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Determination of the diagnostic accuracy of testing for high-risk (HR) human papillomavirus (HPV) types 16, 18 and 45 in precancerous cervical lesions: Preliminary data

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SUMMARY

Aims: In Germany, cervical cancer screening is regulated by the German Federal Ministry of Health and Social Security and is available for all women from the age of 20 on the basis of the Papanicolaou (PAP) smear. The purpose of this study was to determine the positive predictive value of HR-HPV testing for precancerous lesions of the cervix uteri. Therefore, this study especially focused on the diagnostic accuracy of testing for one or more of the HPV types 16, 18 and 45 for all HR-HPV positive women, since HR-HPV infections with subtypes 16, 18 and 45 have demonstrated a higher risk of developing cervical cancer [Bulk S, et al. *Br J Cancer* 2006;**94**:171–5].

Methods: Between 2007 and 2008 a total of 586 women were recruited: a group of 477 women with a history of known cervical lesions and/or HPV infections (eligibility criterion: HR-HPV DNA positive test result with HC2T) and a group of 109 women who were examined as part of their routine cervical cancer screening. Baseline HR-HPV status was measured at enrollment with the FDA-approved Hybrid Capture® 2 HPV DNA Test and the HR-HPV 16/18/45 Probe Set Test (HC2T, PST; QIAGEN, Hilden, Germany). Both tests use hybrid capture hybridization genotyping technology. Cervical smears were classified according to the Second Munich Nomenclature (1989). The results were converted to the nearest equivalent in the Bethesda system. In general, study subjects were followed up semiannually for a period of $1^1/_2$ years. The histopathological endpoint of CIN 2–3 lesion was used as a surrogate endpoint.

Results: Preliminary data for 194 women of the risk group (43.5%) and for the complete control group were available. To date, CIN 2–3 was confirmed in 77 HR-HPV DNA positive women. 85.7% of these lesions were positive for one or more of the HR-HPV types 16, 18 and 45 (PST+). 88.2% (60/68) of the histologically confirmed CIN 3 lesions and six out of nine (66.6%) CIN 2 lesions were positive PST+. Furthermore, all women with a histologically confirmed squamous cell carcinoma (n = 4) were PST+. Besides, three (50%) out of six detected CIN 1 lesions were PST+. Nonetheless, histology confirmed no malignancy in three cases. Two of them were PST+.

Conclusion: These preliminary results demonstrate that starting cervical cancer screening at the age of 20 years remains important as seventeen (25%) of the 68 histologically verified CIN 3 lesions arose in women who were younger than 30 years. Furthermore, our data suggest that adding an HR-HPV test that detects one or more of the HR-HPV types 16, 18 and 45 in conjunction with cytology could help to identify women with an underlying cervical lesion who have an elevated risk of developing severe cervical lesions. This might offer the opportunity of a decrease in incidence and mortality rates that are related with invasive cervical cancer.

1. Introduction

HPV infections are widespread throughout all human populations. They are usually transmitted during sexual intercourse. It is estimated that worldwide 300 million females have an HPV infection and that there are 30 million infected with genital warts, 30 million LG SIL, 10 million with HG SIL and approximately 0.493 million cervical cancers.[1] Most of the time the HPV infections are transient and the related abnormal and precancerous cervical lesions are successfully suppressed by the T-cell system. Nevertheless, a large number of epidemiological studies have demonstrated a relationship between HR-HPV infections and the development of invasive cervical cancer (ICC).[2–6] More than 100 different HPV types are known and about 40 of them infect the genital tract. At least 14 of these subtypes are accepted to be oncogenic.[7] In particular, HPV infections with subtypes 16, 18 and 45 have demonstrated a higher risk of developing cervical cancer.[8] In addition, some well-known cofactors are related with

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1590-8658 /$ – see front matter

an increased risk to develop cervical cancer, e.g. becoming sexually active at a young age, high parity, a prolonged use of oral contraceptives, smoking history and low socioeconomic status.[9,10]

