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Detection of Human Papillomavirus Genotypes Among High-Risk Women: A Comparison of Hybrid Capture and Linear Array Tests

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Aff

Objectives: **To assess the concordance and performance of 2 different assays in detection of human papillomavirus (HPV) genotypes among women with abnormal Pap smear.**

Study Design: **A series of 575 women referred for colposcopy due to an abnormal Pap smear were analyzed with the Linear Array HPV Genotyping test detecting 37 HPV types and compared with Hybrid Capture II (HCII) assay for detection of carcinogenic HPV. Histologic outcomes of cervical intraepithelial neoplasia grade 2 (CIN2) or worse (CIN2+) and CIN3+ were the primary endpoints. Clinical performance, including receiver operating characteristics, was determined for both tests.**

Results: **HCII and linear array (LA) were concordant in 88.1% (433/491; 95% CI 85.3%–91.0%), having a substantial agreement with regular κ (κ = 0.70, 95% CI 0.62–0.77) and almost perfect agreement with weighted κ (ICC = 0.82, 95% CI 0.7–0.85). In detecting CIN2+ and CIN3+, LA is 5% and 6% more sensitive but 9.5% and 8.7% less specific than HCII (area under ROC curve; $P = 0.317$ and $P = 0.875$, respectively).**

Conclusions: **Performance of HCII and LA does not significantly differ in detecting CIN2+ or CIN3+.**

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CONVENTIONAL PAP TEST HAS PROVEN its efficacy as the time-honored means to reduce the incidence and mortality of cervical cancer (CC) in countries, where organized screening programs have been implemented.[1,2] On the other hand, opportunistic screening efforts in several other countries have not been equally successful, because of the problems in implementation and performance of cervical cytology.[2–5] This has prompted a vigorous search for optional diagnostic tests to be used in triage and as potential screening tools.[6–9] These new tools include the testing for human papillomavirus (HPV), the carcinogenic types of which are the etiological agents of CC.[2,10,11] This has led to development of new commercial assays for HPV detection[12–14] and prompted an intense debate on their benefits and shortcomings.[15,16]

It seems now that the following 17 carcinogenic HPV genotypes HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82 are causally related to virtually all CCs, including both the squamous cell carcinoma and adenocarcinoma.[17–19] Availability of commercial assays detecting nearly all carcinogenic HPV types as a group[12–14] has raised the question, whether testing for carcinogenic HPV can improve detection of CC and its precursors (cervical intraepithelial neoplasia).[12,13,14,17–21] There is evidence implicating that carcinogenic HPV testing is more sensitive and has a higher negative predictive value (NPV) in detecting cervical intraepithelial neoplasia grade 2 (CIN2) or worse (CIN2+) when compared with cervical cytology as a screening tool.[8,13,22] Similarly, HPV triage of women with atypical squamous cells of undetermined significance (ASC-US) cytology for colposcopic evaluation has been shown to reduce the number of follow-up smears at the cost of fewer colposcopic referrals making it more cost-effective than evaluation by repeated cytology.[23,24]

Apart from HPV testing for carcinogenic types collectively, specific genotyping has recently been raised as one of the options to improve triage and screening.[25] According to a panel of international experts assembled at EUROGIN 2006 Congress, several important issues remain to be considered before implementing the full HPV genotyping in different settings.[25] These include: (a) test performance; (b) automation and high-throughput; (c) algorithms for clinical interpretations of the viral patterns; and (d) acceptance of a virological model of cervical carcinogenesis. More clinical trials testing the performance of HPV genotyping assays in detection of high-grade CIN are necessary before the implementation of this new technology in routine clinical settings.[25]

To address some of these issues, we extended our recent studies on carcinogenic HPV testing in women attending colposcopy[14] for analysis of HPV genotyping in a similar setting. In this study, we compared the Linear Array HPV Genotyping test (Roche Molecular Systems, ALmeda, CA) with Hybrid Capture II (HCII; Digene Corporation, Gaithersburg, MD) assay in detecting CIN2/3 in women referred for colposcopy because of abnormal Pap smear. The aim was to assess the performance of these 2 commercial assays (with different genotype coverage) in detecting CIN2+ and CIN3+ among these women referred to colposcopy because of an abnormal Pap, and separately among those with ASC-US referral Pap smear.

