Screening for Cervical Cancer in High-Risk Populations: DNA Pap Test or Hybrid Capture II Test Alone?

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Summary: This study was designed to evaluate whether Hybrid Capture II (HC2) test alone refer women to colposcopy as appropriately as DNA Papanicolaou (Pap) test, in the context of a high-risk group of women using the recently validated DNACitoliq® LBC system. Women with suspected cervical disease were included in this cross-sectional study at a tertiary center in São Paulo, Brazil, for further workup. All women had cervical material collected for LBC and HC2 for high-risk human papillomavirus (hrHPV)-DNA test. Irrespective of cytology and HC2 results, colposcopy, and cervical biopsy when applicable, was systematically performed. All tests were performed blindly. Sensitivity, specificity, positive and negative predictive values, and overall accuracy of both methods were computed in relation to histology. A total of 1,080 women were included: 36.4% (393/1080) had ACUS+, 10.2% (110/1080) were high-grade squamous intraepithelial lesions (HSIL) or cancer. Mean age was 33.5 years. All women underwent colposcopy, and cervical biopsies were performed in 38.4% (415/1080): 33% (137/415) of the biopsies were negative, 14.4% (155/415) were low-grade squamous intraepithelial lesions (LSIL), 10.7% (116/415) were HSIL, and 0.6% (7/415) were cancer. HC2 sensitivity to diagnose biopsy-proven HSIL was 100%. Because all HSIL cases had a positive HC2 test, sensitivity could not be improved by adding LBC. Specificity and positive and negative predictive values of DNA Pap were not significantly different from HC2 test alone when considering LSIL+ histology as “gold standard” and HSIL+ histology. As a screening strategy for women with high-risk for cervical cancer, DNA Pap test does not seem to add substantially to HC2 alone in terms of appropriately referring to colposcopy. **Key Words:** Hybrid capture—DCS system—Liquid-based cytology—Papanicolaou test—Cervical cancer—Cervical screening.

The new century has brought new technologies to optimize cervical cancer screening. The introduction of liquid-based cytology (LBC) with preservative liquid medium, allowing for both cell morphology analysis and nucleic acids preservation for molecular tests, has led to

a considerably higher detection rate of high-grade squamous intraepithelial lesions (HSIL) (1,2). Previously, the Hybrid Capture II (HC2) assay for high-risk human papillomavirus (hrHPV) (Digene Corp., Gaithersburg, MD) had been approved as a follow-up test for women who had abnormal or equivocal Papanicolaou (Pap) tests. More recently, the Food and Drug Administration (3) has granted approval to the HPV DNA test to be used simultaneously with LBC Pap test to screen for cervical cancer in women aged 30 years and older. The American Society of Gynecology also has recommended the use of HC2 for equivocal cytology (4). Combination of LBC and HC2 for hrHPV has been reported as a useful technical option for equivocal diagnoses (5) and to enhance the interval

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of screening (6). The high negative predictive value (NPV) of HC2 hrHPV-DNA test (HC2) is assumed to be trustworthy enough to delay the screen interval (5–7). Interestingly, the combined LBC primary smear and HPV testing with a 5-year interval is similar in both cost and effectiveness to the other 3-yearly options of primary smear testing or primary HPV testing alone (8). Despite the recognized success of conventional Pap test in the history of cervical cancer screening, its limitations, namely those related to the high false-negative rates, are well known. Its low sensitivity is a significant problem with public health implications (9). HPV testing has superior sensitivity than cytology, but lower specificity. Taken together, combining Pap test and HPV testing seems promising (10).

The use of HPV test as an adjunct technique to improve cervical screening was first proposed more than a decade ago (11). Ever since, several purposes to change the orthodox prevention organization have been reported to optimize the classic system based on the primary screening with cytology, followed by colposcopy, biopsy, if necessary, and treatment (12). The recently approved DNA Pap test is a hopeful strategy for cervical cancer screening to combine the sensitivity of HC2 test and the specificity of cytology (3). However, is not known whether strategies that have been shown to be cost-effective to screen the general population could be safely recommended to screen special groups of women with high risk for cervical cancer or its precursors.

This study was designed to evaluate whether Hybrid Capture II test alone refer women to colposcopy as appropriately as DNA Pap test, in the context of a high-risk group of women using the recently validated DNACitoliq® LBC system.

