HPV and cervical cancer on Curaçao

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Chapter 4

High prevalence of high-risk HPV genotypes other than 16 and 18 in cervical cancers of Curaçao: implications for choice of prophylactic HPV vaccine

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Outline

Abstract

Background: Curaçao is a Dutch-Caribbean Island located in a high-risk area for cervical cancer. Prior to introduction of a prophylactic HPV vaccine, knowledge of the prevalence of high-risk HPV vaccine genotypes (HPV-16, -18, -31, -33, -45, -52, and -58) in cervical (pre)cancer is required.

Objective: To investigate the prevalence of HPV-genotypes in invasive cervical cancers (ICC) and cervical intraepithelial neoplasia (CIN) grade 1, 2 and 3 on Curaçao.

Methods*:* Paraffin embedded blocks of 104 cervical cancers (89 squamous, 15 adenocarcinoma), 41 CIN3, 39 CIN2, and 40 CIN1 lesions were analysed for the presence of HPV. Sections were stained by Haematoxylin Eosin for histopathological evaluation and DNA was extracted using Proteinase K. HPV-genotypes were detected using SPF10 PCR DEIA & LiPA25-assay.

Results: HPV was found in 92 (88.5%) ICC; 87 (94.6%) had a single HPV infection and 86 (93.5%) were hrHPV-type positive. The three most common HPV types in ICC were 16 (38.5%), 18 (13.5%) and 45 (6.7%), covering 58.7%. HrHPV vaccine genotypes 16, 18, 31, 35, 45, 52, 58 were responsible for 73.1% of ICC. For precancerous lesions the HPV attribution was 85.4% for CIN3, 66.7% for CIN2 and 42.5% for CIN1.

Conclusions*:* Our study, the largest in the Caribbean region, shows that the prevalence of HPV-type 16 and 18 in cervical cancer is lower compared to the world population but no differences in prevalence of these two HPV types are seen in precancerous lesions. When considering HPV vaccination on Curaçao, the relatively high contribution of non-HPV-16/-18 genotypes in ICC should be taken into account.

4.
High prevalence of high-risk HPV genotypes other than 16 and 18 in cervical cancers of Curaçao: implications for choice of prophylactic HPV vaccine

4.1 Introduction

Curaçao is a Dutch-Caribbean island with estimated 155,000 inhabitants last registered in 2014 (CBS Curacao, 2015). It is demographically located in an area with a high incidence and mortality of cervical cancer (Murillo et al., 2008; Consedine et al., 2014; Ferlay et al., 2015; Bosch et al., 2002). known to be caused by high-risk HPV (zur Hausen, 2009; Walboomers et al., 1999). According to The Cancer Registry of Curaçao, the incidence of cervical cancer over 2004-2008 is 13.4 per 100,000 women (personal communication C.M.D Coronel, MD. 2015).

The lack of an organized cervical screening and prophylactic HPV vaccination program largely explains the high incidence of cervical cancer (Banydeen et al., 2015; Lewis Merle, 2004; Martin, 2013).

Presently, three HPV prophylactic vaccines are available. A bivalent HPV vaccine against HPV-16 and -18, a quadrivalent vaccine directed against HPV-16 and -18 with an additional coverage to low risk HPV -6 and -11 and recently a nonavalent vaccine directed against hrHPV-16, -18 -31, -33, -45, -52, -58 and lrHPV-6 and -11. The bi- and quadrivalent vaccines have shown partial to full cross-protection against certain non-vaccine HPV types (Garland et al., 2016; Bosch et al., 2012).

According to report about the situational analysis of cervical cancer prevention and control in the Caribbean, only three Caribbean islands have a

HPV vaccination program up and running (Martin et al., 2013). On Curaçao, the bi- and quadrivalent HPV vaccines are currently available in private practise; however, they are not part of the National Vaccination Programme. In July 2016, the island started with an HPV based national cervical cancer screening programme. Before introducing a prophylactic vaccine to women on Curaçao it is important to know the prevalence of HPV genotypes in cervical precancerous lesions and cancer. This can help in the decision by policy makers which prophylactic HPV vaccine should be used and what the potential impact of the new vaccine might be. Here we describe the prevalence of HPV genotypes in paraffin embedded tissues of cervical cancers and cervical precancerous lesions [cervical intraepithelial neoplasia grade 1, 2 and 3 (CIN1, CIN2, CIN3)] derived from the only pathology laboratory of Curaçao.