Materials and Methods

Patients

In the current study, we examined 575 women referred for colposcopic examination because of an abnormal Papanicolaou (Pap) smear in a colposcopy clinic in Paris (France). All women

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were examined in the Institute Alfred Fournier, during 2006, by 2 certified colposcopists (J.M., G.P.). The mean age of the women was 35.4 years (range 17–71, median 33.0 years, 25th percentile 27 years, 75th percentile 43 years). These women had a Pap smear taken in different clinics in Paris, and referred for colposcopic examination to Institute Alfred Fournier.[14,26] As emphasized before, this cohort represents a "high-risk" population, among whom a high prevalence of both HR-HPV infections and CIN lesions can be anticipated,[14,26] which leads to the test performance indicators substantially different from those in a screening setting.

Methods

Cytology. All women had a previous Pap smear taken within 2 to 3 months before their enrollment in the study (i.e., the referral Pap), performed by community physicians. These baseline smears were examined by cytologists in several different laboratories in Paris, and were not available for reexamination by the authors. The smears were classified according to the 2001 Bethesda system (TBS 2001), and the original interpretation was used as the baseline referral Pap smear diagnoses.

In the referral clinic, a new cervical cytology sample was taken from most of these woman.[14,26] Cervical samples were collected by a specially designed sampling device (Broomlike collection device), which was rinsed into PreservCyt (Cytyc Corporation, Marlborough, MA) and used in the preparation of liquid-based cytology (LBC)(ThinPrep; Cytyc) following the manufacturer's recommendations.

Colposcopy. After sampling for LBC and HPV DNA testing (separately), colposcopic examination of the cervix, vagina, and vulva was performed for all patients by 2 colposcopists, using a jointly agreed protocol. Lesions in the transformation zone (TZ) were assessed by applying 5% acetic acid and iodine solution, using 8× to 12× magnification. If colposcopy proved unsatisfactory, further exploration of the endocervix was systematically carried out using 20× magnification and a Koogan speculum.[27] The international (IFCPC) nomenclature[28] was used to classify the colposcopic patterns as follows: normal (including metaplasia); abnormal TZ (ATZ) with minor changes (with or without features of HPV infection), suggesting low grade CIN (CIN1); ATZ with major changes suggesting CIN2–3; and cancer. For statistical analysis, colposcopic results were dichotomized as either (a) normal, or (b) abnormal.

Biopsy Procedures. All 575 women underwent colposcopic examination and biopsy. Loop electro excision procedure cone biopsy was performed in cases with (a) Pap test showing HSIL and ATZ in colposcopy, (b) regardless of the Pap test result, if the ATZ was large (≥50% of TZ area), (c) an endocervical lesion and unsatisfactory colposcopy, or (d) ATZ and a squamocolumnar junction localized more than 3 mm within the endocervix. Altogether, 89 women underwent treatment by loop electroexcision procedure cone, whereas 431 had a directed punch biopsy taken. The remaining 55 women were not biopsied, because no cervical lesion was detected on colposcopy.

Histology. All biopsies were examined in one pathology laboratory in Paris (Laboratoire Claude-Levy) and reported by one pathologist (R.D.). Histologic assessments were made as masked by the HPV DNA status. In classifying the biopsies, the CIN terminology was adopted.[10] In calculating the performance characteristics of HCII and linear array (LA), biopsy diagnoses were used as the diagnostics endpoint and different cutoff levels (CIN2 or CIN3) were tested.

Hybrid Capture II in LBC Medium. Separate specimens for HCII test were collected into Universal Collecting Medium (Digene, Gaithersburg, MD) using the HCII Collection Device, validated for use with the HCII assay, following the manufacturer's instructions. HCII detects a pool of 13 carcinogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) without distinguishing which type(s) are present. HCII also cross-reacts with another HPV type, HPV66 that was recently designated as carcinogenic.[29] Specimens collected in Universal Collecting Medium were transported to laboratory at 2 to 30°C. Before analysis, specimens may be stored at room temperature for up to 21 days or at 2 to 8°C for up to 8 weeks. The HCII assay was performed according to the instructions of the manufacturer (Digene).[13,30] In estimation of the positive reactions, samples were considered positive, if the relative light units/cutoff were >1.0.[30]

Linear Array HPV Genotyping Test. HPV genotyping was completed using the Roche Linear Array HPV Genotyping test (Roche Molecular Systems, Basel, Switzerland), performed according to the manufacturer's instructions. This assay was done using the samples collected in PreservCyt LBC media.[31] Roche Linear Array HPV Genotyping test is a qualitative PCR technique detecting 37 most prevalent (low, intermediate, and carcinogenic) HPV genotypes: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, and CP6108. This test includes 4 steps[31]: specimen preparation, PCR amplification, hybridization of the amplified products with specific probes, and colorimetric detection of the hybrids on strip.

