI: 75.32-79.23) for hr-HPV. Newly detected HPV at months 6 and 12 was 16.1% (95% CI: 14.45-17.89) for any-type HPV and 12.5% for (95% CI: 11.04-14.14)for lr-HPV and 12.1% (95% CI: 10.67-13.73) for hr-HPV. At months 0, 6, and 12, HPV DNA was persistently detected in 8.7% (95% CI: 7.41-10.06) of all women in the year of follow-up. For lr-HPV and

Table 2.
Demographic characteristics and data on sexual behavior for OCP users and nonusers

	n	OCP users	n	Nonusers	OR (95%CI) of OCP users for hr-HPV
Age	1315	22.8 ± 3.1	495	24.5 ± 3.2	1.13 (0.82-1.58)
Ethnicity	1315		495		
Dutch		1277 (97.1)		471 (95.2)	0.98 (0.71-1.35)
Other		38(2.9)		24 (4.8)	
Current smoking	1308		494		
Yes		231 (17.7)		104 (21.1)	1.01 (0.73-1.40)
No		1077 (82.3)		390 (78.9)	
Living with parents	1309		492		
Yes		264 (20.2)		52 (10.6)	1.02 (0.74-1.41)
No		1045 (79.8)		440 (89.4)	
Relationship	1311		494		
Married		43 (3.3)		35 (7.1)	0.87 (0.62-1.22)
Living together		294 (22.4)		123 (24.9)	
Living apart together		632 (48.2)		122 (24.7)	
Single		342 (26.1)		214 (43.3)	
Sexual activity ever	1314		494		
Yes		1274 (97.0)		428 (86.6)	0.89 (0.64-1.23)
No		40 (3.0)		66 (13.4)	
Age at first intercourse	1274		426		
13 or younger		22 (1.7)		14 (3.3)	0.91 (0.66-1.27)
14–16		626 (49.2)		200 (46.9)	
17–19		524 (41.1)		173 (40.6)	
20 or older		102 (8.0)		39 (9.2)	
Lifetime sexpartner(s)	1269		425		
1		293 (23.1)		63 (14.8)	1.24 (0.88-1.75)
2–5		628 (49.5)		181 (42.6)	
6–10		240 (18.9)		106 (24.9)	
More than10		108 (8.5)		75 (17.7)	
Gender of sex partner(s)	1271		523		
Male		1261 (95.7)		381 (90.1)	0.95 (0.68-1.33)
Female		2 (0.1)		3 (0.7)	
Both		53 (4.2)		39 (9.2)	

Table 2. Continued

	n	OCP users	n	Nonusers	OR (95%CI) of OCP users for hr-HPV
Sex partner(s) in past 6 mo (number)	1268		428		
0		98 (7.7)		56 (13.1)	0.94 (0.67-1.31)
1		990 (78.1)		301 (70.3)	
2		123 (9.7)		46 (10.7)	
More than 2		57 (4.5)		25 (5.9)	
Sexual contact in past 6 mo (frequency)	1234		420		
0		83 (6.7)		50 (11.9)	0.98 (0.71-1.40)
1-6		127 (10.3)		75 (17.9)	
7-24		140 (11.3)		59 (14.0)	
25-54		502 (40.7)		138 (32.9)	
More than 54		382 (31.0)		98 (23.3)	
Ever diagnosed a STI?	1273		425		
No		1168 (91.8)		365 (85.9)	1.00 (0.71-1.38)
Yes		105 (8.2)		60 (14.1)	
Condom use	1270		423		
Never (0%)		639 (50.3)		159 (37.6)	0.88 (0.63-1.24)
Sometimes (0–50%)		351 (27.6)		93 (22.0)	
Most of the times (50-100%)		198 (15.6)		88 (20.8)	
Always (100%)		82 (6.5)		83 (19.6)	
Sexual Age (y)	1273	6.1 ± 3.3	526	8.1 (3.4)	1.06 (0.75-1.49)

OCP, oral contraceptive pill; OR, odds ratio; CI, confidence interval; HPV, human papillomavirus; STI, sexually transmitted infection.
Data are n, mean ± standard deviation, or n (%) unless otherwise specified.

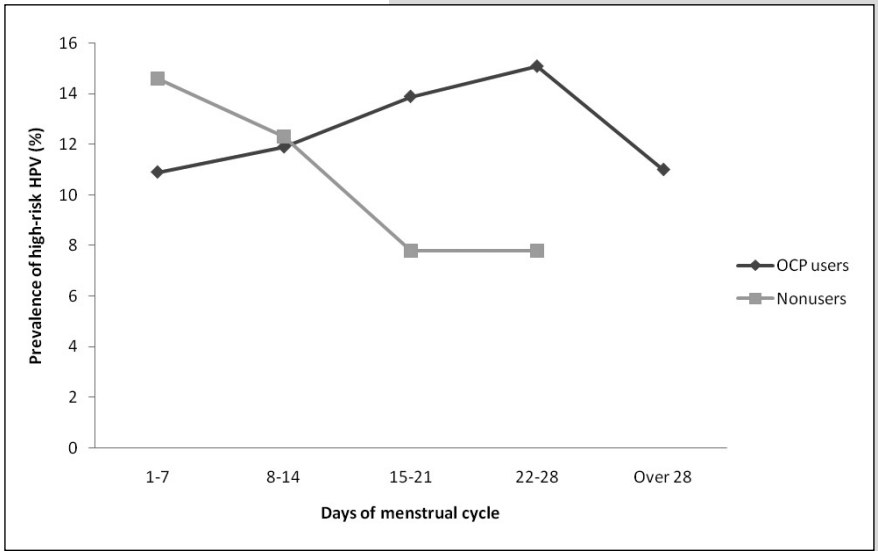
hr-HPV, this was 2.0% (95% CI: 1.40-2.74) and 4.5% (95% CI: 3.62-5.59), respectively. The results on HPV incidence and persistence did not significantly differ between OCP users and nonusers.

Concluding, OCP use did not influence HPV prevalence, incidence or persistence. Additionally, sample timing related to the menstrual cycle had no significant effect on HPV detection in the total group. However, a significant relation between hr-HPV detection and sample timing related to the menstrual cycle was found when separate analysis for OCP users and nonusers was performed. In the second half of the menstrual cycle hr-HPV detection decreased in nonusers (P=.007) and increased in OCP users (P=.021).

Table 3.
Hr-HPV prevalence in sample moments of total group and for OCP users and nonusers divided by LMP sample moments

Day of Last Menstrual Period or Day After Start of Last OCP Withdrawal Bleeding	OCP users (n=3577)	Nonusers (n=1000)
0-14	159/1378 (11.5%)	65/500 (13.0%)
15-28	219/1515 (14.5%)	39/500 (7.8%)
More than 28	75/684 (11.0%)	N.A.
P	0.021	0.007

Figure 1: Human papillomavirus detection for oral contraceptive pill users and nonusers with respect to the last menstrual period and withdrawal bleeding.



Menstrual cycle is divided in 4 weeks for nonusers; the decrease of hr-HPV detection in the second half of the menstrual cycle is significant (P=.049). The increase of hr-HPV detection in the second half of contraceptive cycle lost significance (P=.073). OCP, oral contraceptive pill.

Discussion

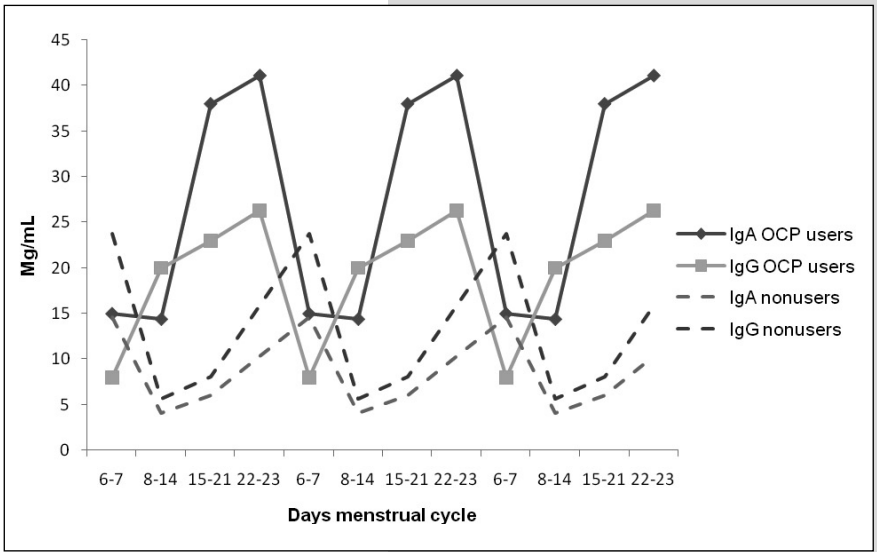
The findings of this longitudinal study among unscreened, young women support the idea that reproductive hormones may influence high-risk HPV detection. In women with a natural menstrual cycle, hr-HPV detection decreased with the duration of the cycle, whereas in OCP users, the hr-HPV detection increased during the second half of the OCP cycle, as depicted in Figure 1. This opposite trend in hr-HPV detection during the menstrual cycle between OCP users and nonusers explains why hr-HPV detection in the total study population was not significantly influenced by the sample timing within the menstrual cycle. Another large study investigating hr-HPV detection within the menstrual cycle showed a small midcycle increase of HPV detection.[9] However, data on OCP use are not reported and therefore it is difficult to compare this study with our results. A smaller study by our group, in nonusers, found a higher rate of HPV-positive samples in the follicular phase. Because the follicular phase is in the first half of the menstrual cycle these data are similar to our findings in the nonusers.[8] This may have implications in the future, when hr-HPV detection may become a primary screening tool in cervical cancer prevention.

An explanation for the fluctuation in hr-HPV detection in the natural menstrual cycle may be found in the changes in the mucosal immunity. The mucosal immunity is influenced by reproductive hormones and reaches the lowest level around ovulation in the natural menstrual cycle.[13-15] A lower mucosal immunity may lead to an increase of HPV replication and therefore HPV detection. However, there is no direct evidence for this assumption in the literature. We hypothesize that there may be some delay between the actual fluctuations in the mucosal immunity and the level of hr-HPV detection. The higher mucosal immunity in the follicular phase may lead to the decrease of hr-HPV detection in the second half of the natural menstrual cycle, whereas the lower mucosal immunity during ovulation may induce increased hr-HPV detection in the first half of the next menstrual cycle. According to this hypothesis, the increase of hr-HPV detection shown during the second half of the OCP cycle may be a delayed effect of the lower mucosal immunity in the week OCP use is stopped. To illustrate this hypothesis, we used the results on the mucosal immunity (IgG and IgA levels) as previously shown by Franklin et al. and displayed them in Figure 2.[13] As shown in this figure, the mucosal immunity shows opposite patterns for OCP users and nonusers.

Another possible explanation for the increase of hr-HPV detection in the second half of the OCP cycle may be a result of the positive influence of estrogens on the viral transcription and the expression of the viral oncogenes (E6 and E7).[21] However, these two explanations do not explain the decrease of hr-HPV detection in women on continuous OCP use. Perhaps the continuous positive stimulation of OCP on the mucosal immunity and the influence of the estrogens on the viral oncogenes balance each other after several weeks.

The lack of evidence for an association between OCP use and hr-HPV prevalence is confirmed in several other studies. In some of these studies only the median number of

Figure 2: Fluctuation of the IgA and IgG levels during the menstrual cycle.



This figure displays the weeks of three menstrual cycles to visualize the fluctuations. OCP, oral contraceptive pill. (Based on data from Franklin RD, Kutteh WH. Characterization of immunoglobulins and cytokine in human cervical mucus: influence of exogenous and endogenous hormones.[13])

sex partners seemed to influence the hr-HPV prevalence significantly.[7;16;22-25] Additionally, studies that did show a significant association between hr-HPV prevalence and OCP use suggested a greater exposure to HPV resulting from a presumed association between OCP use and sexual behavior.[26-28] Because we did not detect a difference in sexual behavior between the groups of OCP users and nonusers, sexual behavior was not a confounding factor in this study.

Mouse models have shown that exogenous estrogens play an essential role in the carcinogenesis of cervical cancer. The estrogens stimulate the viral oncoproteins E6 and E7 and the coexpression of these oncoproteins contributes to the development of cervical cancer.[29] Additionally they have shown that estrogens, besides their role in the development of cervical lesions also contribute in the persistence and continued development of cervical cancer.[30] Because epidemiological studies support the carcinogenic effect of OCP use, it does not prove that OCPs influence hr-HPV prevalence per se.[5;7;16;31] The role of OCPs in the carcinogenesis might be explained with a possible role in facilitating HPV reactivation or persistence.[1;24;30;32] Exogenous hormones enhance HPV transcription and decrease HPV clearance in women, which may lead to more persistent hr-HPV infections resulting in cervical neoplasia and cervical cancer.[4;33]

Despite that HPV persistence is expected to be higher among OCP users, we did not

find any significant difference in HPV incidence and persistence between OCP users and nonusers. This finding is supported by others and may be explained by the short duration of follow-up (1 year), or a lack of data on OCP use in our study.[24;34-36] Because the questionnaire only asked about current OCP use, data on previous use for nonusers or the duration of use for OCP users were not available. Because a longer duration of OCP use is associated with an increased risk of cervical cancer, the lack of data on the duration of OCP use might be a caveat in this study.[37;38] This relatively young group of women may have a shorter duration of OCP use compared to women with cervical abnormalities. However, the increased risk with the duration of OCP use is relative, because the risk is reversible 10 years after use has stopped.[4;38]

In conclusion, hr-HPV detection measured with the highly sensitive SPF_{10} LiPA is influenced by the sampling period related to the LMP or OCP withdrawal bleeding. Further studies need to investigate whether this effect remains with a less sensitive test to elucidate the clinical implications of HPV detection in primary cervical cancer screening. Because OCP use does not significantly influence HPV prevalence, incidence or persistence, its increased risk for cervical cancer may be explained by a direct hormonal effect on the carcinogenesis.

Reference List

1. Castellsague X, Bosch FX, Munoz N. Environmental co-factors in HPV carcinogenesis. Virus Res. 2002;89:191-99.
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;189:12-19.
3. Bosch FX, de SS. The epidemiology of human papillomavirus infection and cervical cancer. Dis.Markers 2007;23:213-27.
4. Appleby P, Beral V, Berrington de GA, Colin D, Franceschi S, Goodhill A et al. Cervical cancer and hormonal contraceptives: collaborative reanalysis of individual data for 16,573 women with cervical cancer and 35,509 women without cervical cancer from 24 epidemiological studies. Lancet 2007;370:1609-21.
5. Green J, Berrington de GA, Smith JS, Franceschi S, Appleby P, Plummer M et al. Human papillomavirus infection and use of oral contraceptives. Br.J.Cancer 2003;88:1713-20.
6. Schneider A, Kirchhoff T, Meinhardt G, Gissmann L. Repeated evaluation of human papillomavirus 16 status in cervical swabs of young women with a history of normal Papanicolaou smears. Obstet.Gynecol. 1992;79:683-88.
7. 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;168:421-25.
8. van Ham MA, Melchers WJ, Hanselaar AG, Bekkers RL, Boonstra H, Massuger LF. Fluctuations in prevalence of cervical human papillomavirus in women frequently sampled during a single menstrual cycle. Br.J.Cancer 2002;87:373-76.
9. Sherman ME, Carreon JD, Schiffman M. Performance of cytology and human papillomavirus testing in relation to the menstrual cycle. Br.J.Cancer 2006;94:1690-96.
10. Fairley CK, Robinson PM, Chen S, Tabrizi SN, Garland SM. The detection of HPV DNA, the size of tampon specimens and the menstrual cycle. Genitourin.Med. 1994;70:171-74.
11. Harper DM, Longacre MR, Noll WW, Belloni DR, Cole BF. Factors affecting the detection rate of human papillomavirus. Ann.Fam.Med. 2003;1:221-27.
12. Wheeler CM, Greer CE, Becker TM, Hunt WC, Anderson SM, Manos MM. Short-term fluctuations in the detection of cervical human papillomavirus DNA. Obstet.Gynecol. 1996;88:261-68.
13. Franklin RD, Kutteh WH. Characterization of immunoglobulins and cytokines in human cervical mucus: influence of exogenous and endogenous hormones. J.Reprod.Immunol. 1999;42:93-106.
14. Safaeian M, Falk RT, Rodriguez AC, Hildesheim A, Kemp T, Williams M et al. Factors associated with fluctuations in IgA and IgG levels at the cervix during the menstrual cycle. J.Infect.Dis. 2009;199:455-63.
15. Nardelli-Haefliger D, Wirthner D, Schiller JT, Lowy DR, Hildesheim A, Ponci F et al. Specific antibody levels at the cervix during the menstrual cycle of women vaccinated with human papillomavirus 16 virus-like particles. J.Natl.Cancer Inst. 2003;95:1128-37.
16. 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:e3743.

17. 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;81:207-12.

18. 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;186:365-73.

19. 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;155:1473-78.

20. 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;44:3122-29.

