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High-risk HPV DNA detected in less than 2% of over 25,000 cytology negative imaged liquid-based Pap test samples from women 30 and older ☆

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ABSTRACT

Objective. The purpose of this study was to document the prevalence of high-risk HPV DNA (HPV) in the largest cohort of woman studied to date with negative ThinPrep Imaging system (TIS)-imaged Pap tests.

Methods. Women with negative (TIS)-imaged ThinPrep Pap Tests (TPPT) who also were tested for HPV were identified between July 1, 2005 and December 31, 2007 from a large women's hospital practice. HPV detection rates were compared for women with either presence or absence of a transformation zone/endocervical cell sample (EC/TZS).

Results. 26,558 negative TPPT also underwent HPV testing. HPV detection was higher in women younger than 30 and sharply declined in women 30–39 ($P<0.001$). Declining HPV detection rates continued in the 40–49 age group (age 30–39 vs. 40–49; 2.8% vs. 1.7%, $P<0.001$) and then levelled off. No statistically significant difference for HPV prevalence was identified comparing women with and without a TZ/ECS.

Conclusion. This is the largest study to date documenting very low HPV detection rates in women screened cytology negative with computer-imaged liquid-based Pap methods now representing a major portion of the U.S. cervical cytology market. Findings of very low rates of HPV detection in 490 (1.9%) of 25,259 cytology negative women 30 and older extend and confirm previously reported findings in smaller study populations. Because HPV testing provides an objective measure of relative residual risk for cervical neoplasia after screening, these data are relevant to discussions on how best to combine cytology and HPV testing in screening low risk populations.

High-risk (hr) human papillomavirus (HPV) infections are now recognized as the dominant worldwide etiology for cervical cancer [1–4]. In 2003, the US Food and Drug Administration (FDA) first approved high-risk human papillomavirus DNA (hrHPV DNA) primary etiology-based testing as an adjunct to routine cytology screening for women 30 and older [5]. Contemporaneous cervical cancer screening guidelines from the American Cancer Society (ACS) and the American College of Obstetricians and Gynecologists (ACOG) in effect acknowledged the extremely high sensitivity of FDA-approved Pap and HPV co-testing by specifically accepting lengthened screening intervals for women who test negative on both cytology and HPV tests [6,7]. However, routine HPV and cytology co-testing are not currently recommended in woman younger than 30, as infections in younger women are much more prevalent and usually reflect transient infections of limited clinical consequence [8]. In women over 30, in contrast, a positive hrHPV test indicates an increased likelihood of a persistent infection and increased risk for a significant cervical cancer precursor lesion [9]. Detection rates reported for hrHPV DNA have varied significantly between different countries and regions and in different local populations and age groups, based on both local infection rates as well as on the frequency and methods of cytologic screening and treatment of cervical lesions [10–12]. A number of international clinical trials [13–15] and modeling studies [16–22] have attempted to further evaluate various cervical cancer screening formulations by using different combinations of conventional and liquid-based cervical cytology (LBC) and hrHPV DNA testing, including primary HPV screening formulations not yet submitted for U.S. FDA premarket approval (PMA).

One recent modeling study by US Army-funded clinical investigators concluded that the most cost-effective FDA-approved combination screening strategy was LBC every 2 years with limited hrHPV DNA testing only after a cytological finding of atypical squamous cells of undetermined significance (ASC-US) [16]. These investigators concluded that routine cytology and hrHPV DNA co-testing, although predicted in their model to be the most successful strategy for preventing cervical cancer deaths, was questionably cost-effective. Few studies, however, have examined the impact of location-guided computer-assisted screening of LBC, approved by the FDA in 2003 and now widely used in the US with the ThinPrep Imaging System (TIS) [23], on evaluations of different possible screening formulations that use both cytology and hrHPV DNA testing. A number of studies now

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indicate that this technology even further enhances [24–27] the already improved ability of LBC [15,28–31] to reliably detect significant precancerous and neoplastic cervical lesions compared to screening with the conventional Pap smear.