However, a study by Östor[11] provides evidence that 57% of CIN 1 lesions demonstrated regression. Persistence was found in 32% and progression to a CIN 3 lesion took place in 11%. In 1% of lesions progression to invasive cancer was detected. The corresponding figures for CIN 2 lesions were 43%, 35%, 22%, and 5%, respectively. The mean time for progression of a cervical lesion to a carcinoma in situ varies: in cases of a high-grade squamous intraepithelial lesions (HG SIL) it may take only one year or it may require up to six years in cases of low-grade squamous intraepithelial lesions (LG SIL).[12]

Cervical cancer remains a major concern for the global prevention health authorities. Approximately 83% of new cases annually arise in the developing world and it is estimated that about 85% of cervical cancer deaths worldwide occur in the developing world.[13] In Germany about 1.7% of all cancer deaths are attributable to cervical cancer. The annual incidence rate of cervical cancer is approximately 3.2% of all new cancer cases. The introduction of an opportunistic screening program in 1971 has led to a significant reduction in the mortality rate, from second place to eleventh place in the common cause of death statistics.[14] The opportunistic cervical screening system was modified in 2005. Cervical cancer screening was available for all women from the age of 20 on the basis of the Papanicolaou (PAP) smear. It was recommended that smears should be taken using either a brush or a spatula.[15] In addition, it was advised on the basis of several systematic reviews [HTA New Zealand 2000[16], HTA Australia 2003[17], HTA Germany 2003[18], USA 1999[19], HTA Canada 2003[20], HTA United Kingdom 2000[21], Karnon (2004)[22]] that liquid-based cytology (LBC) should not be integrated in cervical cancer screening.[23] It was also recommended that HPV testing should not be part of the primary cervical cancer screening program although it is more sensitive, but less specific than cytological screening. One of the main concerns was the question how to take care of the HPV infected women with normal cytology. According to data, the annual participation rate in Germany varies from 36% to 50%.[24,25]

In contrast, the S2k-Guideline of the German Society of Gynecology and Obstetrics advocates for starting cervical cancer screening three years after the sexual debut and for adding HR-HPV testing to primary cervical cancer screening for women ≥30 years with normal cytology. Furthermore, it was suggested to use liquid-based cytology instead of conventional cytology for all women age 20 years and older.[26]

A study by Koliopoulos et al. examined the pooled accuracy of 25 studies for the detection of CIN 2+ and found that the sensitivity for cytology was 72.7% (min. 63.9%, max. 81.5%) and the specificity of cytology was 91.9% (min. 90.2%, max. 93.6%), whereas HR-HPV testing in combination with expert colposcopy had a sensitivity of 90.0% (min. 86.4%, max. 93.7%) and a specificity of 86.5% (min. 83.1%, max. 89.8%) for the detection of CIN 2+.[27] Besides, in 2003 the Food and Drug Administration approved the inclusion of HR-HPV testing as an adjunct to cervical cytology-based screening programs in women aged 30 years and more.[28] Yet, some HR-HPV infections do show an increased prevalence in cervical cancers relative to other HR-HPV types. Therefore this study especially focused on testing for one or more of the HPV types 16, 18 and 45 for all HR-HPV positive women.

2. Methods

2.1. Study design

HR-HPV DNA testing

Two HR-HPV DNA tests were performed on the collected specimens at enrollment. The FDA-approved Hybrid Capture® 2 HPV DNA Test (HC2T; QIAGEN, Hilden, Germany) was performed on all of the specimens. It detects the HPV DNA of one or more HR-HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). The analytical sensitivity of HC2T is clinically validated and has been adjusted to 5,000 copies of HPV DNA. Specimens that were positive with HC2T were retested with the HPV 16/18/45 Probe Set Test (PST), a test "for research use only", that confirms the presence of one or more of the HR-HPV types 16, 18 and 45. The PST was determined to detect the corresponding HPV types with an analytical sensitivity equivalent to that of HC2T, thus indicating that samples tested with PST which demonstrated a relative light unit (RLU) ratio ≥1.0 can be considered to be positive for one or more of the HR-HPV types 16, 18 and 45. Samples with an RLU ratio <1.0 can be evaluated as negative or below the detection limit for one or more of the HR-HPV types 16, 18 and 45. Neither HC2T nor the PST identify specific HPV types. HPV viral load can be detected by HC II method to evaluate the association between viral load and risk of developing CIN and cervical cancer.[29,30] It was measured semiquantitatively as a ratio of RLU of the specimen/mean RLU of the positive controls and therefore this ratio may be used as an equivalent to describe the HPV viral load of the specimen.

