Journal of Clinical Virology 61 (2014) 553–557

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

The concordance of HPV DNA detection by Hybrid Capture 2 and *care*HPV on clinician- and self-collected specimens

Wen Chen[a], Jose Jeronimo[b], Fang-Hui Zhao[a], You-Lin Qiao[a], Melissa Valdez[b], Xun Zhang[a], Le-Ni Kang[a], Pooja Bansil[b], Proma Paul[b], Ping Bai[a], Roger Peck[b], Jing Li[a], Feng Chen[a], Mark H. Stoler[c], Philip E. Castle[d,*]

[a] *Department of Epidemiology, Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, People's Republic of China*
[b] *PATH, Seattle, WA, USA*
[c] *Department of Pathology, University of Virginia, Charlottesville, VA, USA*
[d] *Global Coalition Against Cervical Cancer, Arlington, VA, USA*

ARTICLE INFO

Article history:
Received 18 July 2014
Received in revised form
20 September 2014
Accepted 25 September 2014

Keywords:
Human papillomavirus (HPV)
Cervical intraepithelial neoplasia (CIN)
Cervical cancer
Visual inspection after acetic acid (VIA)

ABSTRACT

Background: *care*HPV is a new, lower-cost DNA test for human papillomavirus (HPV). There are limited analytic comparisons of *care*HPV against a referent HPV DNA test like Hybrid Capture 2 (HC2).
Objective: To assess the test agreement between *care*HPV and HC2 on self- and clinician-collected specimens.
Study design: In a population of 7541 women living in rural China, women provided a self-collected (sc) and two clinician-collected (cc) specimens and underwent visual inspection after acetic acid (VIA). The sc specimen and one cc specimen were tested by *care*HPV and HC2; a random subset of specimens was tested for HPV genotypes.
Results: The percent positive on cc specimens and sc specimens was 14.69% and 14.97% for *care*HPV, respectively, and 15.05% and 18.53% for HC2, respectively; HC2 testing of sc specimens was more likely to test positive than other combinations of tests and specimens ($p < 0.0001$ for all comparisons). The agreement between different tests on the same specimens (kappa = 0.787 and 0.691 for cc and sc specimens, respectively) was better than the same test on different specimens (kappa = 0.653 and 0.649 for HC2 and *care*HPV, respectively). Disagreement between the same test on different specimens increased with increasing participant age ($p_{trend} = 0.0001$ for HC2 and 0.002 for *care*HPV). HC2-positive/*care*HPV-negative specimens were more likely to test positive for non-carcinogenic HPV genotype than test HPV negative whereas the converse was true for HC2-negative/*care*HPV-positive specimens.
Discussion: The agreement for HPV DNA detection between *care*HPV and HC2 was good to very good.

1. Background

Testing for high-risk human papillomavirus (HPV) is now being recommended for cervical cancer screening in both higher-resource settings [1] and lower-resource settings [2]. For the latter, *care*HPV™ (QIAGEN, Gaithersburg, MD, USA), a lower-cost, signal-amplification DNA test for a pool of 14 HPV types, was developed. *care*HPV is based on the same biochemistry as Hybrid Capture 2 (HC2; QIAGEN), a DNA test for a pool of 13 HPV types (the same as *care*HPV minus HPV66) that was U.S. Food and Drug Administration approved in 2003. Several studies have shown that *care*HPV has a sensitivity and specificity for cervical precancer and cancer that can approach that of HC2 [3,4].

We recently conducted a study of lower-cost tests and strategies for screening and triage in China [3–5]. We reported that HC2 and *care*HPV had comparable clinical performance for detection of cervical precancer and cancer, cervical intraepithelial neoplasia grade 2 (CIN2) or more severe diagnoses (CIN2+) and grade 3 (CIN3) or more severe diagnoses (CIN3+), when testing clinician-collected (cc) specimens [3]. However, we found that there was a decrement in performance for *care*HPV when using self-collected (sc) specimens [3], similar to what has been shown in other studies

* Corresponding author. Tel.: +1 7037720611.
E-mail address: castle.philip@gmail.com (P.E. Castle).

http://dx.doi.org/10.1016/j.jcv.2014.09.018
1386-6532/

[6,7]. Using lower positive cutpoints for *care*HPV for both cc and sc specimens could potentially improve sensitive but not without significantly lowering specificity for CIN3+ [5]. A higher positive cutpoint for *care*HPV for cc specimens non-significantly reduced sensitivity but significantly increased specificity for CIN3+ [5].

Here, we describe the analytic agreement for HPV DNA detection for different tests, *care*HPV vs. HC2, on the same specimen, cc or sc, and the agreement for different specimens, cc vs. sc, using the same test, *care*HPV or HC2. These pairwise comparisons have not been explored previously in depth elsewhere. Specifically, we investigated the simple concordance and correlation of signal strengths of these pairwise comparisons. Moreover, we investigated how visual inspection after acetic acid (VIA) results, as a proxy for lesion size, and age influenced discordance (1-concordance) between pairwise results. We considered the concordance of all 4 HPV DNA results, *care*HPV and HC2 on both cc and sc specimens, in the general population and in women with CIN2+ or CIN3+. Finally, in a subset of specimens, we compared pairwise results of *care*HPV and HC2 to results from a third, HPV genotyping test to look at relative predilection of each test to cross-react with non-carcinogenic HPV genotypes, as has been reported for HC2 [8].

