Original Article

HPV DNA Testing With Cytology Triage in Cervical Cancer Screening: Influence of Revealing HPV Infection Status

Lyndsay Ann Richardson, MSc[1]; Mariam El-Zein, PhD[1]; Agnihotram V. Ramanakumar, PhD[1]; Samuel Ratnam, PhD[2,3]; Ghislain Sangwa-Lugoma, MD[4]; Adhemar Longatto-Filho, PhD[5,6,7,8]; Marly Augusto Cardoso, PhD[9]; Francois Coutlée, MD, PhD[10]; Eduardo L. Franco, DrPH[1]; and The PEACHS (Pap Efficacy After Cervical HPV Status) Study Consortium

BACKGROUND: Knowledge of cervical human papillomavirus (HPV) status might influence a cytotechnician's assessment of cellular abnormalities. The authors compared original cytotechnicians' Papanicolaou (Pap) readings for which HPV status was concealed with Pap rereads for which HPV status was revealed separately for 3 screening populations. **METHODS:** Previously collected cervical Pap smears and clinical data were obtained from the Canadian Cervical Cancer Screening Trial (study A), the Democratic Republic of Congo Community-Based Screening Study (study B), and the Brazilian Investigation into Nutrition and Cervical Cancer Prevention (study C). Smears were reread with knowledge of HPV status for all HPV-positive women as well as a sample of HPV-negative women. Diagnostic performance of Pap cytology was compared between original readings and rereads. **RESULTS:** A total of 1767 Pap tests were reread. Among 915 rereads for HPV-positive women, the contrast between "revealed" and "concealed" Pap readings demonstrated revisions from negative to positive results for 109 women (cutoff was atypical squamous cells of undetermined significance or worse) and 124 women (cutoff was low-grade squamous intraepithelial lesions [LSIL] or worse). For a disease threshold of cervical intraepithelial neoplasia of grade 2 or worse, specificity significantly declined at the atypical squamous cells of undetermined significance cutoff for studies A (86.6% to 75.3%) and C (42.5% to 15.5%), and at the LSIL cutoff for study C (61.9% to 37.6%). Sensitivity remained nearly unchanged between readings, except in study C, in which reread performance was superior (91.3% vs 71.9% for the LSIL cutoff). **CONCLUSIONS:** A reduction in the diagnostic accuracy of Pap cytology was observed when revealing patients' cervical HPV status, possibly due to a heightened awareness of potential abnormalities, which led to more false-positive results. ***Cancer (Cancer Cytopathol)*** **2015;123:745-54**. *© 2015 American Cancer Society.*

KEY WORDS: cervical cancer; human papillomavirus (HPV); Papanicolaou test; screening.

Corresponding author: Eduardo L. Franco, DrPH, Division of Cancer Epidemiology, Department of Oncology, McGill University, 546 Pine Ave West, Montreal, QC, Canada H2W 1S6; Fax: (514) 398-5002; eduardo.franco@mcgill.ca

[1]Division of Cancer Epidemiology, Department of Oncology, McGill University, Montreal, Quebec, Canada; [2]Faculty of Medicine, Memorial University, St. John's, Newfoundland and Labrador, Canada; [3]Public Health Laboratory, St. John's, Newfoundland and Labrador, Canada; [4]Department of Obstetrics and Gynaecology, University of Kinshasa, Kinshasa, Democratic Republic of Congo; [5]Laboratory of Medical Investigation 14, Faculty of Medicine, University of Sao Paulo, FMUSP, Sao Paulo, Brazil; [6]Life and Health Sciences Research Institute, School of Health Sciences, University of Minho, Braga, Portugal; [7]Life and Health Sciences Research Institute/3B's-PT Government Associate Laboratory, Braga/Guimaraes, Portugal; [8]Molecular Oncology Research Center, Barretos Cancer Hospital, Barretos, Sao Paulo, Brazil; [9]Department of Nutrition, School of Public Health, University of Sao Paulo, Sao Paulo, Brazil; [10]Department of Microbiology and Infectious Diseases, Montreal University Medical Center, Montreal, Quebec, Canada

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INTRODUCTION

In view of the well-established causal role for human papillomavirus (HPV) in cervical neoplasia, cervical cancer screening practices in most industrialized countries have incorporated molecular testing for DNA of high-risk HPV types (HR-HPV) as an adjunct to or as cotesting with Papanicolaou (Pap) cytology. A third approach that has gradually gained favor for women aged >25 or 30 years is to use HR-HPV testing as the primary screening method, followed by Pap triage of women found to be HR-HPV positive (HPV/Pap triage).[1] This approach is attractive in the sense that it takes advantage of the high sensitivity of HPV testing as the primary screen while relying on the high specificity of Pap cytology to triage those women found to be positive on first screen.[2-5] HPV/Pap triage has been assessed in various settings, including several randomized controlled trials (RCTs) and simulation studies.[1-10] Another advantage of the HPV/Pap triage approach is to reduce the number of tests performed compared with Pap plus HPV cotesting. In practice, cytotechnicians would have a considerably reduced workload (≤10-fold) but would spend more time scanning slides because of the awareness that the Pap tests would have originated from HR-HPV-positive women.