The purpose of our study was to document detection rates of hrHPV DNA in a large, low risk, older than average U.S. population. hrHPV DNA tests results were available from over 26,000 samples with companion negative ThinPrep Imaging System (TIS)-imaged ThinPrep Pap Test (TPPT) results.

Materials and methods

After obtaining institutional review board approval at the University of Pittsburgh Medical Center (UPMC), a retrospective study was initiated. A computer-based search of Copath database (Cerner, Kansas city, MO) of Magee-Womens Hospital (MWH) of the University of UPMC was carried out over a 30-month period between July 1, 2005 and December 31, 2007 to retrieve women with ThinPrep Pap Test (TPPT) reported as negative for intraepithelial lesion or malignancy who also were tested for hrHPV DNA. Vaginal Pap tests were excluded from this study. The MWH/UPMC cytopathology laboratory is a large subspecialized academic hospital laboratory which consistently reports over 100,000 Pap tests per year from a large integrated 20 hospital health system and which serves a metropolitan area with a significantly older age profile than the national average [32]. The reporting profile of the laboratory is now documented in numerous recent publications [27,33–35].

TPPT were prepared according to manufacturer's specifications from PreservCyt (Cytyc, Marlborough, Mass) samples by using an automated processor (ThinPrep 3000). Staining of slides was performed on a Sakura Tissue Tek Automated Slide Stainer (Sakura Finetek USA Inc, Torrance, CA) (Somagen Diagnostics, Edmonton, Alberta, Canada) according to an FDA-approved manufacturer's protocol. Location-guided computer-assisted screening of TPPT slides was accomplished by using the TIS. The TIS performed analysis of batches of up to 250 ThinPrep Pap test slides with specialized imaging software. For each slide, the locations of 22 microscopic fields that contained cells or cell clusters of interest were recorded. The imaged TPPT slides were placed on cytotechnologist review scopes, and the cytotechnologists reviewed the 22 fields in geographic order. If the cytotechnologists found no abnormalities on those 22 fields, the cytotechnologist could sign out the case as negative. In all cases in which any of the 22 fields contained any abnormality, reactive or reparative cellular changes, or microorganisms, the cytotechnologists manually rescreened the entire TPPT slide. All cases interpreted by cytotechnologists as abnormal or as showing reactive or reparative changes were referred to a pathologist for review.

hrHPV DNA testing was ordered by clinicians according to several ordering options as follows: reflex triggered by indeterminate abnormal atypical squamous cell (ASC) Pap test results, co-testing with Pap tests in women 30 and over, and co-testing regardless of age or Pap test results. If hrHPV DNA was detected in negative Pap tests, the Pap test slides were routinely manually rescreened by the screening cytotechnologist, referred for further manual rescreening by a quality-assurance cytotechnologist, and then also reviewed by a pathologist.

hrHPV DNA detection was performed by the commercially available FDA-approved HC2 method [36] (Digene Corp., Gaithersburg, MD), which tests for high-risk and intermediate-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.

The study population was categorized at 10-year interval into various age groups from age 10 to age 80. Age specific hrHPV prevalence and detection rates with either presence or absence of transformation zone/endocervical cell sample (EC/TZS) were compared between women.

Confidence intervals (95% CI) for the different frequencies of hrHPV DNA detection were obtained by a Wald test. The positive rate of hrHPV DNA for each age group was compared with that of the reference group. hrHPV detection rates between women with and without an EC/TZS were compared. Statistical analyses were performed by Chi-square test or Fisher's exact test for small number using SAS 9.1 system (SAS Institute Inc., Cary, NC). P values of <0.05 were considered statistically significant.

