Cancer Biomarkers 34 (2022) 347–358
DOI 10.3233/CBM-210366
IOS Press

Comparison of CINtec PLUS cytology and cobas HPV test for triaging Canadian patients with LSIL cytology referred to colposcopy: A two-year prospective study

Laura Gilbert[a,b], Sam Ratnam[a,c,d,*], Dan Jang[c], Reza Alaghehbandan[e], Miranda Schell[f], Rob Needle[a,b], Anne Ecobichon-Morris[c], Arnav Wadhawan[c], Dustin Costescu[f], Laurie Elit[f], Peter Wang[a], George Zahariadis[a,b] and Max Chernesky[c]
[a]*Memorial University, Faculty of Medicine, St. John's, Canada*
[b]*Eastern Health, Public Health Microbiology Laboratory, St. John's, Canada*
[c]*McMaster University, St. Joseph's Healthcare, Hamilton, Canada*
[d]*McGill University, Faculty of Medicine, Montreal, Canada*
[e]*University of British Columbia, Faculty of Medicine, Vancouver, Canada*
[f]*McMaster University, Hamilton Health Sciences, Hamilton, Canada*

Received 12 July 2021
Accepted 2 December 2021

Abstract.
OBJECTIVES & METHODS: CINtec PLUS and cobas HPV tests were compared for triaging patients referred to colposcopy with a history of LSIL cytology in a 2-year prospective study. Cervical specimens were tested once at enrollment, and test positivity rates determined. Test performance was ascertained with cervical intraepithelial neoplasia grade 2 or worse (CIN2+) and CIN3 or worse (CIN3+) serving as clinical endpoints.
RESULTS: In all ages, (19–76 years, $n = 598$), 44.3% tested CINtec PLUS positive vs. 55.4% HPV positive ($p < 0.001$). To detect CIN2+ ($n = 99$), CINtec PLUS was 81.8% sensitive vs. 93.9% for HPV testing ($p = 0.009$); genotype 16/18-specific sensitivity was 46.5%. Specificity was 52.9% vs. 36.6%, respectively ($p < 0.001$). In all ages, to detect CIN3+ ($n = 44$), sensitivity was 93.2% for both tests; genotype 16/18-specific sensitivity was 52.3%. Specificity was 48.4% for CINtec PLUS vs. 31.1% for HPV testing ($p < 0.001$). In patients < 30 years, CINtec was 91.7% sensitive vs 95.8% for HPV testing ($p = 0.549$).
CONCLUSIONS: CINtec PLUS or cobas HPV test could serve as a predictor of CIN3+ with high sensitivity in patients referred to colposcopy with a history of LSIL regardless of age while significantly reducing the number of LSIL referral patients requiring further investigations and follow-up in colposcopy clinics.

Keywords: p16/Ki-67 dual-stain cytology, CINtec PLUS cytology, cobas HPV test, human papillomavirus (HPV) triage, low-grade squamous intraepithelial lesion (LSIL) triage, cervical intraepithelial neoplasia grade 2 or worse (CIN2+), cervical intraepithelial neoplasia grade 3 or worse (CIN3+)

1. Introduction

While some countries have successfully transitioned to human papillomavirus (HPV) primary cervical cancer screening, Papanicolaou cytology remains the mode

*Corresponding author: Sam Ratnam, St. Joseph's Healthcare, Research Laboratory, Room T3338. 50 Charlton Avenue East, Hamilton, ON, L8N 4A6, Canada. E-mail: sratnam@mun.ca.

of primary screening in many jurisdictions, including Canada, for a variety of reasons. In cytology-based cervical cancer screening, a large number of patients are diagnosed as having borderline or low-grade abnormal cytology who are managed at considerable costs, while only a small fraction is at risk. It could be beneficial to consider currently available triage options, especially in managing patients referred to colposcopy with a history of low-grade squamous intraepithelial lesion (LSIL), in routine colposcopy clinical practice.

LSIL accounts for a large proportion of abnormal cytology in routine screening but it regresses in the majority of cases. However, a small fraction has high grade squamous intraepithelial lesions (HSIL) or could be at risk of progression to HSIL and cervical cancer. Due to this risk, those found to have LSIL in routine screening are either referred to colposcopy directly or managed cytologically, with those having persistent abnormalities being referred to colposcopy [1–3]. In colposcopy clinics, LSIL cases are typically followed with cytology, colposcopy, and biopsy as indicated, for an extended period. With the majority being not at risk, this is excessive and unnecessary for most patients and associated with considerable negative health effects due to distress over prolonged period, increased anxiety at every clinic visit, unnecessary invasive procedures, and overtreatment etc., leading to poorer quality of life [4,5]. An effective triage of LSIL referral patients can identify those at increased risk who need to remain under care and return those not at immediate risk to routine screening [4,6], thus eliminating potential negative health effects and reducing systemic costs.

The CINtec PLUS cytology (Roche Diagnostics) has emerged as an effective biomarker-based adjunct test for triaging patients having atypical squamous cells of undetermined significance (ASCUS) or LSIL in cytology screening [5,7–11] and those testing positive for high-risk human papillomavirus (hr-HPV) in HPV primary screening [12–15]. CINtec PLUS is a dual-stain immunocytochemical test which detects p16 and Ki-67 proteins that are over expressed in cervical cells with transforming HPV infection. As the expression of p16 and Ki-67 is mutually exclusive in normal cells, the co-detection of these proteins simultaneously within the same cervical epithelial cell serves as a specific marker of HPV-mediated oncogenic transformation and predictor of cervical cancer risk [5,7–10,16]. CINtec PLUS has been shown to be more sensitive than cytology with equal specificity, and more specific than HPV testing with relatively comparable sensitivity for detecting cervical intraepithelial neoplasia grade 2 or worse (CIN2+) in LSILs [11,17–20]. While the clinical applications of CINtec PLUS in LSIL triage have been assessed in several studies in Europe and elsewhere [11,18,21,22], there has been limited evaluation of this method to serve as an adjunct test in LSIL triage in North American settings [23].

The ALTS LSIL study precluded LSIL-HPV triage as 83% of LSIL cases tested hr-HPV positive [24]. However, this study was conducted in patients mostly < 30 years. There is evidence that LSIL-HPV triage could be effective in those $\geqslant 35$ years [4,6]. The cobas HPV DNA test (Roche Diagnostics) is a PCR-based qualitative partial genotyping test, identifying genotypes 16/18 specifically and 12 other high-risk (OHR) types collectively in a single analysis, and has been recommended for genotype 16/18-specific risk threshold in HPV primary screening [25]. In this respect, the cobas HPV test also has the potential to serve as an adjunct test for triaging LSIL referral populations within colposcopy clinics, and this could reduce the number of patients requiring additional investigations and follow-ups, and thus aid in better patient care and resource management.