MATERIALS AND METHODS

From January through December 2002, consecutive women with suspected cervical disease on the grounds of an abnormal Pap test, and/or altered visual cervical inspection were referred for further workup at The Pérola Bygnton Hospital, São Paulo, Brazil. All women had cervical material collected for LBC and HC2 for hrHPV-DNA test. Irrespective of cytology and HC2 results, colposcopy, and cervical biopsy, when applicable, was systematically performed. LBC, HC2 tests, and histologic evaluation were processed at the Pathology Division of Adolfo Lutz Institute. All tests were performed blindly. The institutional review boards of both institutions involved in the project approved the study protocol.

The brush from the DNA-Citoliq® System (DCS; Digene Brasil, São Paulo, Brazil) was used to collect

cervical material, and subsequently placed into tubes containing 1 ml of the conservative liquid Universal Collection Medium (UCM®). Once at the laboratory, batches of 12 DCS samples were simultaneously prepared in 10 to 15 minutes. In that system, the specimen in the slide is contained in a 25-mm-diameter circle. Cytology results were reported using the terminology of the Bethesda 2001 System (13), with the exception that atypical squamous (ASC-US) and glandular (AGC) cells of undetermined significance were grouped in one variable referred to as ACUS.

HC2 test was performed according to the instructions of the manufacturer. Only the probe for high-risk HPV was used.

Histologic specimens were initially evaluated according to WHO classification of squamous lesions in three classes (CIN 1, 2, and 3) (14). However, for the purposes of statistical analyzes, results were presented in two categories: low-grade squamous intraepithelial lesions (LSIL) (CIN 1) and HSIL (CIN 2 and 3).

Statistical Analysis

Sensitivity, specificity, positive (PPV), and negative (NPV) predictive values of HC2, LBC, and DNA Pap in diagnosing women with cervical disease were calculated having both HSIL+ and LSIL+ histologic results as “gold standard.” The cutoff for a positive LBC was ACUS or higher (ACUS+). HC2 was deemed positive if the relative light units (RLU) were 1 or greater. The McNemar chi-square test was used to test for statistical differences in diagnostic parameters of the three screening strategies. Impact on diagnostic parameters of changing histologic cutoff from LSIL to HSIL was assessed by the chi-square test. A difference was statistically significant if $p < 0.05$. Data were stored and analyzed using the SPSS® statistical software, version 10.1 (SPSS Inc., Chicago, IL).

RESULTS

Of 1,095 women included in the study, 15 had unsatisfactory LBC preparation and were excluded. Among the remaining 1,080 women, 36.4% (393/1080) had a LBC showing ACUS+, 10.2% (110/1080) being HSIL or cancer. Mean age was 33.5 (range, 16–73) years. All women underwent colposcopy, and cervical biopsies were performed in 38.4% (415/1,080). Thirty-three percent (137/415) of those biopsies were negative, 14.4% (155/415) were LSIL, 10.7% (116/415) were HSIL, and 0.6% (7/415) were cancer.

The diagnostic parameters of HC2, LBC, and combined HC2 and LBC (DNA Pap) in relation to histology are shown in Table 1. HC2 sensitivity to diagnose biopsy-proven HSIL was 100%, whereas it declined to 84.2% in case the definition of a positive histologic result was downgraded to LSIL+ ($p < 0.0001$). In contrast, there is an increment on HC2 specificity when the histologic definition switches from HSIL+ (42.8%) to LSIL+ (59.1%) ($p = 0.002$). The same pattern is observed with LBC and DNA Pap.

To assess the diagnostic contribution of LBC in this high-risk population, sensitivity, specificity, and predictive values of DNA Pap were compared with same diagnostic parameters of HC2 test alone (Tables 2 and 3). Because all HSIL cases had a positive HC2 test, sensitivity could not be improved by adding LBC. Specificity and positive and negative predictive values of DNA Pap were not significantly different from HC2 test alone when considering LSIL+ histology as "gold standard" and HSIL+ histology.

Sensitivity and NPV when diagnosing HSIL+ histology were significantly higher for HC2 test than for LBC, whereas LBC had greater specificity and PPV (Table 2). For LSIL histology, HC2 test and LBC had similar predictive values (Table 3).

