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CINtec PLUS and cobas HPV testing for triaging Canadian women referred to colposcopy with a history of low-grade squamous intraepithelial lesion: Baseline findings

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ABSTRACT

Objective and methods: CINtec PLUS and cobas HPV tests were assessed for triaging women referred to colposcopy with a history of LSIL cytology. Both tests were performed at baseline using ThinPrep cervical specimens and biopsy confirmed cervical intraepithelial neoplasia grade 2 or worse (CIN2+) served as the clinical endpoint.
Results: In all ages, (19–76 years, n = 600), 44.3% (266/600) tested CINtec PLUS positive vs. 55.2% (331/600) HPV positive (p = 0.000). Based on 224 having biopsies, sensitivity to detect CIN2+ (n = 54) was 81.5% (44/54) for CINtec PLUS vs. 94.4% (51/54) for HPV testing (p = 0.039); specificities were, 52.4% (89/170) vs. 44.1% (75/170), respectively (p = 0.129). In women ≥30 years (n = 386), 41.2% (159/386) tested CINtec PLUS positive vs. 50.8% (196/386) HPV positive (p = 0.008). Based on 135 having biopsies, sensitivity to detect CIN2+ (n = 24) was 95.8% (23/24) for both CINtec PLUS and HPV tests; specificities were, 55.0% (61/111) vs. 50.5% (56/111), respectively (p = 0.503).
Conclusions: For women referred to colposcopy with a history of LSIL cytology, CINtec PLUS or cobas HPV test could serve as a predictor of CIN2+ with high sensitivity, particularly in women ≥30 years. Either test can significantly reduce the number of women requiring further investigations and follow up in colposcopy clinics.

1. Introduction

Low-grade squamous intraepithelial lesion (LSIL) is the second most common cytological abnormality found in routine cervical screening. While these lesions regress spontaneously in the majority, a small fraction of women with LSIL cytology have an occult high-grade squamous intraepithelial lesion (HSIL) or will progress to HSIL. Consequently, women found to have LSIL in routine cervical screening are either directly referred to colposcopy or followed cytologically, referring those with persistent cytologic abnormalities to colposcopy [1,2]. In colposcopy clinics, all referred LSIL cases are typically followed with repeat cytology, colposcopy and biopsies for various length of time. This practice increases both unnecessary cost and intervention in patients with a negligible risk of developing cervical cancer; it also subjects many to negative health effects. An effective LSIL triage strategy would identify those women who need to remain in care at the colposcopy clinic and those who can be safely returned to routine screening. In this connection, we previously conducted a multicentre Canadian study for triaging ASCUS and LSIL referral populations using the ProEx C immunoassay (Beckton and Dickinson), an MCM/TOP2a-based biomarker test [3]. In this study, ProEx C sensitivity to detect cervical intraepithelial neoplasia grade 2 or worse (CIN 2+) was found to be unacceptably low in the range of 68%–72% and precluded this assay in triage.

The CINtec PLUS assay (Roche Diagnostics) is a dual-stain

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immunocytochemical test which detects p16 and Ki-67 proteins that are over expressed in cervical cells with transforming HPV infection. This assay has emerged as an effective biomarker-based adjunct test in cervical screening strategy, and is well studied [4–8]. p16 is a tumour suppressor gene which regulates cell cycle through a cascade of biochemical events; Ki-67 is a nuclear protein and a marker of cellular proliferation. In normal cells, the expressions of p16 and Ki-67 are mutually exclusive. In persistent transforming infection with high-risk human papillomavirus (hr-HPV), E7 oncogene disrupts the negative feedback control on p16 expression, resulting in loss of cell cycle control and continued cell proliferation which lead to over expression of both p16 and Ki-67. Thus, the co-detection of p16/Ki-67 simultaneously within the same cervical epithelial cell serves as a specific marker of HPV-mediated oncogenic transformation and predictor of cervical cancer risk. CINtec PLUS assay has been shown to be more sensitive than cytology with equal specificity, and more specific than HPV testing with relatively comparable sensitivity for detecting CIN2+ in women with LSIL cytology [9–13].

