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Comparison of GP5+/6+-PCR and SPF_{10}-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▽

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Using a case control approach, we performed a two-way comparison study between GP5+/6+-PCR and HPV SPF_{10}-Line Blot 25 (SPF_{10}) assays for detection of 14 types of high-risk human papillomavirus (hrHPV) in samples from women with normal cytology results who had or developed grade 3 cervical intraepithelial neoplasia (CIN 3). Samples were pooled from two cohorts, i.e., women participating in population-based screening and women attending a gynecological outpatient clinic. Cases ($n = 45$) were women with histologically confirmed CIN 3 diagnosed within a median follow-up time of 2.7 (range, 0.2 to 7.9) years. Control samples were from women ($n = 264$) who had developed CIN 1 lesions at maximum (median follow-up at 5.8 [range, 0 to 10] years). Identical numbers of cases tested positive for 1 or more of the 14 hrHPV types by both systems (40/45; McNemar; $P = 1.0$). Conversely, SPF_{10} scored significantly more controls as hrHPV positive than did GP5+/6+-PCR (95/264 versus 29/264; McNemar; $P < 0.001$). Consequently, women with normal cytology results and an hrHPV GP5+/6+-PCR-positive test exhibited a risk of CIN 3 that was 4.5 times higher (odds ratio [OR], 65; 95% confidence interval [95%CI], 24 to 178) than that seen for women with an hrHPV-positive SPF_{10} test (OR, 14; 95%CI, 5 to 38)). Similar results were obtained after analysis of both cohorts separately. Discrepancy analysis by viral load assessment for the most common discordant hrHPV types (HPV16, -18, and -52) showed that samples which were SPF_{10} positive only for these types had viral loads significantly lower than those for samples that were positive by both assays (analysis of variance; $P \leq 0.006$). Our data indicate that GP5+/6+-PCR has a better clinical performance than SPF_{10} for women who are diagnosed with CIN 3 after prior normal cytology results. The extra positivity scored by SPF_{10} mainly involved infections characterized by low viral loads that do not result in CIN 3.

Based on the fact that a persistent infection with high-risk human papillomavirus (hrHPV) is the necessary cause of cervical cancer (4, 20), hrHPV testing has been recognized as a potentially valuable tool not only for the triage of women with borderline cytomorphological abnormalities but also for primary cervical screening, either in conjunction with cytology testing or not. Support for this comes from various clinical studies (1, 6, 9, 10, 16, 26). It should be realized, however, that hrHPV tests might detect both transient and persistent hrHPV infections, of which only the latter represent a condition that ultimately may result in the development of grade 3 cervical intraepithelial neoplasia (CIN 3) lesions and cervical cancer (≥CIN 3) and therefore should be considered as clinically relevant. We previously hypothesized that various hrHPV tests, although similarly efficient in detecting persistent infections, may differ considerably in the extent with which transient, clinically irrelevant hrHPV infections are detected (28).

A clinically valuable hrHPV test should perform in such a way that the number of transient infections detected is as low as possible to ensure an optimal balance between clinical sensitivity and specificity for ≥CIN 3. This is important to minimize the number of unnecessary follow-up procedures, particularly for women with normal cytology results. Based on data from longitudinal clinical studies involving large cohorts, so far only the GP5+/6+-PCR and the commercially available hrHPV hybrid capture 2 (hc2) assays have been proven to reach such an optimal balance and therefore can be considered clinically validated (2, 5, 7, 9, 16, 17, 21, 24). In a previous two-way comparison study, we showed that these assays, which both detect hrHPV types as a pool, perform equally well for the detection of ≥CIN 3 in a population-based cervical screening setting (13).

Besides GP5+/6+-PCR and hc2, the consensus HPV SPF_{10}-Line Blot 25 linear probe assay (SPF_{10}-LiPA version 1) PCR assay (15) (referred to herein as SPF_{10}) is a commonly used HPV detection assay, particularly in epidemiological studies and vaccination trials (12). However, this method has not yet

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been tested in a comparison study in relation to the occurrence or development of ≥CIN 3.

