

CONCISE COMMUNICATION

Human Papillomavirus DNA Remains Detectable Longer than Related Cervical Cytologic Abnormalities

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Cervical human papillomavirus (HPV) infections are at high risk of neoplastic progression if they persist. Persistence can be measured by repeated HPV DNA tests or by cytologic testing. Thus, it is useful to understand the relationship between these 2 measurements. To explore the relative timing of HPV DNA clearance versus cytologic regression, data were analyzed from 840 study participants who were followed-up by repeat thin-layer cytology and HPV testing by a hybrid capture test at 6-month intervals for 2 years. On average, HPV DNA detection persisted longer than related cytologic abnormalities ($P < .001$). HPV type-specific data from a subset of 448 women with complete polymerase chain reaction test data confirmed that HPV DNA persisted longer than cytologic abnormalities ($P < .001$). It appears that the natural history of HPV typically includes periods before and after cytologic abnormality, in which HPV DNA is the more sensitive indicator of infection.

Human papillomavirus (HPV) infection causes virtually all cases of cervical cancer worldwide [1] and the full gradation of precursor lesions detected by cytologic screening aimed at preventing cervical cancer [2]. Nonetheless, cervical infections, even with oncogenic types of HPV, are extremely common and usually benign [3]. The apparent contradiction of a very common infection that causes a much less common malignant outcome is resolved by consideration of viral persistence. Cervical HPV infections and the cytologic abnormalities that they produce are usually transient, becoming worrisome only if they persist [4].

HPV persistence and related risk of neoplastic progression can be measured by using repeated DNA tests or cytologic testing. Therefore, it is useful to understand the relationship between the 2 measurements. Specifically, it is not clear whether the clearance of oncogenic HPV infection precedes or follows cytologic regression. Almost no relevant natural history data

have been published. As a notable exception, among 79 women with abnormal Pap smears and detectable oncogenic HPV DNA, Nobbenhuis et al. [5] observed that HPV DNA clearance preceded reversion to cytologic normalcy by an average of 3 months. Therefore, they suggested that HPV testing might be used clinically to predict the fate of cytologic abnormalities. This finding was unexpected.

Current knowledge suggests that productive HPV infection produces the cytomorphologic abnormalities interpreted as mildly abnormal Pap tests. HPV early proteins induce koilocytotic or equivocal cytologic changes that signal creation of new HPV particles. It is not intuitively obvious how HPV-induced cytologic abnormalities could be plausibly detectable 3 months after clearance of HPV DNA, given how quickly the cervical epithelium regenerates. To further explore the relative timing of HPV DNA clearance versus cytologic regression, in this study we analyzed data from 840 women who were followed-up by using repeat cytology and HPV testing at 6-month intervals for 2 years.

Methods

The study population was drawn from the Atypical Squamous Cells of Undetermined Significance (ASCUS)/Low-Grade Squamous Intraepithelial Lesion (LSIL) Triage Study (ALTS), a multicenter trial of women referred with mild cytologic abnormalities [6, 7]. "LSIL" refers to cytologic evidence of HPV infection and includes older cytologic terms, such as mild dysplasia or cervical intraepithelial neoplasia (CIN) 1 [8]. "ASCUS" refers to equivocal interpretations, about half of which are associated with oncogenic HPV infections [7, 8].

Received 18 March 2002; revised 19 June 2002; electronically published 16 September 2002.

Informed consent was obtained from all participants in accordance with guidelines of the US Department of Health and Human Services. Institutional review boards at each clinical center and at the National Institutes of Health (NIH) approved the study.

Financial support: NIH (contracts CN-55153, CN-55154, CN-55155, CN-55156, CN-55157, CN-55158, CN-55159, and CN-55105); Digene; Cytoc; National Testing Laboratories; Denvu. TriPath Imaging provided equipment or supplies used in this study at reduced cost.

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The Journal of Infectious Diseases 2002;186:1169-72

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0022-1899/2002/18608-0017\$15.00

ALTS was conducted in 4 diverse clinical centers to promote the generalizability of findings: University of Alabama at Birmingham, University of Washington (Seattle), Magee–Women’s Hospital of the University of Pittsburgh Medical Center Health System, and University of Oklahoma (Oklahoma City). Participants were referred for community cytologic interpretations of ASCUS or LSIL. They were randomized into 3 trial arms to study the optimal management of ASCUS/LSIL cytology.