We conducted a study to assess positivity rates of CINtec PLUS and cobas HPV tests along with genotype 16/18-specific risk threshold among those referred to colposcopy with a history of LSIL, to identify those at increased risk and thus potentially reduce the proportion requiring further colposcopy clinic visits and follow-up, and prospectively determined clinical efficacy of the two tests to detect CIN2+. The initial study data were obtained at baseline, and the study cohort remaining under care in the colposcopy clinic was followed up to 2 years to ascertain disease outcome. We previously communicated our baseline findings [16], and in this manuscript, we present the complete data obtained in the study.

2. Methods

2.1. Ontario cervical cancer screening guidelines

In the province of Ontario, Canada, liquid-based Papanicolaou cytology is being used for primary cervical cancer screening. In this system, if cytology is normal, triennial screening continues. For those with LSIL cytology, either direct referral to colposcopy or repeat cytology at 6-month intervals is recommended; for those having persistent atypical squamous cells of undetermined significance (ASCUS) or worse in repeat cytol-

ogy, colposcopy is recommended [1]. In colposcopy clinics, all referred patients undergo cytology and colposcopic examination with biopsies of any lesions detected, and further follow-up clinical pathways depend on specific criteria as previously described [16].

2.2. Study design and protocol

The study was designed to assess CINtec PLUS cytology and HPV test positivity at baseline (enrollment) to identify the proportion potentially at increased risk, and therefore, requiring continued follow-up in the colposcopy clinic, and conversely, the proportion that could be returned to routine screening, thus improving overall clinical and systemic efficiency. In relation to this, the study was also designed to determine clinical efficacy of CINtec PLUS and HPV tests to detect CIN2+. This was assessed at baseline, and prospectively during a 2-year follow-up of patients who remained under care in the colposcopy clinic.

The study was conducted within the Ontario cervical screening guidelines. The study population comprised of patients with a history of LSIL cytology referred to the colposcopy clinic at Juravinski Hospital, Hamilton, Canada. All study patients were attended to per standard of care, with cervical specimens collected for cytology, and colposcopy and biopsies performed per routine clinical practice. Cytology was carried out as part of routine patient care, and CINtec PLUS and cobas HPV tests were performed once at enrollment using the residual cervical specimens for the study purpose. Patients' baseline data were recorded, and the study cohort remaining under care in the colposcopy clinic was followed up to 2 years to determine disease outcome. Biopsy confirmed CIN2+ served as the clinical endpoint. CINtec PLUS and HPV testing results obtained at baseline together with that of biopsies performed either at baseline or anytime during the follow-up were recorded as primary study outcomes. CINtec PLUS and HPV positivity rates that would correspond to the proportions requiring further colposcopy clinic visits and follow-up were determined. CINtec PLUS and HPV results obtained at baseline were correlated with biopsy confirmed CIN2+ to ascertain the clinical performance of the tests.

2.3. Ethics

The study was approved by the Hamilton Integrated Research Ethics Board (HiREB) and Newfoundland and Labrador Health Research Ethics Board (HREB). All participants were informed verbally and in writing about the study, use of their residual cervical specimens for CINtec PLUS and HPV testing, and the need to periodically review their medical records during follow-up. Those consenting to participate were enrolled with written informed consent.

2.4. Patient enrolment criteria

Patients with a history of LSIL cytology who had not received treatment were eligible. Enrolment criteria included: 1) patients who had LSIL cytology in routine primary screening and who were directly referred to colposcopy, 2) those who were found to have LSIL cytology initially in routine primary screening and who upon repeat cytology found to have persistent ASCUS or LSIL and referred to colposcopy, and 3) those who were diagnosed as having LSIL among patients being followed in the colposcopy clinic. There were no age limits. Pregnant persons and those without a cervix were excluded. Eligible patients were enrolled consecutively from November 2017 through February 2019.

2.5. Study specimens

Cervical specimens were collected into ThinPrep PreservCyt® (Hologic Inc) cytology medium using their standard collection device for routine cytology at enrolment. Slides were prepared for CINtec PLUS testing using residual cervical specimens at the cytology laboratory, St. Joseph's Healthcare, Hamilton. The slides and aliquots of cervical specimens were shipped to the Public Health and Microbiology Laboratory, St. John's for CINtec PLUS and cobas HPV tests. These tests were carried out as described below no later than 6 weeks post collection.

2.6. CINtec PLUS cytology

Slides were prepared on a ThinPrep processor (T5000, Hologic Inc) using special ThinPrep slides (Hologic, Inc) and stained using CINtec PLUS test kits within 48 hours and processed on BenchMark ULTRA system (Roche Diagnostics) per manufacturer's instructions.

The CINtec PLUS slides were initially evaluated independently by one of two experienced cytotechnologists who were trained to read these slides. Smears were determined to be positive if at least one cervical epithelial cell showed both a brownish cytoplasmic immunostaining for p16 and a red nuclear immunostaining for Ki-67 regardless of cellular morphology. If the dual staining was not observed, the smear was considered

negative. Smears were deemed unsatisfactory if they did not contain an adequate number of cells ($>$ 4 cells per field with a minimum of 10 fields with a 40x objective). All slides were independently reviewed by a study pathologist trained to read CINtec PLUS slides, and the results recorded using the same criteria. Discrepant slides were either internally reviewed by another reader and reconciled or adjudicated independently by an external expert.

2.7. cobas HPV test

The cobas HPV test was performed on the Roche 4800 automated platform per manufacturer's instructions. Results were reported as positive for genotypes 16 and/or 18, and/or 12 OHR types, or negative for 14 hr-HPV types, per standard practice.

2.8. Cervical biopsy

Biopsies were performed by colposcopists per standard clinical practice. Three sections of each biopsy sample were processed with hematoxylin and eosin (H&E) staining per routine practice. p16 immunostaining (CINtec® Histology kit, Roche Diagnostics) was performed as part of the study protocol to provide supporting diagnostic evidence. Biopsies were read by staff pathologists at the originating colposcopy clinic site per standard practice. All biopsy slides together with p16 stained slides were independently reviewed by two study pathologists. Discrepant biopsy results were independently adjudicated by a third pathologist, if needed.

2.9. Results management

CINtec PLUS and HPV tests were conducted independently. Cytotechnologists and the study pathologists were blinded to test results as well as cytology and biopsy results obtained at baseline. Colposcopy clinicians did not have access to CINtec PLUS or HPV results at the time of initial patient evaluation. Only HPV results were provided subsequently to clinicians to aid in patient management.