TABLE 1. Key Characteristics of the Patients and Their Cervical Disease

Variables	Frequency, N (%)
Age; mean, median (range)	35.3, 33.0 (17–71)
Referral pap smear	
Normal	24 (4.5)
ASC-US	211 (39.9)
ASC-H	31 (5.9)
LSIL	203 (38.4)
HSIL	56 (10.6)
AGUS or AGC	4 (0.8)
Colposcopy	
Normal	241 (43.3)
Low-grade	211 (37.9)
High-grade	71 (12.8)
Equivocal	30 (5.4)
Unsatisfactory	1 (0.2)
Biopsy and LEEP	
Normal	68 (15.8)
Metaplasia	105 (24.4)
Flat Condyloma	68 (15.8)
CIN1	87 (20.2)
CIN2	34 (7.9)
CIN3	63 (14.6)
SCC	3 (0.7)
VAIN1–3	3 (0.7)
Linear array	
Positive (all types)	372 (75.2)
Negative	123 (24.8)
Hybrid capture II	
Positive	376 (69.2)
Negative	167 (30.8)

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Statistical Analysis

Statistical analyses were performed using the SPSS and STATA software packages (SPSS for Windows, version 14.1. and STATA/SE 9.2). Frequency tables were analyzed using the χ^2 test, and the likelihood ratio statistics or Fisher exact test (where appropriate) were used to assess the correlation between the categorical variables. Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated as usual. Differences in the means of continuous variables between the groups were analyzed using nonparametric tests (Mann–Whitney). Performance indicators for HPV Linear Array in detection of the outcome variables were calculated using the conventional contingency tables for sensitivity, specificity, positive predictive value (PPV) and NPV, with 95% CI based on the F-distribution. The performance (SE/SP) of 2 tests was compared by using the area under ROC curve comparison test (STATA/SE 9.2). The agreement (reproducibility) between HCII and LA was calculated using Cohen κ and weighted κ [intraclass correlation coefficient (ICC)]. In all tests, the values $P < 0.05$ were regarded statistically significant.

Results

T1, AQ:3 The key characteristics of the patients are given in Table 1. The 2 most common referral Pap diagnoses were ASC-US and LSIL, together comprising almost 80% of the referrals. On colposcopy, however, 43.3% of the women did not demonstrate any cervical lesion, whereas high-grade abnormality was encountered in 71 (12.8%) of the women. Almost 40% of the biopsies only demonstrated a regular metaplastic process or an entirely normal cervix. Altogether, 97 women had CIN2+ lesion, and 3 invasive carcinomas were detected as well. Linear array was positive slightly more frequently than HCII test, 75.2% and 69.2%, respectively ($P = 0.034$).

The prevalence of individual HPV genotypes included in the LA test is shown in Figure 1. Of the 495 samples tested, 123 (24.8%) F1,AQ: 6 remained HPV negative. Following of the 37 genotypes in the assay were not detected: HPV26, 55, 61, 64, 69, 71, 72, 83, and IS39. Altogether, 357 cases (72.1%) were positive for a carcino- AQ: 4 genic HPV type, and 15 cases (3.0%) contained a low-risk HPV type. HPV16 was the single most frequent genotype (49/495; 9.9%), followed by HPV51 (3.2%), HPV66 (2.6%), HPV45 (2.4%), and HPV31 and HPV52 (2.2% both). Multiple infections comprised 36.2% (179/495) of the cases, with a multitude of combinations being discovered as single cases.

Table 2 summarizes the detection rates of carcinogenic HPV as T2 related to cytology, histology, and colposcopy. HCII results (13 carcinogenic types) are significantly related to diagnosis in referral Pap, whereas the LA (all 37 types) is less significantly related ($P = 0.040$). Both tests demonstrate a significant (linear) association with lesion histology, HPV detection reaching 100% in squamous cell carcinoma, and far above 90% in CIN2+ lesions. A major change in colposcopy is significantly associated with HPV detection by the two tests, with OR of 6.5 (95% CI 2.30–18.16) and 8.8 (95% CI 3.15–24.61) for LA and HCII tests, respectively.