21. de Villiers EM. Relationship between steroid hormone contraceptives and HPV, cervical intraepithelial neoplasia and cervical carcinoma. Int.J.Cancer 2003;103:705-08.

22. Deacon JM, Evans CD, Yule R, Desai M, Binns W, Taylor C et al. Sexual behaviour and smoking as determinants of cervical HPV infection and of CIN3 among those infected: a case-control study nested within the Manchester cohort. Br.J.Cancer 2000;83:1565-72.

23. Syrjanen K, Shabalova I, Petrovichev N, Kozachenko V, Zakharova T, Pajanidi J et al. Oral contraceptives are not an independent risk factor for cervical intraepithelial neoplasia or high-risk human papillomavirus infections. Anticancer Res. 2006;26:4729-40.

24. Vaccarella S, Herrero R, Dai M, Snijders PJ, Meijer CJ, Thomas JO et al. Reproductive factors, oral contraceptive use, and human papillomavirus infection: pooled analysis of the IARC HPV prevalence surveys. Cancer Epidemiol.Biomarkers Prev. 2006;15:2148-53.

25. Veress G, Csiky-Meszaros T, Czegledy J, Gergely L. Oral contraceptive use and human papillomavirus infection in women without abnormal cytological results. Med.Microbiol. Immunol. 1992;181:181-89.

26. Cotton SC, Sharp L, Seth R, Masson LF, Little J, Cruickshank ME et al. Lifestyle and socio-demographic factors associated with high-risk HPV infection in UK women. Br.J.Cancer 2007;97:133-39.

27. Rousseau MC, Franco EL, Villa LL, Sobrinho JP, Termini L, Prado JM et al. A cumulative case-control study of risk factor profiles for oncogenic and nononcogenic cervical human papillomavirus infections. Cancer Epidemiol. Biomarkers Prev. 2000;9:469-76.

28. Sasieni P. Cervical cancer prevention and hormonal contraception. Lancet 2007;370:1591-92.

29. Riley RR, Duensing S, Brake T, Munger K, Lambert PF, Arbeit JM. Dissection of human papillomavirus E6 and E7 function in transgenic mouse models of cervical carcinogenesis. Cancer Res. 2003;63:4862-71.

30. Brake T, Lambert PF. Estrogen contributes to the onset, persistence, and malignant progression of cervical cancer in a human papillomavirus-transgenic mouse model. Proc.

Natl.Acad.Sci.U.S.A 2005;102:2490-95.

31. Veress G, Csiky-Meszaros T, Konya J, Czegledy J, Gergely L. Follow-up of human papillomavirus (HPV) DNA and local anti-HPV antibodies in cytologically normal pregnant women. Med.Microbiol.Immunol. 1996;185:139-44.

32. Moreno V, Bosch FX, Munoz N, Meijer CJ, Shah KV, Walboomers JM et al. Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study. Lancet 2002;359:1085-92.

33. 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;18:117-32.

34. Syrjanen K. New concepts on risk factors of HPV and novel screening strategies for cervical cancer precursors. Eur.J.Gynaecol. Oncol. 2008;29:205-21.

35. Bertram CC. Evidence for practice: oral contraception and risk of cervical cancer. J.Am.Acad.Nurse Pract. 2004;16:455-61.

36. Ho GY, Burk RD, Klein S, Kadish AS, Chang CJ, Palan P et al. Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. J.Natl.Cancer Inst. 1995;87:1365-71.

37. 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;18:1060-69.

38. Smith JS, Green J, Berrington de GA, Appleby P, Peto J, Plummer M et al. Cervical cancer and use of hormonal contraceptives: a systematic review. Lancet 2003;361:1159-67.

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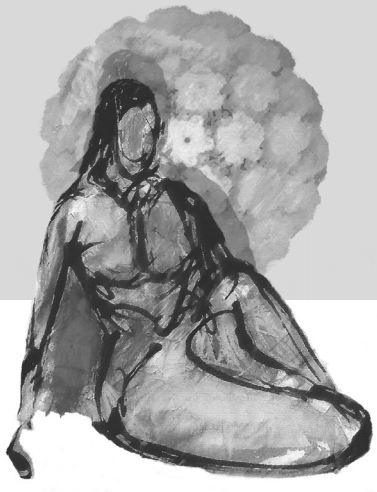

Chapter 6

Human papillomavirus detection in pregnant women, a prospective matched cohort study

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Submitted

Abstract

The objective of this study is to study prevalence, incidence and clearance of human papillomavirus (HPV) in pregnant and non pregnant women.

In this prospective matched cohort study; 51 women, falling pregnant during follow-up of an HPV epidemiology study (n=2065), and 51 matched non-pregnant women were included. All women provided 3-monthly cervico-vaginal self-samples and completed a questionnaire. The PCR SPF_{10} $LiPA_{25}$ was used for HPV testing. Matching was performed using a propensity score. The cumulative prevalence of hr-HPV was 19.6% (n=10) of the pregnant and 17.6% (n=9) of the matched-control women. The time-point prevalence of any-type HPV and high-risk (hr-) HPV was not significantly different for pregnant and matched-control women. After baseline, there were 10 newly detected hr-HPV types in 6 (11.8%) of the pregnant women, and there were 11 newly detected hr-HPV types in 8 (15.7%) of the matched-control women. There was no difference in HPV clearance between pregnant and matched-control women.

In conclusion, this study shows that in a low-parity population of young, unscreened women, pregnancy does not seem to influence HPV prevalence, incidence and clearance.

Introduction

It is now well established that infection with high-risk (hr-) human papillomavirus (HPV) types is the necessary cause of cervical cancer. Although essential, hr-HPV is not the only factor in the multi-step process to cervical cancer development. Two of the co-factors that have been associated with cervical carcinoma include high parity and younger age at first full-term pregnancy.[1-6]

Indeed, the International Agency for Research on Cancer (IARC) reported in a pooled analysis an increased risk of squamous-cell cervical carcinoma for women with high parity. Women with seven full-term pregnancies (FTP) or more had an odds ratio of 3.8 (95% CI: 2.7-5.5) compared to nulliparous women, and 2.3 (95% CI: 1.6-3.2) compared to women with 1-2 FTP.[7]

It has been described that parous women have a slower HPV clearance,[8] but HPV prevalence does not seem to be influenced by high parity and early age at first FTP.[9;10] Moreover, some studies showed that having been pregnant was associated with reduced hr-HPV infection risk.[10;11] Studies that did not find an association between parity and the risk for cervical intraepithelial neoplasia (CIN) or cervical carcinoma were mostly investigating a low-parity population.[12-14]

Hormonal, traumatic, and immunologic mechanisms have been hypothesized as possible explanations for the association between parity and cervical abnormalities in women infected with HPV.[3] The hormonal changes in pregnancy (increased levels of estrogen and progesterone) may influence the immune response to HPV and influence the risk of persistence of HPV or progression to CIN lesions and cervical carcinoma.[3;15]

Studies, reporting HPV prevalence during pregnancy are not conclusive. Some studies did not find evidence for a higher detection rate of HPV in pregnant women compared to non pregnant women,[16-18] whereas others did show a higher HPV prevalence during pregnancy.[19;20] A trend of increasing clearance of HPV post partum was also described.[18;21]

To explain the potential increase of HPV prevalence during pregnancy with a catch-up clearance post partum, it is suggested that the immune-response against HPV is decreased during the first two trimesters of pregnancy with a catch-up in the third trimester and postpartum.[18;22]

Most of the studies performed on the relationship of HPV and pregnancy are cross-sectional, and in the majority of the prospective studies no data were available on the HPV status prior to the pregnancy.

The aim of this prospective matched cohort study is to evaluate the natural HPV prevalence, incidence and clearance during pregnancy, compared with a matched-control group of non pregnant women in an unscreened low parity population.

Materials and Methods

This prospective matched cohort study is part of a longitudinal study on HPV prevalence, incidence, clearance and persistence among young (18-29 years) unscreened women, as described previously.[23] Exclusion criteria at study entry were, being outside the age range

of 18-29 years and pregnancy. Women also had to be fluent in Dutch, because they had to be able to understand and fill-out the questionnaire. In total, 2065 (90%) women were eligible for participation and provided an informed consent. The cervico-vaginal samples for HPV detection were obtained using self-sampling. This study was approved by the local Medical Ethical Committee.
Of the 68 women who became pregnant during the first year of this longitudinal study, 6 women had a miscarriage. The remaining 62 women were invited for follow-up until post-partum, 57 (92%) agreed and participated with this follow-up study. To obtain a matched control group we used a propensity score. The goal of the propensity score analysis is to balance two nonequivalent groups on observed covariates to get more accurate estimates of the effects on which the two groups differ, i.e to remove bias due to all observed covariates in question. The propensity score is the conditional probability of assignment to a particular group (i.e. pregnancy) given the observed covariates, using (multivariable) logistic regression to estimate the strength of each variable. As a consequence, values of a specific covariate may differ between the groups as long as the conditional probabilities are equal. This in contrast to a one to one matching on some covariates itself.[24;25] The propensity score was constructed on the basis of pregnancy as an outcome.
The propensity score was based on variables significantly influencing HPV prevalence that we found previously.[23] This included the following variables; age, smoking, type of current sexual relationship (i.e., married, living together, couple living apart, or single), sexual age (number of years between age of first sexual intercourse and current age), sexual frequency in the past 6 months, number of sex partners in the past 6 months, life-time number of sex partners, gender of sex partners, condom use and whether they were ever diagnosed with an sexually transmitted infection (STI).
We were able to match 51 of the 57 pregnant women with 51 non-pregnant women from the original study population.

Data collection
All women, pregnant and matched-control, received a questionnaire and a self-sample kit by mail. The self-sample kit contained an explanatory letter, an illustrated instruction form on how to perform a cervico-vaginal self-sample, a small brush in a sterile cover (Vibabrush®, Rovers Medical Devices Oss, the Netherlands), and a collection tube containing medium (SurePath™, Tripath Imaging®, Inc., Burlington NC, U.S.A.). To return the self-sample, a leak-proof seal bag, absorption sheet, and a reclosable plastic return envelope (easyslider, Transposafe Systems Holland BV) were included, as described before.[23]
The questionnaire consisted of two parts. One part concerned demographic information and the other part included questions on sexual behavior as well as questions about current or past pregnancy. To ensure privacy the questionnaires were provided with an anonymous code and all the data were entered in a computer program. The self-samples were stored at room temperature and were sent to the Department of Medical

Microbiology for processing and HPV detection.
The matched-control women provided five self-samples with a 3 month interval, one at baseline (month 0), month 3, month 6, month 9 and month 12.
The pregnant women were asked to provide one self-sample in the first trimester, second trimester, third trimester and one within the first 6 months after delivery. The baseline sample was the last sample in the original study before the women became pregnant. To have a complete follow-up until after delivery the women consented to provide samples after the original study stopped. Unfortunately, not all women were completely compliant to the study protocol. Therefore, 4 pregnant women had a missing sample at baseline (3 months prior to falling pregnant) and not all women provided a self-sample at each sample moment. The amendment for extra follow-up after the original protocol for pregnant women was approved by the local Medical Ethical Committee.

Human papillomavirus incidence and clearance
Women were considered to have an incident infection if they tested positive for a type-specific HPV during follow-up, and this HPV type was not detected at baseline or in 2 previous consecutive samples.
When the type-specific infection was not detected in 2 consecutive samples during follow-up, or when the last available sample was negative, the infection was considered cleared. Time to clearance could only be estimated in women negative for that specific HPV type at baseline, since there is no information on the duration of the infection before start of the study.
A persistent infection is defined as a type-specific infection present in ≥2 sample moments, with a maximum of 1 negative sample in between.

HPV testing
Broad-spectrum HPV DNA amplification was performed using a short-PCR-fragment assay. Extracted DNA was used for PCR amplification with the SPF_{10} primer sets, as described earlier.[26;27] The samples were run through an HPV DNA enzyme immunoassay (DEIA) to obtain an OD reading, and categorized as HPV DNA negative, positive, or borderline. The same SPF_{10} amplimers were used on SPF_{10}-DEIA-positive samples to identify HPV genotype by reverse hybridization on a line probe assay (LiPA) (SPF_{10}HPVLiPA$_{25}$, version 1; Labo Bio-Medical Products B.V., Rijswijk, the Netherlands), which detects 25 HPV genotypes: lr-HPV types: type 6, 11, 34, 40, 42, 43, 44, 53, 54, 55, 66, 70, 74, and "X" (DEIA positive and LiPA negative samples); and hr-HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82.

Table 1.
Demographic and sexual behavior baseline characterisitics

	Pregnant women n=51 (%)	Matched-control women n=51 (%)
Age, mean (SD) **[range]**	27.4 years (1.6) [23-30]	27.6 years (1.3) [24-29]
Ethnicity		
Dutch	98	88
Other	2	12
Education		
Lower secondary / Lower vocational training	4.1	8.2
Higher secondary / Vocational training	34.1	26.5
Higher vocational training	61.2	65.3
Current smoking		
Yes	13.7	9.8
No	86.3	90.2
Type of relationship		
Married	45.1	37.3
Living together	51.0	56.9
Couple, living apart	2.0	-
Single	2.0	5.9
Age at first sexual intercourse mean (SD)	17.1 (2.0)	17.1 (1.9)
Sexual agea mean (SD)	10.3 (2.6)	10.5 (2.3)
Number of lifetime sex partners		
1	21.6	23.5
2-5	50.9	56.8
6-10	19.7	13.8
>10	7.8	5.9
Number of sex partners in the past 6 months		
0	2.0	2.0
1	94.1	94.1
2	3.9	3.9
>2	-	-
Sexual frequency in the past 6 months		
0	-	-
1-6	3.9	3.9
7-24	17.6	17.7
25-54	41.2	51.0
>54	37.3	27.4
Ever had STI?		
Yes	11.8	15.7
No	88.2	84.3

Table 1. Continued

	Pregnant women n=51 (%)	Matched-control women n=51 (%)
Condom use		
Never (0%)	71.4	68.6
Sometimes (0-50%)	12.2	9.8
Most of times (50-100%)	8.2	11.8
Always (100%)	8.2	9.8
Gender of sex partner(s)		
Male	98.0	100
Female	-	-
Both	2.0	-

[a] Sexual age: number of years between age of first sexual intercourse and current age

Statistical analyses

The test of McNemar was used to test differences in HPV prevalence at each time point of sampling, between the pregnant women and their matched controls for statistical significance. A non-linear mixed model was used to analyze differences in HPV prevalence between pregnant women and their matched controls over time. The non-linear mixed model was used in order to account for the repeated data in the matched design. The dependent variable was the logarithmic transformed odds of HPV prevalence (any HPV, low-risk HPV and high-risk HPV, respectively) and the dependent categorical variables were pregnancy (yes, no) and time point of sampling (baseline, first, second, third and fourth). The matched stratum was treated as a random variable. Initially the interaction term between the two categorical variables was also included in the model, but was omitted from the final model as this never reached the level of statistical significance. The adjusted odds ratios with the 95% confidence intervals of the final model are presented. Statistical analysis was performed using SPSS 16.0 for Windows and SAS 9.2 for Windows.

Results

Characteristics of participants

Because the pregnant and the matched-control women were matched with the propensity score no significant difference exists at baseline, see Table 1. The mean age of the pregnant women was 27.4 years [23-30 years], and non-pregnant women 27.6 years [24-29 years]. Both groups had a mean age at first sexual intercourse of 17.1 years. Of the pregnant women 21.6% had one lifetime partner and 50.9% had 2-5 lifetime partners, this was 23.5% and 56.8% for the matched-controls, respectively. This difference in number of lifetime partners was not statistical significant. Almost all of the pregnant (96.1%) and matched-control women (94.2%) were married or were living together. At baseline 11.8%

of the pregnant women and 15.7% of the matched-control group reported to ever have had an STI and the majority of both groups reported no condom use in the past 6 months. Forty-five pregnant women reported their number of pregnancies. Of them, 29 (64.4%) were primiparous, 13 (28.9%) were pregnant for the second time, 2 (4.4%) were pregnant for the third time, and 1 (2.2%) was pregnant for the fourth time. Past pregnancy history of the current non-pregnant women is not available.

Human papillomavirus prevalence

The prevalence of any-type HPV at baseline, was 12.8% in the pregnant group and 9.8% of the matched-control group. The prevalence for hr-HPV at baseline was 12.8% and 5.9%, respectively.

During the whole study follow-up, 21.6% (11/51) of the pregnant and 23.5% (n=12) of the matched-control women were ever positive for any-type HPV. For hr-HPV this was 19.6% (n=10) of the pregnant women and 17.7% (n=9) of the matched-control women. In total, the 10 pregnant women with hr-HPV had 18 type-specific hr-HPV infections and the 9 matched-control women with hr-HPV had 13 type-specific hr-HPV infections. The time-point type specific prevalence of any-type HPV, and hr-HPV for the pregnant and matched control women is shown in Table 2. The prevalence was not significantly different between the pregnant and matched-control women (McNemar, $p > 0.05$) at the specific time-points.