DISCUSSION

In the present investigation of high-risk women for cervical cancer and its precursors, the diagnostic performance

TABLE 1. Diagnostic performance of HC2, LBC, and DNA Pap using both LSIL+ and HSIL+ as definition for an altered histology

Screening test	Histology		p value
	LSIL+	HSIL+	
HC2 > 1			
Sensitivity	234/278 (84.2)	123/123 (100)	<0.0001
False-positive	56/137 (40.9)	167/292 (57.2)	0.002
False-negative	44/280 (15.8)	0/123 (0)	<0.0001
Specificity	81/136 (59.1)	125/292 (42.8)	0.002
LBC ACUS+			
Sensitivity	195/278 (70.1)	114/123 (92.7)	<0.0001
False-positive	38/137 (27.7)	119/292 (40.8)	0.01
False-negative	83/278 (29.9)	9/123 (7.3)	<0.0001
Specificity	99/137 (72.3)	173/292 (59.2)	0.01
DNA Pap			
Sensitivity	239/278 (86)	123/123 (100)	<0.0001
False-positive	66/137 (48.2)	182/292 (62.3)	0.008
False-negative	39/278 (14)	0/123 (0)	<0.0001
Specificity	71/137 (51.8)	110/292 (37.7)	0.008

LSIL, low-grade squamous intraepithelial lesions; HSIL, high-grade squamous intraepithelial lesions; HC2, Hybrid Capture II. Data are numbers with percentages in parentheses.

TABLE 2. Efficiency of HC2, cervical cytology (ASCUS+), and DNA PAP[†](HCII or ASCUS+) to diagnose histologically confirmed cervical high-grade lesions (HSIL) or cancer

	HC2	ASCUS+	p value*	DNA PAP [†]	p value [‡]
Sensitivity	100%	92.7%	0.007	100%	1
Specificity	42.8%	59.2%	0.0001	37.7%	0.23
PPV	42.4%	48.9%	0.16	41.7%	0.36
NPV	100%	95.1%	0.03	100%	0.28

PPV, positive predictive value; NPV, negative predictive value.

* HC2 versus LBC comparison.

[†] Positive HC2 or the presence of ASCUS+ considered a positive test.

[‡] HC2 versus DNA PAP comparison.

of HC2 test alone to detect HSIL+ was similar to combined LBC and HC2 test (DNA Pap). All histologically proven HSIL+ were detected by HC2 and DNA Pap, as opposed to nine cases missed by LBC. Furthermore, specificity, PPV, and NPV for HC2 test and DNA Pap were not statistically different. The definition of a positive histologic result (LSIL+ or HSIL+) greatly influences the diagnostic parameters of the three screening strategies. Similarly, if DNA Pap is computed as positive only when both HC2 and LBC are positive also will significantly influence diagnostic parameters of the tests.

In the present study, DNA Pap was computed as positive when HC2 or LBC were positive. All remaining combinations of the two screening tests were computed as a negative DNA Pap. Such definitions prioritize sensitivity at the expense of specificity. Indeed, the observed 100% sensitivity of DNA Pap for detecting HSIL+ would have significantly decreased to 92.7% ($p < 0.0001$) had the definition of a positive DNA Pap been both HC2 test and LBC positive.

In this group of high-risk women for cervical cancer included in this study, adding LBC to HC2 test did not add in terms of subsequent patient management. In fact, considering HSIL+ or LSIL+ as "gold standard," the sensitivity, specificity, and predictive values of HC2 alone and DNA Pap test did differ significantly (Tables 2 and 3). This finding is in sharp contrast to what has been

TABLE 3. Efficiency of HC2, cervical cytology (ASCUS+), and DNA PAP[†](HCII or ASCUS+) to diagnose histologically confirmed cervical low-grade lesions (LSIL), HSIL, or cancer

	HC2	ASCUS+	p value*	DNA PAP [†]	p value [‡]
Sensitivity	84.2%	70.1%	0.0001	86%	0.63
Specificity	59.1%	72.3%	0.03	51.8%	0.27
PPV	80.7%	83.7%	0.44	78.4%	0.55
NPV	64.8%	54.4%	0.09	64.5%	0.92

PPV, positive predictive value; NPV, negative predictive value.

* HC2 versus LBC comparison.

[†] Positive HCII or the presence of ASCUS+ considered a positive test.

