Human Papillomavirus Genotype Distribution in Cytologically Screened Women from Northwest Germany

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Key Words

Human papillomavirus genotyping · Cytology · Histology · Germany

Abstract

Introduction: In this study, we evaluate the distribution of 24 human papillomavirus (HPV) genotypes in a cohort of 3,381 cytologically screened women from a rural area of northwest Germany, in correlation to cytological diagnoses and histological outcomes. **Materials and Methods:** We present a retrospective study in which the HPV-genotyping results of women who attended the German cervical screening program were correlated to cytological diagnosis and histological outcome. **Results:** HPV genotyping showed marked differences among cervical lesions. Although HPV-51 was common in all cervical lesions, it was rarely detected as single-type HPV infection in high-grade cervical intraepithelial neoplasia (CIN)3 (2/118 cases). HPV-16 and 18 were more common in CIN3 (57.6%) than in CIN2 (21.8%), but they were absent in 42.4% of all CIN3 lesions in our cohort. **Discussion:** Our data show that HPV-16, HPV-31, HPV-51, HPV-53 and HPV-42 are the most prevalent HPV types in the different cervical lesions in this region of northwest Germany. HPV genotyping has been shown to be a powerful tool to triage atypical squamous cells of undetermined significance lesions. Considering the observed heterogeneity of HPV

types in CIN2, it could also be useful to triage CIN2+ lesions. Our results underline the usefulness of a morphologically controlled screening program with HPV genotyping as a powerful additional tool.

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Introduction

Persistent human papillomavirus (HPV) infection is the main cause for the development of cervical cancer. About 40 different HPV types can infect the anogenital tract, of which 18 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73 and 82) are defined (as probably) high-risk for the development of high-grade cervical intraepithelial neoplasia (CIN)2+ and cervical cancer [1, 2].

Some high-risk HPV (hrHPV) types are more carcinogenic than others [3]. HPV-16 is the most prevalent type found in 40–70% of cervical cancer cases worldwide, followed by HPV-18 which has an overall prevalence of 15% [4]. HPV genotyping has been shown to have a greater sensitivity with a slightly lower specificity for the detection of CIN2+ lesions compared to cytology [5]. This increased sensitivity can lead to earlier detection and better protection against cervical lesions, although, due to lower specificity, more women need follow-up examinations

because of clinically irrelevant positive results. This is one of the reasons why HPV-DNA testing has not yet been used for primary cervical screening. In the Netherlands, however, hrHPV screening is now being considered as a primary screening method for cervical cancer, with cytology used for triage in case of positive hrHPV results [6–8]. Molecular methods for HPV detection have become a powerful tool for the management of suspicious cervical cytology over the last decades [9], as the presence of specific hrHPV types can be used as an indicator of the risk for developing CIN2+ lesions. The distribution of HPV types varies between countries and little is known about the HPV genotype distribution in correlation to cytological diagnosis and histological outcome in this region of Germany [10–15].

The aim of this study was to investigate the distribution of 24 HPV genotypes in a cytologically screened population coming from a rural area of northwest Germany in correlation to cytological diagnosis and histological outcome, and to evaluate its impact on primary HPV screening and vaccination.

Materials and Methods

Study Population

The HPV-genotyping results of 3,714 women (mean age 38 years) processed between the years 2008 and 2010 were collected and correlated to cytological and histological examinations (4,120 results). Patients came from the rural regions of northwest Germany (Nordrhein-Westfalen, Niedersachsen, Schleswig-Holstein, Bremen and Hamburg) and participated in the German annual cervical screening program for women ≥ 20 years of age [16]. HPV genotyping was performed in the case of normal cytology only if requested by the patient, or else on clinical indication in the case of abnormal cytology, i.e. atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells cannot exclude HSIL (ASC-H), low-grade squamous intraepithelial lesion (LSIL), encompassing mild dysplasia/CIN1, high-grade squamous intraepithelial lesion (HSIL), encompassing moderate and severe dysplasia/CIN2 and CIN3 or atypical glandular cells of undetermined significance (AGUS). No samples were collected solely for this study. Exclusion criteria were no corresponding cytology, a history of total hysterectomy and an age of < 30 years together with an unknown vaccination status.

Currently, there are two approved vaccines for the prevention of cervical cancer, Gardasil[®], a quadrivalent vaccine directed against HPV-16, HPV-18, HPV-6 and HPV-11 and Cervarix[®], a bivalent vaccine directed against HPV-16 and HPV-18 [17, 18].