4.2 Material and Methods

4.2.1 Material

A retrospective study was designed and coordinated by Fundashon Prevenshon (FP), Curaçao, in cooperation with DDL Diagnostic Laboratory, Rijswijk, the Netherlands; the department of pathology, VU University Medical Centre (VUmc) Amsterdam, the Netherlands and the Analytic Diagnostic Centre (ADC), Curaçao, to determine the prevalence of HPV DNA genotypes in formalin fixed paraffin embedded (FFPE) tissues of women with invasive cervical cancer.

All ICC and CIN cases diagnosed in woman from Curaçao were selected in the PALGA system, which is the nationwide network and registry of histo- and cytopathology in the Netherlands and Curaçao. This system includes both private and government insured patients. Only available blocks of good quality, containing enough tissue, were selected from 2012 backwards,

anonymised, relabelled, and analysed at DDL diagnostic laboratory. In total, FFPE blocks of 104 cervical cancers, 40 CIN1, 39 CIN2, and 41 CIN3, were collected from the ADC's department of Pathology, Curaçao. The type of tissue from which the lesion samples are obtained are given in table 1.

Table 4.1
Type of tissue sample of cervical cancer and CIN lesion used for HPV detection

	Cervical ca.	CIN3	CIN2	CIN1
Biopsies	75	30	27	25
LLETZ	1	5	6	4
Intra-cervical curettages	6	4	3	2
Cervix conisations	14	1	2	8
Hysterectomies	8	0	1	2
Total	104	40	39	41

Eight sections were cut from each block according to the sandwich method for histopathology and DNA extraction for HPV testing (Walboomers et al., 1999). In brief, the first and last sections were 4µm and stained with Haematoxilin and Eosin (H&E) for histopathological evaluation of the lesion. In between the two H&E stained sections, six sections of 8µm were cut. The first three were combined in a single tube and used for HPV genotyping, the last three were stored in another tube for back up.

After each 10 blocks that were cut, an empty paraffin block was cut as a control for contamination during the sectioning process. Sections from the control block were tested in parallel with the samples and remained negative. For each sample we also obtained age of the woman at diagnosis, date of sample collection and histological diagnosis.

4.2.2 DNA Isolation and HPV testing

Tissue sections were incubated overnight for DNA extraction with 250 µl of a 0.1% proteinase K solution at 70°C. The standard proteinase volume of 250 µl was reduced to 100 µl for sections containing small tissue samples as observed by visual inspection. After incubation, Proteinase K was inactivated at 95°C for 10 minutes. HPV DNA was amplified by the SPF_{10} PCR in a total volume of 50µl using 10 µl of 10 times diluted DNA. The SPF_{10}-DNA enzyme immunoassay (DEIA) (Labo Bio-medical Products, Rijswijk, The Netherlands) was used for general detection of over 69 known HPV types by hybridization to a mixture of HPV-specific probes in a 96-well format (Kleter et al., 1998; DT. Geraets et al., 2014). Amplimers from positive samples by DEIA were genotyped in the $SPF_{10}LiPA_{25}$ reverse hybridization assay for identification of 25 HPV genotypes (Kleter et al., 1999) (version1; Labo Bio-medical Products, based on licensed Innogenetics technology).

DNA from all samples was also tested for the presence of amplifiable human genomic DNA and PCR inhibition by a duplex real-time PCR targeting the human RNaseP gene and a plasmid spiked into the PCR mix (Luo et al., 2005). In case the Ct value of the plasmid spike-in was elevated, indicating PCR inhibition, the already 10 times diluted sample was diluted 10 more times and re-tested for HPV and PCR inhibition. If the 10 times diluted sample did not show PCR inhibition and the HPV result was negative, the undiluted sample was re-tested for HPV and PCR inhibition.