The cobas HPV DNA test (Roche Diagnostics) is a PCR-based qualitative assay for the detection of 14 hr-HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 in cervical specimens and has been extensively validated [14–16]. This is a high throughput partial genotyping test which differentiates and specifically identifies genotypes 16/18 individually and detects 12 other high-risk (OHR) types collectively in a single analysis. Genotypes 16/18 are far more carcinogenic than any other HPV types, accounting for 70% of cervical cancers; therefore, women infected with these genotypes are at a significantly increased risk for cervical pre-cancer and cancer [17, 18, 19]. This underscores a genotype-specific risk threshold in cervical screening strategies, and the interim clinical guidance in the US recommends direct referral to colposcopy for those testing positive for genotypes 16/18 in primary HPV screening [20]. In this context, the cobas HPV test also has the potential to serve as an adjunct test for triaging LSIL referral population through genotype 16/18-specific risk threshold and reduce the number of women requiring additional investigations and follow up in colposcopy clinics, and thus could aid in better patient care and resource management.

The objectives were to conduct a prospective study to assess positivity rates of CINtec PLUS and cobas HPV tests along with genotype 16/18-specific risk threshold in women referred to colposcopy with a history of LSIL, and to measure each test's ability as well as their clinical performance to predict CIN2+. This report describes the study and presents the data obtained at baseline.

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. If cytology is normal, triennial screening continues. In terms of managing women 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 cytology, colposcopy is recommended [2]. In colposcopy clinics, all referred patients undergo cytology and colposcopic examination with biopsies of lesions detected, and further follow up clinical pathways depend on specific criteria which can take years for some [21]. Cervical screening strategies utilizing HPV triage vary across Canada, with some provinces offering ASCUS-HPV triage for women ≥30 years, and LSIL-HPV triage for those ≥50 years through government funded programs [22]. In Ontario, colposcopy services guidelines incorporate HPV testing into the colposcopy clinical pathways for risk stratification to avoid unnecessary follow up colposcopy visits for HPV-negative women [21]. However, this is not practiced since HPV testing is not offered as part of government funded cervical cancer screening program.

2.2. Study protocol

The study was conducted within the Ontario cervical screening guidelines and has been designed as a prospective study. Study population comprised of women 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 per standard practice, and CINtec PLUS and cobas HPV tests were performed once at baseline using the residual cervical specimens for the study purpose. Patients' baseline data were recorded, and the study cohort was passively followed by review of their medical records to monitor clinic visits and obtain further relevant study data. Biopsy confirmed CIN2+ served as the clinical endpoint. CINtec PLUS and HPV testing results obtained at baseline together with that of biopsy 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. The test results obtained at baseline were correlated with the clinical endpoint observed either at baseline or during follow up to ascertain the clinical performance of the two tests. The study has been designed to follow the cohort for a minimum of one year with a provision to follow them up to three years. This report deals with the results of CINtec PLUS and HPV tests and that of biopsy obtained at baseline.

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 women 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 to obtain relevant study data during follow up. Those consenting to participate were enrolled in the study with written informed consent.

2.4. Patient enrolment

Women with a history of LSIL cytology who had not received treatment were eligible. Enrolment criteria included: 1), women 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 cytology among women being followed in the colposcopy clinic. In all instances, enrolment was limited to women with a pre-enrolment history of LSIL. There were no age limits, and pregnant women and women without a cervix were excluded. Eligible patients were enrolled consecutively from November 2017 through February 2019.

2.5. Study specimens

Cervical specimens were collected in ThinPrep PreservCyt® cytology collection device (Hologic Inc) for routine cytology at enrolment. The residual cervical specimens in the collection vials were stored at ambient temperature and used in the study as follows: the vials were batched on a weekly basis and slides were prepared for CINtec PLUS testing at the cytology laboratory, St. Joseph's Healthcare, Hamilton. A 1 mL aliquot of specimen was pipetted into a tube for cobas HPV testing. Slides and tubes were shipped weekly to the Eastern Health Public Health and Microbiology Laboratory, St. John's for CINtec PLUS and cobas HPV assays. These tests were carried out as described below no later than 6 weeks post collection.

2.6. CINtec PLUS assay

An experienced cytotechnologist prepared smears for CINtec PLUS on ThinPrep processor (T5000, Hologic Inc) using special ThinPrep slides (Hologic, Inc). The slides were fixed in ≥95% reagent grade ethanol and air dried. These were stained using CINtec PLUS assay kit within 48 h and processed on BenchMark ULTRA system (Roche Diagnostics) by trained personnel per manufacturer's instructions.