We here performed a comparison study between GP5+/6+-PCR and SPF_{10} to detect 14 hrHPV types in samples from women with normal cytology results who had or developed CIN 3 lesions. A subsequent discrepancy analysis included viral load analysis by type-specific real-time PCR.

MATERIALS AND METHODS

Study population. For this case control study, cervical samples used were from women with normal cytology results who were recruited from 1988 to 1993 during the course of two studies (18, 24) performed in the Amsterdam area and from whom cervical scrape samples were collected and stored at −80°C. The study groups comprised women who either participated in a population-based cervical screening trial (n = 4,079) or attended a gynecological outpatient clinic (n = 2,311). Women were managed according to guidelines of the Dutch screening program. Briefly, women with moderate dyskaryosis or worse (i.e., high-grade squamous intraepithelial lesions) were directly referred to the gynecologist for colposcopy. Women with borderline or mild dyskaryosis (i.e., atypical squamous cells of undetermined significance/low-grade squamous intraepithelial lesions) were advised to get repeat testing at 6 and 18 months and were referred only in cases of the persistence of borderline or mildly dyskaryotic changes or when cytological progression became manifest. Women with normal cytology results were recalled at the next screening round. Of the population-based screening cohort, 3,471 women had normal cytology results. Of 2,250 of these women, sample material and follow-up data were available, and 61 of these women had a colposcopy-directed biopsy during follow-up. The histological outcomes were no CIN, CIN 1, CIN 2, and CIN 3 in 8, 22, 6, and 25 of these women, respectively. Of the remaining 2,189 women, 1,710 had two consecutive cytologically normal smears at the follow-up. All women with CIN 3 diagnoses were included as cases in this study, and a random sample of 199 women without evidence of CIN 2/3 upon follow-up (2 with no CIN, 11 with CIN 1, and 186 with two consecutive normal smears) were included as controls. The median ages of these cases and controls were 34 (range, 29 to 60) and 41 (range, 34 to 54) years, respectively, and median follow-up times were 2.1 (range, 1 to 8) and 7.0 (range, 1 to 10) years, respectively. In this screening cohort, case women were younger than and had a follow-up time shorter than that of control women (analysis of variance [ANOVA]; both P values were <0.001).

Of the outpatient clinic cohort, 1,566 women had normal cytology results, and sample material and follow-up data were available for 1,020 of these women. Women visited the outpatient clinic for a wide spectrum of gynecological complaints and for their hormonal contraception. One hundred twenty of these women had a colposcopy-directed biopsy; of these women, 42, 30, 28, and 20 were diagnosed with no CIN, with CIN 1, with CIN 2, and with CIN 3, respectively. All 20 women with CIN 3 in this cohort were included as cases as well, and all 72 women with a histologically confirmed absence of CIN 2/3 (42 with no CIN and 30 with CIN 1) were included as controls. The median ages for cases and controls were 32 (range, 22 to 49) and 37 (range, 20 to 53) years, respectively, and median follow-up times were 0.2 (range, 0 to 0.5) and 0.3 (range, 0 to 0.6) years. In the hospital group, ages and follow-up times did not differ significantly between cases and controls (ANOVA; both P values were >0.05). Taking these cohorts together, 45 cases and 271 controls were included in this study. Informed consent was obtained from all participating women, and this study followed the local ethical guidelines of our medical center.

Cytology results and HPV testing. Cervical scrape specimens were taken from the cervix uteri with a cervix brush (Rovers, Oss, The Netherlands). A smear was made on a glass slide for cytomorphological analysis, and the brush with remaining cell material was subsequently placed in a tube containing 5 ml phosphate-buffered saline–0.05% Merthiolate. Upon arrival in the laboratory, the samples were spun down and pellets were resuspended in 1 ml Tris-HCl (pH 8) and stored at −80°C for subsequent HPV analysis. In none of the women were HPV test results used for triage. Cytomorphological classification was performed as described before (33).