2.10. Data analysis

Statistical analysis was performed using SPSS for Windows, versions 23 and 27, Excel, Microsoft Office Professional Plus, 2013, MedCalc, 2021, and Social Science Statistics website, 2020 initially at baseline, as previously described [16], and after two-years of prospective follow-up. Qualitative variables were studied through different frequencies. Descriptive statistics were prepared for test results, distribution of cytology grades and HPV genotypes. Study data were analyzed using contingency tables to determine test positivity rates, and the diagnostic indices of CINtec PLUS and HPV testing. Receiver operating characteristic (ROC) analyses were performed for CINtec PLUS and HPV testing in detecting CIN2+. The area under the curve was calculated for each test as an alternative single indicator of test performance. All tests were two-tailed, and $p< 0.05$ was considered statistically significant.

3. Results

3.1. Study population

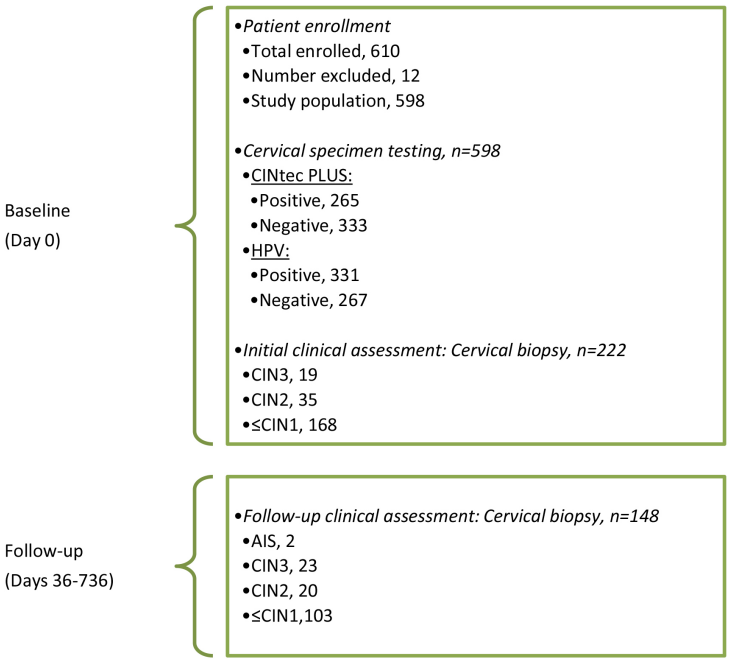
A total of 610 patients meeting the study criteria were enrolled in the study. Of these, 12 were excluded due to insufficient or no cervical specimen for CINtec PLUS and/or HPV testing, or invalid CINtec PLUS or HPV test results, leaving 598 patients in the study with evaluable results (Fig. 1). Age ranged from 19 to 76 years (median, 33.0), with 384 (64.2%) $\geqslant$ 30 years of age (median, 43.0). (In our baseline paper [16], the total number of patients with evaluable results was reported as 600. Upon final review, 2 patients were found not to have met study inclusion criteria and removed from our final analysis, resulting in 598 patients in the study. This review also reduced the number of patients with biopsy from 224 as reported to 222. These changes had no impact on the conclusions drawn in the baseline paper).

Although the index referral cytology was LSIL in all patients enrolled per study criterion, cytology performed at the time of enrollment showed a heterogeneous cytological grade as expected. The time interval between the index referral LSIL cytology immediately prior to the colposcopy clinic visit and cytology performed in the colposcopy clinic at the time of enrollment ranged from $<$ 1 month to $\geqslant$ 18 months with a median of 7 months. During this interval, LSILs regressed in 48.9% and progressed in 9.6% with only 41.5% still having LSIL. The above cytology status of the study population was unknown at the time of patient enrollment. Cytology categories of the study population correlated with CINtec PLUS and HPV results at the time of enrollment were previously described in our baseline paper [16].

Of the 598 patients in the study, per standard practice, biopsies were only performed when clinically indicated. As such, there were 222 evaluable cervical biopsy results available at baseline, and among them

Table 1
CINtec PLUS and HPV test results by age groups

Test	Result	All ages, $n = 598$	< 30 years, $n = 214$	$\geqslant 30$ years, $n = 384$	
CINtec PLUS	Positive	265 (44.3%)[a]	107 (50.0%)[b*]	158 (41.1%)[c*]	$^{*}p = 0.037$, CINtec Plus positivity compared between women < 30 and $\geqslant 30$ years
	Negative	333 (55.7%)	107 (50.0%)	226 (58.9%)	
HPV	Positive	331 (55.4%)[a]	135 (63.1%)[b**]	196 (51.0%)[c**]	$^{**}p = 0.005$, HPV positivity compared between women < 30 and $\geqslant 30$ years
	Negative	267 (44.6%)	79 (36.9%)	188 (49.0%)	
p value		[a]p value < 0.001, CINtec PLUS positivity compared with HPV positivity in all ages	[b]p value $= 0.006$, CINtec PLUS positivity compared with HPV positivity in women < 30 years	[c]p value $= 0.006$, CINtec PLUS positivity compared with HPV positivity in women $\geqslant 30$ years	



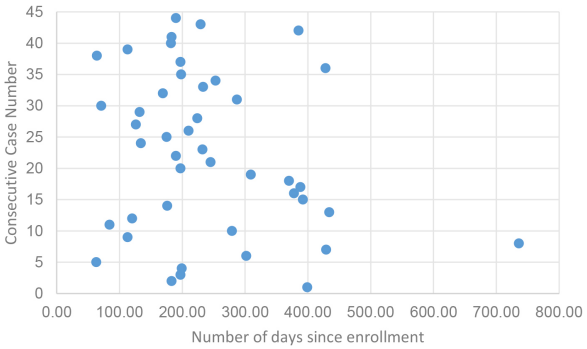
AIS, Adenocarcinoma in situ; CIN3, Cervical intraepithelial neoplasia grade 3; CIN2, CIN grade 2; $\leqslant$ CIN 1, CIN grade 1 or negative.

Fig. 1. Study scheme: patient enrollment, CINtec PLUS and HPV test results and biopsy outcome of clinical assessments at baseline and during follow-up.

54 (24.3%) were diagnosed as CIN2+. The baseline data obtained with the 54 CIN2+ cases were previously described [16]. Having reached the clinical endpoint of the study, the 54 CIN2+ cases were not followed any further in the study, leaving 544 patients for prospective follow-up. Of the 544, 264 patients were mostly discharged following a negative HPV test per Ontario cervical cancer guidelines and some were lost to follow-up, leaving 280 patients remaining under care in the colposcopy clinic, representing the follow-up cohort. The cohort was followed for a median of 202 days (range, 36 days to 736 days). Among the 280, 148 patients underwent biopsy during the follow-up as part of routine patient care per standard practice, and of them, 45 (30.4%) were diagnosed as CIN2+. This yielded a combined total of 99 (27.8%) CIN2+ cases among the 598 patients enrolled in the study, including 42 CIN3 cases and 2 cases of adenocarcinoma in situ, collectively referred to as CIN3+ for analysis purposes. Distribution of the total study population with CINtec PLUS and HPV test results and biopsy outcome of clinical assessments at baseline and during the follow-up are schematically shown in Fig. 1.

