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Equivocal and Weakly Positive Hybrid Capture 2 Tests in Women Aged 50 and Older

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Abstract

The performance of the Hybrid Capture 2 (HC2) test for human papilloma virus (HPV) detection depends on the prevalence of infection. However, the current HC2 manufacturer recommended interpretative algorithm is the same for all women. This test, which may be particularly useful in perimenopausal and postmenopausal women given the morphologic complexity of their Pap tests, could be affected by the overall lower prevalence of HPV infection in this age group. We investigated HC2 equivocal and weakly positive HPV tests in women 50 years and older and the detection of high-grade dysplasia (CIN2+) on their follow-up specimens. All HC2 test data from 1,067 consecutive specimens and 85 additional specimens from women 50-years-old with equivocal and weakly positive HC2 were analyzed. Follow-up specimens from women with HC2 tests within these ranges were reviewed. No CIN2+ was found on follow-up of 49 cases of women 50 with equivocal or weakly positive HC2 results. The current HC2 algorithm resulted in “positive” reports in 63% of specimens with initial equivocal HC2 due to retests mostly within the equivocal range. These results suggest that women 50 years and older may benefit from higher HC2 thresholds. The test could also be reported as HC2 values (RLU/CO) to be interpreted in view of risk factors.

Keywords

human papilloma virus; hybrid capture 2; cervical intraepithelial neoplasia; age

Current guidelines recommend cervical cancer screening of perimenopausal and postmenopausal women up to 65–70 years old.^{1,2} The interpretation of these Pap tests is challenging because of concurrent hormonally determined cytologic changes. While deep atrophy is characterized by hyperchromasia and increased nuclear cytoplasmic ratios resembling high-grade squamous intraepithelial lesions (HSIL) lesser degrees of atrophy and other perimenopausal changes span the spectrum of reactive changes, atypical squamous cells of undetermined significance (ASCUS) and low grade squamous intraepithelial lesions (LSIL).^{3–5} Ancillary tests such as high risk human papilloma virus (HPV) detection could therefore be even more important in older women. Using the Hybrid Capture 2 (HC2) system (Qiagen, Valencia, CA) Johnston and Logani showed that HPV testing may be particularly useful in postmenopausal women with ASCUS because a large proportion will be spared from colposcopy given a negative test.⁶ However, the performance of HC2 in perimenopausal and postmenopausal women is likely to be affected by a lower prevalence of

HPV infection⁷ as demonstrated in several different populations^{8–10} but the current interpretative HC2 algorithm is the same for women of all ages. The HC2 test detects HPV DNA by using probe hybridization and chemiluminescence. The patient's specimen signal as measured in relative light units (RLU) is compared with an average signal of supplied positive reagents. The resulting RLU/cutoff ratio (RLU/CO) is considered positive when ≥ 1 (corresponding to a mean of 1.08 pg/ml of HPV DNA⁷), but results between 1 and < 2.5 on the first run should be confirmed by a positive retest. If a first retest is negative, the result of a second retest is then considered final. We investigated the RLU/CO of 1,067 unselected consecutively performed HC2 tests to compare results in women younger than 50 years with women 50 years and older. We specifically focused on specimens with RLU ratios considered equivocal (1 to < 2.5) or weakly positive (2.5–10)¹¹ and also included 85 additional specimens from women ≥ 50 -years-old with results within these ranges. Histologic and cytologic follow-up was procured for as many cases as possible to determine the frequency of cervical intraepithelial neoplasia 2 or higher (CIN 2+) in women ≥ 50 -years-old.

Materials and Methods

Case Material

To compare women < 50 versus ≥ 50 -years-old we retrospectively reviewed all HC2 RLU/CO data from 1,067 consecutive unselected specimens received during a 3 month period (May 2010–July 2010). To further investigate equivocal and weakly positive HC2 tests in women ≥ 50 -years-old, and given their relative lower number of specimens, we then enriched the study with 85 additional specimens with RLU/CO between 1 and 10 in patients of this age received during the immediately preceding eleven months (June 2009–April 2010). These specimens included reflex testing as well as physician request regardless of cytologic interpretation. The cytologic material included in-house specimens as well as specimens sent from other institutions for HC2 testing. Follow-up cytologic and histologic specimens were reviewed when available in the pathology department. The study was conducted under appropriate Institutional Review Board approval (2002H0089).

Human Papilloma Virus Testing

HC2 was used according to manufacturer's instructions. The specimens were predominantly collected using the SurePath system (BD, Franklin Lakes, NJ). A small proportion of the specimens were collected with the ThinPrep system (Hologic, Marlborough, MA). Results were defined as negative (RLU/CO < 1), equivocal (RLU/CO 1 to < 2.5), weakly positive (RLU/CO 2.5–10), and positive (RLU/CO > 10).