A true estimate of the efficacy of the HPV/Pap triage algorithm before it is rolled out as standard practice would theoretically require knowledge of a woman's HPV infection status by the cytotechnician at the time of the Pap reading. Cytotechnicians who are aware of a woman being HPV positive may perform more meticulous assessments of Pap tests, thus improving the accuracy of cytological triage. Importantly, this could result in decreased false-negative diagnoses, a more thorough evaluation of borderline abnormalities, and a consequent increase in Pap sensitivity as a triage test. Presumably, the longer time spent reading a Pap test would also permit better scrutiny of reactive atypias and thus it is conceivable that knowledge of the HPV status may also impact favorably on the specificity of cytology as a triage tool.

Currently, to our knowledge there is scanty epidemiologic evidence that cytology readings performed in this context are more accurate than the current practice (ie, Pap test reading without knowledge of the patient's HPV positivity status).[11,12] The objectives of the current study were to 1) assess the influence of revealed (rereads) versus concealed (original readings) HPV DNA status on cytotechnicians' appraisal of cervical Pap tests and 2) determine and compare the effectiveness of Pap cytology as a triage test when cytotechnicians are made aware of the woman's cervical HPV status. We hypothesized that the diagnostic performance of Pap cytology would improve if HPV positivity were to be revealed.

MATERIALS AND METHODS

We set up the PEACHS (Pap Efficacy After Cervical HPV Status) Study Consortium to bring together data and specimens from 3 previously conducted epidemiologic studies to compare the performance of Pap rereads with those from each original study. Cervical smear samples were obtained from the Newfoundland study site of the Canadian Cervical Cancer Screening trial,[2,13] the Community-Based Screening Study from the Democratic Republic of Congo,[14,15] and the Brazilian Investigation into Nutrition and Cervical Cancer Prevention.[16-18] These studies are referred to hereafter as studies A, B, and C, respectively. Details regarding these parent studies and the analysis sample for the current study are summarized in Table 1.[2-18] Written informed consent was obtained from all participants.

Cytology and HR-HPV DNA Testing in Parent Studies in PEACHS

Conventional Pap cytology was performed within studies A and B. Study C used liquid-based cytology (LBC); collected samples were released into liquid suspension using the DNA-Cytoliq System (Digene Brazil, Sao Paulo, Brazil). For the detection of HPV DNA in cervical samples, the Digene Hybrid Capture 2 (hc2) HPV DNA assay (Qiagen, Valencia, Calif) was used in studies A and B, according to the manufacturer's recommendations. A polymerase chain reaction protocol (with MY09/11 primers) was used for HPV DNA testing in study C.[16]

Diagnostic Assessment in Parent Studies

Diagnoses were made via colposcopy-directed biopsies. In study A, individuals underwent colposcopy for a positive HPV test (hc2 test ≥1 pg/mL) or positive Pap test classified as atypical squamous cells of undetermined significance (ASC-US) or worse.[19] In study B, gynecologists performed colposcopies on all participants, and biopsy specimens (histopathological verification) were obtained for women in whom lesional tissue was visualized during colposcopy and for a 20% random sample of women with

TABLE 1. Characteristics of Parent Studies and Reread Subsamples: The PEACHS Study Consortium