3.2. CINtec PLUS and HPV test results

Table 1 shows CINtec PLUS and cobas HPV results for the total population of 598 for all ages, and those



*45 CIN2+ detected during follow-up; date of biopsy unavailable for one.

Fig. 2. Temporal distribution of CIN2+ detected during follow-up ($n = 44$)*.

$<$ 30 years of age and $\geqslant$ 30 years. In all ages, CINtec PLUS was positive in 265 (44.3%) vs. 331 (55.4%) testing HPV positive ($p<$ 0.001). Among the 331 HPV positives, genotypes 16/18 were detected in 93 (28.1%). In those $\geqslant$ 30 years, CINtec PLUS was positive in 158 (41.1%) vs. 196 (51.0%) testing HPV positive ($p =$ 0.006). Among the 196 HPV positives, genotypes 16/18 were detected in 57 (29.1%). There were significant differences in both CINtec PLUS and HPV positivity rates between those $<$ 30 years of age and $\geqslant$ 30 years (Table 1).

3.3. *Performance of CINtec PLUS and HPV tests to detect CIN2+ and CIN3+*

Of the 598 patients in all ages, a total of 356 (59.5%) had evaluable biopsy results. Among the 356, as indicated above, biopsy confirmed CIN2+ was diagnosed in a total of 99 (27.8%) patients, comprising of 55 CIN2 and 44 CIN3+. All CIN2+ biopsy diagnoses were substantiated by a positive p16 immunostain result.

Table 2 illustrates the performance of CINtec PLUS in comparison with HPV testing in detecting 99 CIN2+. In all ages, CINtec PLUS was positive in 81 for a sensitivity of 81.8% while HPV was positive in 93 for a sensitivity of 93.9% ($p = 0.009$). Specificity was 52.9% vs. 36.6%, respectively ($p<$ 0.001). In patients $<$ 30 years, CINtec PLUS sensitivity for detection of CIN2+ was 76.0% vs 94.0% for HPV testing ($p = 0.012$). For detection of 55 CIN2, in all ages, CINtec PLUS sensitivity was 72.7% (40/55) vs. 94.5% (52/55) for HPV testing ($p = 0.002$), and in patients $<$ 30 years, these figures were 61.5% (16/26) and 92.3% (24/26), respectively ($p = 0.009$, data not shown).

Table 3 shows the performance of CINtec PLUS compared with HPV testing in detecting 44 CIN3+. In all ages, both CINtec PLUS and HPV tests were positive in 41 of these for an identical sensitivity of 93.2%. Specificity was 48.4% vs. 31.1%, respectively ($p<$ 0.001). Among patients $<$ 30 years, CINtec PLUS sensitivity was similar to that of in all ages at 91.7%. Negative predictive values (NPVs) were $>$ 96.0% in all age groups for both tests.

3.4. *Performance of CINtec PLUS and HPV tests to detect incident CIN2+ during follow-up*

Of the 45 incident CIN2+ detected among 148 having biopsy during the follow-up, 20 were $<$ 30 years and 25 were $\geqslant$ 30 years (range, 22–69; median, 30), and included 20 CIN2 and 25 CIN3+. Of the 45 CIN2+, biopsy diagnostic dates were available for 44, and for these cases, the time intervals from enrollment to detection of CIN2+ ranged from 63 to 736 days with an average of 241 days and a median of 199 days (Fig. 2). In all ages, for detecting CIN2+, CINtec PLUS was 82.2% (37/45) sensitive vs. 93.3% (42/45) for HPV testing ($p = 0.107$). Specificities were 47.6% (49/103) vs. 21.4% (22/103), respectively ($p<$ 0.001). CINtec PLUS showed a positive predictive value (PPV) of 40.7% (37/91) vs. 34.2% (42/123) for HPV testing ($p = 0.327$) (Data not shown). In patients $<$ 30 years, CINtec PLUS sensitivity was similar to that of all ages at 85.0% (17/20) vs. 95.0% (19/20) for HPV test ($p = 0.294$). NPVs were $\geqslant$ 86% in all age groups for both tests, except 81.3% for CINtec PLUS in those $<$ 30 years. Among the 25 CIN3+ in all ages, CINtec PLUS was 92.0% (23/25) sensitive vs. 88.0% (22/25) for HPV testing ($p = 0.638$) (Data not shown).

3.5. *ROC analysis*

Figure 3 shows the results of ROC analysis comparing the overall performance characteristics of CINtec PLUS with HPV testing in detecting CIN2+. For patients in all ages, ROC results showed the areas under the curve for CINtec PLUS and HPV were similar at 0.725 and 0.731 ($p>$ 0.05). Further ROC analyses performed for those $<$ 30 and $\geqslant$ 30 years of age showed similar results. The area under curve was slightly higher for CINtec PLUS among those $\geqslant$ 30 years, although it was not statistically significant in detecting CIN2+ when compared to HPV.

3.6. *Genotype specific risk threshold to detect CIN2+ and CIN3+*

Table 4 shows HPV genotypes 16/18-specific results in comparison with hr-HPV testing to detect CIN2+ and CIN3+. In all ages, to detect CIN2+, genotype

Table 2
Diagnostic indices of CINtec PLUS and HPV tests for detecting CIN2+

Test	Sensitivity			Specificity			Positive predictive value			Negative predictive value		
	n/N	% (95% CI)	p	n/N	% (95% CI)	p	n/N	% (95% CI)	p	n/N	% (95% CI)	p
All ages ($n = 356$)												
CINtec PLUS	81/99	81.8 (72.8–88.9)	0.009	136/257	52.9 (46.6–59.2)	< 0.001	81/202	40.1 (36.3–44.0)	0.407	136/154	88.3 (83.0–92.1)	0.131
HPV	93/99	93.9 (87.3–97.7)		94/257	36.6 (30.7–42.8)		93/256	36.3 (33.9–38.8)		94/100	94.0 (87.7–97.2)	
< 30 years of age ($n = 128$)												
CINtec PLUS	38/50	76.0 (61.8–86.9)	0.012	40/78	51.3 (39.7–62.8)	0.023	38/76	50.0 (43.2–56.9)	0.741	40/52	76.9 (66.1–85.1)	0.159
HPV	47/50	94.0 (83.5–98.8)		26/78	33.3 (23.1–44.9)		47/99	47.5 (43.2–51.8)		26/29	89.7 (73.5–96.5)	
$\geqslant 30$ years of age ($n = 228$)												
CINtec PLUS	43/49	87.8 (75.2–95.4)	0.294	96/179	53.6 (46.0–61.1)	0.003	43/126	34.1 (30.0–38.5)	0.384	96/102	94.1 (88.2–97.2)	0.063
HPV	46/49	93.9 (83.1–98.7)		68/179	38.0 (30.6–45.5)		46/157	29.3 (26.6–32.2)		68/71	95.8 (88.2–98.6)	

Based on 356 patients in all ages with biopsy. CIN2+, cervical intraepithelial neoplasia grade 2 or worse.