Cervical smears

Smears were taken using either a brush or a spatula (German Federal Ministry of Health and Social Security, 19.07.2005). All cervical smears were evaluated and followed-up according to the Second Munich Nomenclature (1989) in one laboratory (Dr. Steinberg und Partner, Laboratory for Cytopathology, Soest, Germany). The results were converted to the nearest equivalent in the Bethesda system.

Subjects and procedures

Women were classified into two groups (Fig. 1): a risk group (RG) of women who already demonstrated cervical lesions and/or had a history of HPV infection and a control group (CG) of women who were attending routine cervical cancer screening. The eligibility criterion for the RG was an HR-HPV DNA positive test result at enrollment. All of the HR-HPV DNA positive specimens were retested with the Probe Set Test (PST) to evaluate if testing for HR-HPV infections with HPV types 16, 18 and 45 would offer a benefit for the women's health in terms of increasing information about how the viral load may influence the progression and remission rates of a cervical lesion. HR-HPV positive study subjects of both groups were followed up semiannually with HC2T, PST and PAP smear for a period of $1^1/_2$ years for the detection of CIN 2–3 lesions. Cases of cleared HR-HPV infection were not followed up.

2.2. Statistical analysis

We evaluated the association between initial HR-HPV test results (PST+ vs. PST− samples) and risk of concurrent CIN 2–3 lesions. Besides, visual inspection via scatter plot was used to analyze if the virus load increases with the severity of the cytological finding. Viral load was measured semiquantitatively as a ratio of RLU of the specimen/mean RLU of the positive controls. This ratio is used as an equivalent to describe the HPV viral load of the specimen. Metric variables were reported as mean, median and range using the statistical software Statistica 8.0. The diagnosis of CIN 2–3 was used as a surrogate endpoint.

3. Results

The prospective cohort study enrolled a total of 586 women (109 control group and 477 risk group). Thirty (30) women of the study population were excluded due to a history of conization or hysterectomy (24), pregnancy (1), age (1), HPV negativity (1) and

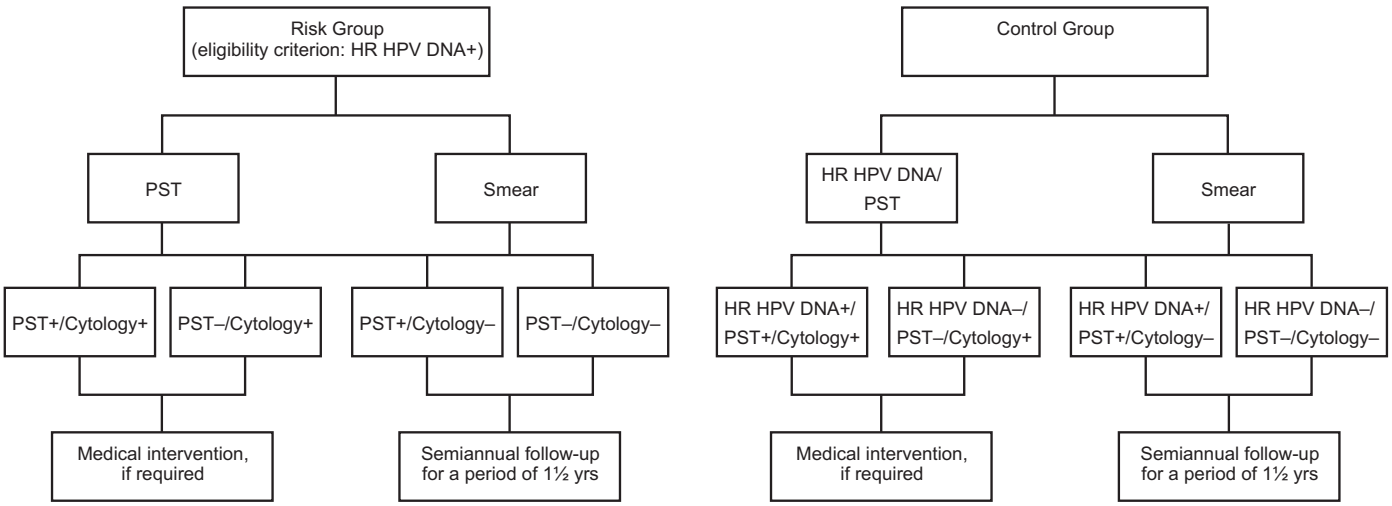

Fig. 1. Framework to illustrate the study settings. See text for detailed description.

