Published in final edited form as:

J Virol Methods. 2013 April ; 189(1): 77–79. doi:10.1016/j.jviromet.2013.01.011.

A Study of Borderline Positive Hybrid Capture 2 Tests in the Kaiser Permanente Northern California Cervical Screening Program: Evidence Against Retesting

Brandon J. LaMere^{a,*}, Philip E. Castle^{b,**}, Barbara Fetterman^c, Nancy Poitras^c, Mark Stanley^c, Jen Shieh^c, Thomas Lorey^c, Walter Kinney^d, and Mark Schiffman^e

^aKaiser Permanente Northern California, Women's Health Research Institute, Division of Research, Oakland, CA

^bAlbert Einstein School of Medicine, Bronx, NY

^cKaiser Permanente TPMG Regional Laboratory, Berkeley, CA

^dDivision of Gynecologic Oncology, Kaiser Permanente Medical Care Program, Oakland, CA

^eDivision of Cancer Epidemiology and Genetics, National Cancer Institute, National Institute of Health, Rockville, MD

1. Introduction

Infection with one or more high-risk HPV (hr-HPV) types is a necessary step in the development of cervical cancer, making its detection a useful tool for cervical cancer screening and prevention (Bosch et al., 2002). Hybrid Capture 2 (HC2, Qiagen, Gaithersburg, MD) was the first FDA-approved and is still the most widely used laboratory test for the presence of high-risk HPV DNA as a predictor of who is or is not at risk for cervical cancer and pre-cancer (defined here as cervical intraepithelial neoplasia grade 2 or worse). While HC2 has remarkable sensitivity for cervical intraepithelial neoplasia grade 2 or worse, the transient nature of most hr-HPV infections reduces its specificity as most infections are benign and will resolve without progressing to cervical intraepithelial neoplasia grade 2 or worse. This can be problematic for cervical cancer screening algorithms in which HPV positivity triggers more intensive follow-up; in such algorithms, good specificity is important for reducing the number of costly colposcopic follow-up visits (Cuzick et al., 2008).

Although manufacturer specifications indicate a relative light unit (RLU) ratio compared with positive control of 1.0 as the threshold for determining positivity, reclassifying HC2 specimens that are equivocal and fall just above the positive cut-off is one potential way to improve the assay's specificity (Rijkaart et al., 2010; Rebolj et al., 2011; Sasieni and Castanon, 2011). Some researchers have looked at increasing the RLU cut-off point because HC2 results at this level predict lower risk than higher values (Lorincz et al., 2002).

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*Corresponding Author: Brandon J. LaMere, Kaiser Permanente TPMG Regional Laboratory, 1725 Eastshore Highway, Berkeley, CA, 94710; (510)559-4854; brandon.j.lamere@kp.org.

**Visiting Professor at Albert Einstein School of Medicine

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However, equivocal results are not that common and raising the cut-point to 2.0 or 3.0 may only yield a modest improvement in specificity at some cost to sensitivity; i.e., misclassification of women with cervical intraepithelial neoplasia grade 2 or worse lesions whose RLU ratios are equivocal (Rijkaart et al., 2010). Rather than raising the cut-point, a more conservative approach is to repeat the HC2 test on specimens with equivocal results and determine the final test result based on a collective assessment of the test results (Knoepp et al., 2007), however this method requires additional laboratory time and cost to implement, weighing against any savings it yields from reducing the number of colposcopic examinations.

In 2001 Kaiser Permanente Northern California added the HC2 assay to its cervical cancer screening program as a triage test for women with a cytologic diagnosis of atypical squamous cells of undetermined significance and, in 2003, as a concurrent test (“cotest”) with cytology for women 30 and older. In the cotesting scheme, women have been managed as follows: 1) women who are HPV-positive with a cytologic diagnosis of atypical squamous cells of undetermined significance, or have more severe (regardless of the HPV test results) are referred for immediate colposcopy; 2) women who test HPV-negative and have cytology of atypical squamous cells of undetermined significance, or who test HPV-positive with negative cytology are rescreened in one year; and 3) if both tests are negative, women are rescreened in three years.