	Study A	Study B	Study C
Study characteristics			
Name	Canadian Cervical Cancer Screening Trial[2,13]	Congo Community-Based Screening Study[14,15]	Brazilian Investigation into Nutrition and Cervical Cancer Prevention[16-18]
Design	Randomized controlled trial	Cross-sectional split-sample screening study	Hospital-based case-control study
Location	St. John's (Newfoundland) and Montreal (Quebec), Canada	Kinshasa, Democratic Republic of Congo	Sao Paulo, Brazil
Recruitment period	September 2002-February 2005	November 2003-April 2004	March 2003-May 2005
Recruitment population	Women attending for regular screening at 30 participating preapproved family medicine and gynecology primary clinics	Women invited to attend a cervical cancer prevention program at a local primary health care center	Women attending for regular screening at 1 of 3 participating public hospitals
Blinding of original test results			
Cytology blinded to HPV	Yes	Yes	Yes
HPV blinded to cytology	Yes	Yes	Yes
Colposcopy blinded to cytology	Yes	Yes	No
Colposcopy blinded to HPV	Yes	Yes	Yes
Histology blinded to colposcopy	Yes	Yes	Yes
Histology blinded to cytology	Yes	Yes	No
Histology blinded to HPV	Yes	Yes	Yes
Pap rereads			
Sample selection[a]	All HPV+ Systematic sample of HPV−	All HPV+ Random sample of HPV−	All HPV+ Random sample of HPV−
Analysis	Eastern Health Cytology laboratory in St. John's Newfoundland, Canada	Groupement de Recherche Cytologique in Lyon, France	Department of Pathology at University of Sao Paulo
Time between readings	3 y	2 y	6 mo
Blinding	Unblinded to HPV status Blinded to original Pap reading, colposcopy, and pathology	Unblinded to HPV status Blinded to original Pap reading, colposcopy, and pathology	Unblinded to HPV status Blinded to original Pap reading, colposcopy, and pathology

Abbreviations: +, positive; −, negative; HPV, human papillomavirus; Pap, Papanicolaou; PEACHS, Pap Efficacy After Cervical HPV Status.
[a] Smears were only obtained from the Newfoundland study site.

normal colposcopy findings. Participants in study C were referred for colposcopy based on Pap test positivity at the cutoff of ASC-US or worse.

Selection Criteria of Subsamples

The selection of subsamples was conditional on a woman's original cervical HPV status. All HPV-positive cases and a sample (systematic sampling in study A and random sampling in studies B and C) of HPV-negative cases were selected. For studies A and B, HPV positivity was defined as a positive result on hc2, whereas for study C it was defined as positivity for 1 of 13 HPV genotypes (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) that were detected in hc2. The selection of subsamples was totally blind with respect to all other information available for each subject, including disease status and original cytology reading.

Cytology Reread Procedures

Cytological results were classified on the basis of The Bethesda System,[19] classifying Pap tests as negative for intraepithelial lesions or malignancy (normal); ASC-US, cannot exclude high-grade squamous intraepithelial lesions; low-grade squamous intraepithelial lesion (LSIL); or high-grade squamous intraepithelial lesion. Histopathological ascertainment of the presence of cervical lesions had been done in the parent studies based on the cervical intraepithelial neoplasia (CIN) terminology with the associated low (CIN1) or high (CIN2 or CIN3) grades. Studies B and C included cases of squamous cell carcinoma.

Original cytology readings and rereads for each of the 3 studies were performed by trained cytotechnologists who worked as regular employees in the accredited laboratories that served the 3 studies (Newfoundland, Canada [study A]; Lyon, France [study B]; and Sao Paulo, Brazil [study C]). Cytology training at all 3 sites was based on local national standards that prevailed at the time of the original studies and when rereads were negotiated. The only exception was the cytopathology laboratory in Lyon, France. France was not a study site but the absence of a collaborating cytology laboratory in Kinshasa (study B) led us to arrange a collaboration with the Lyon laboratory

for convenience. Pap tests were shipped directly to Lyon by study personnel in Kinshasa. Cytotechnologist assignment of the Pap test reading was outside of the study control but all readers were blinded to all clinical and laboratory information except for the HPV status of the sample.