Table 3 summarizes the concordance between HCII test and LA T3 with optional cutoffs and using regular (Cohen) κ and weighted κ (ICC). Using HCII and LA (with all 37 types) gives an overall concordance of 88.1% (433/491; 95% CI 85.3%–91.0%) in HPV detection, resulting in a substantial agreement with regular κ ($\kappa = 0.70$). Using weighted κ, this agreement is increased to almost

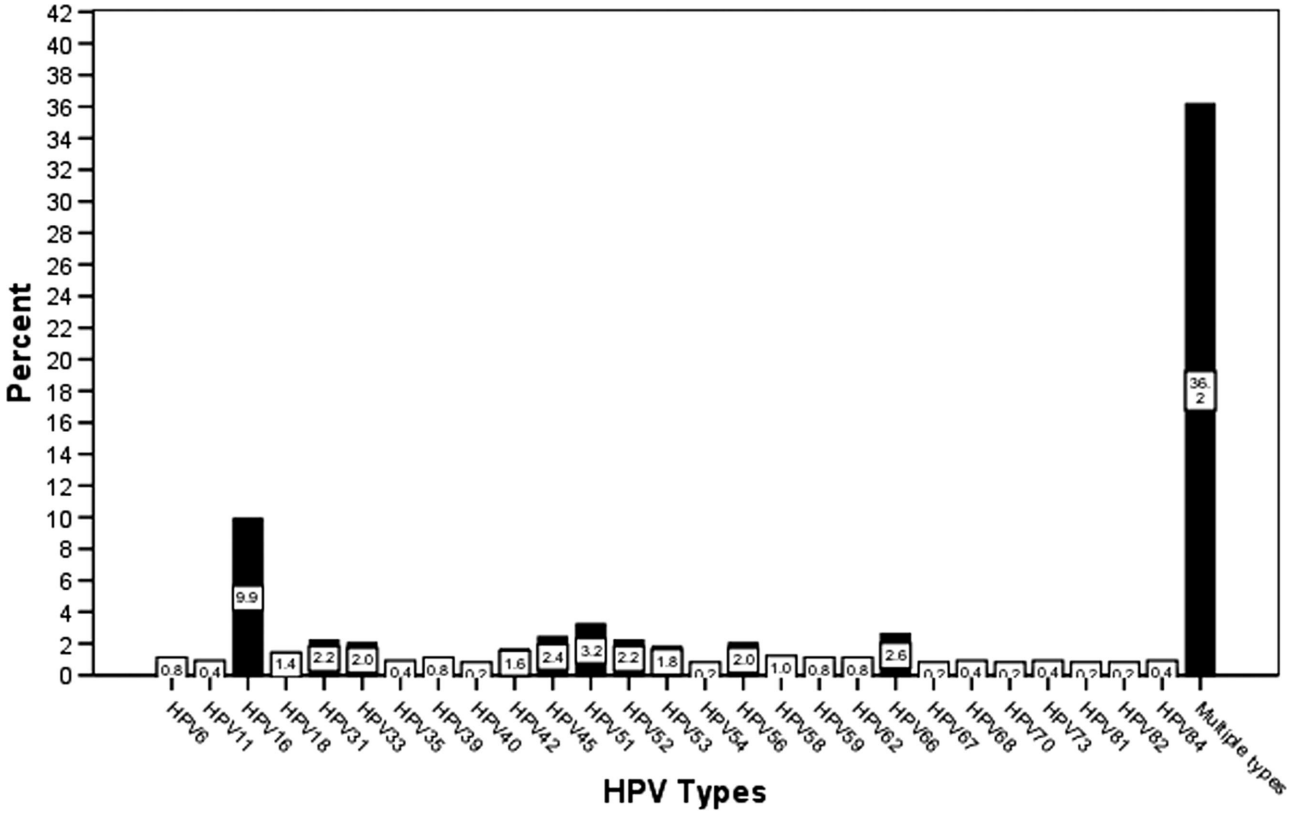

Fig. 1. Prevalence of the different HPV genotypes detected by Linear Array.