Table 3
Diagnostic indices of CINtec PLUS and HPV tests for detecting CIN3+

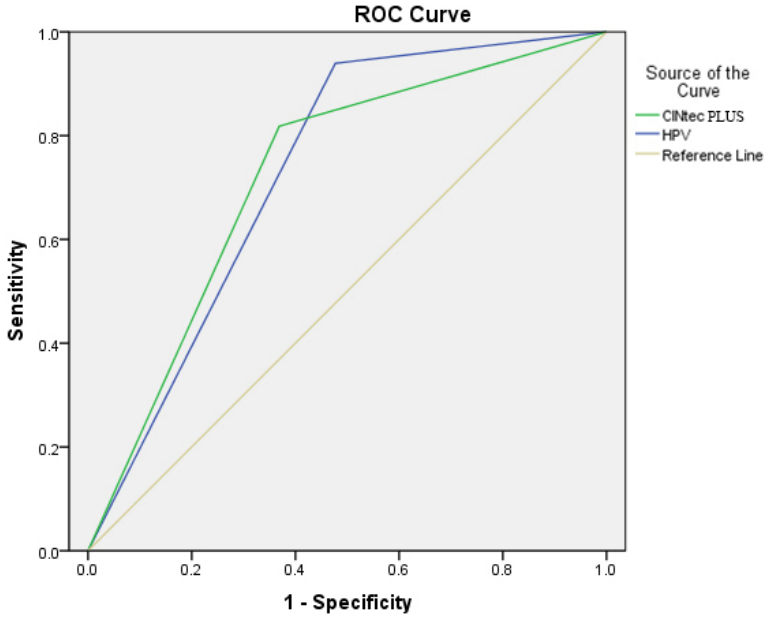
	Sensitivity			Specificity			Positive predictive value			Negative predictive value		
	n/N	% (95% CI)	p	n/N	% (95% CI)	p	n/N	% (95%CI)	p	n/N	% (95% CI)	p
All ages ($n = 356$)												
CINtec PLUS	41/44	93.2 (81.3–98.6)	1.000	151/312	48.4 (42.7–54.1)	< 0.001	41/202	20.3 (18.2–22.6)	0.234	151/154	98.1 (94.4–99.3)	0.589
HPV	41/44	93.2 (81.3–98.6)		97/312	31.1 (26.0–36.6)		41/256	16.0 (14.6–17.5)		97/100	97.0 (91.5–99.0)	
< 30 years of age ($n = 128$)												
CINtec PLUS	22/24	91.7 (73.0–99.0)	0.549	50/104	48.1 (38.2–58.1)	0.002	22/76	29.0 (24.6–33.7)	0.390	50/52	96.2 (86.7–99.0)	0.928
HPV	23/24	95.8 (78.9–99.9)		28/104	26.9 (18.7–36.5)		23/99	23.2 (20.8–25.9)		28/29	96.6 (80.0–99.5)	
$\geqslant 30$ years of age ($n = 228$)												
CINtec PLUS	19/20	95.0 (75.1–99.9)	0.549	101/208	48.6 (41.6–55.6)	0.001	19/126	15.1 (13.1–17.3)	0.368	101/102	99.0 (93.7–99.9)	0.363
HPV	18/20	90.0 (68.3–98.8)		69/208	33.2 (26.8–40.0)		18/157	11.5 (9.8–13.4)		69/71	97.2 (90.1–99.2)	

Based on 356 patients in all ages with biopsy. CIN3+, cervical intraepithelial neoplasia grade 3 or worse (includes 2 cases of adenocarcinoma in situ).

Table 4
HPV genotypes 16/18-specific testing compared with hr-HPV testing to detect CIN2+ and CIN3+

Test	CIN2+						CIN3+					
	Sensitivity			Specificity			Sensitivity			Specificity		
	n/N	%	p	n/N	%	p	n/N	%	p	n/N	%	p
All ages ($n = 356$)												
HPV16/18	46/99	46.5%	< 0.001	225/257	87.5%	< 0.001	23/44	52.3%	< 0.001	257/312	82.4%	< 0.001
hr-HPV	93/99	93.9%		94/257	36.6%		41/44	93.2%		97/312	31.1%	
< 30 years of age ($n = 128$)												
HPV16/18	20/50	40.0%	< 0.001	67/78	85.9%	< 0.001	13/24	54.2%	< 0.001	86/104	82.7%	< 0.001
hr-HPV	47/50	94.0%		26/78	33.3%		23/24	95.8%		28/104	26.9%	
$\geqslant 30$ years of age ($n = 228$)												
HPV16/18	26/49	53.1%	< 0.001	158/179	88.3%	< 0.001	10/20	50.0%	0.006	171/208	82.2%	< 0.001
hr-HPV	46/49	93.9%		68/179	38.0%		18/20	90.0%		69/208	33.2%	

Based on 356 patients in all ages with biopsy. CIN2+, cervical intraepithelial neoplasia grade 2 or worse; CIN3+, cervical intraepithelial neoplasia grade 3 or worse (includes 2 cases of Adenocarcinoma in situ).



Diagonal segments are produced by ties.

Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Limit	Upper Limit
CINtec	0.725	0.026	.000	0.673	0.776
HPV	0.731	0.023	.000	0.686	0.777

The test result variable(s): CINtec, HPV has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

[a] Under the nonparametric assumption

[b] Null hypothesis: true area = 0.5

Fig. 3. ROC curve for CINtec PLUS compared to HPV in detected CIN2+ in all ages ($n = 598$).

16/18-specific testing was 46.5% sensitive vs 93.9% for hr-HPV testing; for CIN3+, these figures were 52.3% and 93.2%, respectively. As previously reported [16], in all genotype 16/18 positive cases, type 16 was predominant as a single type in most cases, and in a few it was detected in combination with type 18 or OHR types.

4. Discussion

Our study showed an overall CIN2+ prevalence of 16.6% (99/598) in all ages in a routine colposcopy referral setting; it was lower at 12.8% (49/384) in patients aged $\geqslant$ 30 years. CIN3+ prevalence was 7.5% (44/598) and 5.2% (20/384), respectively. These figures are within the ranges reported among LSIL referral population in other studies [4,26], and underscore the importance of effective triage to identify the small fraction of LSIL referral patients at increased risk, and also raises the question of following all such patients in colposcopy clinics for an extended period.