The high pure PCR template preparation kit was used for DNA extraction from 100-μl Tris-HCl suspensions of stored samples according to the recommendations of the manufacturer (Roche, Mannheim, Germany), except that samples were eluted with 100 μl of elution buffer. Ultimately, 10 μl of eluate was used for each PCR assay. To assess sample quality, DNA isolates were subjected first to β-globin PCR using the primers PCO3/PCO5 (11). The β-globin PCR assay generates a 209-bp PCR fragment, which exceeds the sizes of the PCR products generated by the SPF_{10} (about 70 bp) and GP5+/6+-PCR (about 150 bp) assays. Subsequently, only β-globin PCR-positive samples were subjected to comparative HPV PCR analyses.

The GP5+/6+-PCR followed by use of the enzyme immunoassay (EIA) readout system with a probe cocktail of 14 hrHPV types (i.e., HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68) was performed at the Department of Pathology, VU University Medical Center (Amsterdam, The Netherlands) as described before (30). Reverse line blotting was used to genotype hrHPV for all EIA-positive samples (30). HPV testing and genotyping using HPV SPF_{10} were performed at the Department of Medical Microbiology, Radboud University Nijmegen Medical Center (Nijmegen, The Netherlands) according to a routinely used protocol in which DNA EIA (DEIA) was the first readout system for SPF_{10} PCR products with a probe cocktail of 14 of the abovementioned hrHPV types plus 24 low-risk HPV types (i.e., 38 types altogether, including HPV6, -11, -16, -18, -26, -30, -31, -33, -34, -35, -39, -40, -42, -43, -44, -45, -51, -52, -53, -54, -55, -56, -58, -59, -61, -62, -64, -66, -67, -68, -69, -70, -72, -73, -74, -82 [MM4], MM7, and MM8) (15, 32). DEIA-positive samples were subsequently subjected to LiPA typing. Both GP5+/6+-PCR and SPF_{10} assays target the L1 region in the HPV genome. Testing with both methods was performed in parallel by different technicians who were unaware of each others' test results and were blinded to the case/control status of the samples.

For final analysis, the GP5+/6+-PCR and SPF_{10} assays were considered hrHPV positive when reverse line blotting and LiPA genotyping, respectively, revealed 1 or more of the 14 abovementioned hrHPV genotypes that can be detected by (D)EIA of both assays. Samples that showed a positive (D)EIA signal but failed to reveal a positive signal in the subsequent typing assays were designated as containing HPV-X, which may indicate (sub)types and/or variants that were not available on the respective typing blots. Genotyping results were considered concordant if there was complete agreement between both assays for 1 or more of the 14 hrHPV types listed above. Results were considered compatible when at least one type was detected with both assays while one or more other types were not detected by either of the assays. Genotyping data were considered discordant when no similarity between genotypes detected by both assays was found.

Viral load analysis. Viral load analysis was performed by type-specific real-time PCR for HPV16, -18, and -52 on the LightCycler instrument essentially as described before (14, 27). Real-time PCR was performed on 5 μl of DNA isolate, representing about 1/200 of the DNA of the total scrape specimen. Primers and probes used for HPV16 and -18 real-time PCR have been described before (14, 27). The oligonucleotide sequences for primers and probes used for HPV52 real-time PCR were as follows: the forward primer was 5′-ATGGACAAGCAG AACAAGCCA-3′ (nucleotide [nt] positions 683 to 704); the reverse primer was 5′-CCCTCCCTTTCGCCCTCT-3′ (nt positions 884 to 902); the donor probe was 5′-TGCGACGGACCTTCGTACTCTACAGC-3′ (nt positions 773 to 799), with the 3′ end labeled with fluorescein; and the acceptor probe was 5′-TGCT GTTGGGCACATTACAAGTTGTG-3′ (nt positions 803 to 828), with the 5′ labeled with LCRed640. Viral loads were expressed as HPV DNA copies per scrape specimen.