With CINtec PLUS assay, the p16 protein appears as a brown cytoplasmic stain and Ki-67 as a red nuclear stain independent of cytomorphology. 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). Smears were screened systematically with a 10x objective and cells showing the dual staining was confirmed 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

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

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 on biopsies per manufacturer's instructions as part of the study protocol to provide supporting diagnostic evidence; p16 results were corroborated with H&E biopsy interpretation. 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 and the study pathologists were blinded to these test results as well as cytology results. Colposcopy clinicians did not have access to CINtec PLUS or HPV results at the time of initial patient evaluation. HPV results were provided subsequently to clinicians to aid in patient management as the benefit of HPV testing in cervical screening strategies is well recognized in routine clinical practice. As CINtec PLUS testing was considered experimental, these results were not released.

2.10. Data analysis

Statistical analysis was performed using SPSS for Windows, version 23, Excel, Microsoft Office Professional Plus, 2013, and Social Science Statistics website, 2020. Qualitative variables were studied through different frequencies. Descriptive statistics were prepared for the data collected at baseline for test positivity rates, distribution of cytology grades and HPV genotypes. Study data were analyzed for all ages, <30 years of age and those ≥30 years, using contingency tables to determine test positivity rates, and the diagnostic sensitivity and specificity of CINtec PLUS and HPV testing by biopsy confirmed clinical endpoint. McNemar's test was used on paired nominal data and a two-tailed z score was used to compare proportions. p-values less than 0.05 were 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, 10 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 600 patients with evaluable results (Flowchart 1). Age ranged from 19 to 76 years (median, 33.5), with 386 (64.3%) ≥30 years of age (median, 43).

3.2. Positivity rates of CINtec PLUS and HPV tests

Table 1 shows in all ages, CINtec PLUS was positive in 266 (44.3%) vs. 331 (55.2%) testing HPV positive (p = 0.000). Among the 331 HPV positives, genotypes 16/18 were detected in 93 (28.1%). In women ≥30 years, CINtec PLUS was positive in 159 (41.2%) vs. 196 (50.8%) testing HPV positive (p = 0.008). Among the 196 HPV positives, genotypes 16/18 were detected in 57 (29.1%). There was a significant difference in both CINtec PLUS and HPV positivity rates in women <30 years of age and those ≥30 years (Table 1). The % agreement between CINtec PLUS and HPV tests was similar (range, 70.7%–70.8%; kappa, 0.416–0.423) in all ages and the two age groups (Data not shown).

3.3. Association of CINtec PLUS and HPV results with biopsy and diagnostic indices

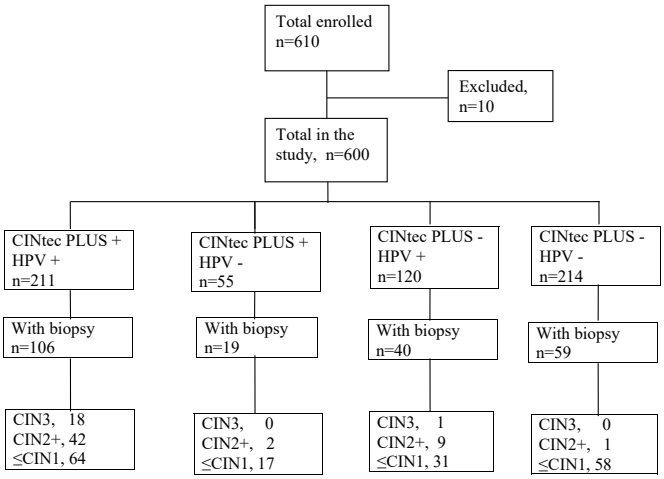
Of the 600 patients in all ages, 232 (38.7%) underwent biopsy per routine clinical practice, and evaluable results were available for 224 (37.3%). Among the 224, biopsy confirmed CIN2+ was diagnosed in 54 (24.1%), including 19 CIN3, with the remaining 170 diagnosed as having ≤ CIN1 (Table 2). In women ≥30 years, biopsy results were available for 135, and 24 (17.8%) had CIN2+, including 9 CIN3. All CIN2+ biopsy diagnoses were substantiated by a positive p16 result.