Results

During the 30-month study interval between July 1, 2005 and December 31, 2007, a total of 26,558 negative TPPT also had hrHPV testing. The age of women with cytologically negative TPPT and hrHPV DNA testing ranged from 11 to 90 years. The comparison of age-specific hrHPV prevalence among women with negative TPPT with and without TZ/ECS (10-year intervals) is shown in Table 1. Cytology negative women who tested positive for hrHPV DNA were significantly more likely to be younger, with peak infection rate occurring in women younger than 30 years, and there was a clear decline in the hrHPV DNA prevalence in older age groups.

Further analysis was carried out on hrHPV DNA prevalence in TIS-imaged cytology negative TPPT; hrHPV DNA prevalence was highest (8.1%) in women ages 11–29 years and decreased to 1.9% in women older than 40 years. hrHPV DNA prevalence was statistically significantly higher in women younger than 30 years compared with women ages 30 years and older (Table 2). The difference in hrHPV DNA prevalence between women ages 11 to 19 years and women ages 20 to 29 years was not statistically significant ($P = 0.977$). A statistically significant decline in hrHPV DNA prevalence from 8.1% in women 20–29 years of age to 2.8% in women in 30–39 years of age ($P<0.001$) was observed. hrHPV DNA prevalence continued a statistically significant decline to 1.7% in women ages 40–49 years (comparison of hrHPV DNA prevalence between women ages 30–39 years vs. 40–49 years, 2.8% vs. 1.7% $P<0.001$). In subsequent age groups, hrHPV DNA prevalence did not significantly continue to decline and remained fairly stable in women after the age of 40 years.

Table 1
Comparison of age-specific hrHPV prevalence among women with negative TPPT with and without TZ/ECS (10-year intervals).

Age group	Tested no.	Positive no.	% (95% CI)	TZ/ECS present			TZ/ECS absent			P value
				Tested no.	Positive no.	% (95% CI)	Tested no.	Positive no.	% (95% CI)	
10–	162	13	8.0 (3.8–12.2)	136	11	8.1 (3.5–12.7)	26	2	7.7 (0–17.9)	1.000[a]
20–	1137	92	8.1 (6.5–9.7)	904	74	8.2 (6.4–10.0)	233	18	7.7 (4.3–11.1)	0.818
30–	6898	190	2.8 (2.4–3.2)	5836	154	2.6 (2.2–3.0)	1062	36	3.4 (2.3–4.5)	0.169
40–	8137	135	1.7 (1.4–2.0)	6810	104	1.5 (1.2–1.8)	1327	31	2.3 (1.5–3.1)	0.035
50–	7026	112	1.6 (1.3–1.9)	5103	79	1.6 (1.3–1.9)	1923	33	1.7 (1.1–2.3)	0.616
60–	2584	39	1.5 (1.0–2.0)	1655	21	1.3 (0.8–1.9)	929	18	1.9 (1.0–2.8)	0.181
70–	522	10	1.9 (0.7–3.1)	292	5	1.7 (0.2–3.2)	230	5	2.2 (0.3–4.1)	0.703
80–	92	4	4.3 (0.2–8.4)	57	3	5.3 (0–11.1)	35	1	2.9 (0–8.5)	1.000[a]
Total	26,558	595	2.2 (2.0–2.4)	20,793	451	2.2 (2.0–2.4)	5765	144	2.5 (2.1–2.9)	0.136

hrHPV indicates high-risk human papillomavirus; TPPT, ThinPrep Pap Test; TZ/ECS, transformation zone/endocervical cell sample; CI, confidence interval.
[a] Fisher's exact test.

Table 2
Comparison of hrHPV DNA-positive rates between women ages ≥30 years and younger with imaged negative TPPT.

Age	Tested no.	Positive no.	% (95% CI)	*P*
<30	1299	105	8.1 (6.6–9.6)	<0.001
≥30	25,259	490	1.9 (1.7–2.1)	

hrHPV indicates high-risk human papillomavirus; TPPT, ThinPrep Pap Test.