Statistical Analysis

Summary statistics were calculated by age group and test performed (initial, first retest, and second retest). Data were described visually by age group and by test performed using box plots and line graphs. The RLU/CO at each test was compared between age groups (< 50 and ≥ 50 years) using a Wilcoxon Rank Sum test.

Results

Initial RLU/CO Ratios by Age

We found 1,067 HC2 tests on women of all ages between May and July 2010 at our institution (846, 79% < 50 -years-old; 221, 21% ≥ 50 -years-old). Seven hundred fiftysix (71%) were negative (RLU/CO < 1), 65 (6%) were equivocal (RLU/CO 1 to < 2.5), 68 (6%) were weakly positive (RLU/CO 2.5–10), and 178 (17%) were positive (RLU/CO > 10). Table I shows the data for women < 50 and ≥ 50 -years-old. There was a statistically

significant difference in initial RLU/CO values between the two age groups ($P < 0.001$, based on a Wilcoxon Rank Sum test). No such difference could be demonstrated in first and second retests, which was an expected effect of a restricted range. Figure 1 shows RLU/CO ratio distribution by age group.

Retesting in Women ≥ 50 -Years-Old with Equivocal Initial RLU/CO Ratios

All cases with equivocal results (RLU/CO 1 to <2.5) underwent retesting according to HC2 manufacturer instructions. If retesting was not possible (i.e., insufficient specimen) HPV testing was then reported as indeterminate. In the ≥ 50 age group 7 (64%) out of 11 retests were positive (RLU/CO ≥ 1) and 4 (36%) negative (RLU/CO < 1). When this group was enriched with 35 additional equivocal specimens retesting was positive (RLU/CO ≥ 1) in 29 (63%) and negative in 14 (30%). All of the latter underwent a second retest with RLU/CO results <1 and were reported negative (Fig. C-1). There were only three indeterminate HPV tests.

Follow-up Specimens on Women 50 Years and Older

One hundred two equivocal (46, 45%) or low positive (56, 55%) HC2 tests on women ≥ 50 were identified (age range 50–83 years, average 58 years). Index Pap test reports were found for 88 cases and consisted of 37 NILM, 43 ASCUS, 2 ASC-H, and 6 LSIL. Follow-up specimens were available for 49 cases (48%) including 30 Pap tests and 33 cervical biopsies or endocervical curettages. Follow-up cytologic specimens were originally reported as 11 NILM, 4 ASCUS, and 1 LSIL. Thirteen biopsy/curettage specimens were negative and six showed CIN1 or postmenopausal squamous atypia. Fourteen cases had both cytology and histology follow-up; 5 NILM/Negative, 4 NILM/CIN1, 3 ASCUS/Negative, 1 LSIL/VaIN1, and 1 case was reported as ASCUS/CIN2. This latter case was reclassified as CIN1 on squamous metaplasia after review including additional p16 and Ki67 immunohistochemistry; it was also negative for HPV by in situ hybridization. All other follow-up specimens were also reviewed and, in keeping with the original diagnoses, none showed CIN2+ or HSIL. None of the latter was considered to require additional ancillary testing for this study. Follow-up specimens were received between 1 and 12 months after the index Pap test (average 3 months).

Discussion

While many cases can be correctly interpreted applying morphologic criteria, ancillary HPV testing may be particularly useful in perimenopausal and postmenopausal women because the cytologic interpretation of their Pap tests is often challenging due to hormonally determined epithelial changes.^{3–5} ASCUS in these women has been shown to have a lower predictive value for SIL¹² probably due to cytologic overcalls. While Johnston and Longani reported the HC2 test to be negative in over 70% of women ≥ 50 -years-old with ASCUS Pap tests, avoiding unnecessary colposcopies in many of these patients⁶; Eltoum et al. reported an increase in HC2 positivity in women > 65 -years-old with ASCUS.¹³ In a large study of 4,855 women ≥ 50 -years-old, Zhao et al. further illustrated the utility of this test, reporting HC2 positivity as 6.3% (overall), 1.2% (NILM), 14.2% (ASCUS), 71% (LSIL), and 89.3% (HSIL).¹⁴ A negative HC2 test had a negative predictive value of 100% for CIN2+ in women ≥ 50 years with LSIL and ASCUS.¹⁴ These studies have demonstrated acceptable results following the manufacturer recommended HC2 interpretative algorithm regardless of age. However, polymerase chain reaction based studies have shown HPV infection prevalence to steadily decrease after peaking between 20 and 30-years-old in several different populations.^{8–10} A second smaller peak was documented in postmenopausal Costa Rican⁸ and British women.¹⁰ In their population based study Herrero et al. showed that this second peak may be associated with HSIL.⁸ These findings highlight