Our CINtec PLUS positivity rate of 44.3% in LSIL was similar to other studies [11,19], while the HPV positivity rate of 55.4% was significantly lower than those reported in concurrent LSIL [27,28] (Table 1). This could be attributed to lesion regression in a large proportion of the LSIL referrals by the time they are seen in the colposcopy clinic, leading to lower HPV prevalence and test positivity rate, thus making HPV testing more effective in this setting as described previously [16]. Based on the above positivity rates, in all ages, CINtec PLUS would reduce the LSIL referral population requiring further investigations and follow-up in a colposcopy clinic by 55.7% (333/598) vs 44.6% (267/598) for HPV testing; these proportions would be higher in those $\geqslant$ 30 years of age. The above difference between the two tests is significant ($p< 0.001$), and the higher reduction rate of CINtec PLUS is due to its higher specificity. The above data demonstrate the potential for incorporating CINtec PLUS or HPV triage for patients referred to colposcopy with a history of LSIL to aid in better patient care and overall efficiency.

For CIN2+ detection, in all ages, CINtec PLUS showed a significantly lower sensitivity of 81.8% vs. 93.9% for HPV testing (Table 2). CINtec PLUS sensitivity was further dropped to 76.0% in patients $<$ 30 years, while remaining higher at 87.8% in those $\geqslant$ 30 years. Further analysis for those $<$ 30 years showed the reduced sensitivity of CINtec PLUS was more associated with CIN2 detection (61.5%) than CIN2+ (76.0%). However, when considering that CIN2 is known to be mostly regressive, and the fact that CINtec PLUS is more predictive of transforming HPV infection, it is likely that CINtec PLUS results are more meaningful and of greater clinical relevance and potential utility than an HPV DNA test. It was further substantiated by the observation that, in all ages, CINtec PLUS showed identical sensitivity of 93.2% as the HPV test for detecting CIN3+ (Table 3), a more definitive predictor of underlying cancer risk. It is also important to note that in patients $<$ 30 and $\geqslant$ 30 years, CINtec PLUS CIN3+ sensitivities were similar to that of HPV at 91.7% and 95.0%, respectively, indicating its clinical utility and value in detecting more severe malignancies regardless of age. Moreover, for the 45 incident CIN2+ cases observed during follow-up, both tests showed similar sensitivity for the total population, without a decrease in CINtec PLUS sensitivity in those $<$ 30 years, with similar PPVs. The significantly higher specificity of CINtec PLUS than HPV test was consistently observed, and in this respect, we note that CINtec PLUS is currently approved for triaging those testing positive in HPV primary screening [29]. The lower sensitivity, albeit with the caveats noted, and higher specificity of CINtec PLUS compared to HPV test we observed is consistent with a screening triage study recently reported in a Canadian population [23] and many other studies [17,21,30,31].

The use of CINtec PLUS in LSIL triage especially for patients $<$ 30 years of age may be of concern considering its lower sensitivity and NPVs in comparison to all ages in detecting CIN2 and CIN2+. Given the option between cytology and CINtec PLUS for LSIL triage of this age group, the latter would still be a better choice as CINtec PLUS is more sensitive than cytology [7,18]. Additionally, CINtec PLUS performance being equal to that of HPV testing for detection of CIN3+ must be considered as noted above. Regardless, further follow-up would be warranted for those testing CINtec PLUS negative in LSIL triage to ensure CIN2+ is not missed.

Although there are differences in the overall sensitivities and specificities of CINtec PLUS and HPV reported in other studies [8,23,31,32] it is important to note that our study showed both tests having an identical high level of sensitivity to detect CIN3+, a better clinical predictor of risk and progression to assess test performance, and as such, both can potentially be used for LSIL triage in all age groups [10,18,21,33]. We also observed high levels of NPV for both CINtec PLUS and HPV tests in all age groups to detect CIN3+ as apparent in identical other studies [4,30,32,34] providing evidence that high grade lesions would be mostly detected in a clinical setting, and reassurance for use of either test for LSIL triage. In this respect we note that the ROC analyses showed no differences in the performance of CINtec PLUS and HPV tests for detection of CIN2+.

We assessed the application of HPV genotypes 16/18-specific threshold in LSIL triage as these genotypes account for approximately 70% of cervical can-

cer [35,36]. Our 16/18 positive proportion of 28% among LSILs is similar to those reported in other studies [23,32]. If this threshold is used, it would mean reducing the number of LSIL referral cases requiring additional follow-up by more than 2/3rds. While this will greatly improve efficiency, it is significantly less sensitive for detecting both CIN2+ and CIN3+ than hr-HPV testing as observed in our study (Table 4) and reported by others [28,37]. Due to this short-coming, if this risk threshold is used in LSIL triage, it would warrant closer follow-up of those testing genotypes 16/18 negative within colposcopy clinics to ensure CIN2+ cases are not missed. As concluded in a meta-analysis, genotypes 16/18-specific risk threshold may be more useful in HPV primary screening than in LSIL triage [28].

In this study, we evaluated the application of CINtec PLUS and HPV tests for triaging LSIL referral patients since their risk stratification and management remain of clinical and programmatic importance in settings where cytology-based cervical screening is used. Overall, triaging LSIL referral populations could help to reduce the number of patients requiring further colposcopy clinic visits and additional investigations, thus decreasing burden on colposcopy clinics and eliminating potential negative health effects, consequently aiding in better patient care and resource management [20]. One aspect that can be difficult to quantify though is the reduction in patients' anxiety and peace of mind by not having to continue colposcopy clinic visits for extended periods [5]. While not the focus of this work, reductions in number of patients requiring further clinic visits would also lead system efficiency by reducing waitlists and wait times for those who truly need colposcopy. Although it may be difficult to quantify the reduction in systems costs due to regional and programmatic differences [5], there are general system cost efficiencies to be found [5,27]. Cost-effectiveness modelling with robust economics methodologies will provide important perspectives when assessing patient management strategies as health systems evolve [22].

Our study was carried out in a real-world colposcopy clinic setting without any intervention for study purpose. This shed some light on the lesion regression rate during the interval between referral LSIL cytology and colposcopy, and this has implications in risk stratification and follow-up, and could also impact the outcome of triage tests. Our data on the delay from referral LSIL to colposcopy may be generalizable in Canadian settings, but this may vary in other jurisdictions. One of the limitations of the study was that only a proportion underwent biopsy as clinically indicated and this prevented ascertaining biopsy-based disease outcome in the total study population. Regardless, in addition to 54 CIN2+ detected at baseline, there were 45 more CIN2+ diagnosed during follow-up. However, the length of our follow-up period was limited; extended follow-up may provide further insight into disease outcome and the PPV of both the CINtec PLUS and HPV tests in LSIL triage [38]. Also, a larger sample size of CIN2+ would be warranted to substantiate our observations.