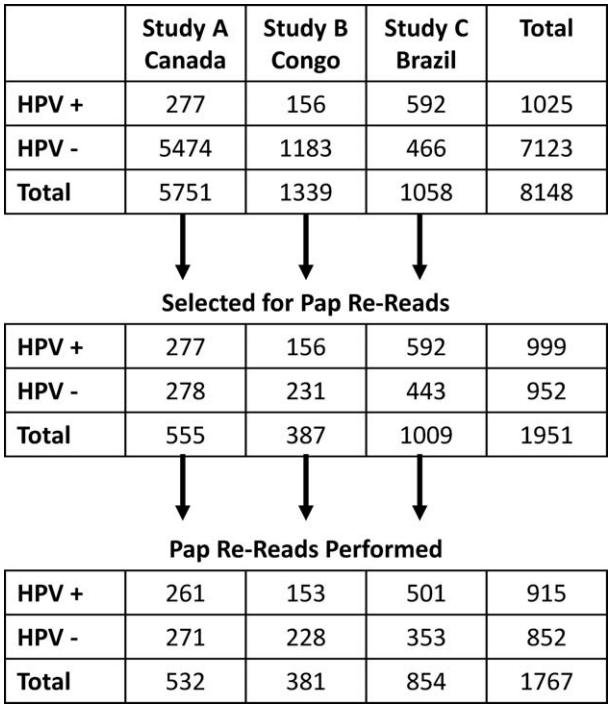

Figure 1. Flow diagram for study-specific selection of Papanicolaou (Pap) test reread samples in the PEACHS (Pap Efficacy After Cervical HPV Status) Study Consortium. The selection of smears for rereading was conditional on human papillomavirus (HPV) status within each parent study. Original cervical slides from parent studies were obtained for all high-risk HPV-positive (HPV+) cases. For study A, Pap tests were obtained only from the Newfoundland study site. A subset of high-risk HPV-negative (HPV−) cases was selected using systematic sampling for study A and random sampling for studies B and C. Pap test rereads were performed by the same cytotechnicians after the HPV status was revealed to them. Both readings were performed with blinding for all other results.

Statistical Analysis

Cytology readings (original vs rereads) were analyzed using 2 binary cutoffs for positivity; borderline result (ASC-US) or worse and LSIL or worse. Agreement between Pap readings (original vs rereads) was assessed using the prevalence-adjusted, bias-adjusted kappa statistic,[20] which accounts for uneven distribution of data across categories of cytology classifications. Binary agreement was measured using the McNemar test. Estimates of sensitivity and specificity were derived by classical 2-by-2 contingency tables, and were also plotted on receiver operating characteristic curves. Verification bias, characterized by an overestimated sensitivity and an underestimated specificity,[21] was corrected for as recommended.[22] Verification bias is best explained by considering the finding that in typical clinical scenarios, the only individuals to undergo the reference standard test for verification of disease are those who are screened as positive.[23,24] This results in a systematic selection bias that, if not accounted for, can lead to biased estimates of sensitivity and specificity of the screening test being evaluated. A method to account for this is to ascertain disease status in a random subsample of disease-negative individuals and apply correction formulae.[22,25] Corrected sensitivity and specificity estimates and associated 95% confidence intervals (95% CIs) were calculated using the Stata macro "Valides."[26] Analyses were performed using the Stata statistical software package (Release 11.0; StataCorp, College Station, TX).

TABLE 2. Study-Specific Disease Outcomes Defined by Histopathology According to HPV Status: The PEACHS Study Consortium

Study (Country)	HPV Status	NILM No. (%)	CIN1 No. (%)	CIN2 No. (%)	CIN3 No. (%)	ICC No. (%)	Unverified No. (%)	Totals No. (%)
Study A (Canada)	HPV+	215 (82.4)	14 (5.4)	10 (3.8)	3 (1.1)	0 (0.0)	19 (7.3)	261 (100.0)
	HPV−	16 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	255 (94.1)	271 (100.0)
Study B (Congo)	HPV+	46 (30.1)	12 (7.8)	5 (3.3)	16 (10.5)	0 (0.0)	74 (48.4)[a]	153 (100.0)
	HPV−	83 (36.4)	3 (1.3)	0 (0.0)	0 (0.0)	1 (0.4)	141 (61.8)	228 (100.0)
Study C (Brazil)[b]	HPV+	104 (20.8)	77 (15.4)	84 (16.8)	188 (37.5)	48 (9.6)	0 (0.0)	501 (100.0)
	HPV−	278 (78.8)	37 (10.5)	19 (5.4)	14 (4.0)	5 (1.4)	0 (0.0)	353 (100.0)

Abbreviations: +, positive; −, negative; CIN, cervical intraepithelial neoplasia; HPV: human papillomavirus; ICC, invasive cervical cancer, includes squamous cell carcinoma and adenocarcinoma; NILM, negative for intraepithelial lesions or malignancy; PEACHS, Pap Efficacy After Cervical HPV Status.
[a] With respect to histopathology, all women underwent a colposcopy.
[b] Individuals in the control group were classified as NILM according to patient history.

TABLE 3. Study-Specific Agreement Between Original Pap Cytology Readings and Rereads According to HPV Status And Pap Cytology Threshold Of Positivity: The PEACHS Study Consortium

Study (Country)	HPV Status	Cutoff	Original Readings/Rereads +/+	+/−	−/+	−/−	McNemar Test *P*	Kappa[a]
Study A (Canada)	HPV+	ASC-US	34	3	31	193	<.0001	0.74
		LSIL	15	3	15	228	.0075	0.86
	HPV−	ASC-US	0	0	0	271	NA	1.00
		LSIL	0	0	0	271	NA	1.00
Study B (Congo)	HPV+	ASC-US	57	0	8	88	.0078	0.90
		LSIL	39	1	17	96	.0001	0.76
	HPV−	ASC-US	7	3	1	217	.6250	0.96
		LSIL	4	3	1	220	.6250	0.76
Study C (Brazil)	HPV+	ASC-US	392	5	70	34	<.0001	0.70
		LSIL	313	9	92	87	<.0001	0.60
	HPV−	ASC-US	33	60	20	240	<.0001	0.55
		LSIL	18	30	5	300	<.0001	0.80