the importance of continued cervical cancer screening and the possibility of adjusting HPV testing according to the epidemiology of the infection in older women. Our study was conducted to analyze lower range HC2 RLU/CO values in older women and the possibility of adjusting the HC2 test in these women. This has already been suggested by several authors including Cuzic et al. who found that in women 35-years-old undergoing routine screening HC2 with cutoff values of 1, 2, and 4 pg/ml had similar sensitivity (95.2–100%) for CIN2+ but the false positives decreased from 4.9% to 2.3 and 2.1% as the threshold increased.¹⁰ The population based ARTISTIC trial documented a significant reduction in positive HC2 tests in women with normal cytology when the RLU/CO threshold was increased from 1 to 2.¹⁵ Furthermore, no significant reduction in HC2 positivity was found in women with CIN2+ when the threshold was increased to 2.¹⁵ However, Ordi et al. in their series of 2,271 women aged 15–92 years-old attending a colposcopy clinic found that increasing the RLU/CO threshold would have led to unacceptable loss of sensitivity for CIN2+ from 2.4% with RLU/CO < 1–28.4% with RLU/CO 5–10.¹⁶

Our population of women 50-years-old showed no cases of high-grade dysplasia (CIN2+) on follow-up (1 to 12 months, average 3 months) specimens after an initial weakly positive (RLU/CO 2.5–10) or equivocal (RLU/CO 1 to < 2.5) HC2 test. This is consistent with a recent report by Jarboe et al. who found CIN2+ in only 3.2% of those with RLU/CO ratios 1–10 but in 17.3% of those with RLU/CO > 10 among 236 women with ASCUS and histologic follow-up (follow-up period similar to this study, no mention of age distribution).¹¹ Furthermore, the current manufacturer recommended interpretative algorithm for the HC2 test resulted in 63% of specimens from women 50 with initial equivocal RLU/CO values to ultimately lead to a “positive” result due to retests that remained mostly within the equivocal range (Fig. C-1). Although we showed that, after an initial RLU/CO value between 1 and <2.5, the distribution of RLU/CO first and second retest values is similar in women < 50 and 50 (Fig. 1), it appears that the predictive value for CIN2+ of the manufacturer recommended algorithm is very low for the latter patients. This may be in contrast to the studies by Knoepp et al., in which even a larger majority of initial equivocal HC2 tests also lead to a “positive” result after retesting but were found to have CIN2+ on 15% of cases.^{17,18} However, these authors do not include the age distribution of their cohort and their studies may represent mostly younger women. Of note, the ARTISTIC trial found that increasing the RLU/CO threshold to 2 in women 35–64 years old would lead to an increase in HPV positivity being confirmed by a second PCR based method from 50.3 to 63.2%.¹⁵

In our population the use of an HC2 RLU/CO threshold up to 10 would not have resulted in missing any cases of CIN2+ in women 50. Our results are limited by cases lacking follow-up (52%), and by the likely inclusion of a mixed population of women, some undergoing routine screening and others undergoing follow-up Pap tests and colposcopies. Also, index Pap tests included NILM, ASCUS, ASCH, and LSIL reflecting the clinician's request of HPV testing regardless of Pap test diagnosis, which is a growing practice in our geographic area. The inclusion of specimens collected in two different media, SurePath and ThinPrep, is unlikely to have altered HC2 results.¹⁹ We believe our data show a valuable picture of some issues involved in HPV testing of older women. Although only 8% of our HC2 tests in women 50 produced RLU/CO ratios within equivocal and weakly positive range, these tests may lead to unnecessary anxiety and colposcopies and increase health care costs. Studies including larger numbers of specimens in older women are to either justify or rule out the use of a new cutoff value for the HC2 test. Alternatively, reporting RLU/CO ratios themselves instead of or in addition to the simplified “Negative” or “Positive” results could be of more use for treating physicians who also have knowledge of each individual's risk factors.

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Fig. 1.

RLU/CO ratio distribution by age, 1,067 consecutive tests. Only initial results showed a statistically significant difference in women < 50 and ≥ 50-years-old ($P < 0.001$). The bottom and top of the box are the 25th and 75th percentiles, the band is the median, the dot is the mean, and the ends of the whiskers are the minimum and maximum values. Notice the logarithmic scale. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Fig. C-1.
RLU/CO value distribution in women 50-years-old including weakly positive specimens (black), equivocal values leading to a “positive” result (red), and equivocal values leading to a “negative” result (blue).

Table I

Initial HC2 Test Results by Age

	Negative	Equivocal	Weakly Positive	Positive	Total
<50-years-old	569 (67%)	54 (6%)	62 (7%)	161 (19%)	846 (79%)
50-years-old	187 (84%)	11 (5%)	6 (3%)	17 (8%)	221 (21%)