5. Conclusions

Either CINtec PLUS cytology or the cobas HPV test could serve as a predictor of CIN3+, with high sensitivity and NPV in patients referred to colposcopy with a history of LSIL cytology regardless of age. However, the reduced sensitivity of CINtec PLUS for detection of CIN2+ in general, and CIN2 in particular, especially in patients < 30 years, needs to be considered in risk assessments if choosing LSIL-CINtec PLUS triage pathways. Nevertheless, CINtec PLUS was consistently more specific than HPV test. Our study data provide a basis to improve patient care and efficiency by significantly reducing the number of LSIL referral patients requiring further investigations and follow-up in colposcopy clinics through CINtec PLUS or cobas HPV triage.

Authors' contributions

LG: Project administration, investigation, methodology, laboratory technical support, resources, data curation and analysis, writing-original draft and writing-review and editing; SR: Conceptualization, funding acquisition, methodology, supervision, data analysis, writing-review and editing; DJ: Project administration, supervision, data curation, resources, laboratory technical support, and writing-review and editing; RA and MS: Investigation, data analysis, validation, and writing-review and editing; RN: Investigation, data curation, laboratory technical support, and writing-review and editing; AEM: Study co-ordination and technical support; AW: Data base management and data analysis; PW: data analysis, writing-review and editing; DC, LE and GZ: Resources, and writing-review and editing; MC: Resources, methodology, supervision, and writing-review and editing.

All authors reviewed and approved the final version for submission. All authors attest they meet the ICMJE criteria for authorship.

Conflict of interest

Sam Ratnam received research grant from Roche Diagnostics.

Max Chernesky received research grant unrelated to this study from Roche Diagnostics.

Other members of the study team declare no conflict of interests.

Funding support

This study was supported by a research grant from Roche Diagnostics, Montreal, Canada. Roche Diagnostics also provided BenchMark ULTRA system, CINtec PLUS and cobas HPV test kits for the study, and training in CINtec PLUS testing, reading and interpretation. The study was investigator initiated and conducted independently. Roche Diagnostics offered suggestions with the study design, but did not have any input in the conduct, data analysis, or preparation and reporting of results of this study.

Acknowledgments

We thank Dr. Michele D'Elia, Roche Diagnostics, Montreal, Canada for his commitment, funding support and provision of BenchMark Ultra system and test kits for the study; Carolynn Frain, Roche Diagnostics, Montreal, Canada for CINtec PLUS cytology training. We also thank Mary Jane Carrier, and other colposcopy clinic medical and nursing staff, Juravinski Hospital, Hamilton for assistance with patient enrolment; Thomas Barrett and Kenneth Melvin, Regional Cytology Laboratory, Eastern Health, St. John's for CINtec PLUS cytology support; Holly Edwards and Tania Smith, Eastern Health, St. John's for administrative assistance. We acknowledge Dr. Diane Lamoureux, Hôpital Pierre Boucher, Longueuil, Quebec for adjudicating discrepant CINtec PLUS results.

References

[1] Cancer Care Ontario, *Ontario Cervical Screening Guidelines Summary*, 2016.

[2] T.C. Wright Jr, I.S. Massad, C.J. Dunton et al., 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests, *J Low Genit Tract Dis* **11**(4) (2007), 201–222.

[3] L.S. Massad, M.H. Einstein, W.K. Huh et al., 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors, *Journal of Lower Genital Tract Disease* **17**(5 Suppl 1) (2013), S1–S27.

[4] J. Cuzick, J.T. Cox, G. Zhang et al., Human papillomavirus testing for triage of women with low-grade squamous intraepithelial lesions, *International Journal of Cancer* **132**(4) (2013), 959–966.

[5] W.A. Tjalma, E. Kim and K. Vandeweyer, The impact on women's health and the cervical cancer screening budget of primary HPV screening with dual-stain cytology triage in Belgium, *Eur J Obstet Gynecol Reprod Biol* **212** (2017), 171–181.

[6] G. Ronco, J. Cuzick, N. Segnan et al., HPV triage for low grade (L-SIL) cytology is appropriate for women over 35 in mass cervical cancer screening using liquid based cytology, *European Journal of Cancer* **43**(3) (2007), 476–480.

[7] W.A. Tjalma, Diagnostic performance of dual-staining cytology for cervical cancer screening: A systematic literature review, *European Journal of Obstetrics, Gynecology, and Reproductive Biology* **210** (2017), 275–280.

[8] H. Sun, K. Shen and D. Cao, Progress in immunocytochemical staining for cervical cancer screening, *Cancer Management and Research* **11** (2019), 1817–1827.

[9] M. Sun, Y. Shen, M.L. Ren and Y.M. Dong, Meta-analysis on the performance of p16/Ki-67 dual immunostaining in detecting high-grade cervical intraepithelial neoplasm, *J Cancer Res Ther* **14**(Supplement) (2018), S587–S593.

[10] L. Yu, L. Fei, X. Liu, X. Pi, L. Wang and S. Chen, Application of p16/Ki-67 dual-staining cytology in cervical cancers, *Journal of Cancer* **10**(12) (2019), 2654–2660.

[11] C. Bergeron, H. Ikenberg, M. Sideri et al., Prospective evaluation of p16/Ki-67 dual-stained cytology for managing women with abnormal papanicolaou cytology: PALMS study results, *Cancer Cytopathology* **123**(6) (2015), 373–381.

[12] M.A. Clarke, L.C. Cheung, P.E. Castle et al., Five-year risk of cervical precancer following p16-Ki-67 dual-stain triage of HPV-positive women, *JAMA Oncology* **5**(2) (2019), 181–186.

[13] P. Guan, R. Howell-Jones, N. Li et al., Human papillomavirus types in 115,789 HPV-positive women: A meta-analysis from cervical infection to cancer, *International Journal of Cancer* **131**(10) (2012), 2349–2359.

[14] T.C. Wright Jr, C.M. Behrens, J. Ranger-Moore et al., Triaging HPV-positive women with k16/Ki-67 dual-stained cytology: Results from a sub-study nested into the ATHENA trial, *Gynecologic Oncology* **144**(1) (2017), 51–56.

[15] N. Wentzensen, M.A. Clarke, R. Bremer et al., Clinical evaluation of human papillomavirus screening with p16/Ki-67 dual stain triage in a large organized cervical cancer screening program, *JAMA Internal Medicine* **179**(7) (2019), 881–888.