Strict adherence to the manufacturer's instructions for the HPV vaccination is a prerequisite for a correct calculation of the immunological response. This requirement was not fulfilled in most of the patients in our study, therefore all vaccinated patients were excluded.

Table 1. Population characteristics

	Total	
	patients	mean age $\pm$ SD
Cytological diagnosis		
Normal cytology	1,463	39.6 $\pm$ 12.5
ASC-US	1,110	40.1 $\pm$ 12.7
LSIL	441	35.5 $\pm$ 12.7
ASC-H	87	39.2 $\pm$ 14.4
HSIL	247	35.7 $\pm$ 13.3
Histological diagnosis		
CIN1	31	36.1 $\pm$ 12.3
CIN2	55	36.6 $\pm$ 11.6
CIN3	118	33.2 $\pm$ 11.5

All Papanicolaou (PAP) smears, biopsies and HPV-genotyping tests were processed at the Institute of Pathology/Cytology Schüt-torf/Leer, an ISO (International Organization for Standardization) 9001:2008 certified laboratory.

Cytological samples were classified according to the Bethesda 2001 system [19]. Cytological samples were taken in gynecology practices using the Rovers[®] Cervex-Brush[®]. Both conventional smears and liquid-based cytology (SurePath[®]) were used.

Histological lesions were classified according the WHO-classification (CIN I–III).

Immunohistochemistry was used to detect overexpression of the cell-cycle protein p16^{INK4a}, a cyclin-dependent kinase inhibitor. Cervical neoplasia expresses high levels of p16^{INK4a} in contrast to other cervical epithelia. This overexpression is linked to oncogenic transformation mediated by persistent hrHPV infection and it has been shown to significantly improve the accuracy of grading CIN lesions [20, 21].

In the case of a PAP-IV (HSIL) lesion or a recurrent PAP-IIID lesion with moderate dysplasia (HSIL) for more than 1 year, a colposcopy together with histological confirmation was advised.

The PapilloCheck[®] assay, a commercially available HPV genotyping assay which has been reported as a compatible test to established PCR assays [22, 23] was used for the qualitative detection and identification of 18 hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73 and 82) and 6 low-risk HPV types (6, 11, 40, 42, 43 and 44/55). The test was performed according to the manufacturer's instructions. A negative control (sample without DNA) was added to each chip to exclude false-positive results, which can occur by contamination during the process. Cytological samples that were correlated to HPV-genotyping results had to be taken within 1 month before or after the HPV-genotyping test was performed. In case of liquid-based cytology, DNA from cervical smears was isolated from residual SurePath[®] samples. When a conventional smear was made, an additional smear using the HC cervical sampler[®] (Digene) was taken for HPV detection. All histological samples were taken within 3 months after cytological diagnosis; we did not separately perform HPV genotyping on histological samples consecutively, HPV genotyping results from the corresponding cytological samples were matched to the CIN lesions.

Table 2. HPV type distribution according to cytological diagnosis

	Cytological diagnosis									
	normal		ASC-US		ASC-H		LSIL		HSIL	
	n	%	n	%	n	%	n	%	n	%
Total	1,463	100	1,110	100	87	100	441	100	247	100
HPV-positive	436	29.8	550	49.5	55	63.2	396	89.8	232	93.9
HR-positive	381	26.0	479	43.2	54	62.1	348	78.9	227	91.9
16	72	4.9	93	8.4	25	28.7	77	17.5	85	34.4
18	24	1.6	22	2.0	5	5.7	23	5.2	19	7.7
31	60	4.1	54	4.9	11	12.6	41	9.3	40	16.2
33	18	1.2	22	2.0	1	1.1	19	4.3	14	5.7
35	3	0.2	9	0.8	1	1.1	7	1.6	7	2.8
39	30	2.1	42	3.8	3	3.4	38	8.6	21	8.5
45	20	1.4	22	2.0	6	6.9	10	2.3	12	4.9
51	99	6.8	95	8.6	9	10.3	53	12.0	47	19.0
52	16	1.1	33	3.0	4	4.6	23	5.2	15	6.1
53	33	2.3	59	5.3	2	2.3	65	14.7	27	10.9
56	52	3.6	40	3.6	1	1.1	52	11.8	24	9.7
58	10	0.7	27	2.4	0	0.0	11	2.5	13	5.3
59	27	1.8	28	2.5	0	0.0	26	5.9	12	4.9
66	19	1.3	27	2.4	1	1.1	48	10.9	10	4.0
68	24	1.6	35	3.2	2	2.3	22	5.0	8	3.2
70	19	1.3	19	1.7	2	2.3	15	3.4	6	2.4
73	4	0.3	13	1.2	2	2.3	6	1.4	12	4.9
82	2	0.1	17	1.5	2	2.3	3	0.7	10	4.0
LR-positive	122	8.3	167	15.0	10	11.5	145	32.9	48	19.4
6	22	1.5	15	1.4	2	2.3	13	2.9	5	2.0
11	7	0.5	4	0.4	0	0.0	3	0.7	1	0.4
40	5	0.3	6	0.5	0	0.0	7	1.6	3	1.2
42	71	4.9	110	9.9	3	3.4	105	23.8	25	10.1
43	8	0.5	5	0.5	0	0.0	9	2.0	4	1.6
44/55	21	1.4	42	3.8	6	6.9	26	5.9	14	5.7
Single infections	279	19.1	365	32.9	36	41.4	209	47.4	118	47.8
Multiple infections	157	10.7	185	16.7	19	21.8	187	42.4	114	46.2