Table 2 shows of the 54 CIN2+ in all ages, CINtec PLUS was positive in 44 for a sensitivity of 81.5%, vs. 51 testing positives by HPV test for a sensitivity of 94.4% (p = 0.039; Table 3). Of the 19 CIN3, CINtec PLUS was positive in 18 for a sensitivity of 94.7%, and HPV test was positive in all 19 (Table 2). Specificity of CINtec PLUS to detect CIN2+ was 52.4% (89/170) vs. 44.1% (75/170) for HPV testing (p = 0.129; Tables 2 and 3). Among women ≥30 years, of the 24 CIN2+, 23 tested positive by both CINtec PLUS and HPV tests for a sensitivity of 95.8% (Table 3), with all 9 CIN3 cases testing positive by both tests (Table 2); specificity to detect CIN2+ was 55.0% (61/111) for CINtec PLUS vs. 50.5% (56/111) for HPV test (p = 0.503: Tables 2 and 3). Distribution of CINtec PLUS and HPV results correlated with biopsy findings are summarized in flowchart 1.

Table 4 shows the comparison of paired results of CINtec PLUS and HPV tests with biopsy results for all ages. Analyses of this data by McNemar test indicated no significant difference between the two tests. Additional data analysis based on age groups, <30 years and ≥30 years, is shown in the Appendix (Table 4a and Table 4b, respectively), indicating a significant difference in CIN2+ detection between the two tests only for women < 30 years.

3.4. Association of HPV genotypes 16/18 with biopsy and diagnostic indices

Table 5 shows association of HPV genotypes 16/18 with biopsy



Flowchart 1. Distribution of CINtec PLUS and HPV results and biopsy outcome in all ages (18-76 years)

results. In women of all ages, among the 51 of the 54 CIN2+ testing HPV positive, genotypes 16/18 were detected in 25 (49.0%), testing for genotypes 16/18 was 46.3% sensitive. Among the 19 CIN3 testing HPV positive, genotypes 16/18 were detected in 11 (57.9%; data not shown). In women <30 years of age, of the 28 CIN2+ testing HPV positive, genotypes 16/18 were detected in 11 (39.3%), testing for genotypes 16/18 was 36.7% sensitive. In women ≥30 years, of the 23 CIN2+ testing HPV positive, genotypes 16/18 were detected in 14 (60.9%), testing for genotypes 16/18 was 58.3% sensitive. There were no significant differences in sensitivities between all ages and the two age groups and between the two age groups. Specificity of testing for genotypes 16/18 was 84.7% in all ages, 86.4% in those <30 years, and 83.8% in women ≥30 years. In all genotypes 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.

3.5. Association of CINtec PLUS and HPV results with cytology

Cytology was performed as part of routine patient care at enrolment, with CINtec PLUS and HPV testing carried out using the residual cervical specimens. The above cytology results were unknown when patients were enrolled and retrieved from patient medical records to assess the association of CINtec PLUS and HPV test results. The time interval between the index referral LSIL cytology immediately prior to enrolment and cytology performed in the colposcopy clinic at initial referral visit ranged from <1 month to ≥18 months with a median of 7 months (average, 7.9 months). Although the index referral cytology was LSIL in all patients enrolled, cytology performed at the time of initial colposcopy clinic visit showed heterogeneous cytological grades as expected (Table 6). In all ages, LSILs appeared to have regressed in 48.9% (291/595) to ASCUS or negative cytology and progressed to HSIL in 8.6% (51/

Table 1
CINtec PLUS and HPV results by age groups.

Test	Result	All ages n = 600	<30 years n = 214	≥30 years n = 386	
CINtec PLUS	Positive	266 (44.3%)[a]	107 (50.0%)[b,*]	159 (41.2%)[c,*]	*p = 0.038, CINtec Plus positivity compared between women <30 and ≥ 30 years
	Negative	334 (55.7%)	107 (50.0%)	227 (58.8%)	
HPV	Positive	331 (55.2%)[a]	135 (63.1%)[b, **]	196 (50.8%)[c, **]	**p = 0.004, HPV positivity compared between women <30 and ≥ 30 years
	Negative	269 (44.8%)	79 (36.9%)	190 (49.2%)	
p value		[a] p value = 0.000, 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 = 0.008, CINtec PLUS positivity compared with HPV positivity in women ≥30 years	

Table 2
Association of CINtec PLUS and HPV results with biopsy by age groups.