Abbreviations: +, positive; −, negative; ASC-US, atypical squamous cells of undetermined significance; HPV, human papillomavirus; LSIL, low-grade squamous intraepithelial lesion; NA, not applicable (no test statistic due to perfect agreement [100%]); Pap, Papanicolaou; PEACHS, Pap Efficacy After Cervical HPV Status.

[a] Prevalence-adjusted, bias-adjusted Kappa statistic.

RESULTS

In total, 1767 Pap tests were reread: 915 from HPV-positive women (89% of original samples) and 852 from HPV-negative women (12%) (Fig. 1). Exclusions were made in the event of inconclusive Pap test results, slide loss or breakage, or inadequacy of the tests at the time of rereading (eg, decay of staining intensity). Disease outcomes from each parent study according to the HPV status of the samples are shown in Table 2. Outcome verification in studies A and B was conditional on positive screening test results, or if selected at random for disease ascertainment. Of the 261 patients in study A with completed rereads, 242 (93%) were ascertained for disease. For study B, 52% of patients were ascertained for disease (79 of 153 available rereads). In study C, disease outcome information was available for all individuals. Collectively, among HPV-positive women, 103 cases of CIN1, 99 cases of CIN2, 207 cases of CIN3, and 48 cases of invasive carcinoma were identified.

Table 3 shows the agreement between original results and those after rereading the same Pap tests for each parent study according to HPV status and cytological threshold of positivity. Using the ASC-US cutoff, 85.8% of 261 smears in study A were originally classified as normal, whereas 75.1% were considered normal at the time of the reread. In study B, 62.7% of 153 tests were classified as normal at the time of the original readings and 57.5% as normal at the reread. In study C, 20.8% and 7.8%, respectively, of 501 Pap tests were classified as normal at the time of the original reading and at the reread. For each study, significant disagreement between smear readings were observed for HPV-positive women (as shown in the columns ± and -/+ in Table 3). In HPV-negative women, cytotechnician readings tended to agree between the original readings and rereads; there was perfect agreement (kappa of 1.0 at the ASC-US cutoff) in study A and very strong agreement (kappa of 0.96 at the ASC-US cutoff) in study B. In study C, agreement was lower (kappa of 0.55 at the ASC-US cutoff) with many discordant cases, mostly revisions from positive on original readings to negative on rereads.

As shown in Table 4, specificity estimates were significantly lower in rereads versus original readings in studies A and C at the outcome of CIN2 or worse. Specificity at the ASC-US cutoff declined from 85.6% (95% CI, 80.4%-89.9%) to 73.4% (95% CI, 67.1%-79%) in study A and from 42.5% (95% CI, 35.2%-50.1%) to 15.5% (95% CI, 10.5%-21.6%) in study C. In addition, specificity significantly declined at the LSIL cutoff for study C, from 61.9% (95% CI, 54.4%-69.0%) to 37.6% (95% CI, 30.5%-45.1%). Sensitivity remained highly consistent between original and reread Pap test results at the ASC-US cutoff. However, at the LSIL cutoff, a significant increase in sensitivity was found in study C (from 79.1% [95% CI, 74.2%-83.4%] to 91.3% [95% CI, 87.6%-94.1%]).

TABLE 4. Diagnostic 2-By-2 Tables: Sensitivity And Specificity Of Pap Cytology To Detect CIN2+ In HPV+ Women According To Pap Cytology Cutoff: The PEACHS Study Consortium