[16] S. Ratnam, D. Jang, L. Gilbert et al., CINtec PLUS and cobas HPV testing for triaging Canadian women referred to colposcopy with a history of low-grade squamous intraepithelial lesion: Baseline findings, *Papillomavirus Research* **10** (2020), 100206.

[17] D. Schmidt, C. Bergeron, K.J. Denton and R. Ridder, p16/Ki-67 dual-stain cytology in the triage of ASCUS and LSIL papanicolaou, *Cancer Cytopathology* **119**(3) (2011), 158–166.

[18] H. Ikenberg, C. Bergeron, D. Schmidt et al., Screening for cervical cancer precursors with P16/Ki-67 dual-stained cytology: Results of the PALMS study, *J Natl Cancer Inst* **105**(20) (2013), 1550–1557.

[19] C. White, S. Bakhiet, M. Bates et al., Triage of LSIL/ASC-US with p16/Ki-67 dual staining and human papillomavirus testing: A 2-year prospective study, *Cytopathology* **27**(4) (2016), 269–276.

[20] E. Peeters, N. Wentzensen, C. Bergeron and M. Arbyn, Meta-analysis of the accuracy of p16 or p16/Ki-67 immunocytochemistry versus HPV testing for the detection of CIN2+/CIN3+ in triage of women with minor abnormal cytology, *Cancer Cytopathology* **127**(3) (2019), 169–180.

[21] J.C. Possati-Resende, J.H.T.G. Fregnani, L.M. Kerr, E.C. Mauad, A. Longatto-Filho and C. Scapulatempo-Neto, The accuracy of p16/Ki-67 and HPV test in the detect of CIN2/3 in women diagnosed with ASC-US or LSIL, *PLOS ONE* **10**(7) (2015), e0134445.

[22] N. Wentzensen, M. Schiffman, T. Palmer and M. Arbyn, Triage of HPV positive women in cervical cancer screening, *Journal of Clinical Virology* **76**(Suppl 1) (2016), S49–S55.

[23] M. El-Zein, W. Gotlieb, L. Gilbert et al., Dual staining for p16-Ki-67 to detect high-grade cervical lesions: Results from the screening tirage ascertaining intraepithelial neoplasia by immunostain testing study, *Int. J. Cancer* **148**(2) (2021), 492–501.

[24] ASCUS-LSIL Triage Study (ALTS) Group, Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance, *Am J Obstet Gynecol* **188**(6) (2003), 1383–1392.

[25] W.K. Huh, K.A. Ault, D. Chelmow et al., Use of primary high-risk human papillomavirus test for cervical cancer screening: Interim clinical guidance, *Gynecological Oncology* **125**(2) (2015), 330–337.

[26] D. Solomon, M. Schiffman and R. Tarone, ALTS Study Group, Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: Baseline results from a randomized trial, *J Natl Cancer Inst* **93**(4) (2001), 293–299.

[27] M. Arbyn, P. Martin-Hirsch, F. Buntinx et al., Triage of women with equivocal or low-grade cervical cytology results: A meta-analysis of the HPV positivity rate, *J Cell Mol Med* **13**(4) (2009), 648–659.

[28] M. Arbyn, L. Xu, F. Verdoodt et al., Genotyping for human papillomavirus types 16 and 18 in women with minor cervical lesions, *Annals of Internal Medicine* **166**(2) (2017), 118–127.

[29] H. Slater, FDA expands use of CINtec PLUS cytology test for women who are HPV-Positive, *Cancer Network*, 2020. Available at: https://www.cancernetwork.com/view/fda-expands-use-of-cintec-plus-cytology-test-for-women-who-are-hpv-positive, accessed April 04, 2021.

[30] K.C.K. Prigenzi, T. Heinke, R.C. Salim and G.R. de Azevedo Focchi, Dual p16 and Ki-67 expression in liquid-based cervical cytological samples compared to pap cytology findings, biopsies, and HPV testing in cervical cancer screening: A diagnostic accuracy study, *Acta Cytol* **62**(2) (2018), 104–114.

[31] Y. Zhu, C. Ren, L. Yang, X. Zhang, L. Liu and Z. Wang, Performance of p16/Ki67 immunostaining, HPV E6/E7 mRNA testing, and HPV DNA assay to detect high-grade cervical dysplasia in women with ASCUS, *BMC Cancer* **19**(1) (2019), 271.

[32] M. McMenamin, M. McKenna and A. McDowell, Clinical utility of CINtec PLUS triage in equivocal cervical cytology and human papillomavirus primary screening, *Am J Clin Pathol* **150**(6) (2018), 512–521.

[33] D. Das, M. Sengupta, K. Basu, M. Tirkey, C. Datta and U. Chatterjee, Role of p16/Ki-67 dual immunostaining in detection of cervical cancer precursors, *Journal of Cytology* **35**(3) (2018), 153–158.

[34] C. Ren, Y. Zhu, L. Yang et al., Prognostic and diagnostic validity of p16/Ki-67, HPV E6/E7 mRNA, and HPV DNA in women with ASCUS: A follow-up study, *Virology Journal* **16** (2019), 143.

[35] N. Munoz, X. Bosch, S. de Sanjose et al., Epidemiologic classification of human papillomavirus types associated with cervical cancer, *The New England Journal of Medicine* **348** (2003), 518–527.

[36] M.J. Khan, P.E. Castle, A.T. Lorincz et al., The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice, *Journal of the National Cancer Institute* **97**(14) (2005), 1072–1079.

[37] J.T. Cox, P.E. Castle, C.M. Behrens et al., Comparison of cervical cancer screening strategies incorporating different combinations of cytology, HPV testing, and genotypeing for HPV16/18: Results from the ATHENA HPV study, *American Journal of Obstetrics & Gynecology* **208**(3) (2013), 184.e1–184.e11.

[38] M.A. Clarke, L.C. Cheung, P.E. Castle et al., Five-year risk of cervical precancer following p16/Ki-67 dual-stain triage of HPV-positive women, *JAMA Oncology* **5**(2) (2019), 181–186.

[39] M. Sun, Y. Shen, M.L. Ren and Y.M. Dong, Meta-analysis on the performance of p16/Ki-67 dual immunostaining in detecting high-grade cervical intraepithelial neoplasm, *Journal of Cancer Research and Therapeutics* (2018), S587–93.

[40] S. de Sanjose, W.G. Quint, L. Alemany et al., Human papillomavirus genotype attribution in invasive cervical cancer; a retrospective cross-sectional worldwide study, *Lancet Oncology* **11**(11) (2010), 1048–1056.

[41] T.C. Wright, M.H. Stoler, C.M. Behrens, A. Sharma, G. Zhang and T.L. Wright, Primary cervical cancer screening with human papillomavirus: End of study results from the ATHENA study using HPV as the first-line screening test, *Gynecologic Oncology* **136**(2) (2015), 189–197.