Statistical analysis. Positivity rates for the GP5+/6+-PCR and SPF_{10} assays of cases and controls were calculated, and Mantel-Haenszel common odds ratios (ORs) were computed to study assay positivity in relation to the development of CIN 3. The sensitivities, specificities, and ORs were relative to the procedure. The McNemar test was used for mutual comparison of the positivity rates of the GP5+/6+-PCR and SPF_{10} assays in the two subgroups, i.e., cases and controls. Both pooled and separate analyses of cases and controls of both cohorts were performed. The level of agreement was determined using kappa statistics. All HPV viral load values were log normalized. One-way ANOVA was used to compare viral loads between the GP5+/6+-PCR-negative/SPF_{10}-positive group and the GP5+/6+-PCR-positive/SPF_{10}-positive group. GP5+/6+-PCR- and/or SPF_{10}-positive samples that were negative in the LightCycler tests, thus containing numbers of HPV copies below the detection level, were set at 10 copies (3, 14), which corresponds to approximately 2,000 copies per scrape specimen. The level of statistical significance was set at 0.05. All analyses were performed using SPSS 11.5 software.

RESULTS

Clinical performance of hrHPV detection by GP5+/6+-PCR and SPF_{10} assays. A case control design was used in order to compare the clinical performances of GP5+/6+-PCR and SPF_{10} assays for the identification of women with normal cy-

TABLE 1. Test results for 14 hrHPV types by GP5+/6+-PCR and SPF_{10} assays for samples from women with normal cytology results

Sample group	GP5+/6+-PCR result	No. (%) with indicated result by SPF_{10} assay		Total (%)	*P* value[a]
		Negative	Positive		
Overall					
Controls	Negative	163 (62)	72 (27)	235 (89)	<0.001
	Positive	6 (2)	23 (9)	29 (11)	
	Total	169 (64)	95 (36)	264	
Cases	Negative	4 (9)	1 (2)	5 (11)	1.0
	Positive	1 (2)	39 (87)	40 (89)	
	Total	5 (11)	40 (89)	45	
Screening cohort					
Controls	Negative	127 (66)	53 (27)	180 (93)	<0.001
	Positive	4 (2)	9 (5)	13 (7)	
	Total	131 (68)	62 (32)	193	
Cases	Negative	2 (8)	1 (4)	3 (12)	1.0
	Positive	1 (4)	21 (84)	22 (88)	
	Total	3 (12)	22 (88)	25	
Hospital cohort					
Controls	Negative	36 (51)	19 (27)	55 (77)	<0.001
	Positive	2 (3)	14 (20)	16 (23)	
	Total	38 (54)	33 (46)	71	
Cases	Negative	2 (10)	0	2 (10)	1.0
	Positive	0	18 (90)	18 (90)	
	Total	2 (10)	18 (90)	20	

[a] McNemar.

tology results who had developed CIN 3. A total of 45 cases (women with CIN 3 diagnosed within a median follow-up time of 2.7 [range, 0 to 8] years) were pooled from two different cohorts (18, 24) of women participating in population-based cervical screening ($n = 25$) and women visiting a gynecological outpatient clinic ($n = 20$), respectively. A random sample of 271 controls (199 from the screening population and 72 from the hospital population) involved women without evidence of CIN 2/3 within a median follow-up time of 5.8 (range, 0 to 10) years.

β-Globin PCR analysis of extracted DNA revealed positivity for samples of all cases and 264 controls (193 and 71 of screening and hospital populations, respectively), and these were considered valid for further HPV PCR analyses. Both HPV PCR assays were subsequently performed on equal amounts of the same DNA isolates of β-globin PCR-positive samples. After (D)EIA detection using cocktail probes, GP5+/6+-PCR gave positive results for 71 samples (42/45 cases and 29/264 controls) and SPF_{10} for 171 samples (42/45 cases and 129/264 controls). However, it should be noted that whereas the cocktail probe for GP5+/6+-PCR EIA detects only 14 hrHPV types (i.e., HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68), that of SPF_{10} detects 24 additional low-risk HPV types as well. Subsequent genotyping by the respective reverse hybridization methods revealed that 2 of the 71 GP5+/6+-PCR EIA-positive samples and 36 of the 171 SPF_{10} DEIA-positive samples did not show a positive typing result for 1 or more of the 14 hrHPV types. These included 2 cases with positivity by both GP5+/6+-PCR and SPF_{10} and an additional 34 controls with positivity by SPF_{10} only. Both of the GP5+/6+-PCR EIA-positive, typing-negative cases could be identified as containing HPV-X, whereas one SPF_{10} DEIA-positive case contained HPV-X and another HPV6. Of the 34 SPF_{10} DEIA-positive, typing-negative controls, 24 contained low-risk HPV and 10 HPV-X.