For this analysis, we ignored the randomization design, which was irrelevant to our topic, and included 840 women with ASCUS or LSIL cytologic abnormalities and oncogenic HPV. Only women with cytologic abnormality and HPV DNA detection at enrollment, as well as complete follow-up, were included in this analysis. Women (median age, 24 years) were followed-up at 6-month intervals for 24 months, for a total of 5 visits each, including the baseline visit. We excluded women with incomplete follow-up and those diagnosed with high-grade lesions at any time in the study (almost all had persistent cytologic abnormalities and HPV DNA until treatment), to look strictly at relative time of regression of abnormal cytology and HPV DNA clearance.

We used a cervical broom (Wallach) to collect specimens for cervical cytology and HPV DNA testing. Specimens were placed directly into PreservCyt, a thin layer cytology fixative (Cytyc). A ThinPrep (Cytyc) cytologic preparation was produced from this medium and was interpreted by clinical center pathologists. HPV DNA testing of a remaining aliquot of the fixed specimen was done by using Hybrid Capture 2 probe B (Digene; hereafter referred to as “hybrid capture”) [9]. The hybrid capture HPV DNA test detects 13 HPV oncogenic types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Detection of other types by hybrid capture is rare and does not substantially affect clinical performance (P.E.C., unpublished data). Ongoing retesting of the same specimens by TaqGold PGMY consensus primer polymerase chain reaction (PCR) [10], using strip test detection, indicates that some infections with very low viral copy numbers are undetected by hybrid capture (C.M.W., unpublished data). Hybrid capture was designed to operate reliably at a detection cutoff point that yields a trade-off of sensitivity and specificity [11].

For the analysis, cytologic abnormalities first were defined as ASCUS or LSIL, as assessed by pathologists at the 4 clinical centers. We defined date of regression of cytologic abnormalities or HPV DNA clearance as the first visit that was negative for that measurement. We compared the visits at which cytologic regression and HPV clearance occurred by asymmetry χ^2 tests. The timing (in days) of regression and clearance were compared by using a paired *t* test. To check the validity of our conclusions, we repeated the analysis with different assumptions. Specifically, we redefined regression and clearance as the midpoint between the date of the last positive result and the date of the second successive negative result. If a patient did not have 2 successive negative results, we assigned the date of the 24-month visit for censoring. We also reanalyzed the data by excluding women who did not have both cytologic regression and HPV DNA clearance (as per the analysis of Nobbenhuis et al. [5]). We recategorized cytologic abnormalities as LSIL or worse. Finally, we repeated the analysis, restricting it to 448 women with complete PCR typing data as of June 2002. This subset was a representative sample of the larger study population of 840 women, because the batch ordering of PCR typing

of the 5-specimen set from each hybrid capture positive woman related merely to her enrollment date.

Results

As shown in table 1, although HPV infection and cytologic abnormalities of ASCUS or worse most often first turned negative at the same visit; when there was discordance, cytologic abnormalities were significantly more likely to become negative earlier ($P < .001$, symmetry χ^2 test). Cytologic abnormalities regressed a mean of 77.0 days before HPV DNA clearance ($P < .001$; median, 0 days; interquartile range [IQR], 0–186 days). When we applied the requirement of 2 successive negative results, the mean number of days from cytologic regression to HPV regression was 38.7 ($P < .001$; median, 0 days; IQR, 0–186 days). However, when we restricted the analysis to women with both cytologic regression and HPV clearance during follow-up, the approach chosen by Nobbenhuis et al. [5], we found no difference between time to cytologic regression and HPV clearance (mean, –5.9 days; $P = .4$).

We found similar results when we defined LSIL, instead of ASCUS, as the threshold for cytologic abnormality. As before, when there was a difference, cytologic abnormalities were significantly more likely to become negative at an earlier visit than detection of HPV DNA (table 2; $P < .001$, symmetry χ^2 test). LSIL regression occurred before HPV regression by a mean of 164.9 days, when a single negative was required, and 173.5 days, when 2 negative visits were required ($P < .001$ for both comparisons). Dichotomizing by age did not alter the conclusions for either the ASCUS or LSIL cytologic threshold.

In reanalyses restricted to type-specific PCR data, we observed similar results. Type-specific HPV DNA still persisted longer than cytologic abnormalities, regardless of cytologic threshold ($P < .001$, symmetry χ^2 test for both ASCUS and LSIL).