lost to follow-up (3). There were 194 women out of 447 (43.4%) who were ≤30 years and 253 were older than 30 years. Preliminary data are available for 194 out of 447 women (43.4%) of the risk group and for the complete control group and therefore it is statistically possible that the final results may lead to different conclusions.

3.1. Baseline status of the control group

The control group (CG) is made up of 109 women (mean 32.8 years, median 32 years, range 18–60 years). There were 106 smears that displayed normal cytology (97.25%) and 3 samples showed signs of cervical lesions (1 LG SIL, 2 HG SIL). Fifteen (15) samples were positive for HR-HPV DNA with the HC2T (13.76%). There were 9 out of 15 samples that were positive for one or more of HPV types 16, 18 and 45 (Table 1): Within normal limits (WNL) (n = 6), LG SIL (n = 1), HG SIL (n = 2).

The initial viral load of the control group demonstrated a variation [WNL: n = 12, mean 377.38 IVL, median 70.35 IVL, IVL range 2.51–1500; LG SIL: n = 1, 1290 IVL; HG SIL: n = 2, n_1 = 1540 IVL, n_2 = 1510 IVL). The average age of the PST+ control group (n = 15) was 28.1 years (median 27, range 19–45) and the average age of the PST– control group (n = 94) was 32.6 years (median 32, range 18–60).

Table 1
PST test results for the control (CG) and risk group (RG) in relation to the Pap status at enrollment

	Risk Group			Control Group		
	PST+	PST–	Σ	PST+	PST–	Σ
WNL	125 (57.87%)	91 (42.13%)	216	6 (5.66%)	100 (94.34%)	106
ASCUS	23 (79.31%)	6 (20.69%)	29	0	0	0
LG SIL	34 (56.67%)	26 (43.33%)	60	1 (100%)	0	1
HG SIL	108 (77.14%)	32 (22.86%)	140	2 (100%)	0	2
CC	2 (100%)	0	2	0	0	0
Σ	292 (65.32%)	155 (34.68%)	447	9 (8.26%)	100 (91.74%)	109

3.2. Baseline status of the risk group

The risk group (RG) consists of 447 HR-HPV positive women with an average age of 34.5 years (median 33 years, range 18–81 years). 48.2% (n = 216) of the samples did not show signs of cervical lesions and 51.8% (n = 231) demonstrated signs of cervical lesions. 65.3% (n = 292) of the samples were PST positive, 34.7% (n = 155) were PST negative. Nearly 43% (n = 125) out of the 292 PST positive samples displayed normal cytology and approximately 57% (n = 167) demonstrated abnormal cytology: ASCUS (n = 23), LG SIL (n = 34), HG SIL (n = 108) and CC (n = 2) (Table 1).

Viral load of the risk group displayed a broad variation (WNL: n = 216, mean 353.5 IVL, median 83.6 IVL, IVL range 2.3–2700; ASCUS: n = 29, mean 250.6 IVL, median 94.4 IVL, IVL range 18.4–1560; LG SIL: n = 60, mean 856.4 IVL, median 586.5 IVL, IVL range 3.2–2810; HG SIL: n = 140, mean 659.1 IVL, median 360.5 IVL, IVL range 8.5–3120; and CC: n = 2, mean 446.3 IVL, IVL range 12.5–880).

3.3. Follow-up data, control group

All of the HR-HPV DNA infected women (PST+, n = 6; PST–, n = 6) who had morphological normal smear at enrollment did not show any signs of progression within an observation period of 0.3–0.8 years. HPV infection was eliminated in three out of six PST– samples. One PST+ sample was classified as LG SIL. It remained morphologically unchanged within 1.2 years. In this case viral load demonstrated a remission from 93.5 to 2.6. Both HG SIL PST+ cases (IVL: n_1 = 1540, n_2 = 1510) demonstrated a remission to normal cytology. One of the specimens became HR-HPV DNA negative within 0.4 years, the other one displayed a reduction in the viral load (n_2 = 255) within 0.2 years.

