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A study of HPV typing for the management of HPV-positive ASC-US cervical cytologic results

Mark Schiffman [a,*], Laurence M. Vaughan [b], Tina R. Raine-Bennett [c], Philip E. Castle [d], Hormuzd A. Katki [a], Julia C. Gage [a], Barbara Fetterman [e], Brian Befano [f], Nicolas Wentzensen [a]

[a] Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Rockville, MD, USA
[b] BD Diagnostics, Sparks, MD, USA
[c] Division of Research, Kaiser Permanente Northern California, Oakland, CA, USA
[d] Department of Epidemiology and Population Health, Albert Einstein College of Medicine, The Bronx, NY, USA
[e] Kaiser Regional Laboratory, Berkeley, CA, USA
[f] Information Management Systems, Inc., Calverton, MD, USA

HIGHLIGHTS

- HPV genotyping might be useful as part of ASC-US triage.
- The absence of the highest-risk genotypes reduces risk sufficiently to consider one-year retesting.
- A fifth of colposcopy referrals might be avoided with little delay in diagnosis of CIN3 or worse lesions.

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ABSTRACT

Background. In US cervical screening, immediate colposcopy is recommended for women with HPV-positive ASC-US (equivocal) cytology. We evaluated whether partial typing by Onclarity™ (BD) might identify HPV-positive women with low enough CIN3+ risk to permit 1-year follow-up instead.

Methods. The NCI-Kaiser Permanente Northern California Persistence and Progression cohort includes a subset of 13,890 women aged 21+ with HC2 (Qiagen)-positive ASC-US at enrollment; current median follow-up is 3.0 years. Using stratified random sampling, we typed 2079 archived enrollment specimens including 329 women subsequently diagnosed with CIN3+, 563 with CIN2, and 1187 with <CIN2. Adjusting for sampling, we computed 3-year cumulative CIN3+ risks for each Onclarity typing channel, using Kaplan–Meier methods.

Results. The 3-year CIN3+ risk for all HC2-positive women with ASC-US was 5.2%; this establishes the "benchmark" risk for colposcopic referral. Hierarchically, 3-year cumulative risks for each typing channel were 16.0% for HPV16, 7.4% for HPV18, 7.0% for HPV31, 7.1% for grouped HPV33/58, 4.3% for HPV52, 3.9% for HPV45, 2.7% for HPV51, 1.6% for HPV39/68/35, and 1.3% for HPV59/56/66.

Discussion. ASC-US linked to HPV16, HPV18, HPV31, or HPV33/58 warrants immediate colposcopy. Optimal management of women with HPV52 or HPV45 is uncertain. Risk of women with only HPV51, HPV39/68/35, or HPV59/56/66 might be low enough to recommend 1-year retesting permitting viral clearance. This strategy would defer colposcopy for 40% of women with HPV-positive ASC-US, half of whom would be cotest-negative at 1-year return. Approximately 10% of those with CIN3 diagnosable at enrollment would be delayed 1 year instead. Cost-effectiveness analyses are needed.

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1. Introduction

Cervical cancer arises via well-established steps including cervical infection with one of approximately a dozen high-risk human papillomavirus (HPV) types, viral persistence (rather than usual clearance), progression of a clone of persistently infected epithelial cells to a precancer, and invasion [1]. Prophylactic vaccination of adolescents against HPV infection will provide the ultimate prevention of cervical cancer but will take decades to achieve this goal. In the meantime in the US, prevention of cancer by screening programs relies on excision or ablation of the cancer-prone cervical transformation zone when precancers (CIN3) are found. Depending on age and parity, women with more equivocal precancers (CIN2) may be treated as well [2].

* Corresponding author at: Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Room 6E544, 9609 Medical Center Drive, Rockville, MD 20850, USA.
E-mail address: schiffmm@mail.nih.gov (M. Schiffman).

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To define which women have treatable precancer, screening in the US relies on a combination of cytology (conventional or liquid-based Pap tests) and HPV molecular tests [3]. Women judged to be at sufficient risk are sent for colposcopic biopsy. However, there is currently no consensus on the most effective combination of the two methods. In some screening programs, HPV testing is limited to clarification (triage) of equivocal cytologic results called atypical squamous cells of undetermined significance (ASC-US) [2]. At the other extreme, an HPV assay has recently been approved by the US FDA for primary standalone screening; in this approach, the use of cytology may be limited to triage of HPV-positive women lacking the 2 most important carcinogenic types, HPV16 and HPV18 (detection of either generally leads to immediate colposcopy) [4].

Although primary HPV testing might eventually be employed broadly, cytology still remains part of most cervical screening in the US, either alone or in the context of HPV/cytology cotesting. But ASC-US (equivocal) is by far the most common non-normal cytology result, which is more common than all other non-normal cytology results combined [5]. An ASC-US result means that there are subtle microscopic abnormalities of the cervical squamous epithelial cells that might or might not represent the effects of HPV infection. ASC-US is not a biological entity; rather it is an expression of interpretive uncertainty [6]. Accordingly, the great majority of ASC-US results are now managed according to results of concurrent or reflex HPV molecular testing.

The use of HPV-positive results to triage ASC-US (colposcopy if positive, normal if not) is complicated by the great differences in cancer risk between different high-risk HPV types that occur commonly in HPV-positive ASC-US. HPV16 is uniquely carcinogenic, causing 60% of cancers and 50% of precancers [7]. At the other extreme, a type like HPV56 rarely causes cancer (and so it is included as "high-risk"), but only in a very small percentage of cases [7,8].