For cotests with borderline positive hr-HPV results, interpretation of the repeat test results is used to determine the final hr-HPV test result and hence the need for rescreening visits versus colposcopy. At the time that HC2 was introduced into the Kaiser Permanente Northern California cervical cancer screening algorithm, based on concern over the meaning of borderline-positive HC2-positive results, it was decided that any specimens with borderline or “equivocal” HC2 results, defined as a RLU ratio greater than or equal to 1.0 but less than 3.0, would be re-tested two additional times. The final hr-HPV result was determined by the majority results of the three tests i.e., only one of the two repeats needed to be positive to have the final positive hr-HPV result.

To assess the utility of Kaiser Permanente Northern California’s re-testing scheme for equivocal HC2 specimens, HC2 data, cytology results, and histological outcomes (less than cervical intraepithelial neoplasia grade 2, cervical intraepithelial neoplasia grade 2 or worse) were compiled for patients undergoing routine cervical cancer screening at Kaiser Permanente Northern California.

2. Methods

This analysis began with an examination of the total number of cotests (966,569) between December 2007 and October 2010 -- the month HC2 data collection started through the latest month with 6 months of histological follow-up data when this analysis was initiated. Cotests were excluded if 1) the HC2 accession number could not be matched to a medical record number (n=36,050); 2) patients were under age 30 or did not have a birth date on file (n=49,251); 3) HC2 results were missing (n=688); or 4) cytology results were missing or unsatisfactory (n=5,154). As a result of these exclusions, a total of 875,426 (90.6%) cotests were available for this analysis.

The percent of equivocal HC2 results and percent of equivocal results in which the final hr-HPV result was negative were calculated by cytologic interpretation (Negative, atypical squamous cells of undetermined significance, low-grade squamous intraepithelial lesion, high-grade squamous intraepithelial lesion, atypical squamous cells cannot rule out high-grade squamous intraepithelial lesion, atypical glandular cells). Statistical differences in these percentages by cytologic interpretation were assessed using Pearson chi-square tests.

Since women with a cytologic diagnosis of low-grade squamous intraepithelial lesion are referred to colposcopy regardless of their HC2 result, it provided an opportunity to assess the association of a final hr-HPV result following an initial equivocal HC2 result with clinical outcomes without selection bias. Results from cervical biopsies collected within 6 months (183 days) of an HC2-equivocal/ low-grade squamous intraepithelial lesion cotest were retrieved from the institutional database. Contingency tables and Fisher's exact test were used to determine if the yield of cervical intraepithelial neoplasia grade 2 or worse was significantly different whether the final hr-HPV result was positive or negative.

3. Results

During a 47-month period (December 2006—October 2010), a total of 12,292 (1.4%) of 875,426 HC2 tests had an equivocal result. The distribution of cytologic results among equivocal HC2 specimens is shown in table 1 (top). Fewer equivocal HC2 than expected by chance were seen in patients with negative cytology and the highest percentages were seen among patients with minor cytologic abnormalities ($p < 0.0001$).

Of the equivocal results, 1,574 (12.7%) had a final negative hr-HPV result after two repeat tests. Table 1 (bottom) shows the percentage of equivocal HC2 specimens with a final hr-HPV negative result by cytologic interpretation. Given an equivocal HC2 tests, a final negative hr-HPV result was more likely when cytology was read as negative ($p < 0.0001$).

To examine the clinical meaning of equivocal specimens eventually testing positive or negative, data were analyzed from 554 patients age 30 and older (median age 41) who had low-grade squamous intraepithelial lesion cytology and an initial equivocal HC2 result. Of these, 431 had retrievable histology results within 6 months of the low-grade squamous intraepithelial lesion /HC2-equivocal cotest. Of the 17 women with a final negative hr-HPV result, 1 (5.9%) had cervical intraepithelial neoplasia grade 2 or worse histology (Table 2). Among the 414 low-grade squamous intraepithelial lesion patients with a final positive hr-HPV result, 28 (6.8%) had histology of cervical intraepithelial neoplasia grade 2 or worse ($P = 0.33$).