Table 3 displays whether hr-HPV detection is related to pregnancy or to the sample moment in time. The overall prevalence of hr-HPV during study follow-up (all sample moments together) was not influenced by pregnancy. Timing of sampling (i.e. the influence of time on HPV prevalence) did also not influence hr-HPV detection. Although, hr-HPV prevalence at the last sample moment (12 months), was slightly lower compared to the second sample moment (3 months) with an odds ratio of 0.23 (95% CI: 0.06-0.99) (see Table 3).

Table 2.
Time-point HPV prevalence

Follow-up time	Any HPV	Hr-HPV
Baseline		
Pregnant	12.8% (6/47)	12.8% (6/47)
Matched-control	9.8% (5/51)	5.9% (3/51)
3 months		
Pregnant	17.4% (8/46)	10.9% (5/46)
Matched-control	19.6% (9/46)	15.2% (7/46)
6 months		
Pregnant	10.5% (4/38)	7.9% (3/38)
Matched-control	8.5% (4/47)	8.5% (4/47)
9 months		
Pregnant	7.0% (3/43)	7.0% (3/43)
Matched-control	8.5% (4/47)	8.5% (4/47)
12 months		
Pregnant	6.2% (3/48)	4.2% (2/48)
Matched-control	15.2% (7/46)	8.7% (4/46)

- There is no significant difference in time-point HPV prevalence (any-type, or high-risk) between pregnant and matched-control women, measured with McNemar
- Not all of the 51 pregnant women and 51 matched-control women provided a sample at each sample moment, therefore the number of samples tested for HPV DNA fluctuate

Table 3.
The adjusted odds ratio with 95% confidence interval of pregnancy and of time point of sampling for the prevalence of high-risk HPV and using non-linear mixed model

	Hr-HPV	
	Adj. OR	CI
Pregnant		
Yes	1.09	(0.20-5.82)
No	1.00 (ref)	-
Timepoint		
Baseline	0.51	(0.14-1.87)
Month 3/ First trimester	1.00 (ref)	-
Month 6/ Second trimester	0.38	(0.09-1.52)
Month 9/ Third trimester	0.38	(0.09-1.52)
Month 12/ Post partum	0.23	(0.06-0.99)

Adj. OR: adjusted odds ratio; adjusting for the other variable in the model
CI: confidence interval
Ref: reference

Human papillomavirus incidence and clearance

All type-specific hr-HPV types detected at baseline and during study follow-up are displayed in Table 4a for the pregnant women, and in Table 4b for the matched-control women.

Duration of detection of prevalent infections is uncertain because we do not know the duration of infection before baseline. In the pregnant women, five of the eight prevalently detected hr-HPV types were cleared the next sample moment (first trimester), one persisted until the first trimester, one until the third trimester and one was still present at the end of study follow-up (post partum). In the matched-control women, one of the three prevalently detected hr-HPV types was cleared the next sample (month 3) but again detected at the last sample (month 12). Another hr-HPV type persisted until sample month 6 and the last one was continuously persistent until the end of study follow-up.

After baseline, there were 10 incident hr-HPV types in 6 (11.8%) of the pregnant women and 11 hr-HPV types in 8 (15.7%) of the matched-control women. These incidence rates were not statistically significant different between pregnant and matched-control women. Seven of the 10 (70.0%) incident hr-HPV infections in pregnant women were only detected once during pregnancy and two incident hr-HPV types were detected during the whole pregnancy, but they were cleared in the post partum sample. One incident hr-HPV type was first detected in the last sample (post-partum) and therefore the duration of infection could not be measured.

In the group of matched-control women seven of the 11 (63.6%) incident hr-HPV types were detected only once during follow-up. One hr-HPV type was detected from month 3 until month 12, and another was detected from month 9 till month 12. Three incident hr-HPV types were newly detected at month 12. Because 5 infections were present at the last sample of the follow-up (month 12), the time to clearance could not be calculated for these infections.

Discussion

No difference in the time-point HPV prevalence between pregnant and matched-control women was detected in this prospective matched cohort study. This is in accordance with other studies, mainly performed among low-risk populations for HPV.[16;17;28-30] Studies that did show a difference in HPV prevalence between pregnant and non pregnant women were cross-sectional and had a higher detection rate (24.9-37.2%) of hr-HPV in pregnant women.[19;20] Additionally, these studies were based on large selected populations, one from a family clinic only matching on age,[19] and the other was a non matched population selected from an STI and gynecology clinic.[20] We performed extensive matching, based on factors influencing HPV prevalence.[23] Therefore we excluded any known potential confounding factors influencing HPV prevalence and thus were able to solely study the influence of current pregnancy on HPV detection.

We did not find an increased HPV prevalence among the pregnant women during the

Table 4a.
Type specific hr-HPV detection in pregnant women

N	Baseline	1st Trimester	2nd Trimester	3th Trimester	Post Partum
1	52	--	52	--	52
1	16, 31	31, 58	--	31	--
1	52	52, 59	--	--	--
1	51	--	--	NA	59
1	31	NA	--	NA	--
1	52, 68	--	--	--	--
1	NA	56	NA	NA	--
1	--	31	31, 58	31	--
1	--	16	NA	16, 52	--
1	--	--	33, 56	--	NA
41	--	--	--	--	--

-- : negative for hr-HP
NA : sample for HPV DNA detection not available

Table 4b.
Type specific hr-HPV detection in matched-control women

N	Baseline	Month 3	Month 6	Month 9	Month 12
1	31	16, 31	31	31	16, 31
1	33	33	33	56	--
1	16	--	--	--	16, 18
1	--	16	16	16	16
1	--	31	--	18	18
1	--	16	--	--	--
1	--	31	--	--	--
1	--	68	--	--	--
1	--	--	33	--	--
42	--	--	--	--	--

-- : negative for hr-HPV

first and second trimester compared to the third trimester and post-partum. Therefore we could not confirm the hypothesis that the changed immune-response during pregnancy influences HPV incidence, prevalence and clearance. A study among pregnant women in Uganda also showed a balance between acquisition and clearance of HPV during and after pregnancy among their young study women.[22] They concluded that pregnancy does not seem to be a period of special vulnerability to HPV infection, despite a high burden of infection (60%) among young primiparous pregnant women in Uganda.[22] In contrast, some studies show that many pregnant patients with detectable HPV will have an increased clearance and become negative for HPV again later in pregnancy or in the post partum period.[16;18;21] Again, confounding factors, mainly associated with sexual behavior, may influence HPV detection in these studies.

The difference in HPV prevalence, incidence and clearance between different studies might also be explained by the HPV DNA detection test used. In this study we used the highly sensitive PCR test $SPF_{10}LiPA_{25}$. This test is known for its high analytical sensitivity and detects HPV with low viral load.[31] If the immune-response during pregnancy influences the replication of an HPV type already present with a low viral load, this HPV type would already have been detected with the $SPF_{10}LiPA_{25}$. Whereas a more clinical sensitive test like the hybrid capture 2 (HC2) would have only detected the HPV type later in time when the viral load is increased.

Another factor potentially influencing the HPV DNA detection is the sampling method that is used. This study is based on self-sampling, which has a comparable sensitivity for hr-HPV detection with physician-sampling.[32;33] Most studies with self-sampling excluded pregnant women by protocol. Only 3 other studies using self-sampling for HPV detection included pregnant women. Two studies compared self-sampling with physician-sampling, including pregnant women (10.7-12.0% of their study population).[34;35] They did not report a difference in performance between pregnant and non-pregnant women. The third study used self-sampling to compare HPV prevalence between pregnant and non-pregnant women in an age-matched study population in Mexico. In contrast to our study, they reported a higher hr-HPV prevalence in pregnant women,[19] but other confounding factors could not be ruled out. Results of these three studies suggest that the use of self-sampling for HPV detection may not explain the lack of difference in HPV detection in our study population.

If pregnancy is not a vulnerable period for HPV infections, the delivery process might be an explanation for the association between multi-parity and cervical carcinoma. Besides hormonal and immunologic mechanisms, traumatic mechanisms have been previously hypothesized as possible explanations.[3;20] During delivery, the cervical canal and the transformation zone will dilate causing many micro abrasions. Therefore any present HPV may easily enter the epithelium and infect the parabasal layer on multiple sites, possibly making these women extra at risk for cervical cancer development.

Currently it is proposed to use HPV DNA testing as a primary screening tool with cytology as triage for HPV DNA positive women.[36-38] Nowadays pregnant women are

excluded from primary screening with cytology because cytology is considered to be of less quality during pregnancy. In the future, when HPV DNA testing is implemented as a primary screening tool, pregnant women may not have to be excluded from screening because we did not find any difference in time-point prevalence between pregnant and matched-control women. Additionally previous data showed that HPV prevalence in cytomorphologically normal smears is hardly influenced by pregnancy.[30] However, further research is needed to confirm our data and to evaluate if pregnant women are willing to participate in screening based on self-sampling.

This prospective matched cohort study shows that, although based on a small study group, pregnancy does not seem to influence HPV detection in a low-parity population of young, unscreened women. Potential confounding factors influencing HPV prevalence were excluded by 1:1 matching and thus we were able to solely study the influence of current pregnancy on HPV detection.

Reference List

1. Yoshikawa H, Nagata C, Noda K, Nozawa S, Yajima A, Sekiya S et al. Human papillomavirus infection and other risk factors for cervical intraepithelial neoplasia in Japan. Br.J.Cancer 1999;80:621-24.
2. Comparison of risk factors for invasive squamous cell carcinoma and adenocarcinoma of the cervix: collaborative reanalysis of individual data on 8,097 women with squamous cell carcinoma and 1,374 women with adenocarcinoma from 12 epidemiological studies. Int.J.Cancer 2007;120:885-91.
3. Castellsague X, Munoz N. Chapter 3: Co-factors in human papillomavirus carcinogenesis--role of parity, oral contraceptives, and tobacco smoking. J.Natl. Cancer Inst.Monogr 2003;20-28.
4. Bosch FX, de SS. The epidemiology of human papillomavirus infection and cervical cancer. Dis.Markers 2007;23:213-27.
5. Hildesheim A, Herrero R, Castle PE, Wacholder S, Bratti MC, Sherman ME et al. HPV co-factors related to the development of cervical cancer: results from a population-based study in Costa Rica. Br.J.Cancer 2001;84:1219-26.
6. Hoyo C, Yarnall KS, Fortney J. Short birth intervals and uterine cervical cancer risk in Jamaican women. Int.J.Gynaecol.Obstet. 2007;98:56-57.
7. Munoz N, Franceschi S, Bosetti C, Moreno V, Herrero R, Smith JS et al. Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case-control study. Lancet 2002;359:1093-101.
8. Molano M, van den BA, Plummer M, Weiderpass E, Posso H, Arslan A et al. Determinants of clearance of human papillomavirus infections in Colombian women with normal cytology: a population-based, 5-year follow-up study. Am.J.Epidemiol. 2003;158:486-94.
9. Cervical carcinoma and reproductive factors: collaborative reanalysis of individual data on 16,563 women with cervical carcinoma and 33,542 women without cervical carcinoma from 25 epidemiological studies. Int.J.Cancer 2006;119:1108-24.
10. Vaccarella S, Herrero R, Dai M, Snijders PJ, Meijer CJ, Thomas JO et al. Reproductive factors, oral contraceptive use, and human papillomavirus infection: pooled analysis of the IARC HPV prevalence surveys. Cancer Epidemiol.Biomarkers Prev. 2006;15:2148-53.
11. Cotton SC, Sharp L, Seth R, Masson LF, Little J, Cruickshank ME et al. Lifestyle and socio-demographic factors associated with high-risk HPV infection in UK women. Br.J.Cancer 2007;97:133-39.
12. Castle PE, Wacholder S, Lorincz AT, Scott DR, Sherman ME, Glass AG et al. A prospective study of high-grade cervical neoplasia risk among human papillomavirus-infected women. J.Natl.Cancer Inst. 2002;94:1406-14.
13. Castle PE, Walker JL, Schiffman M, Wheeler CM. Hormonal contraceptive use, pregnancy and parity, and the risk of cervical intraepithelial neoplasia 3 among oncogenic HPV DNA-positive women with equivocal or mildly abnormal cytology. Int.J.Cancer 2005;117:1007-12.
14. Kruger-Kjaer S, van den Brule AJ, Svare EI, Engholm G, Sherman ME, Poll PA et al. Different risk factor patterns for high-grade and low-grade intraepithelial lesions on the cervix among HPV-positive and HPV-negative

young women. Int.J.Cancer 1998;76:613-19.
15. Brabin L. Interactions of the female hormonal environment, susceptibility to viral infections, and disease progression. AIDS Patient.Care STDS. 2002;16:211-21.
16. Chang-Claude J, Schneider A, Smith E, Blettner M, Wahrendorf J, Turek L. Longitudinal study of the effects of pregnancy and other factors on detection of HPV. Gynecol.Oncol. 1996;60:355-62.
17. Chan PK, Chang AR, Tam WH, Cheung JL, Cheng AF. Prevalence and genotype distribution of cervical human papillomavirus infection: Comparison between pregnant women and non-pregnant controls. J.Med. Virol. 2002;67:583-88.
18. Nobbenhuis MA, Helmerhorst TJ, van den Brule AJ, Rozendaal L, Bezemer PD, Voorhorst FJ et al. High-risk human papillomavirus clearance in pregnant women: trends for lower clearance during pregnancy with a catch-up postpartum. Br.J.Cancer 2002;87:75-80.
19. Hernandez-Giron C, Smith JS, Lorincz A, Lazcano E, Hernandez-Avila M, Salmeron J. High-risk human papillomavirus detection and related risk factors among pregnant and nonpregnant women in Mexico. Sex Transm. Dis. 2005;32:613-18.
20. Fife KH, Katz BP, Roush J, Handy VD, Brown DR, Hansell R. Cancer-associated human papillomavirus types are selectively increased in the cervix of women in the first trimester of pregnancy. Am.J.Obstet.Gynecol. 1996;174:1487-93.
21. Fife KH, Katz BP, Brizendine EJ, Brown DR. Cervical human papillomavirus deoxyribonucleic acid persists throughout pregnancy and decreases in the postpartum period. Am.J.Obstet.Gynecol. 1999;180:1110-14.
22. Banura C, Franceschi S, van Doorn LJ, Arslan A, Kleter B, Wabwire-Mangen F et al. Prevalence, incidence and clearance of human papillomavirus infection among young primiparous pregnant women in Kampala, Uganda. Int.J.Cancer 2008;123:2180-87.
23. 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:e3743.
24. Rosenbaum, P. R. The central role of the propensity score in observational studies for causal effects. Rubin, D. B. 70(1), 41-55. 1983. Biometrika Trust.
Ref Type: Generic
25. Pearl, J. Causality: Models, Reasoning, and Inference. second. 2009. Cambridge Univeristy Press.
Ref Type: Serial (Book,Monograph)
26. 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;155:1473-78.
27. 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;44:3122-29.
28. Smith EM, Johnson SR, Jiang D, Zaleski S, Lynch CF, Brundage S et al. The association between pregnancy and human papilloma virus prevalence. Cancer Detect.Prev. 1991;15:397-402.
29. Kemp EA, Hakenewerth AM, Laurent SL, Gravitt PE, Stoerker J. Human papillomavirus prevalence in pregnancy. Obstet.Gynecol.

1992;79:649-56.

30. de Roda Husman AM, Walboomers JM, Hopman E, Bleker OP, Helmerhorst TM, Rozendaal L et al. HPV prevalence in cytomorphologically normal cervical scrapes of pregnant women as determined by PCR: the age-related pattern. J.Med.Virol. 1995;46:97-102.

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

32. 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;105:530-35.

33. 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;81:207-12.

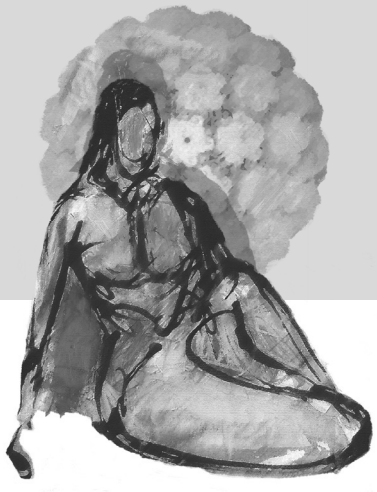
34. Lorenzato FR, Singer A, Ho L, Santos LC, Batista RL, Lubambo TM et al. Human papillomavirus detection for cervical cancer prevention with polymerase chain reaction in self-collected samples. Am.J.Obstet.Gynecol. 2002;186:962-68.

35. Safaeian M, Kiddugavu M, Gravitt PE, Ssekasanvu J, Murokora D, Sklar M et al. Comparability of self-collected vaginal swabs and physician-collected cervical swabs for detection of human papillomavirus infections in Rakai, Uganda. Sex Transm.Dis. 2007;34:429-36.

36. Cuzick J, Arbyn M, Sankaranarayanan R, Tsu V, Ronco G, Mayrand MH et al. Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. Vaccine 2008;26 Suppl 10:K29-K41.

37. Naucler P, Ryd W, Tornberg S, Strand A, Wadell G, Elfgren K et al. Human papillomavirus and Papanicolaou tests to screen for cervical cancer. N.Engl.J.Med. 2007;357:1589-97.

38. 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;124:516-20.