The relationship between hrHPV DNA and the presence or absence of EC/TZS was also calculated. No statistically significant difference of hrHPV prevalence was present between women with and without a TZ/ECS except for age 40–49 years in which hrHPV rate was slightly higher in TZ/ECS absent group than that in TZ/ECS present group.

The total number of negative Pap tests with hrHPV DNA testing significantly increased for the women age 30 and over in 2007 [2007 (12 months) vs. July 2005–December 2006 (18 months), 17256 vs. 8003], reflecting increased physician orders for DNA with Pap co-testing in women 30 and older.

Discussion

This is the largest reported study to date documenting hrHPV DNA detection rates in women with negative cytology results utilizing computer-assisted screening methods which now represent a major portion of the U.S. cervical cytology market. We detected hrHPV DNA in only 490 of 25,259 (1.9%) negative cytology specimens in women 30–90 years old. In one other academic laboratory, a very low hrHPV DNA rate of 3.9% was reported in 1000 TIS-imaged cytology negative women aged 30–45 years (38.9 ± 4.7 years) [37]. We also reported earlier an even lower hrHPV prevalence of 2.4% in a smaller study population of 7426 women 30 and older with negative imaged Pap tests [38]. The current study confirms and extends those observations with an over three times larger data set of over 26,000 imaged cytology negative patients.

Worldwide prevalence of HPV DNA in women with normal cytology utilizing both conventional and liquid-based cytology has been reported to vary from 1% to 35.4% [10–12,39–52]. This wide variation reflects in part the wide variety of Pap test preparations and HPV testing methods used and also the absence of age stratification data in many of the reports. In some studies Paps were prepared using liquid-based methods, but most reported data reflects use of conventional smear cytology. In some reports, smears were even obtained using cotton swabs, a cell-trapping, false negative-promoting methodology discouraged today in the U.S. [53]. HPV testing methods have also been variable. Some investigators have used the HC2 method, but most have used PCR. In general, lower rates of prevalent HPV in different populations of women with negative cytology results reflect some combination of lower population infection rates and increased rates of screening detection and ablation of HPV-associated cervical lesions in screened populations [11].

Our results document that overall no statistically significant difference in hrHPV prevalence was present when comparing imaged cytology negative patients with and without an EC/TZS. This observation is consistent with our previous report that detection of hrHPV DNA in TPPT vials is independent of sampling of the transformation zone [34]. We have also previously reported that although cytologic detection rates for low grade squamous intraepithelial lesions (LSIL) and high grade squamous intraepithelial lesions (HSIL) may be significantly higher in women with an EC/TZS compared to women without an EC/TZS, when hrHPV DNA detection rates in LSIL and HSIL are compared in women with and without an EC/TZS no statistically significant difference is present [35]. Therefore, we believe that HPV prevalence in cytology negative women in screened populations provides a valuable objective measure of relative residual risk for cervical neoplasia following different methods of screening.

In 2003, the FDA approved the HC2 HPV DNA test for adjunctive use along with Pap testing in cervical screening of women ages 30 years and older [5]. The high rate of hrHPV DNA test results in women younger than 30 years of age was judged, at that time, to preclude broader routine application of adjunctive co-testing in younger women. A major rationale for FDA approval for women 30 and older was the low risk for development of cervical intraepithelial neoplasia CIN3+ lesions in women who tested negative with both Pap testing and HC2 hrHPV DNA testing [54]. Cost-benefit analyses available at that time also suggested that the increased cost of combining HPV DNA testing with cytology could be somewhat offset by increasing the screening interval for double-negative women over 30 years [55].