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TABLE 2. Detection of HPV Types by Linear Array (All Types) and HCII Test as Related to Cytology, Histology, and Colposcopy

	Linear Array Positive for Carcinogenic HPV*		HC Assay for Carcinogenic HPV	
Test	N (%)	*P*	N (%)	*P*
Referral pap				
Normal	10/12 (83.3)		10/20 (50.0)	
ASC-US	138/193 (71.5)	0.040†	132/206 (64.1)	0.001†
ASC-H	22/29 (75.9)		17/31 (54.8)	
LSIL	136/179 (76.0)		143/196 (73.0)	
HSIL	41/44 (93.2)		47/52 (90.4)	
AGUS/AGC	2/3 (66.7)		2/4 (50.0)	
Histology				
Negative	39/63 (61.9)		35/68 (51.5)	
Metaplasia	60/89 (67.4)		61/102 (59.8)	
HPV-NCIN	43/55 (78.2)		42/62 (67.7)	
CIN1	60/68 (88.2)	0.0001†	63/82 (76.8)	0.0001†
CIN2	26/28 (92.9)		29/32 (90.6)	
CIN3	55/56 (98.2)		55/60 (91.7)	
SCC	2/2 (100)		3/3 (100)	
VAIN1–3	3/3 (100)		3/3 (100)	
Colposcopy				
Normal	300/416 (72.1)	0.0001	304/464 (65.5)	0.0001
Abnormal‡	67/71 (94.4)		67/71 (94.4)	
	OR = 6.47 (95% CI 2.30–18.16)		OR = 8.81 (95% CI 3.15–24.61)	

*Carcinogenic types as defined by Munoz et al.[17]
†Fisher exact test.
‡Major change as cutoff.

perfect, ICC = 0.82. When only the carcinogenic HPV types[17,18] are included in LA test, this agreement further increases. If only the 13 genotypes of the HCII test are considered in Linear Array, the κ between HCII and LA falls below 0.60, which is the lower limit for substantial agreement.

The performance results of HCII and LA in detection of CIN2+ or CIN3+ is shown in Table 4. When HCII and LA performance is compared by area under ROC curve test, the only significant differences are obtained with LA-13 HCII types ($P = 0.0002$) or LA-HPV16 ($P = 0.0145$). Importantly, the performance of HCII and LA (with all 37 genotypes) is practically identical for CIN2+ and CIN3+; $P = 0.317$ and $P = 0.875$, respectively.

Performance indicators of HCII and LA as well as their comparison were separately calculated for those 211 women with ASC-US referral Pap test, as shown in Table 5. The only significant difference between HCII and LA is obtained when the latter is restricted to the 13 HCII genotypes in detection of CIN3+ ($P = 0.015$), and CIN2+ ($P = 0.055$). However, 100% sensitivity and 100% NPV is reached for LA, LA-HR-types, and LA-13 HCII types in detecting CIN3+ among these ASC-US patients, which is superior to HCII assay, and never reached in analysis of the whole series. The numbers in this table are relatively small, however, and not too much emphasis can be put on these 100% sensitivity figures.

Discussion

ROCHE Linear Array HPV Genotyping test (LA) detects 37 most frequent HPV genotypes,[31] whereas HCII assay used here as comparison detects 13 carcinogenic HPV types collectively.[13,30–36] So far, LA has been tested in a few studies where it was compared with other PCR-based HPV detection techniques or direct DNA sequencing.[37–41] Until now, HCII and LA have been directly compared only in one previous study.[42] The concordance between HCII, AMPLICOR (AMP), and LA tests in detecting carcinogenic HPV among a cohort of 1679 women with previous abnormal Pap smears was evaluated. Concordance was substantial between HCII/AMP (84.4% agreement, $\kappa = 0.64$) and HCII/LA (84.0% agreement, $\kappa = 0.63$), and near perfect between AMP/LA (97.8% agreement, $\kappa = 0.94$). These figures are very similar to those reported in the current study, with HCII/LA concordance of 88.1% (433/491; 95% CI 85.3%–91.0%; $\kappa = 0.70$; 95% CI 0.62–0.77).

We went on further to modify these calculations by setting different test positivity options for LA, i.e., for carcinogenic HPV types only, for the 13 HCII types, and for HPV16 only. The latter was defined test positive when HPV16 was present as a single type or included in any of the multiple-type combinations. Somewhat unexpectedly, when the 13 HCII genotypes were used as the cutoff for LA positivity, the HCII/LA concordance fell down to $\kappa = 0.57$, which is below the lower boundary of substantial concordance (Table 3). This must be an indication of different analytical sensitivity of these two tests, being higher for LA than that of HCII.[13,30,31,37,39,41] Evidently, LA misses some of the HCII genotypes, and on the other hand, cross-reacts with some others that are not included in the HCII panel, e.g., HPV66.[29] Accordingly, because of the different genotype coverage of these two assays, the intertest agreement is not perfect. Modifying the type coverage of LA, however, upgrades the concordance of these two tests to the level of almost perfect. It should be emphasized that any direct comparison of this assay must also take into account the prevalence of the individual genotypes, which varies from one geographic region to another.[17,19] The prevalence of the 37 individual