The data obtained after restricting the analysis to the 14 hrHPV types are summarized in Table 1. Overall, the GP5+/6+-PCR assay scored a significantly low number of samples as hrHPV positive ($n = 69$) compared to the SPF_{10} assay ($n = 135$; McNemar; $P < 0.001$). Restricting the analysis to cases, the hrHPV positivity rates of the GP5+/6+-PCR and SPF_{10} assays were similarly high, namely, 89% (40/45; 95% confidence interval [95%CI], 80 to 98), and did not significantly differ (McNemar; $P = 1.0$). Similar results were obtained after analysis of samples from case women from both cohorts separately (Table 1). The assays scored 88% (22/25; 95%CI, 76 to 100) and 90% (18/20; 95%CI, 77 to 100) of samples from case women from the screening and hospital cohorts, respectively, as hrHPV positive.

The overall higher hrHPV positivity of the SPF_{10} assay was due to a significantly high hrHPV positivity rate for control women (36%; 95/264; 95%CI, 31 to 43) compared to that obtained by the GP5+/6+-PCR assay (11%; 29/264; 95%CI, 7 to 15) (McNemar; $P < 0.001$). The difference between both assays was evident for samples of control women of both cohorts (Table 1). For the screening cohort, the hrHPV positivity

of the SPF_{10} versus the GP5+/6+-PCR assay was 32% (62/193; 95%CI, 25 to 39) versus 7% (13/193; 95%CI, 3 to 11). For the hospital cohort, the corresponding figures were 46% (33/71; 95%CI, 34 to 58) versus 23% (16/71; 95%CI, 13 to 33). For both cohorts, these differences were statistically significant (McNemar; $P < 0.001$).

The strength of the overall agreement in assay positivity was moderate (kappa values of 0.42). When stratification was done according to cases and controls, this strength was poor for controls (kappa value of 0.24) but good for cases (kappa value of 0.78). The significantly high positivity rate for controls assayed by SPF_{10} compared to that assayed by GP5+/6+-PCR was reflected in the ORs of both assays for CIN 3. The overall ORs (Mantel-Haenszel) were 14 (95%CI, 5 to 38; $P < 0.001$) for the SPF_{10} assay and 65 (95%CI, 24178; $P < 0.001$) for the GP5+/6+-PCR assay. This indicates that women with normal cytology results who tested hrHPV positive by the GP5+/6+-PCR assay were 4.5 times more likely to have or develop CIN 3 lesions than those who were hrHPV positive by the SPF_{10} assay. The agreement figures and differences in ORs did not differ meaningfully when calculated for the two cohorts separately (data not shown).

Comparison of hrHPV genotypes detected by the GP5+/6+-PCR and SPF_{10} assays. As specified in Table 2, the samples that were scored as hrHPV positive by the SPF_{10} assay but negative by the GP5+/6+-PCR assay displayed a broad variety of hrHPV genotypes, although HPV52 infections were relatively frequent. Among 23 control samples that were hrHPV positive by both assays, 17 (74%) showed concordant, 2 (9%) compatible, and 4 (17%) discordant typing results. For case samples, these numbers were 31 (79%), 7 (18%), and 1 (3%), respectively. Thus, one or more of the same hrHPV types were detected by both assays in 83% of the control samples and 97% of the case samples that were positive by both assays. The single discordant case had HPV16 as determined by the GP5+/6+-PCR assay and HPV52 according to the SPF_{10} assay. Furthermore, one case sample that was hrHPV negative by the SPF_{10} assay was positive for HPV16 by the GP5+/6+-PCR assay, and one case sample that was negative for hrHPV by the GP5+/6+-PCR assay contained HPV52 according to the SPF_{10} assay.