4.2.3 Statistical Analyses

HPV type attribution per group was assessed in different ways to deal with multiple infections (Supplementary table 4.1). Firstly, we considered only single HPV infections. That is, women with multiple HPV infections were not included in the calculation, underestimating the genotype attributable fraction. Secondly, we considered each infection as a causal infection, i.e. for a woman

infected with HPV-16 and HPV-31 both 16 and 31 were counted as separate attribution, overestimating the genotype attributable fraction (Quint et al., 2012). Finally, we used hierarchical and proportional attribution to account for multiple HPV infections (Wentzensen et al., 2010). In short, in a woman with multiple infections either the HPV infection with the highest prevalence within the population (hierarchical method) or each HPV infection proportional to the total prevalence within the population (proportional method) is assumed to have attributed to the lesion.

Ethical approval of the study was obtained from the Institutional review board of the medical ethics committee of Fundashon Prevenshon, Curaçao, (IRB board's approval number 0001/14).

4.3 Results

Table 4.2 presents the mean age at which cervical cancer and CIN lesions were diagnosed, and the prevalence of HPV in these lesions. Since the results of hierarchical attribution analysis were similar to the proportional attribution analysis, we only present here the hierarchical distribution. The prevalence of HPV genotypes in multiple infections in cervical cancer and CIN lesions is shown in Supplementary Table 4.1.

Table 4.2
The prevalence of HPV, single HPV-and multiple HPV infections and hrHPV in cervical cancer and CIN lesions

	N	Age (mean)	Age (range)	HPV pos.	%	HPV neg.	%	HPV single	%	HPV multiple	%	hrHPV* pos.	%
CIN1	40	32.7	(20-51)	31	77.5	9	22.5	21	67.7	10	32.3	24	77.4
CIN2	39	34.4	(19-72)	35	89.7	4	10.3	27	77.1	8	22.9	34	97.1
CIN3	41	38.6	(25-74)	39	95.1	2	4.9	31	79.5	8	20.5	39	100.0
Cancer (ICC)	104	52.6	(27-86)	92	88.5	3	20.0	87	94.6	5	5.4	86	93.5
SCC + ASC	89	52.2	(27-86)	80	89.9	9	10.1	75	93.8	5	6.3	74	92.5
ADC	15	51.9	(32-74)	12	80.0	12	11.5	12	100.0	0	0	12	100.0

** Percentage of hrHPV is calculated from the number of HPV positive cases*

4.3.1 Carcinomas

Most of the histotypes, were squamous cell carcinoma [SCC n=85 (81.7%)], followed by adenocarcinoma ([ADC n= 15 (14.4%)] and adenosquamous carcinoma [(ASC n=4 (3.8%)] were (Table 4.2). Because of the small number of ASC, for statistical analysis we added the 4 ASC cases to the SCC. The large majority (88.5%) of cervical carcinoma were HPV positive of which only 5.4% had a multiple infection. The majority of positive ICC cases (93.5%) contained one hrHPV type (Table 4.2). The most prevalent HPV types in ICC were 16 (38.5%), 18 (13.5%) and 45 (6.7%), covering 58.7% of the carcinomas (Table 3). Four out of the five multiple hrHPV-infections in cervical cancer had HPV-16 in combination with another high-risk type (Supplementary Table 4.2). Eighty percent of the ADC (n=15) were HPV positive. Only single HPV type infections were detected, and all samples were

high-risk HPV types. HPV-18 was the most prevalent HPV type (40.0%) followed by HPV-16 (26.7%) and -45 (13.3%) (Table 4.3). In total, 12 of the ICC samples were HPV negative: 9 SCC and 3 ADC.

4.3.2 CIN lesions

The percentages of HPV prevalence increased with the grade of CIN lesion namely CIN1 77.5%, CIN2 89.7% CIN3 (95.1%). (Table 4.2). The percentages of multiple HPV infections decreased from CIN1 (32.3%), to CIN3 (20.5%) and were lowest in cervical carcinomas (5.4%). HPV-16 was the most prevalent HPV type in all degrees of CIN and its prevalence increased substantially with greater severity of CIN. The most prevalent HPV type in CIN3 samples were HPV-16 (46.3%) and HPV 31(12.2%). HPV types 18, 52 and 58 each covered 7.3%. In CIN 2 the most common types were HPV-16 (30.8%) while HPV-18, -35 and -51 were the second most common, each covered 10.3%. In CIN 1 the most common HPV types were HPV-16 (15.0%), -31 (12.5%) and -51 (7.5%) (Table 4.3).