TABLE 3. Concordance Between HCII Test and Linear Array With Different Cutoffs

	Cohen's Kappa		Weighted Kappa (ICC)	
	κ (95% CI)	*P*	ICC (95% CI)	*P*
HCII + Linear array all 37 types*	0.695 (0.621–0.769)	0.0001	0.821 (0.786–0.850)	0.0001
HCII + Linear array carcinogenic HPV types†	0.725 (0.655–0.795)	0.0001	0.841 (0.810–0.867)	0.0001
HCII + Linear array 13 HCII types‡	0.569 (0.495–0.643)	0.0001	0.726 (0.639–0.787)	0.0001
HCII + Linear array HPV16 alone§‖	0.771 (0.689–0.853)	0.0001	0.871 (0.828–0.903)	0.0001

*McNemar test, $P = 0.149$.
†McNemar test, $P = 0.289$.
‡McNemar test, $P = 0.0001$.
§HPV16 detected as single-type infection and in any combinations (LA negative test as reference).
‖McNemar test, $P = 0.001$.
ICC indicates intra-class correlation coefficient (absolute agreement definition).

TABLE 4. HCII and Linear Array (With Different Cutoffs) in Detection of CIN2+ and CIN3+ in the Whole Series

Outcome Cutoff	Predictive Test and Cutoff	Sensitivity	Specificity	PPV	NPV	OR (95% CI)	Area Under ROC Curve
CIN2 histology	HCII assay	91.8 (84.5–96.4)	36.0 (30.7–41.6)	30.9 (25.7–36.6)	93.4 (87.4–97.1)	6.32 (2.96–13.50)	0.639 (0.601–0.677)*†‡§
	Linear array						
	All types	96.6 (90.5–99.3)	26.5 (21.4–32.2)	29.9 (24.6–35.5)	96.1 (88.9–99.2)	10.36 (3.17–33.77)	0.616 (0.584–0.648)*
	HR-types	95.5 (88.9–98.8)	33.1 (27.6–39.0)	31.6 (26.1–37.5)	95.8 (89.6–98.8)	8.30 (3.25–21.19)	0.643 (0.608–0.678)†
	13 HCII types	92.1 (84.5–96.8)	46.9 (40.9–53.0)	36.0 (29.7–42.6)	94.9 (89.7–97.9)	10.35 (4.61–23.20)	0.695 (0.654–0.736)‡
	HPV16 only‖	30.2 (20.8–41.1)	92.6 (88.0–95.8)	63.4 (46.9–77.9)	75.7 (69.9–80.9)	5.40 (2.68–10.86)	0.614 (0.562–0.666)§
CIN3 histology	HCII assay	92.4 (83.2–97.5)	33.5 (28.6–38.8)	21.0 (16.4–26.1)	95.9 (90.6–98.6)	6.15 (2.40–15.74)	0.630 (0.589–0.670)¶#**††
	Linear array						
	All types	98.4 (91.2–100)	24.8 (20.0–30.0)	20.8 (16.3–26.0)	98.7 (92.9–100)	19.73 (2.68–144.7)	0.616 (0.586–0.645)¶
	HR-types	98.4 (91.2–100)	31.0 (25.9–36.6)	22.3 (17.5–27.8)	98.9 (94.3–100)	27.40 (3.74–200.7)	0.647 (0.616–0.678)#
	13 HCII types	96.7 (88.7–99.6)	44.2 (38.5–50.0)	25.9 (20.3–32.1)	98.5 (94.8–99.9)	23.39 (5.61–97.48)	0.705 (0.669–0.741)**
	HPV16 only‖	35.0 (23.1–48.4)	91.2 (86.8–94.6)	51.2 (35.1–67.1)	84.2 (79.1–88.5)	5.60 (2.77–11.29)	0.631 (0.568–0.695)††

*AUC (area under ROC curve) comparison, $P = 0.317$.
†AUC comparison, $P = 0.620$.
‡AUC comparison, $P = 0.0056$.
§AUC comparison, $P = 0.071$.
‖HPV16 detected as single-type infection and in any combinations (LA negative test as reference).
¶AUC comparison, $P = 0.875$.
#AUC comparison, $P = 0.158$.
**AUC comparison, $P = 0.0002$.
††AUC comparison, $P = 0.0145$.