Study (Country)	Pap Cutoff	Pap Readings	True-Positive No. (%)	False-Positive No. (%)	False-Negative No. (%)	True-Negative No. (%)	Sensitivity Estimate (95% CI)	Specificity Estimate (95% CI)
Study A (Canada)	ASC-US	Original	4 (1.7)	33 (13.6)	9 (3.7)	196 (81.0)	30.80 (9.1-61.4)	85.60 (80.4-89.9)
		Reread	4 (1.7)	61 (25.2)	9 (3.7)	168 (69.4)	30.80 (9.1-61.4)	73.40 (67.1-79.0)
	LSIL	Original	2 (0.8)	16 (6.6)	11 (4.5)	213 (88.0)	15.40 (1.9-45.4)	93.00 (88.9-96.0)
		Reread	4 (1.7)	26 (10.7)	9 (3.7)	203 (83.9)	30.80 (9.1-61.4)	88.60 (83.8-92.4)
Study B (Congo)	ASC-US	Original	18 (22.8)	18 (22.8)	3 (3.8)	40 (50.6)	85.70 (63.7-97.0)	69.00 (55.5-80.5)
		Reread	18 (22.8)	22 (27.8)	3 (3.8)	36 (45.6)	85.70 (63.7-97.0)	62.10 (48.4-74.5)
	LSIL	Original	13 (16.5)	11 (13.9)	8 (10.1)	47 (59.5)	61.90 (38.4-81.9)	81.00 (68.6-90.1)
		Reread	18 (22.8)	20 (25.3)	3 (3.8)	38 (48.1)	85.70 (63.7-97.0)	65.50 (51.9-77.5)
Study C (Brazil)	ASC-US	Original	293 (58.5)	104 (20.8)	27 (5.4)	77 (15.4)	91.60 (88.0-94.4)	42.50 (35.2-50.1)
		Reread	309 (61.7)	153 (30.5)	11 (2.2)	28 (5.6)	96.60 (93.9-98.3)	15.50 (10.5-21.6)
	LSIL	Original	253 (50.5)	69 (13.8)	67 (13.4)	112 (22.4)	79.10 (74.2-83.4)	61.90 (54.4-69.0)
		Reread	292 (58.3)	113 (22.6)	28 (5.6)	68 (13.6)	91.30 (87.6-94.1)	37.60 (30.5-45.1)

Abbreviations: +, positive; 95% CI, 95% confidence interval; ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HPV: human papillomavirus; LSIL, low-grade squamous intraepithelial lesion; Pap, Papanicolaou; PEACHS, Pap Efficacy After Cervical HPV Status.

Visual representation of sensitivity and specificity estimates is shown in Figure 2, with directional arrows plotting the changes from original readings to rereads at the outcome of CIN2 or worse for the ASC-US and LSIL cutoffs. Figure 2 clearly shows that, at the ASC-US cutoff, rereads had decreased specificity compared with original readings. Specificity estimates also declined in rereads at the LSIL cutoff; however, gains in sensitivity were observed in each parent study. Similar results were found using CIN3 or worse as disease endpoint (data not shown).

DISCUSSION

The current study addresses an important question of whether a cytotechnician's awareness that a Pap test to be read came from a HR-HPV-positive woman will lead to an improvement in the accuracy of the ensuing Pap report, which is correlated with disease status. In practice, this is the scenario that will prevail in settings that adopt the HPV/Pap triage approach to cervical cancer screening because Pap tests will only be read (or prepared from LBC) if the initial screen with a validated HR-HPV test is positive. We used previous molecular epidemiologic or screening studies that had preserved Pap tests and data regarding HPV positivity and cervical disease status. Our hypothesis was that the extra time and attention devoted by a reader who is aware that the patient harbors a cervical HPV infection would have resulted in more meticulous reading. The current study findings demonstrated that revealing HPV positivity had a marked influence on the cytotechnician's grading of cervical Pap tests. There was a tendency to overcall abnormalities in rereads of HPV-positive women, which translated in more false-positives findings at both the ASC-US and LSIL cutoffs with consequent losses in specificity compared with the original smear readings (blinded to HPV status). Conversely, in all 3 parent studies, there was a gain in sensitivity that was pronounced when based at a threshold of LSIL to define a positive smear.

The HPV/Pap triage serial screening algorithm is best described as a testing-in-series design, specifically a "test-if-positive" design; the second test (Pap cytology) is performed based on positive results of the first test (HPV testing). Therefore, derived Pap estimates are entirely conditional on HPV positivity, and differ from unconditional, or stand-alone, Pap estimates.[27] This prevents us from comparing our sensitivity and specificity estimates with previously derived estimates of Pap as a stand-alone test, such as the often quoted 51% benchmark of Pap sensitivity.[28] Doing so could lead to erroneous inferences regarding the performance of screening tests.