2. Objective

To describe the agreement for detection of HPV DNA between *care*HPV and HC2

3. Study design

3.1. Enrollment

The recruitment and enrollment of 7500 women aged 25–65 years and living in rural China has been previously reported in detail [3,4]. All eligible women were then asked to complete the written, informed consent in order to participate in the study. The PATH, Cancer Institute and Hospital, Chinese Academy of Medical Sciences (CICAMS), and US National Cancer Institute institutional review boards (IRBs) approved the study.

Women were screened by 6 screening tests: HC2 and *care*HPV testing on cc and sc specimens, OncoE6^{TM} testing (Arbor Vita Corporation, Freemont, CA, USA) on a second cc specimen, and VIA. Women underwent colposcopy and a 4-quadrant microbiopsy protocol if any screening test was positive or was selected as a random sample of approximately 10% screen-negative women as previously described [3,4].

3.2. HPV DNA tests

*care*HPV and HC2 testing was done on both cc and sc specimens as previously described [3,4].

For HPV genotyping, QIAmp 96 DNA Blood Kits (96 wells, 12 plates/kit) (QIAGEN, Hilden, Germany) were used for DNA isolation on CCM samples according to the manufacturer's protocol. Ten μl was used for HPV genotyping by INNO-LiPA HPV Genotyping Extra (Innogenetics NV, Ghent, Belgium), which uses SPF10 primers for DNA amplification by PCR [9,10] and detected HPV genotypes by reverse hybridization. This system targets 28 HPV types (HPV6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 43–45, 51–54, 56, 58, 59, 66, 68–71, 73, 74 and 82).

HPV genotyping results on self- or clinician-collected specimens were categorized as carcinogenic HPV positive (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and/or 68) [11] to match the same types targeted by *care*HPV and by HC2 plus HPV66, which HC2 detects due to cross-reactivity [8], else carcinogenic HPV negative and positive for non-carcinogenic HPV (any other type or tests positive for HPV but no HPV type detected), or HPV negative.

3.3. Pathology

The primary histopathologic diagnosis was provided by two CICAMS pathologists after reaching agreement, and the worst of the biopsies or surgical specimen was used for the final diagnosis in these analyses.

3.4. Analysis

Test agreement statistics (percent agreement, percent positive agreement, and kappa values) between 6 pairwise combinations of the two tests (*care*HPV and HC2) done on the two specimens (cc and sc) were calculated. McNemar chi-square test was used to test for statistical significance between test results. The Spearman correlations between signals (rlu/pc) were calculated for those pairwise results in which one or both results were positive. The percent disagreement and its relationship to subject age, categorized into quartiles (25–38, 38–43, 44–50, and 51–65 years), was evaluated using a test of trend [12]. Agreement and correlations were also stratified on whether women were VIA positive or negative. The distribution for all combinations of the 4 tests was calculated overall, among women with CIN2+, and among women with CIN3+.

4. Results

Fig. 1 shows the scatter plots of the rlu/pc values (signal strength) for 6 pairwise combinations of specimen types and HPV test results. The spearman correlations among HPV positive for either test (excluding the HPV negatives for both) ranged from 0.432 for HC2 testing on cc specimens vs. *care*HPV testing on sc specimens to 0.842 for both tests done on the cc specimens. In general, there was poorer correlation of the signal strengths when two different specimens were used than when different tests were run on the same specimen; there was better correlation on cc specimens than on sc specimens for the two assays (0.842 vs. 0.710, respectively). There was a stronger signal for HC2 on sc specimens than cc specimens ($p < 0.0001$) while the reverse was true for *care*HPV ($p < 0.0001$).

The paired positive/negative results for HPV detection for the two specimens and two tests are shown in Table 1. The percent positive was 14.69% for *care*HPV on cc specimens and 14.97% on sc specimens and 15.05% for HC2 on cc specimens and 18.53% on sc specimens. Only the percentage of positive HPV test results for HC2 on sc specimens was significantly different from HPV results for the other combinations ($p < 0.0001$ for all comparisons). The percent agreement for all pairwise results was ~90% but the positive agreement and the kappa values differ greatly, reflecting the patterns for the signal strengths described above. There was better agreement between different tests on the same specimens (kappa = 0.787 and 0.691 for cc and sc specimens, respectively) than the same test on different specimens (kappa = 0.653 and 0.647 for HC2 and *care*HPV, respectively).

Shown in Fig. 2 are the (A) age quartile-specific percent HPV positive (prevalence) for each test and specimen combination and (B) discordant HPV results by test (HC2 vs. *care*HPV) for a given specimen type (self-collection or clinician-collection) and by specimen type for a given test. For each combination of test and specimen, the percent HPV positive increased with older age groups ($p_{trend} < 0.0001$), with HC2 testing of sc specimens consistently having a 2–4% higher test positive than other combinations. While there was a significant trend of increasing discordance with older age group for HPV with the two specimen types for the same test