4. Discussion

Although Kaiser Permanente Northern California adopted the HC2-equivocal re-testing strategy to improve the accuracy of their cervical cancer screening effort, the data suggests there is limited practical value for this approach. First, equivocal HC2 results are rare. Second, the vast majority have at least one positive repeat test and thus only a small proportion, <1 in 500, have a final negative result.

Because Kaiser Permanente Northern California's screening algorithm does not require colposcopy for HC2-negative atypical squamous cells of undetermined origin positive cotests, it was not possible to assess the effect of HC2 re-testing among patients with atypical squamous cells of undetermined significance cytology, in whom additional risk stratification would have an impact on follow-up care. However, with histological data for both HC2-positive and -negative women with low-grade squamous intraepithelial lesion cytology, there were no significant differences in histological outcomes for women in this population by the final hr-HPV result following the initial equivocal HC2 result. These results are consistent with those of Knoop et al. (2007), who showed repeat tests of equivocal HC2 specimens provide little practical information for improving patient care and management. These analyses differ in that Kaiser Permanente Northern California's equivocal re-testing algorithm requires both repeat tests to have an RLU ratio <1.0 in order to be deemed negative, while the strategy evaluated by Knoop et al. requires only one RLU ratio <1.0 of the two repeat tests. Despite a more rigorous testing algorithm for HC2

equivocals to be called negative, there was no disease association observed with the final hr-HPV result.

The results of this study indicate that retesting of equivocal HC2 test results is probably not a cost-efficient practice and offers no practical benefit to patients. A policy of using a RLU cutoff of equal to or greater than 1.0 as positive on the first HC2 result is a more reasonable approach that may potentially avoid reclassifying a small percentage of women who have cervical intraepithelial neoplasia grade 2 or worse as HPV negative and resulting in a delay in management.

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Highlights

Despite a theoretical concern, few HC2 specimens have borderline results in realistic practice.

Few HC2 specimens with borderline-positive results have two consecutive negative results when the specimen is re-tested twice.

For patients with LSIL cytology whose HC2 specimens were re-tested, histological outcomes for those with a negative final HC2 result were not significantly different from those whose final HC2 result was positive.

Our results support categorizing all borderline HC2 positive specimens as positive immediately without repeat.

Number and percent of HC2-equivocal tests out of all cotests by cytology; Number and percent of HC2-equivocal tests with a final HC2-negative result by cytology

Table 1

	Benign	Cytology					Total
		ASC-US ^a	ASC-H ^b	LSIL ^c	HSIL+ ^d	AGC ^e	
Equivocal/Total	9309/823,674	2098/34,813	157/2155	554/9903	104/2272	70/2609	12,292/875,426
%	1.1	6.0	7.3	5.6	4.6	2.7	1.4
HC2-/Equivocal	1413/9309	94/2098	8/157	33/554	7/103	9/70	1564/12,292
%	15.2	4.5	5.1	6.0	6.8	12.9	12.7

^a atypical squamous cells of undetermined significance;
^b atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesion;
^c low-grade squamous intraepithelial lesion;
^d high-grade squamous intraepithelial lesion or worse;
^e atypical glandular cells

Table 2

Histological outcomes for equivocal Hybrid Capture-2 positive and negative women with low-grade squamous intraepithelial lesion cytology (P=0.33)

Final Hybrid Capture-2 Result	Histology		Total
	<CIN2 ^a , n (%)	CIN2+ ^b , n (%)	
Negative	16 (94.1)	1 (5.9)	17
Positive	386 (93.2)	28 (6.8)	414
Total	402	29	431

^a less than cervical intraepithelial neoplasia grade 2;

^b cervical intraepithelial neoplasia grade 2 or worse