In a large-scale RCT performed in Finland, primary HPV testing was evaluated in women as part of a mass organized screening program.[29] Women were randomized to either conventional Pap screening or HPV/Pap triage. Pap triage was found to alleviate declines in

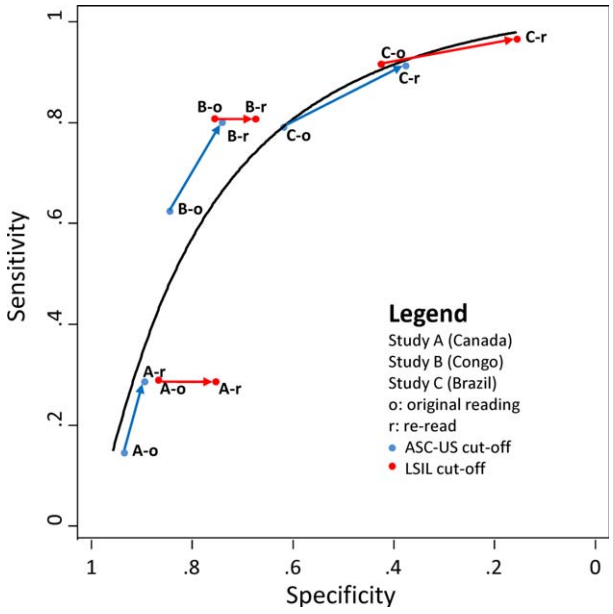

Figure 2. Summary receiver operating characteristic curve of sensitivity and specificity estimates in human papillomavirus-positive women in the PEACHS (Pap Efficacy After Cervical HPV Status) Study Consortium. Study outcome was cervical intraepithelial neoplasia of type 2 or worse. Drawn arrows represent paths from original readings to rereads. A-o indicates Canada study original readings; A-r, Canada study rereads; B-o, Congo study original readings; B-r, Congo study rereads; C-o, Brazil study original readings; C-r, Brazil study rereads. Red arrows represent differences from original readings to rereads at the cutoff of low-grade squamous intraepithelial lesion (LSIL). Blue arrows represent differences from original readings to rereads at the cutoff of atypical squamous cells of undetermined significance (ASC-US). Estimates shown for studies A and B were corrected for verification bias.

specificity compared with stand-alone HPV testing (CIN2 or worse: specificity of 91.7% for HPV alone vs 98.9% for HPV/Pap triage). At follow-up, using HPV/Pap triage resulted in significant gains in relative sensitivity.[3] Given the absence of a direct comparison of blinded estimates of sensitivity and specificity in the Finnish study, we can only speculate regarding the potential level of bias in specificity. However, the Finnish study was performed within a structured organized screening program, and therefore the quality control practices in place may have counteracted the level of biases that we observed with knowledge of HPV status.

Another RCT that evaluated the HPV/Pap triage approach was performed in British Columbia, Canada (HPV FOCAL study).[30] In contrast to the Finnish trial, LBC was used in the FOCAL trial. Initial (round 1) results suggested that the use of HPV/Pap triage increases specificity over high-risk HPV testing alone.[31]

More recently, a substudy of the New Technology in Cervical Cancer RCT evaluated the influence of cytotechnicians' informed knowledge of HPV positivity.[11] Comparing their uninformed Pap cytology readings with their informed Pap cytology readings, a relative sensitivity of 1.58 (95% CI, 1.22-2.01) at a cutoff of CIN2 or worse was identified, which underscores the gain in Pap cytology sensitivity that comes from revealing the HPV positivity status. A gain in sensitivity with a minor loss in specificity was also observed by Benoy et al in a study in Belgium.[12]

The low prevalence of disease in negative Pap tests is a well-known contributing factor to the tendency toward false-negative rates associated with Pap cytology.[32] The foremost rationale for HPV/Pap triage lies in the premise that narrowing the caseload of Pap tests to only HPV-positive women will alleviate the redundancy of reading many negative Pap tests and enrich the caseload with tests that are more likely to harbor precancerous lesions.[1,32]

Truly alleviating subjectivity may necessitate the incorporation of newer technologies in the form of improved preparation of Pap tests with enhanced readability (ie, LBC rather than conventional cytology) or alternatively through automated cytology technologies. Although LBC is increasingly being incorporated into screening programs, a certain level of speculation exists with regard to the true benefit offered. As part of a cervical screening RCT conducted in Italy, LBC (performed in 22,708 cases) did not appear to offer statistically significant improvements in sensitivity over conventional cytology (performed in 22,466 cases).[33] In the current study, LBC was used only in study C, which might explain in part the relatively higher accuracy of sensitivity estimates in that study. However, using an LBC medium for HPV testing and the triage with Pap cytology of HPV-positive women appears to improve the feasibility of an HPV/Pap triage strategy by avoiding the need to obtain a new sample for a Pap cytology from HPV-positive women at a follow-up visit.

