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Age-specific detection of high risk HPV DNA in cytologically normal, computer-imaged ThinPrep Pap samples

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Abstract

Objective. Recent cervical cancer screening guidelines for women over age 30 seek to improve the sensitivity of cytology by incorporating high-risk (HR) human papillomavirus (HPV) DNA testing into the screening algorithm, a recommendation based largely on data that utilized the conventional Pap smear and were not stratified by patient age. Data on the rate of HR HPV among women over age 30 undergoing liquid-based Pap test screening are limited. The objective of this study was to determine the rate of HR HPV DNA positivity in women ages 30 and over with a cytologically negative liquid-based Pap test result.

Methods. Consecutive residual ThinPrep Pap samples from women with a cytologically negative result following computer-assisted screening were tested for HR HPV using the Hybrid Capture 2 (HC2) method. All HC2-positive samples were additionally tested with the Linear Array (LA) HPV Genotyping Test.

Results. 1000 cytologically negative specimens from women aged 30 to 45 years (38.9±4.7 years) were evaluated. The overall HC2 HR HPV positivity rate in this age group was 3.9% (confidence interval 2.8–5.3%). When stratified by age group, the rate was inversely proportional to age (ages 30–35: 6.7%; 36–40: 3.0%; 41–45: 2.6%) and lower than most previous reports (1–17%). Some of the cases that were positive for HR HPV by HC2 were negative by LA, or showed only low-risk virus.

Conclusions. The HR HPV rates in women ages 30–45 with a cytologically negative, computer-imaged ThinPrep test result are low. If these findings are confirmed in future studies, the added benefit of HPV testing to liquid-based cytology for women ages 30 and over should be critically evaluated.

Keywords: Cytology; Pap test; Human papillomavirus; Cervical cancer; Cancer screening

Introduction

Recent cervical cancer screening guidelines of the American Cancer Society (ACS) and the American College of Obstetricians and Gynecologists (ACOG) seek to improve the sensitivity of the Pap test by incorporating adjunct testing for the oncogenic, or high risk (HR), types of the human papillomavirus (HPV) in women ages 30 and over [1,2]. HPV infections in women younger than 30 are very common and often transient, and thus often have no clinical implications. In a woman over 30, however, a positive HR HPV test, even if the Pap were normal, might suggest a chronic HPV infection that is more likely to lead to a significant cervical cancer precursor lesion.

The Hybrid Capture 2 (HC2) test was approved by the Food and Drug Administration (FDA) in 2003 for just such an indication in women 30 years of age or older. To date, published data on the added value of HR HPV DNA analysis have looked primarily at the conventional Pap smear [3]. Studies that examined HPV testing in the context of liquid-based Paps used polymerase chain reaction (PCR) technology rather than HC2, the only FDA-approved HPV technology for women aged 30

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and over [4–17]. The distinction is important because liquid-based Paps are more common than conventional smears in the U.S. and may be more sensitive in detecting cervical cancer precursors [18–21].

The purpose of the current observational study is to establish the baseline rates of HR HPV DNA within 3 age groups in women ages 30 and over with a cytologically negative ThinPrep Pap. Knowledge of these rates might be useful in the development of future screening guidelines.

Methods

Consecutive liquid-based cervical cytology samples that met the following inclusion criteria were identified at the Brigham and Women's Hospital (BWH): cases were obtained from patients 30 to 45 years of age; prepared using the ThinPrep method (Cytyc Corp., Marlborough, MA); interpreted as "negative for an intraepithelial lesion or malignancy" (NILM) by a cytotechnologist whose cytological evaluation was aided by the ThinPrep Imaging System (TIS); contained greater than 10 ml residual sample volume; less than or equal to 16 days since specimen collection; no prior HC2 DNA analysis on the sample; not subjected to glacial acetic acid reprocessing. Samples were obtained in the following numbers from these age groups: 300 specimens from women aged 30 to 35; 200 specimens from women aged 36 to 40; 500 specimens from women aged 41 to 45. Institutional review board approval for research on discarded materials was obtained.

The TIS (Cytyc Corp., Marlborough, MA), an FDA-approved computer-based imaging system, was used to assist the cytotechnologist (CT) in the evaluation of Pap slides [22]. The TIS guided the CT via the review microscope to selected areas of interest identified during the computer imaging process. Based on programmed algorithms, the TIS selected a subset of approximately 20% of the total cell spot on the slide for the CT to review. If those areas appeared normal, a full review of the entire cell spot was not required and the Pap was reported as "negative for an intraepithelial lesion or malignancy". If any abnormal cells were identified during the TIS-guided review, however, a full review of the entire cell spot was performed.