Test	Result	Biopsy result								
		All ages, n = 224			<30 years, n = 89			≥30 years, n = 135		
		≤CIN1, n = 170	CIN2+, n = 54	CIN3, n = 19	≤CIN1, n = 59	CIN2+, n = 30	CIN3, n = 10	≤CIN1, n = 111	CIN2+, n = 24	CIN3, n = 9
CINtec PLUS	Positive	81	44	18	31	21	9	50	23	9
	Negative	89	10	1	28	9	1	61	1	0
HPV	Positive	95	51	19	40	28	10	55	23	9
	Negative	75	3	0	19	2	0	56	1	0

Table 3
Diagnostic indices of CINtec PLUS and HPV tests for detection of CIN2+.

Diagnostic index	CINtec PLUS test			HPV test			p value, All ages
	All ages	<30 years	≥30 years	All ages	<30 years	≥30 years	
Sensitivity % (95% CI)	81.5 (76.4–86.6)	70.0 (60.5–79.5)	95.8 (92.5–99.2)	94.4 (91.4–97.4)	93.3 (88.2–98.5)	95.8 (92.5–99.2)	p = 0.039, CINtec Plus sensitivity compared with HPV sensitivity
Specificity % (95% CI)	52.4 (45.8–58.9)	47.5 (37.1–57.8)	55.0 (46.6–63.3)	44.1 (37.6–50.6)	32.2 (22.5–51.4)	50.5 (42.0–58.9)	p = 0.129, CINtec Plus specificity compared with HPV specificity
PPV % (95% CI)	35.2 (28.9–41.5)	40.4 (30.2–50.6)	31.5 (23.7–39.3)	34.9 (28.7–41.2)	41.2 (31.0–51.4)	29.5 (21.8–37.2)	p = 0.960, CINtec Plus PPV compared with HPV PPV
NPV % (95% CI)	89.9 (86.0–93.8)	75.7 (66.8–84.6)	98.4 (96.3–100.0)	96.2 (93.6–98.7)	90.5 (84.4–96.6)	98.2 (96.0–100.0)	p = 0.114, CINtec Plus NPV compared with HPV NPV

CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Table 4
Comparison of CINtec PLUS and HPV tests by biopsy result: All ages (n = 224).

		HPV test result					
		≤ CIN1, n = 170			CIN2+, n = 54		
		Positive	Negative	Total	Positive	Negative	Total
CINtec PLUS result	Positive	64	17	81	42	2	44
	Negative	31	58	89	9	1	10
Total		95	75	170	51	3	54
McNemar		p = 0.059			p = 0.065		

Table 5
Association of HPV genotypes 16/18 with biopsy and diagnostic indices.

		Biopsy result					
		All ages, n = 224		<30 years, n = 89		≥30 years, n = 135	
		≤CIN1, n = 170	CIN2+, n = 54	≤CIN1, n = 59	CIN2+, n = 30	≤CIN1, n = 111	CIN2+, n = 24
HPV 16/18	Positive	26	25	8	11	18	14
	Negative	144	29[a]	51	19	93	10
HPV 16/18 genotyping[b]	Sensitivity	25/54 = 46.3%		11/30 = 36.7%		14/24 = 58.3%	
	Specificity	144/170 = 84.7%		51/59 = 86.4%		93/111 = 83.8%	
	p value, sensitivity: 0.395, All ages compared to <30 years, 0.327, All ages compared to ≥30 years; 0.112, <30 years compared to ≥30 years						

[a] Among 29 testing genotype 16/18 negative, 26 tested positive for 12 other high-risk (OHR) genotypes, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.
[b] Sensitivity/specificity based on CIN2+ detection.

595), with only 41.5% (247/595) still having LSIL at the time of CINtec PLUS and HPV testing. The cytologic lesion regression and progression rates were similar at 50.1% (193/381) and 6.6% (25/381), respectively, in women aged ≥30 years (Data not shown). The positivity rates of both CINtec PLUS and HPV tests uniformly decreased with decreasing cytologic lesion severity (Table 6), and this was similar also in those ≥30 years (Data not shown). There were no significant differences in positivity rates between CINtec PLUS and HPV tests per any of the cytology group.

Table 6
Cytology status at enrolment and association with CINtec PLUS and HPV results All ages, n = 595*.