3.4. Follow-up data, risk group

So far, follow-up data are available for 194 out of 447 women of the risk group. Therefore, the final result of the study may lead to different conclusions. As different progression and remission rates are associated with the severity of a cervical cytological finding, we decided to perform sub-analyses for WNL, ASCUS, LG SIL and HG SIL cases.

WNL

For 96 out of 216 women with normal cytology follow-up data were available. One or more of the HPV DNA types 16, 18 and 45 accounted for HR-HPV DNA positivity in 54 specimens (56.3%). Histology verified a progression to a severe cervical precursor lesion (CIN 2+) in eight women (PST+, n = 5, PST–, n = 3). In one case a progression to HG SIL was detected within 1.2 years. In this case, histology verified a squamous cell carcinoma (SCC). 88 women (PST+, n = 49, PST–, n = 39) remained with a normal cervical cytology after 1.3 years. HPV infection was eliminated in 20 women (20.8%) with normal cytology (PST+, n = 8, PST–, n = 12) within 0.8 years (range 0.23–1.3 years).

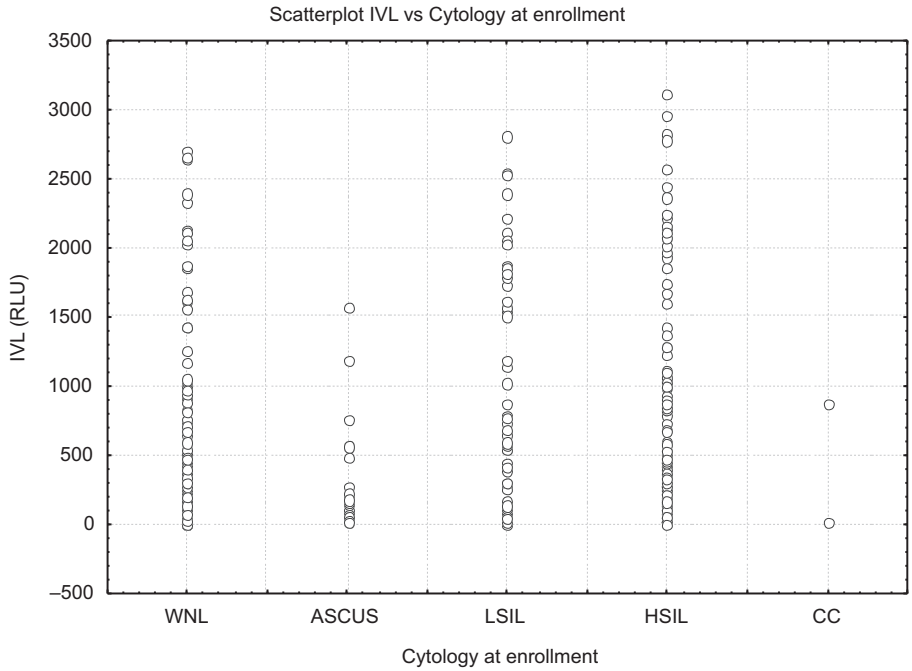

Fig. 2. Initial viral load vs. Pap status coded in Bethesda Nomenclature at enrollment of the risk group demonstrates a broad variation and cannot be associated with the severity of the cervical lesion.

ASCUS

In 19 out of 29 cases (65.5%) follow-up data were available. Regression from ASCUS to normal cytology was detected in 5 women. Three of them were PST+ and two were PST−. HPV infection was eliminated in one woman who was negative for PST. Histology was triggered by cytology in 14 cases and displayed one PST+ SCC, 10 PST+ CIN 3 lesions, one PST− CIN 2 lesion and one PST+ CIN 1 lesion. One PST+ case did not demonstrate any signs of malignancy.

LG SIL

Follow-up data for 35 out of 60 women (58.3%) who were considered to have a LG SIL at enrollment were available. The cervical cytology of 24 women (PST+, n = 12; PST−, n = 12) demonstrated a regression to normal cytology within a mean observation period of 0.8 years (range 0.7–1.4 years). Seven women who were initially PST+ remained HR-HPV+, but became PST−. Histology was triggered by cytology in 8 cases and displayed 7 PST+ CIN 3 lesions and one PST+ CIN 1 lesion.