Analysis of discordant GP5+/6+-PCR and SPF_{10} test results. To investigate whether the nondetection of hrHPV by the GP5+/6+-PCR assay in samples positive by the SPF_{10} assay might be due to low viral loads, we determined the type-specific viral loads of three types (HPV16, -18, and -52 in both single and multiple infections) that most commonly revealed discordant typing results. For this purpose, we tested a total of 56 cervical scrape specimens of which sufficient sample material was left for real-time PCR. These included samples of case and control women with both discordant (13 for HPV16, 14 for HPV18, and 15 for HPV52) and concordant (6 for HPV16 and 4 for both HPV18 and HPV52) SPF_{10} and GP5+/6+-PCR test results. The median HPV16, -18, and -52 DNA loads for the GP5+/6+-PCR assay-negative but SPF_{10} assay-positive group were 7.0×10^3 (range, 2.0×10^3 to 4.4×10^4), 2.0×10^3 (range, 2.0×10^3 to 4.8×10^4), and 2.0×10^3 (range, 2.0×10^3 to 2.1×10^8) HPV copies/scrape specimen, respectively, and were significantly lower (ANOVA; all P values were ≤ 0.006) than those for the GP5+/6+-PCR assay-positive/SPF_{10} assay-positive group, i.e., 1.1×10^6 (range, 1.6×10^5 to 7.7×10^6), 7.4×10^7 (range, 4.0×10^5 to 8.0×10^7), and 3.7×10^6 (range, 2.4×10^5 to 3.7×10^6), respectively (Fig. 1). Only three samples that were HPV52 positive by the SPF_{10} assay and GP5+/6+-PCR assay negative had viral load levels that overlapped with those of SPF_{10} and GP5+/6+-PCR assay double-positive samples for this type. These samples, all from control women, might contain certain HPV52 variants that are less efficiently detected by the GP5+/6+-PCR assay than by the SPF_{10} assay (8). The viral load of the CIN 3 case missed by the GP5+/6+-PCR assay, which was positive for HPV52 by the SPF_{10} assay, revealed 1.0×10^4 copies/scrape specimen. After the restriction of calculations to control samples, only for HPV16 were sufficient samples with concordant GP5+/6+-PCR and SPF_{10} results available. This analysis revealed similar findings. Thus, the exclusion of cases had no influence on the relation between lower viral loads and GP5+/6+-PCR assay-negative but SPF_{10} assay-positive samples (ANOVA; $P < 0.001$; data not shown). Altogether, these data indicate that the extra positivity scored by the SPF_{10} assay in relation to the GP5+/6+-PCR assay mainly involved relatively low viral loads.

DISCUSSION

Comparison of the clinical performances of hrHPV detection by the GP5+/6+-PCR and SPF_{10} assays showed that both assays had similar overall clinical sensitivities (i.e., 89%) for the prediction of finding CIN 3 lesions (i.e., via detection of hrHPV) in women with cytomorphologically normal smears. It is of note that the actual overall sensitivity values of these assays would be slightly higher (i.e., 93% [95%CI, 86 to 100] for the GP5+/6+-PCR assay and 91% [95%CI, 83 to 99] for the SPF_{10} assay) in this set when the outcome is based on hrHPV detection using a pool by (D)EIA, since two GP5+/6+-PCR EIA-positive cases and one SPF_{10} DEIA-positive case were designated as HPV-X and were not included in the final analysis. In practice, in clinical and experimental screening settings, scoring of hrHPV GP5+/6+-PCR assay positivity is based on the EIA readout only, showing a good sensitivity (2, 5–7). The positivity rate in controls was significantly low for the GP5+/6+-PCR assay compared to that for the SPF_{10} assay. The latter could be attributed mainly to the fact that SPF_{10} scored as positive significantly more samples from control women that had viral load levels apparently falling below the detection level of the GP5+/6+-PCR assays. Consequently, women with normal cytology results and a GP5+/6+-PCR-positive test result exhibited a 4.5 times higher risk of CIN 3 than those with a positive SPF_{10} test result. Altogether, these data confirm our previous concept (28) that a too-high analytical sensitivity of an hrHPV test results in a marked decrease in clinical specificity without having an impact on the clinical sensitivity for detecting the ultimate development of ≥CIN 3. This is in line with data from several studies indicating that not hrHPV DNA presence per se but increased viral loads confer an increased risk of ≥CIN 3 (19, 27, 31, 34).