Cytology		CINtec PLUS result		HPV result	
Status	Number of cases (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)
HSIL	51 (8.6)	45 (88.2)	6 (11.8)	47 (92.2)	4 (7.8)
ASC-H	6 (1.0)	5 (83.3)	1 (16.7)	4 (66.7)	2 (33.3)
LSIL	247 (41.5)	148 (59.9)	99 (40.1)	184 (74.5)	63 (25.5)
ASCUS	101 (17.0)	27 (26.7)	74 (73.3)	43 (42.6)	58 (57.4)
Negative	190 (31.9)	39 (20.5)	151 (79.5)	50 (26.3)	140 (73.7)
Total	595	264 (44.4)	331 (55.6)	328 (55.1)	267 (44.9)

*There were 5 cases with unsatisfactory cytology and excluded from the total of 600 study patients.
HSIL, High-grade squamous intraepithelial lesion; ASC-H, Atypical squamous cells of undetermined significance-cannot exclude HSIL; LSIL, Low-grade squamous intraepithelial lesion; ASCUS, Atypical squamous cells of undetermined significance

4. Discussion

An effective strategy is needed for triaging women referred to colposcopy with a history of LSIL since only a small proportion is at risk for cervical pre-cancer and cancer. The premise of our study was that both CINtec PLUS assay and cobas HPV test with genotype 16/18-specific risk threshold have the potential to identify those at increased risk requiring further investigations and follow up in colposcopy clinics and safely return those not at immediate risk to routine screening. In this context, we also assessed the clinical performance of CINtec PLUS and cobas HPV tests to detect CIN2+.

Although LSIL is a common cytologic finding in cervical screening, it accounts for only 10–20% of CIN2+ [23]. Regardless, women with LSIL cytology in primary screening are considered at high enough risk for referral to colposcopy, and the ALTS study concluded LSIL cytology is best managed by colposcopy initially [1]. It should be noted that in the US, a risk for CIN3+ greater than 5.2% is considered the threshold for colposcopy referral whereas in Europe it is greater than 10% [24,25]. Our baseline study data showed a CIN2+ prevalence of 9% (54/600) in all ages in a routine colposcopy referral setting; it was lower at 6.2%

(24/386) in women aged ≥30 years. This emphasizes the importance of an efficient triage to identify the small fraction of women at increased risk among the LSIL referral population. On the other hand, it also raises question of following all women with a history of LSIL cytology with further investigations in colposcopy clinics even though the risk is limited to only a few.

The baseline results from our ongoing study provided some insight into the positivity rates and relative performance of CINtec PLUS and cobas HPV tests for triaging Canadian women referred to colposcopy with a history of LSIL cytology. Based on the test positivity rates, CINtec PLUS identified 44.3% would be at increased risk, vs. 55.2% by HPV test in women of all ages. In women ≥30 years, these figures were non-significantly lower at 41.2% vs. 50.8%, respectively (Table 1). This implies, in all ages, cutting the size of the LSIL referral population requiring further investigations and follow up in colposcopy clinics slightly over one half by CINtec PLUS assay, and slightly under one half by HPV test. Also, regardless of the test, the proportion requiring further investigations would be lower in women ≥30 years than those <30. In this regard, we note as a strength of our study that our data provide evidence for the benefit of incorporating LSIL-HPV triage for risk threshold and to reduce unnecessary follow up colposcopy clinic visits for HPV-negative women as recommended in the Ontario colposcopy services guidelines [21].