Clinical use of HPV typing might exploit the heterogeneous gradient of risk by type, and possibly create a more finely tuned management approach to ASC-US if such precision is desired and resources permit. At present, HPV typing is recommended by guideline committees only to help guide the management of HPV-positive women with negative cytology [2]. In general, such women are retested in 1 year, but finding HPV16 or HPV18 elevates risk estimates, and mandates colposcopy instead. We examined a complementary possibility, i.e., whether HPV partial typing using the BD Onclarity™ assay might uncover a sizable subset of women with HPV-positive ASC-US having *low* enough precancer/cancer risk to permit follow-up in 1 year with the expectation of viral clearance, thus avoiding colposcopy.

2. Materials and methods

2.1. The PaP cohort

As described previously in detail, Kaiser Permanente Northern California (KPNC) initiated cervical screening with cytology-HPV cotesting in 2003 at 3-year intervals for women 30 and older [9]. HPV triage of ASC-US was already routinely performed at all ages 21 and older. The Persistence and Progression (PaP) cohort study was created as a very large "convenience sample" by storing residual cervical specimens from approximately 55,000 mainly HPV-positive women co-tested or triaged at KPNC from 2007 to 2010 (with <1% enrolled in late 2006 or January 2011) [10]. Approximately 8% of women opted out of specimen use.

Clinical HPV testing was performed using Hybrid Capture 2 (HC2, Qiagen, Germantown, MD). Cytology tests varied by time and KPNC laboratory: during the study period, conventional Pap smears were replaced by liquid-based cytology (SurePath, BD Diagnostics, Sparks, MD). HC2 results were known at the time of cytology review, which was also informed by FocalPoint (BD Diagnostics) automated prescreening for slightly more than half of the readings. A residual exfoliated cervical specimen in specimen transport medium (STM, Qiagen) left after HC2 testing was stored for study use. The specimen was neutralized and stored at −70 to 80 °C, and transported on dry ice until HPV typing was performed [11]. The clinical cut-off for the STM sample type was deduced using a pre-established cut-off for 0.5 mL of PreservCyt liquid-based cytology (LBC) media. Briefly, equal numbers of C-33 A cells harboring eight different HPV E6/E7 target sequences were spiked into both collection vials (1 mL STM; 20 mL ThinPrep) and the Ct response with increasing amounts of an STM specimen was compared to 0.5 mL of ThinPrep media. 25 μL of the STM specimen was found to give a similar Ct response to 0.5 mL of LBC medium and had a similar clinical performance to Hybrid Capture 2 [12]. An equivalent aliquot of a neutralized specimen was used in this study.

2.2. Study population

During the years 2007–2010 at KPNC, 809,315 women aged 21 or older had at least one cotest (concurrent cytology and HC2, including for this analysis those performed reflexively for management of cytologic results in women aged 21–29). The cytologic result of the first cotest in the 4-year enrollment period was ASC-US for 39,305, of which 17,190 (43.7%) were HC2 positive. The percentage of HC2 positivity decreased substantially with age (from >60% at ages 21–24 to ~30% at ages 60+). As shown in Fig. 1, we successfully collected residual HC2 specimens from 13,890 women with enrollment HC2-positive ASC-US, or about 81% of the 17,190 eligible specimens. The missed fraction was random with regard to risk of disease, e.g., we did not collect specimens from women attending Friday afternoon clinics because prompt processing was not possible. Thus, the PaP collection is highly likely to represent the full KPNC population.

For Onclarity testing, we drew a random sample of half of women whose worst histologic diagnosis during follow-up to date was CIN3+, half of those diagnosed with CIN2, and approximately 10% of those never (to date) diagnosed with CIN2+ (≤CIN1). A subsequent data update of the cohort after the initial draw in mid-2014 revealed several additional, interval incident cases of CIN2 or CIN3+; they were switched from the control group to the applicable incident case category for the data analyses.

To permit a methodologic ancillary analysis, we also selected a random sample of 200 specimens from women with HC2-negative ASC-US at enrollment. The objective was to estimate crudely the impact had we used Onclarity rather than HC2 on HPV prevalence and colposcopy referrals.

2.3. Follow-up of the PaP cohort

Information on subsequent histologic diagnoses of women in the PaP cohort was obtained. The median length of follow-up of the women sampled for this analysis who were not diagnosed with CIN2+ was 3.6 years (IQR of 1.2 to 5.0 years, maximum 7.7 years). Median time until the screening visit leading to diagnosis of CIN2 was 0.0 years (prevalent diagnosis, IQR of 0.0 to 0.5 years, maximum 6.0 years); median time until the screening visit leading to diagnosis of CIN3+ was 0.0 years (IQR of 0.0 to 1.1 years, maximum 6.3 years). Thus, the majority of cases of precancer/cancer diagnosed in the PaP cohort to date among women with an HPV-positive ASC-US result were prevalent cases diagnosed based on enrollment screening. The average age at enrollment of women in the three diagnostic groups was very similar, with means of 35–36 years of age and the majority of women in all three groups between 25 and 40.

2.4. BD Onclarity HPV typing

The BD Onclarity HPV Assay is a multi-plex real-time PCR assay that targets HPV type-specific E6 or E7 sequences [13]. The assay detects 14 HPV types, providing extended genotyping information. The assay was designed to distinguish the most carcinogenic HPV types from those