Chapter 7

The indicating FTA elute cartridge: a solid sample carrier to detect high-risk HPV and high-grade cervical lesions

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Abstract

The clinically validated high-risk human papillomavirus (hr-HPV) Hybrid Capture (HC2) and GP5+/6+-PCR assays were analyzed on an Indicating FTA elute cartridge (FTA cartridge). The FTA cartridge is a solid dry carrier that allows safe transport of cervical samples. FTA cartridge samples were compared with liquid-based samples for hr-HPV and high-grade cervical intraepithelial neoplasia (CIN) detection. One cervical sample was collected in a liquid-based medium and one was applied to the FTA cartridge. DNA was eluted directly from the FTA cartridge by a simple elution step. HC2 and the GP5+/6+-PCR assay were performed on both the liquid-based and the FTA-eluted DNA of 88 women. Overall agreement between FTA and liquid-based samples for the presence of hr-HPV was 90.9% with GP5+/6+-PCR and 77.3% with HC2. The sensitivity for high-grade CIN of hr-HPV testing on the FTA cartridges was 84.6% with GP5+/6+-PCR and only 53.8% with HC2. By comparison, these sensitivities on liquid-based samples were 92.3% and 100% for GP5+/6+-PCR and HC2, respectively. Therefore, the FTA cartridge shows a reasonably good overall agreement for hr-HPV detection with liquid-based media when using GP5+/6+-PCR, but not for HC2 testing. Even with GP5+/6+-PCR, the FTA cartridge is not yet capable of detecting all high-grade CIN lesions.

Introduction

Infection with human papillomavirus (HPV) is indicated as the causal role in cervical cancer development. Primary high-risk (hr) HPV screening appeared to be more sensitive than cytology in detecting cervical intraepithelial neoplasia grade 2 or worse (CIN2+) and, therefore, displayed superior protection against cervical precancer and cancer.[1,2] Interestingly, material from cervico-vaginal lavages or cervico-vaginal brushes proved to be highly representative of cervical hr-HPV status.[3,4,5,6,7,8] Moreover, analysis of cervico-vaginal self-samples appears to be as reliable as physician taken samples for detecting cervical (pre)malignant disease following hr-HPV analysis.[9,10]

Screening via self-samples obtained in the privacy of women's own homes, is likely to result in a better attendance than screening via samples obtained by physicians or other health care providers.[11,12] Therefore, cervico-vaginal self-sampling is an attractive alternative for physician taken cervico-vaginal material.[5,13]

Most previous studies used cervical samples with liquid-based collection systems.[3,11,12,14,15,16] In principle, the use of liquid-based self-samples has the impractical consequence that fluids may leak; in addition, special precautions have to be taken for transport. In case alcohol-containing preservation fluids are used, problems like inflammability and harm to eyes and skin may occur. These problems may be circumvented when applying self-sampled specimens to a solid dry carrier, the Whatman® Indicating FTA® Elute cartridge (FTA cartridge). FTA cartridges are biohazard free, because the sample is denaturized on application. These properties solve storage and transport problems often seen with liquid samples. More important, the FTA cartridge indicates dye changes from purple to white when a sample is applied, thereby confirming that women performed the procedure properly. This solid dry carrier might be beneficial for specimens collected by non-physicians in remote areas, which would need transportation to the laboratories. A proof-of-principle study was previously performed to assess the potential of HPV detection directly on eluted DNA from the FTA cartridge. The SPF_{10} Line Blot 25 assay was used, and 98% agreement with physician-obtained samples was found.[17] However, the SPF_{10} Line Blot 25 assay is sensitive in HPV detection, and it is unknown how clinically validated hr-HPV assays with a lower analytical sensitivity would perform on FTA cartridge samples. In the current study, we evaluated the clinically validated Hybrid Capture 2 (HC2) and GP5+/6+-PCR[18] methods on physician-obtained cervical samples applied to the FTA cartridge for the detection of hr-HPV and cervical premalignancies in women visiting a gynecological outpatient clinic.

Materials and Methods

Study subjects

Between May 25 and December 18, 2009, 94 women were recruited at the department of Obstetrics and Gynecology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands. The cohort consisted of women with different risk factors for HPV infection and CIN. All women visited the gynecologist at the outpatient department and two cervical samples, for liquid-based and cartridge collection were obtained, as specified later. The volume of liquid-based samples of six women was not sufficient to perform the two different hr-HPV tests in addition to liquid-based cytology. These women were therefore excluded. The remaining 88 patients constituted the study population.

Sample collection

Two Cervex-brushes (Rovers Medical Devices B.V., Oss, the Netherlands) were used to obtain cervical samples. The first brush was rinsed in a Thinprep vial (Cytyc corp. Boxborough MA, USA) on which regular liquid-based cytological examination and HPV testing by HC2 and GP5+/6+-PCR was performed. The second brush was applied to the FTA cartridge (the Indicating FTA elute micro card, Whatman/GE Healthcare, Kent, UK), as previously described.[17] Again, HC2 and GP5+/6+-PCR HPV testing was performed on the DNA eluted from these FTA cartridges.[17]
In to assess the samples anonymously, all FTA cartridge samples and cervical liquid-based samples were labeled with a unique patient code before sending them to the laboratory. Histological results were retrieved from the medical records in case a biopsy specimen was obtained from the cervix during colposcopy or in case of surgery. Histological results were considered superior to cytological results.

Liquid-based samples

All Thinprep vials were used for regular cytological examination. Papanicolaou smear abnormalities were interpreted and classified by using the Bethesda system.
For the HC2 assay, 5 ml liquid-based homogenized medium was used according to the manufacturer's instructions (Qiagen, Gaithersburg, MD).
For the hr-HPV GP5+/6+-PCR, DNA was isolated out of 500 µl medium of the liquid-based cervico-vaginal samples, using the MagNAPure LC Isolation station (Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany). Nucleic acids were resuspended in a final volume of 50 µl; 10 µl were used for the GP 5+/6+ assay, as previously described.[19,20]

FTA cartridges

The Indicating FTA Elute matrix contains an indicating dye that changes from purple to white upon application of a colorless sample, such as a cervico-vaginal swab. The white parts on the FTA cartridges were punched using a sterilized perforator specifically designed for the FTA cartridges (3mm Harris Uni-core device, Whatman). DNA was

recovered from the FTA elute matrix through a simplified elution process using heat and water. Inhibitory components, such as hemoglobin, are retained on the FTA elute matrix. For elution, four 3 mm punches from the matrix were transferred into a 2.0 ml Microfuge tube; and 2.0 ml of sterile H_2O was added to the punches and immediately pulse vortexed three times, for 5 seconds each. The H_2O was removed with a sterile fine-tipped pipette. Sterile H_2O, 75 µl, 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. Subsequently, the tube was centrifuged for 30 seconds and the supernatant with eluted DNA was transferred to a new microcentrifuge tube. The eluted DNA was stored at -80°C for further use. Finally, according to protocol, 10 µl of the eluate was used for the GP5+/6+-PCR, and 50 µl for the HC2.

HPV detection by Hybrid Capture 2 and GP 5+/6+-PCR

Liquid-based homogenized medium, 5 ml, and separate 50 µl of eluted DNA from the cartridges were used for the HC2 according to the manufacturer's instructions. The HC2 assay included a mixture of probes for the HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. RNA/DNA hybrids were captured with antibodies and a secondary signaling antibody created a chemiluminicent signal that was ultimately expressed as relative light units per cut-off value (RLU/CO), representing the ratio between the emission from a sample to the average of three positive controls (i.e. 1 pg/ml of cloned HPV 16 DNA). Samples were considered HC2 positive in case of an RLU/CO value ≥ 1.0 (equivalent to signal of 1 pg/ml HPV 16 DNA). Repeat testing is recommended for RLU/CO ratios between 1.0 and 2.5. Because priority was given to clinical testing, in the context of this study material was limited. Therefore, the primary test result was considered definitive.
Separately, 10µl of isolated DNA from the liquid-based specimens and 10µl of DNA eluted from the FTA cartridges were used for HPV testing by the GP5+/6+-PCR assay. The GP5+/6+-PCR was performed using the enzyme immunoassay readout system with a probe cocktail of 14 hr-HPV types (i.e. HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) as described previously.[19,20] The standard cutoff value of three times the mean OD value of the PCR-negative controls was used.[20,21]

Statistics

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

Results

The study group consisted of 88 women (median age, 37 years; SD, 10 years; range 24 to 72 years). In 18 of the 88 cases, histological specimens of the cervix were obtained. Histological feature was indicated by a liquid-based cytological result of at least a low-grade squamous intraepithelial lesion (LSIL). However, in 10 LSIL cases, no biopsy specimen were obtained because of nonsuspicious colposcopy results. Of the 18 histological specimens, four showed normal tissue, one showed a low-grade CIN lesion (CIN1), and 13 specimens showed a high-grade CIN lesion (CIN2-3). In total, the cervical samples were within normal limits in 46 cases (52.3%), and atypical squamous cells (ASC-US) in 18 cases (20.5%); in 24 cases (27.3%), the smear appeared to be LSIL or more severe.

Table 1.
Liquid-based and FTA cartridge samples analyzed with the two different hr-HPV assays (HC2 and GP5+/6+-PCR), according to cytological/histological features

	liquid-based samples				FTA cartridge samples	
	cytology	histology	HC2 (RLU/CO)	GP5+/6+-PCR	HC2 (RLU/CO)	GP5+/6+-PCR
1	WNL	-	**1.23**	neg	neg	neg
2	WNL	-	**1.23**	**pos**	neg	**pos**
3	WNL	-	**2.91**	neg	neg	neg
4	WNL	-	**2.93**	**pos**	neg	neg
5	HSIL	Normal	**4.50**	neg	neg	neg
6	WNL	-	**8.98**	**pos**	neg	neg
7	WNL	-	**76.00**	neg	neg	**pos**
8	WNL	-	**213.00**	**pos**	**16.24**	**pos**
9	LSIL	Normal	**783.58**	**pos**	**2.61**	**pos**
10	ASC-US	-	**1.09**	neg	neg	**pos**
11	ASC-US	-	**1.45**	neg	neg	**pos**
12	ASC-US	-	**2.99**	**pos**	neg	**pos**
13	ASC-US	-	**4.36**	neg	neg	neg
14	ASC-US	-	**95.00**	neg	neg	neg
15	ASC-US	-	**460.00**	**pos**	**19.41**	**pos**
16	ASC-US	-	**543.00**	**pos**	**218.43**	**pos**
17	ASC-US	-	**635.00**	**pos**	**34.83**	**pos**
18	ASC-US	-	**737.00**	**pos**	**140.70**	**pos**
19	LSIL	-	neg	**pos**	neg	neg

Table 1. Continued

	liquid-based samples				FTA cartridge samples	
	cytology	histology	HC2 (RLU/CO)	GP5+/6+-PCR	HC2 (RLU/CO)	GP5+/6+-PCR
20	LSIL	-	**2.24**	**pos**	neg	neg
21	LSIL	-	**2.73**	**pos**	**1.47**	**pos**
22	LSIL	-	**13.59**	neg	neg	neg
23	LSIL	-	**35.92**	**pos**	**4.49**	**pos**
24	LSIL	-	**196.00**	**pos**	**6.38**	**pos**
25	LSIL	-	**238.94**	**pos**	**2.61**	**pos**
26	LSIL	-	**318.00**	**pos**	**185.67**	**pos**
27	LSIL	-	**552.60**	**pos**	**10.34**	**pos**
28	LSIL	-	**771.27**	**pos**	**1.37**	**pos**
29	ASC-US	CIN1	**4.74**	**pos**	**4.94**	**pos**
30	LSIL	CIN2	**11.15**	neg	neg	neg
31	L/HSIL	CIN2	**13.40**	**pos**	**1.73**	**pos**
32	L/HSIL	CIN2	**73.19**	**pos**	**1.43**	**pos**
33	ASC-US	CIN2	**1085.49**	**pos**	neg	**pos**
34	HSIL	CIN3	**1.34**	**pos**	neg	**pos**
35	HSIL	CIN3	**1.90**	**pos**	neg	neg
36	HSIL	CIN3	**27.07**	**pos**	**18.28**	**pos**
37	HSIL	CIN3	**100.75**	**pos**	**4.20**	**pos**
38	ASC-US	CIN3	**108.72**	**pos**	**3.04**	**pos**
39	HSIL	CIN3	**294.65**	**pos**	**8.12**	**pos**
40	HSIL	CIN3	**422.95**	**pos**	neg	**pos**
41	HSIL	CIN3	**467.28**	**pos**	**8.85**	**pos**
42	HSIL	CIN3	**794.08**	**pos**	neg	**pos**
43-79	WNL	-*	neg	neg	neg	neg
80-88	ASC-US	-	neg	neg	neg	neg

Bold text indicates positive result
-, no histological specimen taken; * = histology; ASC-US, atypicalsquamous cells; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial; Neg, negative result; Pos, positive result; WNL, within normal limits.

GP5+/6+-PCR

Table 1 shows that in 32 (36.4%) of the 88 liquid-based samples, hr-HPV was detected using the GP5+/6+-PCR. By comparison, 30 (34.1%) of the 88 FTA cartridge eluted DNA samples, were hr-HPV positive with GP5+/6+-PCR. Of the 32 GP5+/6+-PCR positive liquid-based samples, 27 (84.4%) showed concordant hr-HPV positive results on the eluted DNA from the corresponding FTA cartridge samples. Five women had GP5+/6+-PCR positive liquid-based samples but negative FTA cartridge samples. Two of these five women had normal cytological results, two had LSIL cytological results, and one had a histological CIN3 lesion. In three women, hr-HPV was detected on the eluted DNA from the cartridge but was negative on the liquid samples with GP5+/6+-PCR. One of these women had a normal cytological result, and the other women had ASC-US. In the total group of 88 samples, 80 FTA cartridge samples showed concordant hr-HPV test results with the liquid-based samples [concordance, 90.9%; κ, 0.80; 95% confidence interval (CI), 0.67 to 0.93] when the GP5+/6+-PCR was used for detection (Table 2).
There were 13 histologically confirmed high-grade CIN lesions. The liquid-based samples of 12 of these 13 lesions were hr-HPV positive with GP5+/6+-PCR (sensitivity 92.3%). The FTA cartridges showed 11 hr-HPV positive samples with GP5+/6+-PCR (sensitivity 84.6%).

Hybrid Capture 2 Assay

Using the HC2 assay for the detection of hr-HPV, 41 (46.6%) of 88 liquid-based samples were found positive (Table 1). These included all samples with high-grade CIN (sensitivity, 100%). Of the positive samples, only 21 were also positive with HC2 using the eluted DNA from the FTA cartridge samples. Of the 67 women with a negative FTA cartridge sample, 54 had a normal or ASC-US cytological result and four had a normal histology result. However, of 13 HC2 hr-HPV liquid-based-positive women with hystologically confirmed high-grade CIN lesions, six were missed using the HC2 on eluted DNA (sensitivity, 53.8%). The RLU of the FTA cartridge samples detected with four punches was low (mean, 33.1; SD, 63.9) compared with the corresponding RLU of the liquid-based samples (mean, 225.3; SD, 296.7). Indeed, 13 of the 20 hr-HPV positive liquid-based samples with a negative result on the FTA cartridge samples had a low RLU (< 10). In 68 of the total 88 samples, concordant hr-HPV HC2 results were found between the liquid-based samples and the FTA cartridge samples (concordance, 77.3%; κ, 0.53; 95% CI, 0.37 to 0.69) (Table 2).

Table 2.
Concordant and discordant results for lesion type, sample type, and assay used

	FTA versus LB (HC2)		FTA versus LB (GP5+/6+)		GP5+/6+ versus HC2 (LB)		GP5+/6+ versus HC2 (FTA)	
cytology/ histology	**c**	**d**	**c**	**d**	**c**	**d**	**c**	**d**
normal*	39	7	43	3	42	4	44	2
ASC-US*	13	5	16	2	14	4	15	3
LSIL*	8	2	8	2	8	2	10	0
CIN1†	1	0	1	0	1	0	1	0
CIN2†	2	2	4	0	3	1	3	1
CIN3†	5	4	8	1	9	0	6	3
Total	68	20	80	8	77	11	79	9
	concordance 77.3% κ 0.53, CI 0.37-0.69		concordance 90.9% κ 0.80, CI 0.67-0.93		concordance 87.5% κ 0.75, CI 0.61-0.88		concordance 89.8% κ 0.76, CI 0.61-0.90	

c=concordant, d=discordant, FTA=FTA cartridge sample, LB=liquid-based sample, HC2=Hybrid capture 2
* cytology, without subsequent histology
† histology after abnormal cytology

GP5+/6+-PCR versus HC2

Only one liquid-based sample was negative by HC2 but positive by GP5+/6+-PCR. This was a LSIL sample. Ten samples were positive with HC2 but negative with the GP5+/6+-PCR. Most (8 of 10) of these HC2-positive but GP5+/6+-PCR-negative liquid-based samples were within the normal cytological or histological group (n = 4) or ASC-US (n = 4). One high-grade CIN lesion (ie, CIN2), positive by HC2 on liquid, was negative by GP5+/6+-PCR on liquid. Absolute agreement between the two hr-HPV tests was 87.5% (κ, 0.75; 95% CI, 0.61 to 0.88) for the liquid-based samples (Table 2).