HG SIL

Follow-up data for 92 out of 140 women (65.7%) who were considered to have an HG SIL at enrollment were available. The cervical cytology of 19 women who were positive for one or more of the HPV types 16, 18 and 45 (PST+, n = 15; PST−, n = 4) demonstrated a regression (<HG SIL) within a mean observation period of 1.1 years (range 0.8–1.4 years). HR-HPV infection was eliminated in 8 women (PST+, n = 4, PST−, n = 4). Histology was triggered by cytology in 48 cases and demonstrated one PST+ CIN 1 lesion, two PST− CIN 1 lesions, 6 PST+ CIN 2 lesions, 38 PST+ CIN 3 lesions and 7 PST− CIN 3 lesions. One PST+ case did not demonstrate any signs of malignancy.

4. Discussion

Before we go into detail, we have to inform about some methodological aspects that require discussion. A potential source of bias may be the split sample approach of the study. Therefore it is possible that cervical lesions may not be detected or that HPV-infected cells may not be extracted, although collection of HPV samples and PAP smears was performed on the same day. In addition, we performed a HR-HPV DNA test. HPV DNA levels may fluctuate during the course of infection. If the HR-HPV DNA is below the analytical sensitivity of HC2T, the test result may be negative although an HPV infection is prevalent. Furthermore, it may take more than 10 years to develop cervical cancer.[31] So, there is the possibility that some of the normal cytological samples will progress to a high-grade lesion during the follow-up.

In this study we used an HPV test that detects one or more of the HR-HPV types 16, 18 or 45 as an adjunct to cytology. We tried to improve information about the ongoing debate about whether the viral load affects the severity of a cytological finding or whether it does influence the progression or regression rate of a cervical precursor lesion. Our data on the viral load display a broad variation and therefore the viral load does not seem to influence the severity of the cervical lesion. It would appear that viral load detected by HC2T cannot directly indicate severity of the cervical lesion and that testing for different levels of viral loads is not of diagnostic value. Some authors also found a negative association[32,33] between viral load and the severity of a cervical lesion. Nonetheless, there are reports that have demonstrated positive associations.[34,35]

Furthermore, in our study 25% (17/68) of the histologically confirmed CIN 3 lesions arose in women younger than 30 years. This may reflect a change in sociocultural behavior, such as having the sexual debut at a younger age as well as promiscuity. Both are well described cofactors that may contribute to the development of severe cervical lesions. So, screening for cervical cancer at the age of 20 will be important and it may soon be necessary to start at an earlier age to prevent cervical cancer from becoming truth.

However, as to date 66.7% of the CIN 2 lesion and 88.2% of CIN 3 lesions of the study were PST positive (Table 2), it seems that testing for HPV types 16, 18 and 45 may be a biomarker that is helpful in identifying women with an underlying cervical lesion who have an elevated risk of developing severe cervical lesions. Nevertheless, cytological screening for cervical lesions needs to continue for at least two reasons: Firstly, because not the entire severe cervical lesions are associated with HPV 16, 18 and 45 infections and, secondly, not every HPV infection, even not every HPV infection

Table 2
Preliminary overall results for the Risk Group of PST testing vs. histological outcome

Histological outcome	PST+	PST−	Σ
No malignancy	2 (66.67%)	1 (33.33%)	3
CIN 1	3 (50%)	3 (50%)	6
CIN 2	6 (66.67%)	3 (33.33%)	9
CIN 3, CIS	60 (88.24%)	8 (11.76)	68
SCC	4 (100%)	0	4
Σ	75 (83.33)	15 (17.67%)	90

with one of the HR-HPV types 16, 18 and 45, will lead to a severe cervical lesion. Sometimes, these infections will be cleared by the T-cell system. Nonetheless, women with an HPV infection with one of these types should be carefully monitored using cytology and HPV testing as there is an increased risk to develop severe cervical lesions.

In conclusion, to date only data of 43.4% (n = 194) of the whole risk group are available. So, it is possible that conclusions may change, once the complete data set is analyzed.

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