Table 4.3
HPV-type distribution in HPV positive cervical cancer, subdivided in squamous cell carcinoma/ adenosquamous carcinoma (SCC/ASC), adenocarcinoma and CIN 3, 2, 1 lesions.

type	Hierarchical attribution*													
	Cancer		SCC/ASC		ADC		CIN3		CIN2		CIN1		Total	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
hrHPV*														
16	40	38.5%	36	40.4%	4	26.7%	19	46.3%	12	30.8%	6	15.0%	77	34.4%
18	14	13.5%	8	9.0%	6	40.0%	3	7.3%	4	10.3%	1	2.5%	22	9.8%
31	4	3.8%	4	4.5%			5	12.2%	2	5.1%	5	12.5%	16	7.1%
33	3	2.9%	3	3.4%			1	2.4%	2	5.1%	1	2.5%	7	3.1%
35	3	2.9%	3	3.4%					4	10.3%			7	3.1%
39	1	1.0%	1	1.1%					1	2.6%			2	0.9%
45	7	6.7%	5	5.6%	2	13.3%	2	4.9%	1	2.6%	2	5.0%	12	5.4%
51	3	2.9%	3	3.4%			2	4.9%	4	10.3%	3	7.5%	12	5.4%
52	4	3.8%	4	4.5%			3	7.3%	2	5.1%	1	2.5%	10	4.5%

(Table 4.3 continued)

type	Cancer		SCC/ASC		ADC		CIN3		CIN2		CIN1		Total	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
hrHPV*														
56											1	2.5%	1	0.4%
58	4	3.8%	4	4.5%			3	7.3%	1	2.6%	2	5.0%	10	4.5%
59	1	1.0%	1	1.1%							1	2.5%	2	0.9%
66							1	2.4%	1	2.6%			2	0.9%
68	2	1.9%	2	2.2%							1	2.5%	3	1.3%
lrHPV$														
6									1	2.6%	2	5.0%	3	1.3%
44														
53											1	2.5%	1	0.4%
54														
61											1	2.5%	1	0.4%
67	1	1.0%	1	1.1%									1	0.4%
70											2	5.0%	2	0.9%
71														
74														
82	1	1.0%	1	1.1%									1	0.4%
91														
X	4	3.8%	4	4.5%							1	2.5%	5	2.2%
Total	92	88.5%	80	89.9%	12	80.0%	39	95.1%	35	89.7%	31	77.5%	197	87.9%
negative	12	11.5%	9	10.1%	3	20.0%	2	4.9%	4	10.3%	9	22.5%	27	12.1%

**hrHPV= high risk Human papillomavirus, $lrHPV= low risk Human papillomavirus*

4.4 Discussion

This is the first study from Curaçao to show systematic data on HPV prevalence in cervical cancer and CIN lesions. Interestingly although the prevalence of HPV-16 (38.5%) and HPV-18 (13.5%) in cervical cancer of women from Curaçao are still the most prevalent HPV genotypes their prevalence is lower compared to the world prevalence (ICO HPV Information Centre, 2016; de San Jose et al., 2016; Guan et al., 2012)

The prevalence of Vaccine types HPV-31, -52, -58 and the untypable HPV-X (DEIA pos. and LiPA neg.) was for each HPV type 3.8%, in total 15.2%. In ADC (n=15) HPV-18 (40.0%), -16 (26.7%) and -45 (13.3%) were the most prevalent HPV types. Twelve cervical cancers were found to be HPV negative.