All FTA cartridge samples that were positive with HC2 were also positive with the GP5+/6+-PCR. Nine FTA cartridge samples were negative with HC2 but positive with GP5+/6+-PCR. These comprised samples that were cytologically classified as normal (n = 2) or ASC-US (n=3) or histologically classified as CIN2 (n = 1) or CIN3 (n = 3). Absolute agreement between the two hr-HPV tests results was 89.8% (κ, 0.76; 95% CI, 0.61 to 0.90) for the FTA cartridge samples (Table 2).

Table 1 and 2 show a summary of the hr-HPV results of the HC2 and GP5+/6+-PCR tests of both the liquid-based and the FTA cartridge samples, as well as the concordances.

Discussion

We aimed to test whether the clinically validated HC2 and GP5+/6+-PCR systems could be performed on the FTA cartridge material to detect high-grade CIN lesions. We found a reasonably good concordance in hr-HPV detection between liquid-based and FTA cartridge systems when using the GP5+/6+-PCR. This concordance was much lower using HC2. Moreover, the sensitivity of hr-HPV detection on the FTA cartridge for high-grade CIN lesions was 84.6% with GP5+/6+-PCR but only 53.8% with HC2.

Although more liquid-based samples were hr-HPV positive with HC2 than with GP5+/6+-PCR (41 versus 32), only 21 of the 41 FTA cartridge samples were hr-HPV HC2 positive. A possible explanation for this is that the amount of DNA eluted from the punches is insufficient to allow reliable HC2 testing, a method that does not use target amplification. Optimization of the processing steps (eg, by increasing the number of punches) may improve the HC2 outcome. However, increasing the number of punches is labor intensive and the H_2O volume used needs to be increased equivalently. In addition, we used the accepted RLU/CO value of 1.0 for signifying a positive HPV HC2 test result. This is arbitrary because these values were developed and validated in conjunction with liquid-based tests. It might be possible that values <1.0 RLU/CO represent positive test results when other media and different amounts of DNA are used. Moreover, results with RLU/CO values between 1.0 and 2.5 are not repeated within this study, as recommended according to protocol. Because priority was given to clinical tests, there was limited availability of sampled material. These samples could have been negative with repeat testing.

Based on the results so far, HC2 might not be the preferred method for hr-HPV detection using FTA cartridges. To obtain sufficient (clinical) sensitivity, amplification based methods might be more suitable. Results for the FTA cartridge with the SPF_{10}LiPA-PCR were previously.[17] With an overall agreement for hr-HPV between the FTA cartridges and the liquid-based samples of 98% (κ, 0.94), the SPF_{10}LiPA proved to be a highly reliable method for hr-HPV testing on the FTA cartridges. In addition, Gustavsson et al[22] reported an agreement in hr-HPV positivity between the Cytobrush and FTA samples of 94% (κ, 0.88; 95% CI, 0.748 to 1), again by using a real-time PCR-based assay. Although the results of the clinically validated GP5+/6+-PCR in this study are promising, they do not completely confirm previous findings on PCR-based hr-HPV testing on FTA cartridges.

Because the sensitivity of detecting hr-HPV and high-grade cervical lesions on the FTA cartridge is still not sufficient, there are aspects that need further consideration. Transferring a sample collected with a sampling device onto the surface of a solid sample carrier, such as the FTA cartridge, might cause the same problems that plagued the conventional Papanicolaou test (ie, not all cells collected are transferred, but they remain on the sampling device). This may result into a nonrepresentative sample. Moreover, in case of high-grade CIN, the HPV copy number per cell tends to be lower as the HPV tends to be integrated. Especially then, showing that there is DNA in the sample does not necessarily mean that the HPV-infected cells are transferred to the FTA cartridge.

Previous studies have shown that cells from dysplastic cervical lesions are more likely to show aberrant expression of adhesion molecules and might fail to exfoliate. Similarly, such cells might be relatively less likely to be transferred to a solid substrate, like the FTA cartridge.

Given the cross-sectional nature of this study, only 18 women had a histological specimen. Women who do not have colposcopically detectable lesions, regardless of whether they are hr-HPV positive, are not usually subjected to blind biopsies. The biopsy specimens would be highly unlikely to show significant pathological features.

Furthermore, only physician-collected cervical samples were used in this study to enable an optimal comparison between liquid-based and FTA cartridge samples. The aim of this study was to compare the use of two collection methods (ie, FTA cartridge versus liquid) and to avoid any influence from sampling different sides. Therefore, only physician-collected cervical samples were analyzed. For self-sampling, vaginal rather than cervical samples are brushed. Consequently, no direct applicability of this study to self-sampling is legitimate. In addition, the fact that the two Cervex brushes were obtained consecutively because of diagnostic reasons might have induced a bias against the FTA cartridge method. The second brushes were used for the FTA cartridges. Most of the relevant material might already have been sampled by the first brush.

This study concerns a population of women who visited the gynecological outpatient department and consequently involved an hr-HPV group. Therefore, to consider the use of the FTA cartridge in a screening population, a thorough analysis of the FTA cartridge in such a population is necessary.

Nevertheless, our data have shown that, in the context of GP5+/6+-PCR, but not HC2 testing, the FTA cartridge holds promise as a collector of cervical specimens for screening. However, an ultrahighly or a highly sensitive method for HPV detection, such as PCR-based HPV DNA testing, is required. The clinical implication of those tests must be further assessed. The sensitivity is still not equal to that of hr-HPV testing by HC2 on Thinprep vials; therefore, further optimization is recommended.

Reference List

1. Bulkmans NW, Berkhof J, Rozendaal L, van Kemenade FJ, Boeke AJ, Bulk S, et al. Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. Lancet 2007, 370:1764-1772
2. Ronco G, Giorgi-Rossi P, Carozzi F, Confortini M.; Dalla Palma P, Del Mistro A, et al. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. Lancet Oncol 2010, 11:249-257
3. 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, 44:2518-2523
4. 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, 17:2163-2168
5. 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, 186:365-373
6. 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, 55:435-439
7. 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, 163:513-518
8. Winer RL, Feng Q, Hughes JP, Yu M, Kiviat NB, O'Reilly S, Koutsky LA. 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, 34:371-377
9. 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, 81:207-212
10. 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, 105:530-535
11. 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, 120:1505-1510
12. Gok M, Heideman DA, van Kemenade FJ, Berkhof J, Rozendaal L, Spruyt JW, et al. HPV testing on self-collected cervicovaginal lavage specimens as screening method for women who do not attend cervical screening: cohort

study. BMJ 2010, 340:c1040

13. Waller J, McCaffery K, Forrest S, Szarewski A, Cadman L, Austin J, Wardle J: Acceptability of unsupervised HPV self-sampling using written instructions. J Med Screen. 2006, 13:208-213

14. Bulkmans NW, Rozendaal L, Snijders PJ, Voorhorst FJ, Boeke AJ, Zandwijken GR, et al. POBASCAM, a population-based randomized controlled trial for implementation of high-risk HPV testing in cervical screening: design, methods and baseline data of 44,102 women. Int J Cancer 2004, 110:94-101

15. Sanner K, Wikstrom I, Strand A, Lindell M, Wilander E. Self-sampling of the vaginal fluid at home combined with high-risk HPV testing. Br J Cancer 2009, 101:871-874

16. Wright TC, Jr, Denny L, Kuhn L, Pollack A, Lorincz A. HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer. JAMA 2000, 283:81-86

17. 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, 47:2564-2570

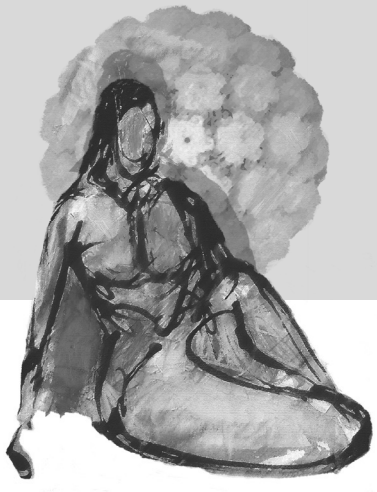
18. Meijer CJ, Berkhof J, Castle PE, Hesselink AT, Franco EL, Ronco G, et al. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. Int J Cancer 2009, 124:516-520

19. Hesselink AT, van Ham MA, Heideman DA, Groothuismink ZM, Rozendaal L, Berkhof J, et al. Comparison of GP5+/6+-PCR and SPF10-Line Blot assays for detection of high-risk human papillomavirus in samples from women with normal cytology results who develop grade 3 cervical intraepithelial neoplasia. J Clin Microbiol 2008, 46:3215-3221

20. van den Brule AJ, Pol R, Fransen-Daalmeijer N, Schouls LM, Meijer CJ, Snijders PJ. GP5+/6+ PCR followed by Reverse Line Blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. J Clin Microbiol 2002, 40:779-787

21. Jacobs MV, Snijders PJ, van den Brule AJ, Helmerhorst TJ, Meijer CJ, Walboomers JM. A general primer GP5+/GP6(+)-mediated PCR-enzyme immunoassay method for rapid detection of 14 high-risk and 6 low-risk human papillomavirus genotypes in cervical scrapings. J Clin Microbiol 1997, 35:791-795

22. Gustavsson I, Lindell M, Wilander E, Strand A, Gyllensten U. Use of FTA card for dry collection, transportation and storage of cervical cell specimen to detect high-risk HPV. J Clin Virol 2009, 46:112-116



Chapter 8

General discussion

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In this thesis the dynamics of human papillomavirus (HPV) in young women in the pre-vaccine era is described. This thesis thereby provides insight into the short-term natural history of HPV infections. Particularly, chapter 3 and 4 evaluate the acquisition of infection, its clearance, and persistence and chapter 5 and 6 evaluate the potential co-factors like pregnancy, oral contraceptive pill (OCP).[1] These results on HPV dynamics represent a baseline for future research on the potential shift in HPV epidemiology due to HPV vaccination.

In most individuals, the immune response eventually leads to clearance of the virus, or to its maintenance in a latent or asymptomatic state in epithelial basal cells. It remains to be elucidated which proportion of HPV eventually clears or is maintained in a latent state. Latency implies that the virus may remain within basal epithelial cells, either arrested or very slowly replicating, but hardly detectable by current DNA technology. The existence of a latent state is supported by studies in immunosuppressed individuals. Particularly studies among women with defective cell-mediated immunity as with HIV infection or use of immunosuppressive medication, showed an increase in cervical precancerous lesions or genital warts without new exposure to HPV infection.[2;3] This indicates that an HPV type may be in a latent state for a certain period and may become reactivated and (again) detected.

Whether the presence of HPV may be detected depends, among other factors, upon the sensitivity of the HPV DNA test used.[4] Based on the cut-off value of the viral load level still detected by the HPV test, Hybrid Capture 2 (HC2) has the lowest sensitivity and SPF_{10} the highest, whereas GP5+/6+-PCR and PGMY-PCR have intermediate sensitivities.[5] When HPV DNA tests are applied into clinical practice and routine population-based screening programs, they should be able to detect women at greater risk of having high-grade cervical intraepithelial neoplasia (CIN),[6] and an HPV negative result should provide sufficient reassurance against development of a high-grade CIN during the screening interval. Snijders et al. introduced the terms analytical and clinical sensitivity. Analytical sensitivity of a test refers to the proportion of HPV-positive women correctly identified. Clinical sensitivity indicates the proportion of women with disease (i.e., women with ≥CIN3) correctly identified by a positive HPV test.[5]

The short-term fluctuations in HPV (described in chapter 3 and 4) and the fluctuations in HPV within the menstrual cycle (chapter 5),[1;7] raise new questions; (1) When does a woman have a productive (replicating) infection in case an HPV type is detected? (2) When is the detected HPV type infectious? (i.e., when is there a high risk of transmission to a sexual partner?) (3) When is a newly detected HPV type truly a new infection and when does it represent a re-infection or reactivation? In order to answer these questions we must consider the role of the immune system and the role of the detected viral load.

Productive human papillomavirus infection

The presence of HPV may indicate a productive (replicating) infection or not. It is important to realize that an infection is more than simply the detection of a pathogen. Infection

is invasion by and multiplication of pathogenic microorganisms in body tissue, which may produce subsequent tissue injury and progress to overt disease through a variety of cellular or toxic mechanisms.
When studying the HPV life cycle, initial infection requires access of infectious particles to cells in the basal layer through micro abrasions. After cell division, one daughter cell migrates and undergoes differentiation. This induces the productive phase in which expression of E6 and E7 deregulates cell cycle control, allowing viral genome amplification. When reaching the uppermost layer of the epithelium the virions are shed.[8;9] At this point, depending on the amount of virions shed (i.e., viral load), the virus may be detected and the virus may be transmitted to others. In women who do not successfully resolve their chronic productive HPV infection, CIN may develop, progress to CIN3 and eventually to cervical carcinoma (i.e., clinically relevant infection).[10;11]
Some studies showed that an increased viral load is associated with an increased risk of clinically relevant cervical lesions.[12;13] However, this potential relationship between viral load and disease is not shown for every HPV type. Studies mainly showed that HPV type 16 viral load increases with increasing disease severity.[13-15] Snijders et al. showed that viral load was also significantly higher for HPV types 18, 31 and 33, in scrapes of women with ≥CIN2 when compared with those of women with normal cytology.[12] On the other hand, longitudinal studies showed that baseline viral load did not relate to outcome of CIN2/3 and that only changes in viral load correlated with risk of CIN 2/3 development.[16;17] Furthermore, there exists a wide range in viral DNA load levels and a substantial overlap between women with and without CIN3.[13;18] Setting a clinical significant viral load cut-off value, predictive for disease, is therefore difficult and viral load is not a preferable tool in clinical practice.

Transmission of the human papillomavirus

Our immune system is important in the natural control of the spread of HPV-associated disease. The immune system may clear the infection or control the virus by keeping it at a low copy number.
Transmission dynamics are dependent on both pathogen and host factors and are defined by three components: (1) transmissibility from an infected to an uninfected partner upon exposure, (2) the likelihood of exposures between infected and uninfected persons, and (3) the duration that a person is infectious.[19] When studying genital HPV prevalence, the main factors associated with HPV prevalence are related to sexual behaviour.[20] In a simulation model, based on likelihood of sexual exposures between infected and uninfected persons, and the duration of the infection, the transmissibility of genital HPV from an infected to an uninfected partner upon exposure was estimated to be at the median of 40% per sexual act. The probability of male-to-female transmission would reach virtually 100 percent with only 11 acts of intercourse. This estimated high rate of transmission of HPV implicates that the potential protective effect of condoms would disappear over multiple intercourse acts.[19] However, condom use does partially prevent

HPV transmission and therefore reduces the spread of HPV in a sexually active population. It even may promote HPV clearance and regression of CIN and penile lesions.[21;22]
In a meta-analysis by Reiter et al., it was shown that in 37.7% of couples, both partners were infected with any type of HPV. Of these couples, 63.2% were infected with 1 or more of the same HPV types.[23] The mucosal epithelium of the cervix is likely to be more susceptible to HPV infection, which may cause this difference in concordance. Additionally, positive concordance was higher for studies using PCR and for the studies that recruited men with HPV related penile lesions.[23] However, Bleeker et al. showed that the concordance rate of HPV in couples is also higher when cervical lesions are present. They also showed that concordance is higher in couples in which one of the partners has a high genital viral load than in couples with low viral load. [24;25] This implicates that infections with a higher viral load are more likely to be transmitted.[26]
These results on HPV transmission underline the importance of the sex partner in the viral dynamics of HR HPV infection of the genital tract. Longitudinal studies on genital HPV transmission dynamics should follow-up sexual couples from first contact. This way insight on transmission, re-infection, and back-and-forward transmission between couples, may be obtained.[23] Additionally data should be recorded on cervical and penile lesions and sexual behavior, frequency and condom use.

Human papillomavirus; when is it newly acquired, a re-infection or a reactivation?