The ability of a cytotechnician to reliably discern true borderline abnormalities could presumably be refined with more years of experience. The American ASC-US and LSIL Triage Study found that, at the time of reassessment of cervical smears and independent revision by a cytopathology quality control team, the greatest discordance between readings was found in those originally

classified as ASC-US.[34] Approximately 39% of cytological tests deemed as ASC-US at the time of the original reading were revised to negative on quality control review, with moderate agreement observed between cytological readings (kappa of 0.46). In contrast, the declines in agreement in the current study were driven down by changes from negative on original readings to ASC-US on rereads. One may speculate whether the loss in specificity due to upgrades to ASC-US from negative could be counteracted by a well-organized quality control program. The current study differed in that both of our readings were performed by cytotechnicians, with no formal quality review performed other than the laboratories' accreditation of parent studies, and the fact that we considered a group entirely composed of HPV-positive women compared with a 37% HPV-positivity rate in the ASC-US and LSIL Triage Study trial.[34] Had our positive reread reports been verified via a cytopathologist's quality review, we might not have observed such a decline in specificity. Conversely, cytotechnicians may have been more perceptive of minor HPV-associated cellular changes and decided to call the Pap tests positive despite the fact that such changes were not important enough to translate into histopathologically recognizable lesions.

We assessed the influence of HPV-revealed rereads in random samples of HPV-negative patients to give us a better understanding of reproducibility in a situation in which little change would be expected, as well as to assess for differences between readings that may be representative of personnel changes and a potential decline in test quality over time. Evidently, we found agreement to be much higher in HPV-negative compared with HPV-positive individuals, given the high percentages of negative Pap tests, which is expected for HPV-negative women. Knowledge that women are negative for HPV infection may provide reassurance to cytotechnicians in reaffirming what are more easily discerned as negative results.

A strength of the current study was the opportunity to compare findings across 3 vastly different study settings, which reflect the reality of 3 markedly different countries with respect to the incidence of cervical cancer: low (Canada), intermediate (Brazil), and high (Congo). Similar trends were observed across studies despite sample size limitations, substantial heterogeneity across studies, and a relatively small sample for disease outcomes. Greater precision and accuracy was permitted in study C, in which 47% of patients were HPV-positive women with high-grade CIN3 or worse. The findings of the current study possibly reflect the various levels of influence that knowledge of HPV status could have within different screening settings. Of greatest clinical interest was our observed diagnostic performance of a Pap test at the LSIL threshold; specificity estimates were not found to be substantially affected on rereading but there were gains in sensitivity. This finding was expected given the subjectivity associated with the ASC-US "borderline" category.

The findings of the current study are provocative and counterintuitive. We failed to confirm our original hypothesis of across-the-board gains in performance that we expected to occur with an artificially high disease prevalence (ie, the tray of Pap tests from HPV-positive women) and reduced workload (ie, $<10\%$ of all Pap tests). We expected that awareness of HPV positivity would have improved the accuracy of readings because cytotechnicians would be compelled to meticulously focus on abnormalities that would have otherwise gone unnoticed if the Pap test reading workload were not enriched (ie, had a low prevalence of lesions). Actually, heightened attention appears to have led to more false-positive results, which adversely affected specificity with the consequence of more women been referred for colposcopy.

The current study findings underscore the importance of maintaining meticulous quality control practices for cytology, even when it is serving as a triage test subsequent to primary HPV testing. Our observation of better preserved estimates at the higher LSIL threshold suggests that off-setting the loss in specificity may be achieved through the use of LSIL as a cutoff for Pap positivity within the context of diagnostic triage. Third-party review by cytopathologist appraisal may also counter the tendency toward overcalls observed due to revealed HPV positivity.

Because cohorts of vaccinated women will reach screening age over the next few decades, declines in oncogenic HPV infections that vaccines protect against could be anticipated. Although our data sets do not include vaccinated women, the current study provided a unique setting in which to assess the diagnostic triage value of Pap cytology for HPV-positive women. Avoidance of subjectivity may ultimately necessitate the consideration of molecular markers, including HPV genotyping, to augment the value of cytology.

In practice, the HPV/Pap triage approach has been successfully implemented in some real-world settings,

with gains in efficiency noted in the detection of high-grade precancerous lesions and cancer and reduced wait times for Pap test processing and scheduling of colposcopies.[35] These findings indicate that in the new strategy of HPV/Pap triage cytology, quality control will have to incorporate safeguards to protect against a tendency to overcall cytological grades. p16 staining and other markers may provide the same benefit.[36] However, after 20 to 30 years, nearly all women entering screening age will have been vaccinated and lesion prevalence will have been reduced to levels that will be so low as to affect the overall efficiency of any cervical cancer screening program, irrespective of technology. A reassessment of cervical cancer screening in all its dimensions will have to be made with due attention to the balance of risks and benefits that will prevail in an era of very low risk for cervical cancer and its precursors.[1,32]

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CONFLICT OF INTEREST DISCLOSURES

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