TABLE 5. HCII and Linear Array (With Different Cutoffs) in Detection of CIN2+ and CIN3+ Among Women (n = 211) With ASC-US Referral Pap Smear

Outcome Cutoff	Predictive Test and Cutoff	Sensitivity	Specificity	PPV	NPV	OR (95%CI)	Area Under ROC Curve
CIN2 histology	HCII assay	91.7 (73.0–99.0)	39.3 (30.6–48.6)	22.9 (15.0–32.6)	96.0 (86.3–99.5)	7.13 (1.60–31.73)	0.655 (0.584–0.726)*†‡§
	Linear array	95.7 (78.1–99.9)	29.1 (20.8–38.5)	22.0 (14.3–31.4)	97.0 (84.2–99.9)	9.02 (1.16–69.81)	0.624 (0.563–0.684)*
	All types						
	HR-types	95.7 (78.1–99.9)	36.4 (27.4–46.1)	23.9 (15.6–33.9)	97.6 (87.1–99.9)	12.57 (1.63–96.81)	0.660 (0.598–0.722)†
	13 HCII types	91.3 (72.0–98.9)	50.9 (41.2–60.6)	28.0 (18.2–39.6)	96.6 (88.1–99.6)	10.88 (2.43–48.69)	0.711 (0.636–0.786)‡
	HPV16 only‖	27.3 (10.7–50.2)	94.9 (87.4–98.6)	60.0 (26.2–87.8)	82.2 (72.7–89.5)	6.93 (1.75–27.45)	0.611 (0.512–0.709)§
CIN3 histology	HCII assay	91.7 (61.5–99.8)	36.6 (28.4–45.3)	11.5 (5.9–19.6)	98.0 (89.4–99.9)	6.34 (0.79–50.61)	0.641 (0.550–0.733)¶#**††
	Linear array	100 (73.5–100)	27.3 (19.6–36.1)	12.0 (6.4–20.0)	100 (89.4–100)	NC	0.636 (0.597–0.676)¶
	All types						
	HR-types	100 (73.5–100)	33.9 (25.5–43.0)	13.0 (6.9–21.7)	100 (91.4–100)	NC	0.669 (0.627–0.712)#
	13 HCII types	100 (73.5–100)	47.9 (38.8–57.2)	16.0 (8.6–26.3)	100 (93.8–100)	NC	0.740 (0.695–0.784)**
	HPV16 only‖	33.3 (9.9–65.1)	93.2 (85.7–97.5)	40.0 (12.2–73.8)	91.1 (83.2–96.1)	6.83 (1.58–29.38)	0.633 (0.491–0.774)††

*AUC (area under ROC curve) comparison, $P = 0.629$.
†AUC comparison, $P = 0.455$.
‡AUC comparison, $P = 0.055$.
§AUC comparison, $P = 0.567$.
‖HPV16 detected as single-type infection and in any combinations (LA negative test as reference).
¶AUC comparison, $P = 0.851$.
#AUC comparison, $P = 0.357$.
**AUC comparison, $P = 0.015$.
††AUC comparison, $P = 0.369$.
NC indicates noncomputable (one cell empty).

types in our cohort follows the general European pattern (Fig. 1). Similarly, more and more data are emerging, suggesting that HCII assay cross-reacts with several HR-types not included in the HCII test panel, e.g., HPV66 and HPV53.[29] Detailed discussion about these implicated cross-reactivity issues (e.g., HPV18 with HPV45) is not possible in this context, however, but explains in part the different concordance between HCII and LA, when the type coverage is changed, as done here to demonstrate this fact (Table 3).

Particular attention was paid to women referred due to equivocal (ASC-US) smear, which comprise a particular problem in clinical management practice, and analyzed as a special subgroup in the current study. In the only previous study, where HCII and LA were directly compared, carcinogenic HPV prevalence among women with cytologic or histologic high-grade disease (CIN2 or greater) detected by LA was significantly higher ($P = 0.0001$) when compared with detection by HCII.[42] This was also confirmed in the current study (Table 2); LA detected more HPV among LSIL and HSIL lesions than did HCII, and the same was true with all grades of CIN lesions. In the present series, these differences were not remarkable, however, and did not reach statistical significance in any of the SIL or CIN grades. The same holds true if only carcinogenic HPV types are considered in the LA assay. Importantly, both tests are identical in detecting HPV in women with normal and abnormal (major change) colposcopy (Table 2). This implicates that both HCII and LA detect HPV in an equivalent manner across the spectrum of SIL and CIN categories. Because the test performance is also critically dependent on prevalence of HPV and CIN in the population, the only feasible means to compare individual tests is to run them in parallel in the same study setting.[43,44]