Short term fluctuations of HPV in an individual suggest that some detected HPV types might be newly acquired whereas others might have been acquired in the past and remained latent below detection level for a certain period and are reactivated.
Reactivation of latent HPV infections was observed in HIV-infected women. However, few reactivation events were identified in HIV-uninfected women. The most important factor consistently associated with reactivation in HIV-infected women is a CD4 count less than 200/mm3.[2] This suggests that functional immune systems keep HPV infections in a sub-clinical state and that they may be reactivated by immunosuppressive conditions.
Unfortunately we do not know how frequently latency occurs among immune-competent individuals, how long it may last, what causes reactivation into a detectable state, and what fraction of cancer arises after a period of HPV latency. Because of the apparent low rate of reactivation, large studies would be needed to adequately address reactivation in a immune-competent group.[2] Studying HPV reactivation in cervical infection is also complicated by the inability to distinguish reactivation of an existing infection from re-infection with the same HPV type through sexual contact with an infected partner. Therefore, knowledge of previous infection, sexual behavior within the HPV testing interval, and testing the sexual partner, is required to exclude the possibility of re-infection.
It is unclear if clearance of carcinogenic HPV infections may result in type specific immunity and whether it needs boosting overtime. Studies do not coincide whether a

sustained natural high level of IgG antibodies reduces the risk for subsequent infection with the same HPV type.[27-29] Moreover, only about 50-60% of women with carcinogenic HPV infection develop detectable serum antibodies.[27;30] Potentially a certain number of seroconversions might be missed due to testing interval, short time of seroconversion, and a low titre that may not be detected by tests used.[27] Suggesting that HPV serology is a poor marker of current infections or related lesions.
Some studies report a relation between viral load and seroconversion of IgG,[15;27] whereas others identified a persistent HPV infection to be a significant factor.[30;31] A high viral load may provide an acute and rapid immune response, whereas a persistent infection maintains a slow gradual boosting effect.[27] Another factor, potentially related to seroconversion of IgG , was OCP use.[27;31] Hormones, may induce transcription of the integrated viral oncogenes and influence the mucosal immunity in the genital tract.[32] As discussed in chapter 5, the influence of the endogenous and exogenous hormones on the mucosal immuneresponse most likely explained the differences in HPV detection within and between the OCP cycle and the natural menstrual cycle.[1]
This variety in data on seroconversion demonstrates that the host immune response against HPV is only partially understood and that the relation with clearance, re-infection and reactivation still needs to be resolved.[27;29]

Human papillomavirus DNA detection in cervical cancer screening

Until now HPV DNA testing has been used in most cervical screening programs as a triage test for women who have abnormal cytology. However, HPV DNA based primary screening has proven to be more sensitive than cytology in detecting high-grade CIN, but with a lower specificity.[33;34] Additionally, HPV DNA based primary screening may also improve the participation rate, especially when self-sampling is used for specimen collection, and therefore increasing the overall effectiveness of the screening program.[35;36]
Until now, however, self-sampling has proven to have a sufficient sensitivity to screen women otherwise not screened, but further research is necessary before wide implementation in a national screening program is possible. (chapter 2)
HPV dynamics should be taken into account to improve the specificity of HPV DNA based primary screening.[4] Indicating that, as discussed in chapter 3 and 4, a detected high-risk HPV type may be considered as (1) a newly acquired infection, or re-infection, or reactivation, that could be a transient infection or potentially persisting and causing CIN within several years, (2) an already persistent infection, likely to cause/have caused a CIN or cervical carcinoma (i.e., a chronic productive infection) or (3) an accidental pick-up of a latent infection.
The available data on cytology/histology and hr-HPV status in chapter 4 of this thesis underline the value of HPV persistence as a clinical marker to identify women who are at high risk of cervical cancer. Repeat (genotype specific) HPV DNA testing at 12 months may therefore be a valuable way to identify women at increased risk of CIN and cervical cancer.[37] HPV genotyping might be used as additional tool to decide whether to treat or

not, because some types are more likely to persist than others (chapter 4).[37] Especially for women <30 years of age, it will be important for clinicians to be conservative and wait for evidence of a persistent (chronic productive) hr-HPV infection rather than act on the first positive test for hr-HPV. Because these women are more likely to have a transient infection and the lesion (if present) may regress.[37-40]

Novel biomarkers, immunologic and genetic or viral factors, may be useful for triage in HPV DNA detection based primary screening, to support decision making on treatment or wait and see policy.[41;42] Currently developed biomarkers are; E6 and E7 HPV mRNA transcripts; methylation of several genes; markers for viral integration and of chromosomal instability; and markers of increased cell proliferation such as Ki-*67*, and p16.[43-45]

The high negative predictive value (NPV), of an HPV DNA test for developing CIN3, means that very few relevant lesions are missed.[33;46] However, as shown in chapter 3 and 4, we know that in natural HPV dynamics a sequence of positive hr-HPV samples may be interrupted with a single hr-HPV negative sample.[47;48] Within these fluctuations we may assume that either the viral load is below detection level (depending on the replication rate of the virus, latency, or the sensitivity of the used HPV DNA test), or that there may be a sampling error. When a non-productive hr-HPV infection is 'missed' there are most probably no clinical consequences. When an active hr-HPV infection is missed due to low sensitivity of HPV test or sampling error, this leads to inadequate follow-up and treatment if necessary. Therefore the sensitivity of the HPV DNA detection test used in primary cervical cancer screening should be sufficiently high to detect CIN3 or a chronic productive hr-HPV infection likely to produce CIN3 within the screening interval, in order to safely extend this cervical screening interval in case of a negative test.

Influence of HPV vaccination on HPV dynamics

As an increasing proportion of the population is vaccinated, the prevalence of cervical abnormalities will decrease. However, given the lag in time between HPV infection and the development of cervical cancer, (universal) vaccination is likely to have only a minimal impact on CIN and cervical cancer rates until 10-20 years from now.[49] Additionally, vaccination against HPV cannot provide 100% protection against cervical carcinoma and its precursor lesions, and still little is known about the potential long-term benefit of (cross-) type immune response. Therefore vaccinated women still need to be followed, and cervical cancer screening programs must continue.

The potential impact of HPV vaccination on the epidemiology of HPV and the rate of abnormal cytology depends on the following variables: (1) vaccination coverage, (2) the duration of vaccine induced immune protection, (3) the target high-risk HPV types of the vaccine, (4) rate of cross-protection, and (5) whether HPV type-replacement takes place. In 2009, the UK and Australia used school-based vaccination programs and achieved a high three-dose completion rate of approximately 80% and 70%, respectively.[50;51] In the Netherlands, however, only 52% of the girls in the target age-group received the HPV vaccine (HPV nieuws nr3;8march2011;www.rivm.nl). When vaccination coverage

is less than 90%, herd immunity obtained by only vaccinating women may be insufficient to eradicate the targeted HPV types.[52] When considering vaccinating boys, to increase herd immunity, the potential gain in a further reduction of cervical cancer must also be carefully weighed against extra costs. It appears that when female programs obtain high (>75%) coverage, the vaccination of males provides only small additional benefit and is not cost effective.[53]

In the future, long-term follow-up studies should determine the true efficacy and duration of both vaccines. However, up till now, there were no cases of infection or cytohistological lesions associated with HPV 16/18 observed in 7.3 years follow-up after vaccination with the bivalent vaccine (HPV 16/18 AS04-adjuvanted; Cervarix; GlaxoSmithKline).[54] Vaccination with the quadrivalent vaccine (HPV 6/11/16/18; Gardasil; Merck) had a high prophylactic efficacy against low grade cervical and vulvovaginal neoplasia and condylomata associated to the vaccine types through 42 months of follow-up. Vaccine efficacy against CIN 1 was 96%.[55]

The percentage of all cervical cancers attributable to HPV 16/18 in Europe has been estimated at 71%.[56] The percentage of prevented cervical cancer is potentially higher as both vaccines have reported cross-protection against other high-risk HPV types. As summarized by Szarewski;[57] the efficacy provided by the bivalent vaccine against CIN2+ lesions was 100% for HPV 31 or 45, 66.1% for non-HPV 16 species 9, and 77.3% for non-HPV 18 species 7;[58] and the efficacy provided by the quadrivalent vaccine against CIN2+ lesions was 58.7% for HPV 31 or 45, 35.4% for non-HPV 16 species 9, and 47.0% for non-HPV 18 species 7.[59] Although these results are encouraging, the duration of cross-protection is unknown.

Furthermore, currently the efficacy of a broad spectrum vaccine, against nine HPV types including seven oncogenic HPV types, is studied in a randomized Phase III trial (Merck, USA). When a polyvalent vaccine against 9 HPV types is implemented, the prevalence of the majority of cervical cancer associated HPV types will be drastically decreased and the discussion on cross-protection will change.

There is a possibility that the distribution of HPV types may gradually change in vaccinated populations to fill the vacated ecologic niches after the elimination of HPV type 16 and 18.[60;61] Type replacement is a viral population dynamics phenomenon and is defined as elimination of some types causing an increase in incidence of other types. This effect can only occur if two conditions apply: (1) there exists partial competition among different types during natural infection and (2) the vaccine does not afford cross-protection against types affected by this natural competition.[61] Future epidemiologic studies are needed to show whether type replacement occurs.

Challenges to primary cervical cancer screening after vaccination

The potential impact of HPV vaccination needs to be taken into account when planning for future screening guidelines. It is estimated that there will be a 50-60% reduction in colposcopy referrals due to prevention of HPV 16 and 18 lesions. This will lower the

positive predictive value (PPV) of any screening test for cervical cancer.[62] However, HPV testing is automated and therefore more objective and thus less likely to be influenced by this effect than cytology.[63]
Due to the high costs of vaccination it is important to restrict the costs of cervical screening programs and implement an approach based on the vaccine implementation.[64] Later and less frequent screening will probably only become an acceptable worldwide policy if vaccination uptake is high across social and economic strata.[65]
In the future, provided there is a high vaccination coverage with a polyvalent vaccine or a high level of cross-protection, a long duration of protection or a validated booster vaccination schedule, screening will likely be superfluous.

What should be monitored in the post-vaccine era?

The HPV dynamics described in this thesis represent a baseline for future research on the potential shift in HPV epidemiology due to HPV vaccination.
Future studies should monitor HPV DNA type-specific prevalence in sexually active young adults to analyze whether type-replacement takes place and to measure the level of protection against HPV type 16 and 18, and the level of cross-protection to related types. Furthermore, population coverage of HPV vaccination and duration of protection should be monitored in order to estimate the impact of vaccination on cervical screening results. This will eventually provide information on the need to screen in this vaccinated cohort, and if so, it will help to develop new guidelines for cervical cancer screening algorithms.

Reference List

1. Schmeink CE, Massuger LF, Lenselink CH, Quint WG, Melchers WJ, Bekkers RL. Effect of the menstrual cycle and hormonal contraceptives on human papillomavirus detection in young, unscreened women. Obstet.Gynecol. 2010;116:67-75.
2. Theiler RN, Farr SL, Karon JM, Paramsothy P, Viscidi R, Duerr A et al. High-risk human papillomavirus reactivation in human immunodeficiency virus-infected women: risk factors for cervical viral shedding. Obstet. Gynecol. 2010;115:1150-58.
3. Garland SM, Smith JS. Human papillomavirus vaccines: current status and future prospects. Drugs 2010;70:1079-98.
4. 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;14:95-105.
5. Snijders PJ, van den Brule AJ, Meijer CJ. The clinical relevance of human papillomavirus testing: relationship between analytical and clinical sensitivity. J.Pathol. 2003;201:1-6.
6. Hesselink AT, van Ham MA, Heideman DA, Groothuismink ZM, Rozendaal L, Berkhof J et al. Comparison of GP5+/6+-PCR and SPF10-line blot assays for detection of high-risk human papillomavirus in samples from women with normal cytology results who develop grade 3 cervical intraepithelial neoplasia. J.Clin.Microbiol. 2008;46:3215-21.
7. van Ham MA, Melchers WJ, Hanselaar AG, Bekkers RL, Boonstra H, Massuger LF. Fluctuations in prevalence of cervical human papillomavirus in women frequently sampled during a single menstrual cycle. Br.J.Cancer 2002;87:373-76.
8. Doorbar J. The papillomavirus life cycle. J.Clin. Virol. 2005;32 Suppl 1:S7-15.
9. Moody CA, Laimins LA. Human papillomavirus oncoproteins: pathways to transformation. Nat.Rev.Cancer 2010;10:550-60.
10. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. Vaccine 2006;24 Suppl 1:S1-15.
11. Kjaer SK, Frederiksen K, Munk C, Iftner T. Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. J.Natl.Cancer Inst. 2010;102:1478-88.
12. Snijders PJ, Hogewoning CJ, Hesselink AT, Berkhof J, Voorhorst FJ, Bleeker MC et al. Determination of viral load thresholds in cervical scrapings to rule out CIN 3 in HPV 16, 18, 31 and 33-positive women with normal cytology. Int.J.Cancer 2006;119:1102-07.
13. Swan DC, Tucker RA, Tortolero-Luna G, Mitchell MF, Wideroff L, Unger ER et al. Human papillomavirus (HPV) DNA copy number is dependent on grade of cervical disease and HPV type. J.Clin.Microbiol. 1999;37:1030-34.
14. Gravitt PE, Burk RD, Lorincz A, Herrero R, Hildesheim A, Sherman ME et al. A comparison between real-time polymerase chain reaction and hybrid capture 2 for human papillomavirus DNA quantitation. Cancer Epidemiol.Biomarkers Prev. 2003;12:477-84.
15. Tabora N, Ferrera A, Bakkers JM, Massuger LF, Melchers WJ. High HPV 16 viral load is associated with increased cervical dysplasia in Honduran women. Am.J.Trop.Med.Hyg. 2008;78:843-46.
16. Clavel C, Masure M, Levert M, Putaud I, Mangeonjean C, Lorenzato M et al. Human papillomavirus detection by the hybrid

capture II assay: a reliable test to select women with normal cervical smears at risk for developing cervical lesions. Diagn.Mol.Pathol. 2000;9:145-50.

17. Crum CP, Beach KJ, Hedley ML, Yuan L, Lee KR, Wright TC et al. Dynamics of human papillomavirus infection between biopsy and excision of cervical intraepithelial neoplasia: results from the ZYC101a protocol. J.Infect. Dis. 2004;189:1348-54.

18. Snijders PJ, Steenbergen RD, Heideman DA, Meijer CJ. HPV-mediated cervical carcinogenesis: concepts and clinical implications. J.Pathol. 2006;208:152-64.

19. 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;163:534-43.

20. 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:e3743.

21. Bleeker MC, Berkhof J, Hogewoning CJ, Voorhorst FJ, van den Brule AJ, Starink TM et al. HPV type concordance in sexual couples determines the effect of condoms on regression of flat penile lesions. Br.J.Cancer 2005;92:1388-92.

22. 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;354:2645-54.

23. Reiter PL, Pendergraft WF, III, Brewer NT. Meta-analysis of human papillomavirus infection concordance. Cancer Epidemiol. Biomarkers Prev. 2010;19:2916-31.

24. Bleeker MC, Hogewoning CJ, Berkhof J, Voorhorst FJ, Hesselink AT, van Diemen PM et al. Concordance of specific human papillomavirus types in sex partners is more prevalent than would be expected by chance and is associated with increased viral loads. Clin.Infect.Dis. 2005;41:612-20.

25. Bleeker MC, Hogewoning CJ, Voorhorst FJ, van den Brule AJ, Berkhof J, Hesselink AT et al. HPV-associated flat penile lesions in men of a non-STD hospital population: less frequent and smaller in size than in male sexual partners of women with CIN. Int.J.Cancer 2005;113:36-41.

26. Veldhuijzen NJ, Snijders PJ, Reiss P, Meijer CJ, van de Wijgert JH. Factors affecting transmission of mucosal human papillomavirus. Lancet Infect.Dis. 2010;10:862-74.

27. Ho GY, Studentsov YY, Bierman R, Burk RD. Natural history of human papillomavirus type 16 virus-like particle antibodies in young women. Cancer Epidemiol.Biomarkers Prev. 2004;13:110-16.

28. Viscidi RP, Schiffman M, Hildesheim A, Herrero R, Castle PE, Bratti MC et al. Seroreactivity to human papillomavirus (HPV) types 16, 18, or 31 and risk of subsequent HPV infection: results from a population-based study in Costa Rica. Cancer Epidemiol. Biomarkers Prev. 2004;13:324-27.

29. Frazer IH. Interaction of human papillomaviruses with the host immune system: a well evolved relationship. Virology 2009;384:410-14.

30. Carter JJ, Koutsky LA, Hughes JP, Lee SK, Kuypers J, Kiviat N et al. Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. J.Infect.Dis. 2000;181:1911-19.

31. Wideroff L, Schiffman MH, Hoover R, Tarone

RE, Nonnenmacher B, Hubbert N et al. Epidemiologic determinants of seroreactivity to human papillomavirus (HPV) type 16 virus-like particles in cervical HPV-16 DNA-positive and-negative women. J.Infect.Dis. 1996;174:937-43.

32. Brabin L. Interactions of the female hormonal environment, susceptibility to viral infections, and disease progression. AIDS Patient.Care STDS. 2002;16:211-21.

33. Dillner J, Rebolj M, Birembaut P, Petry KU, Szarewski A, Munk C et al. Long term predictive values of cytology and human papillomavirus testing in cervical cancer screening: joint European cohort study. BMJ 2008;337:a1754.

34. Cuzick J, Sasieni P, Davies P, Adams J, Normand C, Frater A et al. A systematic review of the role of human papilloma virus (HPV) testing within a cervical screening programme: summary and conclusions. Br.J.Cancer 2000;83:561-65.

35. 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;100:451-54.

36. Gok M, Heideman DA, van Kemenade FJ, Berkhof J, Rozendaal L, Spruyt JW et al. HPV testing on self-collected cervicovaginal lavage specimens as screening method for women who do not attend cervical screening: cohort study. BMJ 2010;340:c1040.

37. Castle PE, Rodriguez AC, Burk RD, Herrero R, Wacholder S, Alfaro M et al. Short term persistence of human papillomavirus and risk of cervical precancer and cancer: population based cohort study. BMJ 2009;339:b2569.