Figures in bold type are the most frequently detected HPV types.

Statistical analysis software SPSS 17.0 (SPSS Inc., Chicago, Ill., USA) and SAS 9.3 (SAS Institute, Inc., Cary, N.C., USA) were used. The Pearson χ^2 test calculated p values, with $p < 0.05$ being considered statistically significant.

Results

In total, 3,381 of 3,714 women fulfilled the inclusion criteria. Population characteristics of our cohort are summarized in table 1. AGUS was diagnosed in 33 of 3,381 specimens; HPV distribution in these cervical lesions will not be discussed in this article.

The HPV distribution according to cytological diagnosis is shown in table 2. Increasing severity of cytological lesions was associated with higher prevalence rates of HPV infection (but not significant for LSIL vs. HSIL, $p = 0.0654$) and especially with higher prevalence rates of hrHPV infection ($p < 0.001$).

Low-risk HPV was significantly more common ($p < 0.0001$) in LSIL (32.9%) compared to normal cytology (8.3%), ASC-US (15.0%), ASC-H (11.5%) and HSIL (19.4%).

The distribution of HPV type varied among cytological and histological lesions. The most common HPV types in cytological lesions are in recorded in bold type in

Fig. 1. Type-specific HPV prevalence in CIN2 (a) and CIN3 (b) lesions.

table 2. The most prevalent HPV types were: HPV-51 (6.8%) in normal cytology, HPV-42 (9.9%) in ASC-US, HPV-16 (28.7%) in ASC-H, HPV-42 (23.8%) in LSIL and HPV-16 (34.4%) in HSIL.

HPV-6 (varying from 1.5 to 2.9%) and HPV-11 (varying from 0.0 to 0.7%) were uncommon in our cohort.

In histological lesions, the most prevalent HPV types were: both HPV-16 and HPV-42 (25.8%) for CIN1, HPV-

51 (25.5%) for CIN2 and HPV-16 (52.5%) for CIN3. The distribution of HPV types in both CIN2 and CIN3 is shown in figure 1. With regard to CIN3, HPV-16, HPV-18 and HPV-31 were more common as single-type than multiple-type infections compared to the other HPV types. Low-risk HPV types and HPV-35, HPV-59 and HPV-68 were not found as single-type HPV infections in CIN2+ lesions. HPV-66, HPV-70 and HPV-73 were only

detected as single-type HPV infections in CIN2, but not in CIN3.

The prevalence of HPV-16 and HPV-18 in cytological and histological lesions is shown in figure 2. Infections with HPV-16 and 18 were more common in HSIL (39.7%) and in ASC-H (32.2%) compared to in ASC-US (10.0%) and in LSIL (21.1%) ($p < 0.0001$). Regarding histology, HPV-16 and HPV-18 were significantly more common in CIN3 (57.6%) than in CIN2 (21.8%) ($p < 0.0001$). In contrast, hrHPV types other than HPV-16 and HPV-18 were significantly more common ($p < 0.0001$) in CIN2 (76.4%) than in CIN3 (39.8%).