We performed several experiments to substantiate absence of HPV in cervical cancer and exclude experimental causes. Review of the slides by two pathologists did not change the diagnosis and repeated HPV testing and genotyping with SPF10 yielded identical results. Inadequate DNA quality could be excluded by parallel testing with a duplex real-time PCR targeting the human RNaseP gene. Potential PCR inhibition could be excluded by a plasmid spiked into the PCR mix.
All 12 samples contained amplifiable human genomic DNA and stable Cq values of the plasmid spike showed absence of PCR inhibition. Another explanation for a negative HPV result in cervical carcinoma might be integration of HPV DNA in the L1 region because the SPF10 target the L1 gene. We did not perform HPV detection assays targeting the E6/E7 region to exclude this possibility (Hessenlink et al., 2014). However published data show that less than 1 % of cervical cancers has integration in the L1 region (Wentzensen et al., 2004; Hu et al., 2015).

Our HPV-16/-18 prevalence data in cervical cancer are in agreement with Martinique (57% in 131 ICC) (Dos Santos et al. 2015) but differ from those in Trinidad 83.9% (Hosein et al., 2013).
The ranking order of HPV-genotypes found in cervical cancer on Curaçao match with those from Trinidad HPV-16 (66.1%), -18 (17.8%) and -45 (8.9%)(Hosein et al. 2013). Comparison with HPV genotype studies from Jamaica was difficult due to pooling of the small sample size, (9 ICC and 30 CIN3 lesions) (Rattray et al., 1996).

Compared to HPV-16 and- 18 in cervical cancer from the Caribbean region according to the ICO data (57.6%), our estimated HPV-16 and -18 prevalence data in cervical cancer are somewhat lower (52.0%), but our HPV-16 and -18 prevalence data in high-grade lesions (CIN2/3;47.6%) are higher compared to the ICO data (CIN2/3;34.6%) (ICO HPV Information Centre, 2016).

However, it should be noted that most of the published HPV prevalence data from CIN lesions in the Caribbean region are obtained from studies in women with normal cytology where these lesions were discovered incidentally during the trials. In Jamaica (n= 840) an HPV-16 and -18 prevalence of 50% was found in 14 HSIL and 21.3% in 61 ASC-US and LSIL (Lewis-Bell et al., 2013).
Data from Guadeloupe (n=618), only described HPV-16 and -18 prevalence in LSIL: 9.7% in LSIL (n=31) and 11.3% of ASC-US (n=106). HPV-16 and -18 prevalence in HSIL were not described because of the small sample size (n=8) (Cordel et al., 2015)

An explanation for the differences in HPV prevalence between the Caribbean region may be the very heterogeneous ethnical background, which consists of a mix of African and European descent and different social and cultural behaviours in this population.
Our HPV genotype prevalence data argue for the use of a prophylactic HPV vaccine with broad coverage of the non-HPV-16/-18 genotypes, as present in the nonavalent vaccine. The use of HPV vaccines with a smaller HPV genotype coverage such as the bivalent vaccine can also be considered when longitudinal data show that the cross-protection of the HPV-16/-18 vaccines provides also long lasting protection against non-vaccine HPV types such as 45, 31 and 33, present in relevant percentages in the population (Harper et al., 2006; Malagón et al., 2012)

The strength of our study is that all samples are derived from the only Pathology laboratory on Curaçao where all cases were handled according to one protocol. Three pathologists did histological review and HPV typing was carried out following a single standardised protocol in DDL, which has ample experience with HPV genotyping in very large epidemiological studies on genotyping (de San Jose et al., 2016)

A limitation of the study is the limited number of CIN lesions studied because only available blocks of good quality and containing enough tissue were included. Consequently, this should be taken into account when interpreting the HPV prevalence data. However, this is the largest series of CIN from the Caribbean area in which HPV prevalence was assessed.

In conclusion: our study shows that compared to the world population the prevalence of HPV type 16 and 18 in cervical cancer is lower and consequently the contribution of the HPV genotypes 31, 45, 51, 52 and 58 is higher. When considering prophylactic HPV vaccination on Curaçao, these HPV genotype prevalence data in cervical (pre)cancer should be taken into account.