38. Moscicki AB. Conservative management of adolescents with abnormal cytology and histology. J.Natl.Compr.Canc.Netw. 2008;6:101-06.

39. Ostor AG. Natural history of cervical intraepithelial neoplasia: a critical review. Int.J.Gynecol.Pathol. 1993;12:186-92.

40. Moscicki AB, Ma Y, Wibbelsman C, Darragh TM, Powers A, Farhat S et al. Rate of and risks for regression of cervical intraepithelial neoplasia 2 in adolescents and young women. Obstet.Gynecol. 2010;116:1373-80.

41. Schiffman M, Wentzensen N, Wacholder S, Kinney W, Gage JC, Castle PE. Human Papillomavirus Testing in the Prevention of Cervical Cancer. J.Natl.Cancer Inst. 2011.

42. van HD, Bulten J, Shirango H, Melchers WJ, Massuger LF, de Wilde PC. Biological behavior of CIN lesions is predictable by multiple parameter logistic regression models. Carcinogenesis 2008;29:840-45.

43. Cuschieri K, Wentzensen N. Human papillomavirus mRNA and p16 detection as biomarkers for the improved diagnosis of cervical neoplasia. Cancer Epidemiol. Biomarkers Prev. 2008;17:2536-45.

44. Wentzensen N, Sherman ME, Schiffman M, Wang SS. Utility of methylation markers in cervical cancer early detection: appraisal of the state-of-the-science. Gynecol.Oncol. 2009;112:293-99.

45. Boulet GA, Horvath CA, Berghmans S, Bogers J. Human papillomavirus in cervical cancer screening: important role as biomarker. Cancer Epidemiol.Biomarkers Prev. 2008;17:810-17.

46. Ronco G, Giorgi-Rossi P, Carozzi F, Confortini M, Palma PD, Del MA et al. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. Lancet Oncol. 2010.

47. Insinga RP, Perez G, Wheeler CM, Koutsky LA, Garland SM, Leodolter S et al. Incidence, duration, and reappearance of type-specific

cervical human papillomavirus infections in young women. Cancer Epidemiol.Biomarkers Prev. 2010;19:1585-94.

48. Moscicki AB, Ma Y, Jonte J, Miller-Benningfield S, Hanson E, Jay J et al. The role of sexual behavior and human papillomavirus persistence in predicting repeated infections with new human papillomavirus types. Cancer Epidemiol.Biomarkers Prev. 2010;19:2055-65.

49. Cuzick J. Long-term cervical cancer prevention strategies across the globe. Gynecol.Oncol. 2010.

50. 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;32:457-61.

51. Cuzick J, Castanon A, Sasieni P. Predicted impact of vaccination against human papillomavirus 16/18 on cancer incidence and cervical abnormalities in women aged 20-29 in the UK. Br.J.Cancer 2010;102:933-39.

52. Berkhof J, Bogaards JA. Vaccination against human papillomavirus types 16 and 18: the impact on cervical cancer. Future.Oncol. 2010;6:1817-21.

53. Marra F, Cloutier K, Oteng B, Marra C, Ogilvie G. Effectiveness and cost effectiveness of human papillomavirus vaccine: a systematic review. Pharmacoeconomics. 2009;27:127-47.

54. De CN, Teixeira J, Roteli-Martins CM, Naud P, De BP, Zahaf T et al. Sustained efficacy and immunogenicity of the HPV-16/18 AS04-adjuvanted vaccine up to 7.3 years in young adult women. Vaccine 2010.

55. Dillner J, Kjaer SK, Wheeler CM, Sigurdsson K, Iversen OE, Hernandez-Avila M et al. Four year efficacy of prophylactic human papillomavirus quadrivalent vaccine against low grade cervical, vulvar, and vaginal intraepithelial neoplasia and anogenital warts: randomised controlled trial. BMJ 2010;341:c3493.

56. Clifford G, Franceschi S, Diaz M, Munoz N, Villa LL. Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases. Vaccine 2006;24 Suppl 3:S3-26-S3/34.

57. Szarewski A. HPV vaccine: Cervarix. Expert. Opin.Biol.Ther. 2010;10:477-87.

58. 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;374:301-14.

59. Brown DR, Kjaer SK, Sigurdsson K, Iversen OE, Hernandez-Avila M, Wheeler CM et al. The impact of quadrivalent human papillomavirus (HPV; types 6, 11, 16, and 18) L1 virus-like particle vaccine on infection and disease due to oncogenic nonvaccine HPV types in generally HPV-naive women aged 16-26 years. J.Infect.Dis. 2009;199:926-35.

60. Franco EL, Trottier H. Reassessing the epidemiology of human papillomavirus infection: back to basics. Sex Transm.Dis. 2008;35:283-85.

61. Stanley M, Lowy DR, Frazer I. Chapter 12: Prophylactic HPV vaccines: underlying mechanisms. Vaccine 2006;24 Suppl 3:S3-106-S3/113.

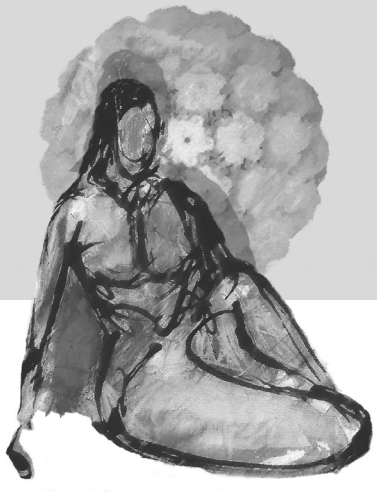
62. van Hamont D., Bekkers RL, Massuger LF, Melchers WJ. Detection, management, and follow-up of pre-malignant cervical lesions and the role for human papillomavirus. Rev. Med.Virol. 2008;18:117-32.

63. Cuzick J, Arbyn M, Sankaranarayanan R,

Tsu V, Ronco G, Mayrand MH et al. Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. Vaccine 2008;26 Suppl 10:K29-K41.

64. Basu P, Chowdhury D. Cervical cancer screening & HPV vaccination: a comprehensive approach to cervical cancer control. Indian J.Med.Res. 2009;130:241-46.

65. Massad LS, Einstein M, Myers E, Wheeler CM, Wentzensen N, Solomon D. The impact of human papillomavirus vaccination on cervical cancer prevention efforts. Gynecol.Oncol. 2009;114:360-64.



Chapter 9

Summary
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Summary

Chapter 1

Genital human papillomavirus (HPV) infection is the causal factor in the cervical carcinogenesis. Genital HPV infections are sexually transmitted and the lifetime risk of an infection is estimated to be about 80%. Acquiring an HPV infection is the initial step, but a persistent HPV infection is necessary for the further development of precursor lesion and/or cervical carcinoma. Fortunately most women (80-85%) clear their HPV infection spontaneously. On the basis of epidemiological criteria 15 anogenital HPV types are classified as high risk.

It is still not clearly understood why HPV infections are transient in some women and persist in others. The main identified co-factors are; sexual behaviour, long-term oral contraceptive pill (OCP) use, smoking, parity, specific HPV type, co-infection with other HPV types or other sexually transmitted diseases and intrinsic host factors like endogenous hormones and genetics.

Detection of HPV may be influenced by demographics and occurrence of the above mentioned co-factors in the study population. As well as the HPV DNA test used that may also influence HPV detection.

Chapter 2

Based on the high sensitivity and objectivity of HPV detection, testing for HPV has recently been advocated as a primary cancer screening tool. Currently, physician-collected cervical samples are considered the gold standard for HPV detection. However, self-sampling is an inexpensive, well accepted, method for human papillomavirus detection that may increase participation of non responders in current screening programs.

We reviewed the studies comparing self-sampling with physician-sampling to determine if self-sampling could be implemented in cervical cancer screening as a tool for HPV detection, and which combination of sampling device and HPV test may be used.

Self-sampling showed a good overall agreement with physician-sampling when a swab or a brush was used in combination with a PCR-based test for HPV DNA detection. The agreement was only moderate when using other sampling devices or HC2 for HPV DNA detection. However, the clinical sensitivity of self-sampling with PCR based tests needs to be validated. Future studies should focus especially on general screening populations to determine if self-sampling combined with PCR based HPV testing is a valid tool for national screening.

In high-risk populations, although based on only a few studies, physician-sampling had a higher sensitivity for CIN2+ detection than self-sampling.

Today self-sampling for HPV detection has proven that its sensitivity is sufficient to screen women otherwise not screened, and may be implemented in national screening programs to reach non-responders. Further research is necessary before a wide implementation in an already successful national screening program is possible.

Chapter 3

In this chapter the HPV type-specific incidence and clearance and its relation with age and sexual behaviour is analyzed. Incidence rates of any-type high-risk HPV and low-risk HPV were 17.0 per 1000-person months, and 14.3 per 1000-person months, respectively. HPV types 16, 52, 51, and 31 had the highest type specific incidence rates. HPV incidence rates in this young Dutch study population are comparable to incidence rates in young women in other western countries and not related to age. Within this 12 month follow-up study, the overall clearance of the newly detected type-specific high-risk HPV infections and low-risk HPV infections was 61.2% and 69.0%, respectively. Independent factors significantly influencing incidence and clearance were all related to past or current sexual behaviour, except for the clearance of HPV 16. Women with HPV 16 and co-infection with other high-risk HPV types had a lower proportion of clearance than women only infected with HPV 16.

The fact that incidence and clearance were not only related to current sexual behavior, but also to past sexual behavior indicates that accidental pick-up or re-activation of a latent HPV may be quite common. HPV clearance is also likely to be related to host immune factors, as clearance did show a relation with multiple infections.

Chapter 4

The detection of a persistent hr-HPV infection represents an important marker of an increased risk for CIN and cervical carcinoma. However, there is no consensus on the definition of persistence. Most investigators define a persistent HPV infection as detection of the same HPV type, or group of types, on two consecutive visits, but these visits could be from 2 months up to 72 months apart. Several risk factors have been identified that are associated with HPV persistence, especially viral load, type-specific HPV and smoking. This chapter describes the analysis of hr-HPV persistence and associated risk factors in a prospective cohort of young unscreened women. Additionally, the relation between hr-HPV status and cytology/histology results is examined.

We showed that hr-HPV infections are more likely to persist the longer they have been present. HPV genotypes 16, 18, 31, and 45 were most likely to persist. Other co-factors influencing persistence were, multiple HPV infections, smoking and multiple lifetime sexual partners. Women with a persistent hr-HPV infection have a higher rate of HSIL/CIN2+ detected in the following year. Thus, women with a persistent hr-HPV infection should be monitored for HSIL/CIN2+ development.

Chapter 5

There are several indications that hormonal factors might influence HPV dynamics. Long-term oral contraceptive pill (OCP) use is identified as risk factor for cervical cancer and a woman's' last menstrual period (LMP) is found to be related to HPV detection. The mucosal immunity of the female genital tract, which is influenced by reproductive hormones, may explain the possible effect of OCP use and the menstrual cycle on HPV detection.

In this chapter the potential influence from OCP use and sample timing within the menstrual cycle on HPV detection is analysed. Furthermore we investigated the potential difference in the HPV prevalence, incidence, and persistence between OCP users and non users.
The baseline HPV prevalence, the annual HPV incidence, and annual persistence did not differ between OCP users and nonusers. High-risk HPV detection was significantly influenced by the timing of sampling within the menstrual cycle when OCP users and nonusers were analyzed separately. In the second half of the menstrual cycle high-risk HPV detection decreased in nonusers and increased in OCP users. When women used OCPs continuously, high-risk HPV detection returned to the level of the first half of the menstrual cycle. Further studies are needed to investigate whether this effect remains with a less sensitive HPV detection test to elucidate the clinical implications of HPV detection in primary cervical screening. Because OCP use does not significantly influence HPV prevalence, incidence, or persistence, its increased risk for cervical cancer may be explained by a direct hormonal effect on the carcinogenesis.

Chapter 6
Two other co-factors that have been associated with cervical carcinoma are high parity and younger age at first full-term pregnancy. The aim of the study described in this chapter is to study the prevalence, incidence and clearance of human papillomavirus (HPV) in a prospective case control study of pregnant and non-pregnant women using a highly sensitive HPV detection and genotyping method. The 51 pregnant women and 51 non-pregnant women were matched 1:1 with a propensity score. The time-point prevalence of any-type HPV, high-risk-HPV, and low-risk-HPV did not significantly differ between the pregnant and matched-control women. Furthermore the number of women with a newly detected hr-HPV type during follow-up was also similar between both groups. Many of the infections were cleared in both groups, however due to small numbers statistical analysis to identify any potential difference could not be performed. This prospective case-control study shows that pregnancy does not influence HPV detection in a low-parity population of young, unscreened women.

Chapter 7
Most studies on self-sampling used liquid based collection systems. The use of liquid based self-samples contains the risk of leakage. In case of alcohol-containing preservation fluids, this may cause problems like inflammability and harm to eyes and skin. These problems may be avoided when applying self-sampled specimens to a solid dry carrier like the Indicator FTA elute cartridge (FTA cartridge). Previously, the FTA cartridge was found to be highly reliable when using the ultrasensitive SPF10 LiPA25 assay. In this chapter the performance of the FTA cartridge was evaluated using the clinically validated Hybrid Capture 2 (HC2) and GP5+/6+-PCR tests for HPV DNA detection.
A good concordance between liquid based and FTA cartridge systems in high-risk HPV

detection was found, when using the GP5+/6+ PCR. However, this concordance was much lower using HC2. The sensitivity of high-risk HPV detection on the FTA cartridge for high-grade CIN lesions was 84.6% with GP5+/6+ PCR, but only 53.8% with HC2. Therefore the use of the FTA cartridge with GP5+/6+ PCR is promising. It is necessary to evaluated the performance of the FTA cartridge with a clinically validated HPV DNA test like GP5+/6+ PCR on cervicovaginal self-samples obtained in a screening population, before implementation in national cervical screening program is possible.

Chapter 8

The previous chapters provide insight into the short-term natural history of HPV infections. These results, however also raise new questions. In the general discussion of this thesis, we formulate and discuss these new questions on the HPV dynamics; (1) When does a woman have a productive (replicating) infection in case an HPV type is detected? (2) When is the detected HPV type infectious? (i.e., when is there a high risk of transmission to a sexual partner?) (3) When is a newly detected HPV type truly a new infection and when does it represent a re-infection or reactivation?

Furthermore the role of HPV DNA detection in cervical cancer screening and HPV vaccination are discussed.

Samenvatting

Hoofdstuk 1

In de carcinogenese van het cervixcarcinoom is een genitale infectie met het humaan papillomavirus (HPV) een noodzakelijke factor. HPV is seksueel overdraagbaar en het risico om ooit in het leven deze infectie te krijgen wordt geschat op 80%. Het oplopen van een HPV infectie is de eerste stap in de carcinogenese, maar voor het ontwikkelen van een voorstadium en/of cervixcarcinoom is het noodzakelijk dat het virus persisteert. Gelukkig klaren de meeste vrouwen (80-85%) een HPV infectie spontaan. Op basis van epidemiologische criteria zijn 15 anogenitale HPV typen als hoog-risico typen voor het ontstaan van cervixcarcinoom gedefinieerd.

Het is nog steeds niet duidelijk waarom HPV infecties bij sommige vrouwen van voorbijgaande aard zijn en waarom HPV infecties bij andere vrouwen persisteren. De factoren die hierin een rol lijken te spelen zijn: seksueel gedrag, langdurig gebruik van orale anticonceptie, roken, multi-pariteit, specifieke HPV typen, co-infectie met andere HPV typen of andere seksueel overdraagbare ziektes. Daarnaast spelen ook intrinsieke factoren als endogene hormonen en genetische predispositie, een rol.

De mate van detectie van HPV binnen een populatie wordt bepaald door demografische kenmerken en bovenstaande risicofactoren binnen de populatie, maar zeker ook door het soort HPV DNA test dat gebruikt wordt.

Hoofdstuk 2

Vanwege de hoge sensitiviteit en de objectiviteit van HPV-testen heeft de gezondheidsraad recent het advies uitgebracht aan de Minister van Volksgezondheid, Welzijn en Sport (VWS) om, in plaats van cytologie, HPV detectie te gebruiken als primaire test voor het bevolkingsonderzoek baarmoederhalskanker. Tot op heden wordt een sample (uitstrijk) van de cervix afgenomen door een arts ("arts-sample"), als de gouden standaard beschouwd. Een sample door de vrouw zelf afgenomen ("zelf-sample") is een door vrouwen zeer geaccepteerde methode, die de deelname aan het bevolkingsonderzoek zou kunnen vergroten.

In hoofdstuk 2 zijn literatuurstudies beschreven die zelf-samples en arts-samples vergelijken om te bepalen of een zelf-sample voor HPV detectie geïmplementeerd kan worden in het bevolkingsonderzoek baarmoederhalskanker. Daarnaast wordt geanalyseerd welke combinatie van materiaal voor afname en HPV DNA test, het beste resultaat geeft.