Discussion

The overall HPV prevalence in women with normal cytology was 29.8%. This rate is high compared to the study of Klug et al. [12] and the WHO/ICO HPV Information Centre [24] which reported HPV prevalence rates of 4.4 and 6.9%, respectively, in women with normal cytology in Germany [12, 24], or compared to the meta-analysis of de Sanjosé et al. [10], in which a worldwide overall HPV prevalence of 10.8% was found in women with normal cytology, with a corresponding estimate by region of 8.1% for Europe. The study of Kovács et al. [25] reported an overall HPV prevalence rate of 24.2% among 5,934 women (2,217 specimens with normal cytology) who only received one HPV test; however, since this study did not separately analyze the cytological diagnosis, the results are difficult to compare. A possible explanation for the high HPV prevalence found among women with normal cytology in our study population is the fact that they underwent primary cytological screening and were only tested for HPV if the patient or clinician wished, which might have introduced a selection bias. Furthermore, we did not exclude women with previous abnormal PAP smears. Other possible explanations for the observed differences among the studies could be the use of different HPV genotyping assays with different sensitivities for HPV detection [26, 27]. In the review of Snijders et al. [26], analytical performances of the Hybrid capture II method and the consensus PCR assays PGMY-PCR, GP5+6+-PCR and SPF10 were discussed. It was seen that the estimated HPV prevalence rates, particularly in women with normal cytology, varied dramatically depending on the method used. They also discussed a study comparing the SPF1/2 (a former version of SPF10) with the GP5+6+-PCR assay, in which significantly more HPV-DNA was detected in cervical scrapes from women

Fig. 2. Comparison between the prevalence of HPV-16 and HPV-18 compared to other high-risk types. Every lesion positive for HPV-16 and/or HPV-18 was counted to the category HPV-16/18, irrespective to other hrHPV types. Every lesion positive for other high-risk types (31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73 and 82) without the presence of HPV-16 or HPV-18 was counted to other high-risk types. The error bars indicate the 95% confidence intervals.

with normal cytology when using the SPF1/2, whereas HPV prevalence rates found in abnormal smears did not significantly differ between the assays. These differences might be explained by a wide range in HPV viral load, particular in women with normal cytology, and the estimated HPV prevalence rate in this group may be proportional to the analytical sensitivity of the genotyping test used [26].

Recent studies reported significant variation in hrHPV prevalence rates for women diagnosed with ASC-H (33.3–85.6%), ASC-H is a relatively uncommon diagnosis and although cytomorphologic criteria have been described, there is still a great interobserver and even intra-observer variability, resulting in limited reproducibility. Considering that the highest HPV-positive rates have been reported in multicenter trials using consensus panel diagnoses compared to most local practice settings which reported lower HPV-positive rates using the interpretation of a single pathologist, interinstitutional variation in PAP test diagnosis could also partly explain the high HPV prevalence rate found; however, interpretation of normal cytology is generally a less interpretive challenge [28–31].

Other explanations could be the different demographic and geographic characteristics of the cohorts and the

different screening models in each country. We also included women <30 years of age; HPV prevalence is higher in this age category, so this could also partly explain the finding of an unusually high prevalence [32].

In contrast to the high HPV prevalence rate in normal cytology, hrHPV prevalence rates in abnormal cytological lesions were mostly consistent with other national and international studies [11, 13, 33], although discrepancies were observed with the study of Evans et al. [14] who reported lower hrHPV prevalence rates for LSIL (55.0 vs. 78.9% in our cohort) and higher hrHPV prevalence rates for ASC-H (80.0 vs. 62.1% in our cohort).

The fact that ASC-US cytology had a considerably lower hrHPV positivity rate (43.2%) compared to other abnormal cytological lesions ($p < 0.0001$) makes women with ASC-US good candidates for triage by HPV genotyping, which is also advised in the European Guidelines for Quality Assurance in Cervical Cancer Screening [9].