[‡] HC2 versus DNA PAP comparison.

observed in the general population, in which the prevalence of HSIL is far lower than the 10.2% encountered in our study. Thus, in the general population of women, screening programs based on primary HPV testing and combined HPV and LBC would give rise to a far greater risk of inappropriate referral to colposcopy, as result of high proportion of false-positive tests (8). However, in groups of women with high prevalence of cervical disease, 40.9% of the women with no cervical lesions would be referred to colposcopy on the grounds of a positive HC2 alone, whereas 48.2% would be referred based on a LBC showing ASC-US+ (McNemar test: $p < 0.002$), or 13.7% more inappropriate referral with DNA Pap. At this high disease prevalence level, despite having superior specificity, LBC missed 7.3% of HSIL, as opposed to HC2, which detected all cases.

On the other hand, because all cases with HSIL would have been appropriately referred to colposcopy by HC2 test alone, adding LBC would be of no benefit. Similarly, a negative HC2 test alone would fail to appropriately refer 15.8% of women with LSIL, whereas DNA Pap would not refer 14% of those women (McNemar test: $p = 0.06$).

The histologic cutoff chosen as "gold standard," LSIL+ or HSIL+, has a clear impact on the diagnostic parameters of the screening tests being assessed (Table 1). Sensitivity increases when HSIL is the cutoff and consequently specificity will decrease. Which cutoff to choose will depend on the goal set for the screening program that is being evaluated. A possible objective could be to detect all HSIL, whereas a more comprehensive goal would be the detection of all lesions. In that case, the cutoff should be LSIL+.

In conclusion, as a screening strategy for women with high-risk for cervical cancer, DNA Pap test does not seem to add substantially to HC2 alone in terms of appropriately referring to colposcopy.

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REFERENCES

1. Lörincz A, Anthony J. Advances in HPV detection by Hybrid Capture®. *Papillomavirus Report* 2001;12:145–54.
2. Nonogaki S, Alda Wakamatsu A, Longatto Filho A, et al. Hybrid Capture II and polymerase chain reaction for identifying HPV infections in samples collected in a new collection medium: a comparison. *Acta Cytol* 2004;48:514–20.
3. FDA news. FDA approves expanded use of HPV test. March 31, 2003. Available at: www.fda.gov/bbs/topics/news/2003/new00890.html. Accessed.
4. Cox JT. American Society for Colposcopy and Cervical Pathology. The clinician's view: role of human papillomavirus testing in the American Society for Colposcopy and Cervical Pathology Guidelines for the management of abnormal cervical cytology and cervical cancer precursors. *Arch Pathol Lab Med* 2003;127:950–8.
5. Arbyn M, Buntinx F, Ranst MV, et al. Virologic versus cytologic triage of women with equivocal Pap smears: a meta-analysis of the accuracy to detect high-grade intraepithelial neoplasia. *J Natl Cancer Inst* 2004;96:280–93 W.
6. Witt A, Hudelist G, Gregor H, et al. The detection of HPV DNA improves the recognition of cervical intraepithelial lesions. *Arch Gynecol Obstet* 2003;268:29–34.
7. Nieminen P, Vuorma S, Viikki M, Hakama M, Anttila A. Comparison of HPV test versus conventional and automation-assisted Pap screening as potential screening tools for preventing cervical cancer. *BJOG* 2004;111:842–8.
8. Sherlaw-Johnson C, Philips Z. An evaluation of liquid-based cytology and human papillomavirus testing within the UK cervical cancer-screening programme. *Br J Cancer* 2004;91:84–91.
9. Franco E. Primary screening of cervical cancer with human papillomavirus tests. *J Natl Cancer Inst Monogr* 2003;31:89–96.
10. Clavel C, Cucherousset J, Lorenzato M, et al. Negative human papillomavirus testing in normal smears selects a population at low risk for developing high-grade cervical lesions. *Br J Cancer* 2004;90:1803–8.
11. Reid R, Greenberg MD, Lorincz A, et al. Should cervical cytologic testing be augmented to cervicography or human papillomavirus deoxyribonucleic acid detections? *Am J Obstet Gynecol* 1991;164:1461–9.
12. Solomon D. Role of triage testing in cervical cancer screening. *J Natl Cancer Inst Monogr* 2003;31:97–101.
13. Solomon D, Davey D, Kurman R, for the Forum Group Members and the Bethesda 2001 Workshop. The Bethesda 2001 System: terminology for reporting results of cervical cytology. *JAMA* 2002;287:2114–9.
14. Tavassoli FA, Deville P. Tumours of the breast and female genital organs. *World Health Organization Classification of Tumours*. Lyon IARC Press, 2003.