Discussion

Our data suggest that clearance of oncogenic types of HPV DNA, as defined by hybrid capture testing, occurs later than the regression of cytologic abnormalities. For purely metho-

Table 1. Cross-tabulation of human papillomavirus (HPV) clearance and cytologic regression at enrollment (0) and at follow-up visits (months 6, 12, 18, and 24) shows tendency of HPV DNA to persist longer than cytologic abnormalities (cytologic threshold for abnormality of atypical squamous cells of undetermined significance [ASCUS]).

Last visit HPV positive, month	Last visit with ASCUS or LSIL diagnosis					Total
	0	6	12	18	24	
0	291	70	18	4	7	390
6	58	64	18	4	3	147
12	21	21	30	7	4	83
18	27	20	10	9	5	71
24	48	30	19	15	37	149
Total	445	205	95	39	56	840

NOTE. LSIL, low-grade squamous intraepithelial lesion.

Table 2. Cross-tabulation of human papillomavirus (HPV) clearance and cytologic regression at enrollment (0) and at follow-up visits (months 6, 12, 18, and 24) shows tendency of HPV DNA to persist longer than cytologic abnormalities (cytologic threshold for abnormality of low-grade squamous intraepithelial lesion [LSIL]).

Last visit HPV positive, month	Last visit with LSIL diagnosis					Total
	0	6	12	18	24	
0	250	6	0	0	0	256
6	65	32	2	0	0	99
12	26	14	8	1	0	49
18	26	9	7	1	0	43
24	53	20	5	6	3	87
Total	420	81	22	8	3	534

dologic reasons, this result might be expected. Diagnosis of HPV by a molecular test is logically more sensitive than microscopic recognition of cytologic abnormalities [12]. Apart from methodologic issues, the finding also makes sense epidemiologically. In prospective studies, HPV DNA detection precedes and predicts subsequent cytologic abnormalities [13]. During infection, HPV DNA assays consistently detect a higher percentage of the same exfoliated specimens than cytologic examination [14]. If, as we observed, HPV DNA detection lasts longer than cytologic abnormalities, the latter conceptually may be the “tip of the iceberg” of HPV infections, occurring in the middle of the natural history of some, but not all, infections in association with peak virion production [11].

We cannot explain the difference in findings between Nobbenhuis et al. [5] and ALTS. Nobbenhuis et al. minimized the findings of differences in persistence between HPV and cytologic abnormalities by requiring that both resolved within the observation period. When we copied this approach, which assesses only rapid resolution of infection and cytologic abnormalities, we still did not corroborate their finding of HPV DNA resolving before cytologic abnormalities.

HPV assay choice is an important variable to consider in any study of this kind. Our study population was defined by hybrid capture positivity and cytologic abnormality at baseline. Hybrid capture and the general primer PCR test used by Nobbenhuis et al. [5] yielded roughly similar levels of analytic sensitivity [15]. Still, because PCR is slightly more sensitive than hybrid capture, perhaps some very low viral copy infections (more likely with ASCUS than with LSIL, in our experience) were not included in the study, thereby influencing the results unpredictably. However, more sensitive detection of HPV infection by PCR during follow-up would only lengthen measurable viral persistence and strengthen the conclusions we reached by using hybrid capture.

The hybrid capture technique does not distinguish among the 13 oncogenic types that it detects, and it is possible to mistake new infections for persistent ones. New infections in ALTS could affect both the hybrid capture DNA and cytologic abnormality data we present here in that successive infections could simulate persistence at both levels. Of note, our findings

were not appreciably altered among older women, who tend to have fewer new infections. The requirement of 2 successive negative tests, 1 complete year of HPV negativity, also minimized this concern. Most importantly, when we restricted our definition of HPV persistence to type-specific persistence, as measured by PCR, our conclusions were unchanged.

It is possible that international variation in cytologic diagnoses, which is profound [16], could help explain the difference in our 2 studies. However, we think this unlikely, given the direction of the results and our inclusion of equivocal (ASCUS) interpretations as abnormal in table 1 to maximize cytologic sensitivity. Finally, our study group was very large, reducing chance effects, although Nobbenhuis et al. [5] had a more intensive follow-up schedule and somewhat longer follow-up. Differences aside, we conclude from ALTS, as Nobbenhuis et al. [5] did from their accumulated data, that a negative result with a sensitive test for oncogenic HPV DNA indicates extremely low risk of underlying or incipient high-grade CIN or cancer [7]. This property makes HPV testing promising for the triage of equivocal cytologic abnormalities and perhaps ultimately for general screening.

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