It is noteworthy that differences in the population characteristics could influence the relative performance of each assay. However, in this study similar differences in assay performance were found for control women of both the screening and the

TABLE 2. HPV genotyping results for 14 hrHPV types of SPF_{10} versus GP5+/6+-PCR

hrHPV type by SPF_{10} assay	No. of samples of indicated type			
	Concordant	Compatible (genotype by GP5+/6+-PCR assay)	Discordant (genotype by GP5+/6+-PCR assay)	hrHPV negative by GP5+/6+-PCR assay
Control sample type(s)				
None			6 (3 of type 31, 1 of 39,	163
16	3		2 of 16, 1 of 18)	10
18				8
31	2			5
33	2			3
35			1 (45)	1
39	1			4
45				1
51	1		1 (16, 18, 39)	5
52	3			12
56	1			4
58			1 (31)	
59		1 (56, 59)		
66	3			1
68				1
16,18	1			8
16, 18, 66				1
16, 31				1
16, 39, 52				1
16, 68				1
18, 31				1
18, 39			1 (16)	
18, 52				1
31, 52				1
35, 39				1
52, 56				1
51, 52, 56		1 (45, 56)		
Total	17	2	10	235
Case sample type(s)				
None			1 (16)	4
16	11	1 (16, 58)		
18	3	1 (18, 31)		
31	5			
33	2			
35	3			
39	1			
45		1 (18, 45)		
51	1			
52		1(52, 56)	1 (16)	1
56	1			
59	1			
16, 18	1			
16, 31	1			
16, 52		1 (16)		
18, 33		1 (18)		
31, 51	1			
39, 52, 68		1 (39, 52)		
Total	31	7	2	5

gynecological outpatient populations. The fact that among the first group of women the median age was significantly higher for controls (i.e., 41 years) than for cases (i.e., 34 years) is unlikely to have contributed markedly to the observed difference between both assays, since in the hospital population the median ages of cases and controls (32 versus 37 years) did not differ significantly. Our data nevertheless seem to contradict those found in a study reported by Safaeian et al. (25). That study revealed that for women with normal cytology results enrolled for a vaccination trial, there was no difference in hrHPV positivities between the SPF_{10} and hc2 assays, the latter of which is considered to be compatible with the GP5+/6+-PCR assay (25). A likely explanation for this apparent discrepancy is that Safaeian et al. studied young women under 30 years of age (median age, 21 years; range, 18 to 25 years). It is therefore tempting to speculate that hrHPV positivity in women under 30 years of age with normal cytology results is often accompanied by relatively high viral loads, giving rise to