In laag-risico populaties, zoals screeningspopulaties, toonden zelf-samples en arts-samples een goede overeenkomst over de aanwezigheid van HPV wanneer voor de afname een wattenstaafje of een borsteltje werd gebruikt, in combinatie met een HPV PCR test. Deze overeenkomst voor HPV detectie was middelmatig wanneer andere afnamematerialen werden gebruikt of in combinatie met de hybrid capture 2 (HC2). De klinische sensitiviteit van een zelf-sample gecombineerd met een HPV test, dient echter

nog gevalideerd te worden.
In hoog-risico populaties, alhoewel gebaseerd op slechts enkele studies, had een arts-sample een hogere sensitiviteit dan een zelf-sample voor de detectie van CIN2+.
Concluderend heeft het zelf afnemen van een vaginaal sample door de vrouw een bewezen voldoende hoge sensitiviteit voor HPV detectie. Zelf-afname voor HPV detectie zou in het bevolkingsonderzoek baarmoederhalskanker gebruikt kunnen worden om vrouwen die niet reageren op de oproep toch te kunnen bereiken. Om zelf-samples voor HPV detectie breed te kunnen implementeren in een reeds succesvol bevolkingsonderzoek is meer onderzoek nodig.

Hoofdstuk 3
In hoofdstuk 3 werd de HPV type-specifieke incidentie en klaring in relatie met leeftijd en seksueel gedrag bestudeerd. De incidentie van alle typen hoog-risico HPV en laag-risico HPV was respectievelijk 17.0 per 1000-persoon maanden, en 14.3 per 1000-persoon maanden. De HPV typen 16, 52, 51, en 31 hadden de hoogste type-specifieke incidentie. De HPV incidentie in deze jonge Nederlandse studiepopulatie is vergelijkbaar met de incidentie in andere westerse landen. Binnen deze studiegroep was de klaring van nieuwe HPV infecties hoog- respectievelijk laag-risico HPV types 61.2% respectievelijk 69.0% gedurende de studieduur van 12 maanden. Factoren die de incidentie en klaring van HPV significant beïnvloedden, waren alle gerelateerd aan het huidige seksuele gedrag of het seksuele gedrag in het verleden. Dit gold niet voor de klaring van HPV 16. Vrouwen die een HPV 16 hadden en tegelijkertijd een infectie met een ander HPV type, hadden een lager klaringspercentage dan vrouwen die alleen met HPV 16 geïnfecteerd waren.
Het feit dat incidentie en klaring niet alleen gerelateerd waren aan het huidige seksuele gedrag, maar ook aan het seksuele gedrag in het verleden, kan er op wijzen dat 'accidental pick-up' of reactivatie van een latente HPV infectie regelmatig voorkomt. De klaring van HPV lijkt ook gerelateerd te zijn aan de werking van het immuunsysteem van de vrouw, omdat het hebben van meerdere infecties negatief gerelateerd was aan klaring.

Hoofdstuk 4
De aanwezigheid van een persisterende hoog-risico HPV infectie is een belangrijke risicofactor voor cervicale intraepitheliale neoplasie (CIN) of cervixcarcinoom. Desondanks is er geen consensus over de definitie van persistentie. De meeste onderzoekers definiëren HPV persistentie als de detectie van hetzelfde HPV type, of HPV groep, op twee opeenvolgende afname momenten. Het interval tussen deze afnamen kan echter 2 tot 72 maanden zijn. Verscheidene risicofactoren voor HPV persistentie zijn virale load, HPV genotype en roken.
Hoofdstuk 4 beschrijft de analyse van HPV persistentie en gerelateerde risicofactoren in een prospectief cohort van jonge ongescreende vrouwen. Daarnaast is de relatie tussen detectie van hoog-risico HPV en cytologie/histologie uitslagen onderzocht.
Uit deze studie bleek dat hoe langer hoog-risico HPV infecties reeds aanwezig waren,

hoe hoger de kans dat de HPV infectie persisteert. De HPV genotypen 16, 18, 31, en 45 hadden de hoogste persistentie percentages. Andere risicofactoren voor HPV persistentie waren het hebben van meerdere HPV typen, roken en het hebben van meerdere seksuele partners gedurende het leven. In de groep vrouwen met een persisterende hoog-risico HPV infectie werd een verhoogd percentage hoog gradige squameuse intraepitheliale laesie (HSIL) /CIN2+ gevonden. Vanwege deze hoge kans op ontwikkeling van HSIL/ CIN2+ moeten vrouwen met een persisterende hoog-risico HPV infectie vervolgd worden.

Hoofdstuk 5

Er zijn verschillende aanwijzingen dat hormonale factoren de HPV dynamiek beïnvloeden. Langdurig gebruik van de orale anticonceptie (OAC) is geïdentificeerd als risicofactor voor cervixcarcinoom en de menstruele cyclus lijkt gerelateerd te zijn aan verschillen in HPV detectie. De mucosale immuniteit van het vrouwelijke geslachtsorgaan, welke beïnvloed wordt door hormonen, kan mogelijk het potentiële effect van OAC gebruik en de menstruele cyclus op de HPV detectie verklaren.

In hoofdstuk 5 werd de potentiële invloed van OAC gebruik en de timing van sample afname binnen de menstruele cyclus op de HPV detectie onderzocht. Tevens werd het potentiële verschil in HPV prevalentie, incidentie en persistentie tussen OAC gebruiksters en niet-gebruiksters geanalyseerd. De HPV prevalentie bij start van de studie, de jaarlijkse incidentie en persistentie, waren niet significant verschillend tussen beide groepen. De detectie van hoog-risico HPV werd significant beïnvloed door het afnamemoment binnen de menstruele cyclus wanneer de resultaten van OAC gebruiksters en niet-gebruiksters afzonderlijk werden geanalyseerd. In de tweede helft van de menstruele cyclus nam de detectie van hoog-risico HPV af bij vrouwen met een natuurlijke menstruele cyclus en toe bij OAC gebruiksters. Bij vrouwen die continu OAC gebruikten, dus zonder stopweek, zagen we dat deze toename van HPV detectie weer normaliseerde naar het niveau van de eerste twee weken van de cyclus. Om de klinische relevantie te bepalen van dit verschil in HPV detectie binnen de menstruele cyclus, dient verder onderzoek gedaan te worden met een klinisch gevalideerde (dus minder gevoelige) HPV test.

Hoofdstuk 6

Twee andere factoren die geassocieerd worden met een verhoogd risico op cervixcarcinoom zijn hoge pariteit en jonge leeftijd bij de eerste voldragen zwangerschap. In hoofdstuk 6, een prospectieve case-control studie, werd het verschil in prevalentie, incidentie en klaring van HPV tussen zwangere en niet-zwangere vrouwen getest. 51 zwangere en 51 niet-zwangere vrouwen werden 1 op 1 aan elkaar gekoppeld middels een propensity score. De prevalentie van alle HPV typen, hoog-risico HPV, en laag-risico HPV was niet significant verschillend tussen zwangere en niet-zwangere vrouwen. Het aantal nieuw gedetecteerde HPV typen in beide groepen was eveneens vergelijkbaar. Het grootste deel van de HPV infecties werden gedurende de studieperiode geklaard,

maar de aantallen waren te klein om een mogelijk verschil statistisch te verklaren. Deze prospectieve case-control studie laat zien dat in deze populatie van jonge ongescreende vrouwen met een lage pariteit, zwangerschap geen invloed heeft op de HPV detectie.

Hoofdstuk 7

De meeste studies die zelf-samples voor HPV detectie bestuderen gebruiken collectie systemen met vloeistof. Het gebruik hiervan kan risico bevatten op lekkage en, in het geval van vloeistoffen die alcohol bevatten, risico op ontvlamming en op schade aan ogen en huid. Deze mogelijke problemen kan men vermijden door een droog collectiesysteem, zoals de Indicator FTA elute cartridge (FTA cartridge), te gebruiken. In eerder onderzoek bleek de FTA cartridge in combinatie met SPF_{10} $LiPA_{25}$ assay zeer betrouwbaar voor HPV detectie. In hoofdstuk 7 werd in een hoog-risico populatie het gebruik van de FTA cartridge in combinatie met de klinisch gevalideerde HC2 en GP5+/6+ PCR HPV test onderzocht.

Met de GP5+/6+ PCR werd, voor detectie van hoog-risico HPV DNA, een goede overeenkomst gevonden voor de detectie van HPV tussen het op vloeistof gebaseerde systeem en de FTA cartridge. De sensitiviteit van hoog-risico HPV detectie op de FTA cartridge voor detectie van hooggradige CIN laesies was echter 84.6% met GP5+/6+ PCR, en slechts 53.8% met HC2. Het gebruik van de FTA cartridge in combinatie met de GP5+/6+ PCR lijkt veelbelovend. Voor een mogelijke implementatie in het bevolkingsonderzoek naar baarmoederhalskanker dient het gebruik van de FTA cartridge met een klinisch gevalideerde test als GP5+/6+ PCR onderzocht te worden op zelf-samples in een screeningspopulatie.

Hoofdstuk 8

De voorgaande hoofdstukken geven inzicht in het natuurlijke gedrag van HPV infecties in een periode van één tot twee jaar en deze resultaten leiden opnieuw tot vragen over de HPV dynamiek. In de algemene discussie van dit manuscript worden deze vragen uitgewerkt: (1) Wanneer is er sprake van een productieve (replicerende) infectie bij detectie van een HPV type?; (2) Wanneer is het gedetecteerde HPV type infectieus? (d.w.z. wanneer is transmissie van het virus naar de seksuele partner hoog?); (3) Wanneer is een nieuw gedetecteerd HPV type werkelijk een nieuwe infectie en wanneer is dit een re-infectie of reactivatie?

Tenslotte wordt in hoofdstuk 8 de rol van HPV detectie in het bevolkingsonderzoek baarmoederhalskanker en HPV vaccinatie bediscussieerd.

Bibliography

Publications

Use of oral contraceptives and increased risk of cervical cancer
Schmeink CE, Lenselink CH, Bekkers RLM. *Ned Tijdschr Geneeskd.* 2008 Aug 2;152(31):1717-8. Dutch

Young adults and acceptance of the human papillomavirus vaccine
Lenselink CH, **Schmeink CE**, Melchers WJ, Massuger LF, Hendriks JC, van Hamont D, Bekkers RLM. *Public Health.* 2008 Dec;122(12):1295-301. Epub 2008 Jul 10

An individual decision aid for sexually active women considering HPV vaccination / HPV vaccination and individual risk factor assessment
Lenselink CH, **Schmeink CE**, Hendriks JCM, Melchers WJG, Quint WGV, Massuger LFAG, Bekkers RLM. *Proefschrift:* Sexual behaviour and HPV in Young women, the pre-vaccine era, Lenselink CH, March 2010

Effect of the menstrual cycle and hormonal contraceptives on HPV detection in young unscreened women
Schmeink CE, Massuger LFAG, Lenselink CH, Quint WGV, Melchers WJG, Bekkers RLM. *Obstet Gynecol.* 2010 Jul;116(1):67-75

The potential role of self-sampling for hr-HPV detection in cervical cancer screening
Schmeink CE, Bekkers RLM, Massuger LFAG, Melchers WJG. *Rev Med Virol.* 2011 May;21(3):139-53. doi: 10.1002/rmv.686. Review

The indicator FTA elute cartridge: a solid sample carrier to detect high-risk HPV and high-grade cervical lesions
de Bie RP, **Schmeink CE**, Bakkers JMJE, Snijders PJF, Quint WGV, Massuger LFAG, Bekkers RLM, Melchers WJG. *J Mol Diagn.* 2011 Jul;13(4):371-6. Epub 2011 Apr 29

Co-administration of Human papillomavirus-16/18 AS04-Adjuvanted vaccine with Hepatitis B vaccine in healthy girls aged 9-15 years: results of a randomized, controlled study
Schmeink CE, Bekkers RLM, Josefsson A, Richardus JH, Berndtsson Blom K, David MP, Dobbelaere K, Descamps D. *Vaccine.* 2011 Nov 15;29(49):9276-83. Epub 2011 Aug 19

Young adults awareness of HPV and vaccine acceptance after introduction of the HPV vaccine in the Dutch national vaccination program
Schmeink CE, Gosens KCM, Melchers WJG, Massuger LFAG, Bekkers RLM. *Eur J Gynaecol Oncol*. 2011;32(5):481-6

Human papillomavirus persistence in young unscreened women, a prospective cohort study
Schmeink CE, Melchers WJG, Siebers AG, Quint WGV Massuger LFAG, and Bekkers RLM. *PloS ONE*. 6 (11): e 27937. Epub 2011 Nov 23

Dynamics of human papillomavirus and cervical cancer screening
Schmeink CE, Massuger LFAG, Melchers WJG and Bekkers RLM. *European Obstetrics & Gynaecology, 2011;6(2):107-10*

Human papillomavirus detection in a prospective case control study of pregnant women
Schmeink CE, Melchers WJG, Hendriks JCM, Quint WGV, Massuger LFAG, Bekkers RLM. *Submitted*

Prospective follow-up of 2065 young unscreened women to study human papillomavirus
incidence and clearance
Schmeink CE, Massuger LFAG, Lenselink CH, Quint WGV, Witte BI, Berkhof J, Melchers WJG and Bekkers RLM. *Submitted*

Oral presentations

The effects of hormonal contraceptives and the menstrual cycle on single point prevalence of human papillomavirus in young women
EUROGIN 2008, Nice

Individual risk-factor assessment of sexually active women prior to HPV vaccination; how effective is your HPV vaccination?
16th International Meeting of the European Society of the Gynaecological Oncology (ESGO) Belgrade, Serbia, October 11-14, 2009

Poster presentations

A small majority of young adults accepts Human papillomavirus vaccination
23th International Papillomavirus conference (IPV), Prague 2006

The effects of hormonal contraceptives and the menstrual cycle on single point prevalence of human papillomavirus in young women
25th International Papillomavirus conference (IPV), Sweden, Malmö, May 2009

Co-administration of AS04-adjuvantes human papillomavirus-16/18 vaccine with hepatitis B vaccine in healthy female subjects aged 9-15 years: Month 7 data
28th meeting of European Society for Peadiatric Infectious Diseases (ESPID), France, Nice, May 2010

The influence of pregnancy on hr-HPV detection
26th International Papillomavirus conference (IPV), Canada, Montreal, July 2010

Increased awareness of the human papillomavirus does not increase vaccine acceptance in young adults
13th Biennial Meeting of the International Gynecologic Cancer Society (IGCS 2010), Prague, October 2010

Effect van de menstruele cyclus en gebruik orale anticonceptiva op de detectie van het humaan papillomavirus bij jonge, ongescreende vrouwen
38ste Gynaecongres, NVOG, Papendal, november 2010

Human papillomavirus persistence in young unscreened women, a prospective cohort study
27th International Papillomavirus conference (IPV), Germany, Berlin, September 2011

Dankwoord

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Curriculum Vitae

Channa Schmeink werd geboren op 28 april 1983, te Huissen. Haar basisschooltijd bracht zij door in Wageningen en Huissen. In 2001 behaalde zij haar Gymnasium-diploma aan het Olympuscollege te Arnhem.
Door een gunstige loting kon Channa direct starten met haar studie Geneeskunde in Nijmegen. Tijdens haar studententijd was ze niet alleen druk met studeren, vrienden, hardlopen, dans, reizen, en verschillende bijbaantjes, maar was ze ook actief binnen enkele commissies van de Medische Faculteit Vereniging Nijmegen (MFVN). De combinatie van reizen en reeds bestaande interesse in de gynaecologie zorgden ervoor dat ze voor haar co-schappen twee maanden naar Malawi ging voor een IFMSA-stage: Mother and Child health care.
Tijdens haar wetenschappelijke stage deed Channa onderzoek naar 'Kennis omtrent cervixcarcinoom, uitstrijkjes en HPV onder jonge mannen en vrouwen' bij dr. Dennis van Hamont. In het laatste jaar van haar co-schappen werkte ze mee met dr. Charlotte Lenselink aan de opzet van het onderzoek 'Het vóórkomen van het Humaan papillomavirus bij jonge mensen'. Beide onder leiding van dr. Ruud Bekkers. Hiermee werd de basis van het promotieonderzoek van Channa gelegd. Haar co-schappen sloot ze af met een tropen co-schap en een rondreis in Tanzania. Daarnaast deed ze bij terugkomst nog een extra keuze co-schap gynaecologie om haar liefde voor dit vak te bevestigen.
Vanaf maart 2008 werkte zij onder begeleiding van haar promotor prof. dr. Leon Massuger, en de co-promotoren dr. Ruud Bekkers en dr. Willem Melchers, als arts-onderzoeker fulltime aan het onderzoek, hetgeen resulteerde in dit proefschrift.
In april 2011 is Channa gestart als ANIOS Gynaecologie in het Rijnstate Ziekenhuis te Arnhem. Sinds 1 januari 2012 is zij als AIOS Gynaecologie in ditzelfde ziekenhuis werkzaam (opleiders Dr. F.P.H.L.J. Dijkhuizen, dr. R.L.M. Bekkers en prof. dr. D.D.M. Braat).
Channa woont sinds 2006 samen met Sander Beikes in Arnhem.