Key messages

- The incidence of cervical cancer on Curaçao is high (13.1) and cervical cancer prevention programmes are lacking.
- The prevalence of HPV-16 and -18 in cervical cancer in this large study from Curaçao is lower compared to the world population and Trinidad
- The prevalence of HPV genotypes in CIN lesions on Curaçao is comparable with the world population
- In the selection of a prophylactic HPV vaccine the high proportion of non HPV-16/-18 genotypes in cervical cancer should be taken into account

Contributorship statement

Design of the study: Hooi, Witte, Kenter, Meijer, Quint

Data collection: Hooi, Pinedo

HPV detection and genotyping: de Koning, Quint, Hooi

Statistics: Hooi, Witte, Meijer, de Koning, Quint

Writing: Hooi, Meijer, Kenter, Witte, Quint

All authors critically commented on all versions of the manuscript.

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Supplementary tables

On the following pages:

Supplementary table 4.1
HPV type attribution in relation to cervical (pre) cancer

	*Single type infection										**Proportional attribution										***Any infection									
	cancer		CIN3/CIS		CIN2		CIN1		total		cancer		CIN3/CIS		CIN2		CIN1		total		cancer		CIN3/CIS		CIN2		CIN1		total	
type	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
hrHPV																														
16	36	34,6%	14	34,1%	8	20,5%	1	2,5%	59	26,3%	39,3	37,8%	18,4	44,9%	11,3	29,0%	5,4	13,6%	74,5	33,3%	40	38,5%	19	46,3%	12	30,8%	6	15,0%	77	34,4%
18	14	13,5%	3	7,3%	3	7,7%			20	8,9%	14,2	13,7%	3,0	7,3%	3,9	10,0%	0,77	1,9%	21,9	9,8%	15	14,4%	3	7,3%	5	12,8%	1	2,5%	24	10,7%
31	4	3,8%	4	9,8%	2	5,1%	4	10,0%	14	6,3%	4,0	3,8%	4,8	11,7%	2,2	5,6%	4,6	11,6%	15,6	7,0%	4	3,8%	6	14,6%	3	7,7%	6	15,0%	19	8,5%
33	3	2,9%	1	2,4%	2	5,1%	1	2,5%	7	3,1%	3,1	3,0%	1,6	3,8%	2,4	6,0%	1,4	3,6%	8,5	3,8%	4	3,8%	3	7,3%	3	7,7%	3	7,5%	13	5,8%
35	3	2,9%			4	10,3%			7	3,1%	3,0	2,9%			4,0	10,3%			7,0	3,1%	3	2,9%			4	10,3%			7	3,1%
39	1	1,0%			1	2,6%			2	0,9%	1,0	0,96%			1,0	2,6%	0,10	0,24%	2,1	0,94%	1	1,0%			1	2,6%	1	2,5%	3	1,3%
45	7	6,7%	2	4,9%			1	2,5%	10	4,5%	7,1	6,9%	2,0	4,9%	0,68	1,8%	1,8	4,5%	11,6	5,2%	8	7,7%	2	4,9%	1	2,6%	2	5,0%	13	5,8%
51	2	1,9%	2	4,9%	4	10,3%	3	7,5%	11	4,9%	2,5	2,4%	2,0	4,9%	4,0	10,3%	3,1	7,9%	11,7	5,2%	3	2,9%	2	4,9%	4	10,3%	4	10,0%	13	5,8%
52	4	3,8%	2	4,9%	2	5,1%	1	2,5%	9	4,0%	4,1	4,0%	2,8	6,9%	2,1	5,5%	1,0	2,5%	10,1	4,5%	5	4,8%	4	9,8%	3	7,7%	1	2,5%	13	5,8%
56														0	0,57	1,5%	0,75	1,9%	1,3	0,59%					1	2,6%	3	7,5%	4	1,8%
58	4	3,8%	2	4,9%	1	2,6%	2	5,0%	9	4,0%	4,5	4,3%	2,8	6,7%	1,0	2,6%	2,3	5,7%	10,5	4,7%	5	4,8%	3	7,3%	1	2,6%	3	7,5%	12	5,4%
59	1	1,0%					1	2,5%	2	0,9%	1,0	0,96%					1,0	2,5%	2,0	0,89%	1	1,0%					1	2,5%	2	0,9%
66			1	2,4%					1	0,4%			1,2	2,9%	0,50	1,3%			1,7	0,75%			2	4,9%	1	2,6%			3	1,3%
68	2	1,9%					1	2,5%	3	1,3%	2,0	1,9%					1,0	2,5%	3,0	1,3%	2	1,9%					1	2,5%	3	1,3%
lrHPV																														
6							2	5,0%	2	0,9%					0,43	1,1%	2,0	5,0%	2,4	1,1%					1	2,6%	2	5,0%	3	1,3%
44													0,06	0,15%					0,06	0,03%			1	2,4%					1	0,4%
53							1	2,5%	1	0,4%					0,50	1,3%	1,2	3,0%	1,7	0,75%					1	2,6%	2	5,0%	3	1,3%
54															0,04	0,10%	0,47	1,2%	0,50	0,22%					1	2,6%	2	5,0%	3	1,3%
61																	0,33	0,83%	0,33	0,15%							1	2,5%	1	0,4%
67	1	1,0%							1	0,4%	1,0	0,96%							1,0	0,45%	1	1,0%							1	0,4%
70							2	5,0%	2	0,9%			0,39	0,95%	0,38	0,97%	2,0	5,0%	2,8	1,2%			2	4,9%	2	5,1%	2	5,0%	6	2,7%
71																	0,33	0,83%	0,33	0,15%							1	2,5%	1	0,4%
74													0,01	0,03%					0,01	0,01%			1	2,4%					1	0,4%
82	1	1,0%							1	0,4%	1,0	0,96%							1,0	0,45%	1	1,0%							1	0,4%
91																	0,33	0,83%	0,33	0,15%							1	2,5%	1	0,4%
X	4	3,8%					1	2,5%	5	2,2%	4,0	3,8%					1,0	2,5%	5,0	2,2%	4	3,8%					1	2,5%	5	2,2%
Total	87	83,7%	31	75,6%	27	69,2%	21	52,5%	166	74,1%	92	88,5%	39	95,1%	35	89,7%	31	77,5%	197,0	87,9%										