Cytologic interpretations were rendered using the Bethesda System terminology [23]. Residual ThinPrep Pap vials were de-identified and assigned a unique study identification number at BWH. This identification number was used throughout the study for tracking purposes. The age of patient, the cytologic interpretation, and the date of specimen collection were provided with each vial.

All specimens were tested using the Hybrid Capture® 2 High-Risk HPV DNA Test (HC2) (Digene Corporation, Gaithersburg, MD) within 21 days from the date of specimen collection. Testing was performed according to procedures outlined by the manufacturer. ThinPrep specimens were prepared for HPV testing using the HC2 Sample Conversion Kit. Six milliliters (ml) from each residual specimen were processed with the conversion kit, ensuring enough processed specimen to complete three HC2 tests. This allowed for retesting to resolve equivocal results when necessary. Converted specimens were then used in the HC2 assay as per standard procedures. Each HC2 batch consisted of only initial test specimens, only retest specimens, or a combination of both initial test and retest specimens (although not from the same vial).

Table 1
Frequency of HC2-detected high risk HPV by age group in women with negative ThinPrep Paps

Age group	*N*	# HPV positive	% HPV positive	95% confidence interval
30–35	300	20	6.7	4.1–10.1
36–40	200	6	3.0	1.1–6.4
41–45	500	13	2.6	1.4–4.4
30–45	1000	39	3.9	2.8–5.3

HC2 = Hybrid Capture 2.
HPV = human papillomavirus.

Table 2
Results of Linear Array genotyping on the cases positive for high risk HPV by HC2

Age group	HC2 HPV positive	LA HPV negative	LA low risk positive	LA high risk positive
30–35	20	7 (35%)	2 (10%)	11 (55%)
36–40	6	3 (50%)	0	3 (50%)
41–45	13	4 (31%)	2 (15%)	7 (54%)
30–45	39	14 (36%)	4 (10%)	21 (54%)

HC2 = Hybrid Capture 2.
HPV = human papillomavirus.
LA = Linear Array.

All specimens that yielded HR HPV DNA positive results by HC2 underwent HPV genotyping using the Linear Array (LA) HPV Genotyping Test (Roche Molecular Systems, Inc., Brandenburg, NJ). Testing was performed according to the manufacturer's instructions. DNA was extracted within 21 days of the specimen collection date using the Qiagen QIAamp® MinEluteis™ Media Kit (Qiagen Inc., Valencia, CA). The extracted DNA was frozen in two 60 uL aliquots to allow re-testing if necessary, without having to extract additional DNA from the original residual specimen. Extracted DNA from specimens was stored at −20°C until batches of 10 or 22 specimens were accrued for a LA test. Because extracted DNA is stable for 8 weeks at −20°C, at the end of 8 weeks from the first DNA extraction an LA test was performed even if fewer than 22 specimens had accrued. If more than 22 specimens required typing, specimens were batched in groups of 10 or 22 (when appropriate) or fewer. All specimens were tested within the 8-week window. Assays for high and low β-globin were performed as internal controls.

Results

Specimens were collected from September to December of 2005. During this time period, the Cytopathology Laboratory at BWH examined an annual volume of 57,585 Pap tests, of which 28,040 (48.7%) were examined using the ThinPrep Imaging System. Approximately 20% of the cases submitted to the Laboratory were from health care clinics that serve a predominantly indigent patient population. In 2005, 88.7% of cases from the Laboratory were reported as NILM, including cases with reactive changes.

In total, 1001 specimens were examined. One was excluded from analysis because of a cytologic interpretation of atypical squamous cells of undetermined significance (ASCUS), which did not meet inclusion criteria. A total of 1000 cytologically negative specimens from women aged 30 to 45 years (38.9±4.7 years) were evaluated. 39 samples were positive for HR HPV DNA by HC2. The overall HC2 HR HPV positivity rate in this age group was 3.9% (confidence interval 2.8–5.3%) (Table 1). When stratified by age group, the rate was inversely proportional to age (ages 30–35: 6.7%; 36–40: 3.0%; 41–45: 2.6%).

Not all the cases positive for HR HPV DNA were confirmed positive by LA analysis. Some of the cases that were positive for HR HPV by HC2 were negative by LA, or showed only low-risk virus. Of the 39 cases positive for HR HPV by HC2, 21 (54%) were positive for HR HPV DNA by LA analysis (Table 2). 14 cases (35%) were negative, and 4 (10%) showed only low risk HPV types. The list of HPV genotypes is shown in Table 3. Of the 25 HPV-positive cases by LA, 16 showed a single genotype, 7 showed 2 genotypes, and 2 showed 3 or