Until present, there is only one previous study, where the performance indicators of HCII and LA have been directly compared.[42] In that study, LA showed higher sensitivity but lower specificity than HCII for detecting carcinogenic HPV among women with high-grade disease. These observations were fully confirmed in the current study. Indeed, LA showed some 5% higher sensitivity when compared with HCII assay, irrespective of which LA cutoff was used, and applied similarly to the CIN2+ and CIN3+ cutoffs (Table 4). On the other hand, specificity of HCII assay (33.5%–36.0%) was some 8% to 9% higher than that of LA (37 genotypes) test in detecting both CIN2+ and CIN3+. With different "modifications" of the LA test, however, its specificity could be increased up to 46.9%, still maintaining the sensitivity level of HCII test. One of these"modifications" was to consider HPV16 genotype (single-infection, n = 49) as the cutoff for LA+ test. When compared with the full LA, there was a dramatic increase in specificity (up to 92.6%) and PPV (up to 63.4%) in detection of CIN2+. Negative LA as reference, HPV16 positive test predicts CIN3+ with OR = 78.75 (95% CI 9.97–621.48) and shows 95.5% SE, 78.9% SP, 51.2% PPV, and 98.7% NPV (data not in Table).

These figures were even better when only women with ASC-US cytology were analyzed, as done in this study as a special subgroup (Table 5). When HPV16 genotype was used as positive LA test, there was an even more accentuated increase in specificity (up to 94.9%) and PPV (up to 60.0%) in detection of CIN2+. In such a setting, HPV16 positive test predicts CIN3+ with 100% SE, 84.6% SP, 87.0% PPV, and 100% NPV (OR not calculable). It should be borne in mind, however, that these calculations are based on small numbers of cases, and because of that, may be of limited statistical power. However, this observation is of potential value in management or triage of women with ASC-US cytology. Among these women, clinical decisions are usually made according to the risk state, i.e., women at the greatest risk are managed more aggressively than those at low risk.[25] One potential concern of this line of thinking, however, is that such a risk stratification will lead to more aggressive management of all carcinogenic women but without concomitant less aggressive management of low-risk women.[25,36] This approach also raises the important question concerning the minimum risk for developing CIN3+ that should warrant colposcopic evaluation. Current US guidelines suggest HPV triage of ASC-US and carcinogenic HPV positive ASC-US or LSIL+ cytology warrants colposcopic evaluation.[45] This is based on the experience from the ALTS trial, where the 2-year risk of CIN3+ in this group of women was ≥15%. This increased risk of high-grade CIN was clearly confirmed in our series as well, where HPV16+ women with ASC-US cytology had OR = 48.00 (95% CI 4.53–507.56) for CIN2+ (OR for CIN3+ not calculable). Thus, our data implicate that including HPV16 genotyping in the diagnostic repertoire of these women would lead to highly specific detection of CIN2/3+ lesions, which is not achieved if the test is used for all 37 genotypes or even for the carcinogenic HPV types (Table 5).

To conclude, despite their different type coverage, LA and HCII tests show substantial agreement. Among women with ASC-US cytology, high specificity is obtained by HPV16 genotyping only. As emphasized in a recent consensus statement,[25] genotyping may prove useful in stratifying HPV-positive women according to their risk of developing high-grade CIN, which enables tailored management strategies.[25] To achieve the full benefit to the patients, however, addition of HPV genotyping to screening and management protocols should not be compromised by excessive referrals for colposcopy, which can be a real danger, if poorly validated HPV tests are used.[25] Furthermore, with the introduction of prophylactic HPV vaccines (against HPV16 and HPV18), we can anticipate a reduction of 60% to 70% of abnormal Pap smears, and genotyping for these 2 HPV types should have implications in monitoring these vaccine effects as well.

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AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES 1

AQ1— Kindly note that Refs. 27–29 are renumbered in both the text and the reference list as they are not in sequence. Please check.

AQ2— Please provide expansions for PCR, LSIL, and ALTS.

AQ3— Kindly check the minor edits made in all the tables.

AQ6— Production Editor: Please note that there was a phrase "A separate file. . . . . ." at the end of the figure legend and this has been deleted. Should author be queried for this?

AQ4— Kindly check the sentence "Following of the 37 genotypes. . . " for clarity.

AQ5— Kindly check whether the volume number and the page range are Ok as given.