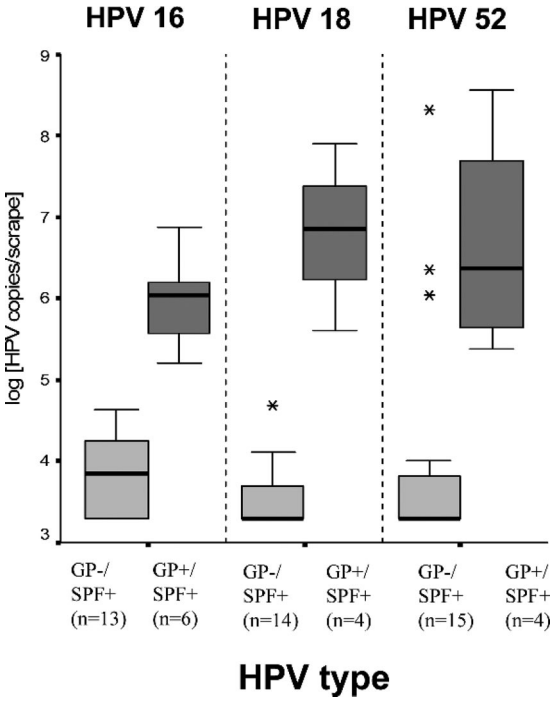

FIG. 1. Box plot of HPV16, -18, and -52 DNA loads in GP5+/6+-PCR assay-negative (GP−)/SPF_{10} assay-positive (SPF+) (light boxes) and GP5+/6+-PCR assay-positive (GP+)/SPF_{10} assay-positive (dark boxes) samples. Viral load values are shown on the *y* axis as HPV copies/scrape specimen and are log transformed. For all three HPV types, the viral loads were significantly higher in the GP5+/6+-PCR assay-positive/SPF_{10} assay-positive samples than in the GP5+/6+-PCR assay-negative/SPF_{10} assay-positive samples (ANOVA; *P* values were <0.001, <0.001, and 0.006 for HPV16, -18, and -52, respectively). The upper and lower boundaries of the boxes represent the 75th and 25th percentiles, respectively. The thick black line represents the median, the whiskers represent the minimum and maximum values, and the asterisks represent outliers.

positivity rates that are equal for assays displaying different analytical sensitivities for hrHPV. This is supported by findings of a different comparison study between SPF_{10} and hc2 on samples from a population of women visiting a colposcopic clinic (22). The latter study mainly included women above 30 years of age and displayed for women with normal cytology results a difference between hrHPV positivity by SPF_{10} versus that by hc2 that was similar to that seen for SPF_{10} versus GP5+/6+-PCR assays for control samples from our study.

Taken together, these data indicate that the threshold for the positivity of a given hrHPV test should be at such a level that an optimal balance between clinical sensitivity and specificity for identifying women at risk of having or developing ≥CIN 3 is reached. This concept finds support by recent recommendations of the American Society for Colposcopy and Cervical Pathology (ASCCP) (29), which indicate that in a cervical screening setting, clinical sensitivities for ≥CIN 3 of at least 92% ± 3% seem satisfactory only when clinical specificities are above 85% at the same time. Therefore, the GP5+/6+-PCR assay can be advocated above SPF_{10} for screening purposes involving women over 30 years of age.

Apart from the difference in clinical performances, both tests showed common characteristics, since there was a high concordance in hrHPV genotyping, especially for samples from case women. We found a relatively high number of infections with HPV52 among the women for whom samples were SPF_{10} positive and GP5+/6+-PCR negative. This cannot be explained solely by a potentially low sensitivity of the GP5+/6+-PCR assay for HPV52 compared to that for HPV16 and HPV18 (23). The minimal HPV52 viral load value determined for SPF_{10} assay-positive but GP5+/6+-PCR assay-negative samples did not differ markedly from those for HPV16 and -18. In fact, only three discordant HPV52 control samples might have resulted from a lower detectability by the GP5+/6+-PCR assay, possibly because these infections involve HPV52 variants. Thus, the majority of HPV52 infections detected by the SPF_{10} assay alone are due to lower copy numbers of HPV52, which are apparently relatively common among SPF_{10}-positive women with normal cytology results.

In conclusion, the application of hrHPV detection assays with a too-high analytical sensitivity for hrHPV results in a markedly reduced specificity for CIN 3 without being beneficial for clinical sensitivity. Therefore, hrHPV test requirements, as currently under preparation in The Netherlands, should be incorporated in cervical screening guidelines to prevent overdetection, which would counteract the benefits of implementing hrHPV testing in screening programs.

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