We observed a strong variability in the most prevalent HPV types detected in the different cytological and histological lesions, which was less pronounced in high-grade lesions (ASC-H, HSIL and CIN2+), especially when only single-type HPV infections were considered. The most prevalent types considering single-type HPV infections in CIN3 were HPV-16, HPV-31 and HPV-18. These types together with HPV-33 and HPV-45 are the most commonly detected in cervical cancer in Europe [4]. In Germany, the 5 most prevalent HPV types detected in cervical cancer are HPV-16 (58%), HPV-18 (18.8%), HPV-52 (5.6%), HPV-68 (5.6%) and HPV-31 (2.9%) [24]. Except for HPV-68 which was not detected as single-type infection in CIN2+ lesions, these HPV types were the most prevalent when considering single-type HPV infection in CIN3 in our study population (fig. 1). This indicates that although there are marked differences in HPV prevalence within low-grade lesions (ASC-US, LSIL and CIN1) between regions and countries [10, 11, 13–15, 24], the HPV types most often causing cervical cancer probably remain the same. It is important to take this into account, since both the cost-effectiveness and the specificity for the detection of CIN2+ lesions will decrease when infections that only rarely progress to cancer undergo screening [15]. In our population, HPV-51 was one of the most commonly detected hrHPV genotypes for every lesion. However, it was rarely detected as a single infection in CIN3 ($n = 2/58$). There are no studies that reported the Papillocheck® to have an unusually high sensitivity for the detection of HPV-51. Furthermore, this was not the first study that found high prevalence rates for HPV-51 in Germany [12, 34, 35]. Therefore, we assume that HPV-

51 is a common genotype in our cohort which is not biased by test characteristics.

Screening for HPV-51 would not be effective in our cohort, as it would yield many irrelevant positive results since an infection with HPV-51 alone rarely causes cervical cancer. The issue for which oncogenic screening for HPV type should be useful becomes difficult when taking into consideration that the most prevalent HPV types are dependent on geography and age [10, 12, 32, 34, 36]. This also underlines the usefulness of a morphologically controlled screening program with HPV genotyping as a powerful additional tool or opting to triage HPV-positive screening results in the future.

Another very common HPV type in our cohort was HPV-42. The Papillocheck® has proven to be more sensitive than other assays in the detection of HPV-42 [34], which might be an explanation for the high prevalence of HPV-42 in our cohort. This demonstrates the importance of standardizing HPV genotyping assays.

The prevalence of HPV-16 and HPV-18 increased with more severe cytological and histological lesions. These types were detected in 57.6% of the CIN3 lesions, which corresponds to other studies [12, 14, 37, 38]. We observed a marked difference between CIN2 and CIN3 regarding the contribution of HPV-16 and HPV-18, compared to other high-risk types. HPV-16 and 18 were significantly more common in CIN3 than in CIN2, whereas other high-risk types were more common in CIN2 than in CIN3. Although it is known that CIN2 lesions are more heterogeneous and more likely to regress [39], many studies combine these two cervical lesions when determining HPV distribution because the European Guidelines for Quality Assurance in Cervical Cancer Screening advise treating both CIN2 and CIN3 by excision of the transformation zone without delay in the presence of high-grade intraepithelial neoplasia (CIN2+) or suspicion of early stromal invasion or microinvasion [9, 12, 14].

Our data, however, show that HPV genotyping could be useful for triage of women with CIN2 lesions to assess which women are harboring the HPV genotypes that are commonly detected in cervical cancer. Doing so could prevent overtreatment, especially when considering that only 5% of the CIN2 lesions indeed progress to invasive cancer [39]. A possible explanation for the large differences between CIN2 and CIN3 in the distribution of HPV type, as observed in our study, could be the additive use of immunohistochemistry to detect overexpression of the cell cycle protein p16^{INK4a} for histological grading of CIN lesions. This immunohistochemical method has been

shown to improve the accuracy of grading CIN lesions significantly [20, 21].

HPV-31 was three times as common as HPV-18 in CIN3. Although it is demonstrated that the current vaccine gives a certain cross-protection against HPV-31 [40], a direct vaccination against HPV-31 would have been useful in our cohort.

A vaccine directed against HPV-51 would have prevented many abnormal cytological lesions but only a few high-grade histological lesions. It would not have prevented many invasive cervical cancers either, as only 1% (28/2,058) of all cervical cancer in Europe is caused by HPV-51 [4]. Therefore, a vaccine against HPV-51 would have reduced the diagnostic follow-up of women with abnormal cytology, but it would not have prevented many high-grade histological lesions and consequent surgical treatments. A vaccine against HPV-42 would also have

been effective in the prevention of low-grade cytological and histological lesions (ASC-US, LSIL and CIN1), especially when considering that almost all HPV-42 infections show the histological characteristics of genital condylo-mata or flat papillomas [41].

In particular, vaccination would have prevented high-grade lesions as these most often contained HPV-16 and HPV-18. Because other hrHPV types were more common in low-grade lesions, many women would probably still have developed abnormal cytology.

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