In our laboratory, hrHPV DNA was detected by HC2 in only 1.9% of over 25,000 women 30 years of age or older with cytology negative TIS-imaged TPPT. The ability of newer cytology-based methods to identify negative Pap test slides in women with very low residual hrHPV DNA rates suggests that the cost effectiveness of adding routine hrHPV DNA co-testing to TIS-imaged Pap testing should be re-evaluated against a more selective strategy of reflex hrHPV DNA testing limited to patients with indeterminate (atypical) Pap test results. Bidus' US Army evaluation, for example, showed that a strategy of LBC and reflex-limited HPV testing every 2 years was clearly cost-effective with an estimated incremental cost-per-life-year saved of $56,728 [16]. This cost-effective strategy dominated routine hrHPV DNA and Pap co-testing at both 2-year and 3-year intervals because the significantly higher costs associated with routinely combining hrHPV DNA testing (CPT code 87621; 2007 Medicare payment $49.04) with LBC (CPT code 88142; 2007 Medicare payment $28.31) for routine primary screening. The cost disadvantage for routine primary hrHPV DNA co-testing is not significantly different when HPV testing is combined with TIS-imaged Pap screening (CPT code 88175; 2007 Medicare payment $37.01). In one preliminary cost-effectiveness model evaluation, the TIS was judged to be a highly cost-effective screening strategy [56].

The likelihood of cytology negative women with a single positive HC2 hrHPV DNA test of developing highly significant (CIN3+) precancerous or malignant lesions in long-term (10-year) follow-up appears generally to be very low in North America [57–61]. Specifically, in the Portland, Oregon, National Cancer Institute prospective risk study that used conventional Pap smears rather than LBC, CIN3+ lesions developed over 10 years in only 88 of 2941 women (3%) who tested HPV positive at enrollment with any Pap result other than HSIL or cancer [58]. For the 2562 HPV positive women with negative enrollment cytology [60], only 52 of the 2562 (2.0%) developed CIN3+ lesions over the 10-year study period [61]. Finally, for the 2562 HPV positive women with negative enrollment cytology [60], only 30 of the 2562 (1.2%) who also tested positive for HPV 16 or HPV 18 developed CIN3+ lesions over the 10-year study period [61]. In screening programs where women are traditionally screened at lengthier intervals [62] and where cytologists are significantly less likely than North American cytopathologists to interpret cytologic findings on any given Pap test as abnormal [63], different follow-up findings can be expected. For example, in a recent Danish prospective risk study that used conventional Pap smears, the 10-year CIN3+ risk reported in cytology negative hrHPV DNA-positive patients was somewhat higher and was highest in older women [64].

Results recently reported from the U.K. ARTISTIC trial, the first randomized trial to report a comparison of LBC plus HPV testing against LBC alone, surprised some observers when it showed that LBC and HPV co-testing did not detect a higher rate of CIN2+ or CIN3+ than manually screened LBC alone [15], findings different from similar trials using the conventional Pap smear [13,14]. The authors also observed that "LBC and automated slide presentation of fields at higher risk of abnormality might result in a more consistent range of sensitivity between laboratories" [15]. The very low hrHPV DNA

prevalence documented here in TIS-imaged, LBC-negative women 30 and older are consistent with this data. In fact, our 1.9% negative imaged LBC-positive hrHPV rate is almost 50% lower than the 3.99% negative conventional smear-positive hrHPV rate reported from a 5-year experience HPV DNA and Pap smear co-testing documented from Kaiser Permanente in Northern California [65]. Future prospective trials in the U.S. and elsewhere may need to compare the very low risk for undetected significant cervical disease after imaged LBC screening with the risk for undetected significant cervical disease in patients with negative HC2 hrHPV DNA test results [14,66]. A recent editorial, authored by high profile experts involved in the ALTS trial, stated that according to today's "achievable standards," a range of 89%–95% hrHPV DNA test sensitivity for CIN3+ is "acceptable" [67]. Our recent report of verification bias-adjusted histopathological outcomes from over 400,000 patients screened at MWH using computer-imaged LBC over 4 years indicates that this level of performance is achievable utilizing modern state of the art cytology-based methods [27]. We are now several years into a long-term follow-up study of TIS-imaged, cytology negative, hrHPV DNA-positive women in this large integrated health plan practice [68].

Conflict of interest statement

The authors declare that they have no competing interests.

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