* "Single type infection" is calculated by the frequency of each HPV genotype.
** *"Proportional attribution" of a specific HPV genotype in a multiple i nfection is assessed according to the frequency of that genotype at the respective disease category. (Wentzensen et al. 2010)*
*** *In "any infection attribution" each infection is accounted fully to the lesion. (Wentzensen et al. 2010)*

Supplementary table 4.2
HPV genotypes in multiple HPV infections in cervical (pre)cancer.

	SCC (n=85)+				
	Total *n*= 224	**ASC (n=4)**	**CIN 3 (n=41)**	**CIN 2 (n=39)**	**CIN 1 (n=40)**
HPV types	**(100%)**	***n*** (%)	***n*** (%)	**n** (%)	***n*** (%)
6,56	1 (0.4)			1 (2.6)	
16, 18	2 (0.09)	1 (25.0)		1 (2.6)	
16,31	3 (1.3)		1 (2.4)	1 (2.6)	1 (2.5)
16, 33	3 (1.3)	1 (1.2)	1 (2.4)		1 (2.5)
16,45	1 (0.4)	1 (1.2)			
16,51	1 (0.4)				1 (2.5)
16,52	2 (0.09)	1 (1.2)	1 (2.4)		
16, 54	2 (0.09)			1 (2.6)	1 (2.5)
16,56	1 (0.4)				1 (2.5)
16, 70	1 (0.4)		1 (2.4)		
16, 74	1 (0.4)		1 (2.4)		
18,33	1 (0.4)			1 (2.6)	
31, 33	1 (0.4)		1 (2.4)		
45,53	1 (0.4)				1 (2.5)
45, 70	1 (0.4)			1 (2.6)	
51,58	1 (0.4)	1 (1.2)			
52, 70	1 (0.4)		1 (2.4)		
53,66	1 (0.4)			1 (2 .6)	
54,56	1 (0.4)				1 (2.5)
16,52, 70	1 (0.4)			1 (2.6)	
18,39,56	1 (0.4)				1 (2.5)
31,33,58	1 (0.4)				1 (2.5)
44,58, 66	1 (0.4)		1 (2.4)		
61, 71, 91	1 (0.4)				1 (2.5)
Total	**31**	**5**	**8**	**8**	**10**