The reported sensitivity and specificity of CINtec PLUS in detecting CIN2+ among those with ASCUS or LSIL cytology varies in different studies and populations [4,6,7,10–13], and our observations were comparable to the range of published figures, in that CINtec PLUS showed a significantly lower sensitivity and non-significantly higher specificity in comparison to HPV testing in women of all ages. CIN2+ sensitivity was 81.5% for CINtec PLUS vs. 94.4% for HPV test in women of all ages, and these were 70.0% and 93.3%, respectively, in women <30 years (Table 3). But, both tests showed identical CIN2+ sensitivity of 95.8% in women ≥30 years. Further, the sensitivities were in the range of 95–100% for both tests in detecting CIN3 in all ages and in those ≥30 years (Table 2). This indicates CINtec PLUS could be more reliably used for triaging women ≥30 years than <30 years, whereas HPV testing could be equally reliable in women of all ages, regardless of age groups, referred to colposcopy with a history of LSIL cytology. Further analyses of paired CINtec PLUS and HPV results in women of all ages by McNemar test showed no difference between the two (Table 4), indicating that both tests could serve as a predictor of CIN2+ with high sensitivity while conferring a significant reduction in the number of women requiring further colposcopy clinic visits. Since CINtec PLUS sensitivity was found to be lower in women <30 years, this may be of concern if considering CINtec PLUS in LSIL triage for this age group. However, CIN2+ is known to be mostly regressive [26], and CINtec PLUS-negative results may in fact be reflective of regressing lesions, and therefore, clinically more relevant than the higher HPV positivity which in many cases likely represents transient infection. Regardless, the reduced CIN2+ sensitivity of CINtec PLUS should be considered if using this test for LSIL triage in women <30 years. Given the option between Pap 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 [4,10]. Nevertheless, a further follow up would be warranted for those testing CINtec PLUS negative to ensure CIN2+ is not missed. Alternatively, HPV triage could be an option for this age group, and if using a partial genotyping test such as the cobas HPV assay, there is an added advantage of providing a secondary triage result with genotypes 16/18 information for risk stratification.

HPV genotypes 16/18 dominate in high grade lesions accounting for 70% of cervical cancer world-wide [17,18,27]. Therefore, testing for these two genotypes has been proposed as an additional tool to allow for more fine-tuned patient management [28]. This is now technologically supported by several currently available next generation HPV testing platforms such as the cobas HPV test which offer high throughput one-step partial genotyping for 16/18, thus providing immediate access to this information. Based on a meta-analysis of 24 studies involving more than 5000 women with LSIL, Arbyn et al. [28] reported the average risk for CIN3+ to be 19% in genotypes 16/18-positive women compared to 5% in hr-HPV-positive but genotypes 16/18-negative women. Further, the pre-test probability of CIN3+ was 8.6% whereas the post-test probabilities after triage were 10.6% in hr-HPV positive women, 19.3% in genotypes 16/18-positive women and 3.8% in genotypes 16/18-negative women. This analysis also showed testing for genotypes 16/18 was substantially more specific but less sensitive than testing for hr-HPV in detecting CIN2+. This is relevant when considering genotypes 16/18-specific threshold for further investigations and follow up of LSIL populations. In our study, among those testing HPV positive, genotypes 16/18 were detected in 28.1% (93/331) in all ages, and 29.1% (57/196) in women ≥30 years. If genotype 16/18-specific risk threshold were to be used for further follow up in colposcopy clinics, based on the above percentages, the proportion requiring additional investigations would be cut down by more than 2/3 rds among those testing HPV positive. This would have detected 49% (25/51) of CIN2+ among those testing HPV positive in all ages, with a sensitivity of 46.3%, and 60.9% (14/23) of CIN2+ among those testing HPV positive in woman ≥30 years, with a sensitivity of 58.3% (Table 5). The above sensitivity rates were similar to the pooled genotype 16/18-specific CIN2+ sensitivity of 55.5% reported in the meta-analysis by Arbyn et al. [28]. Our specificity rates of 84.7% and 83.8%, respectively, for these two age groups (Table 5), were also like the 76.3% reported in the meta-analysis. It is important, however, to point out while testing for genotypes 16/18 increases efficiency, it is significantly less sensitive than testing for hr-HPV as shown in our study (Tables 3 and 5) and consistent with the data reported in the meta-analysis [28]. Whether continued colposcopy follow up or cytological follow up in primary care is considered for hr-HPV-positive, but genotypes 16/18-negative women would depend on local decision thresholds, this can be derived from pre- and post-test probability plots [28]. Regardless, the above observation takes cue from the US interim clinical guidance document that recommends immediate colposcopy referral for those testing positive for genotypes 16/18 in primary HPV screening, and reflex cytology for those positive for OHR HPV types [20]. Although the average risk for CIN2+ is significantly lower in OHR HPV positive women, continued follow up of such cases is warranted to ensure detection of any additional CIN2+ cases that would otherwise be missed by genotype 16/18-based risk stratification if this option is considered. Ongoing studies evaluating triage strategies for HPV positive women should provide further guidance in this regard [20].

Both CINtec PLUS and cobas HPV tests showed a close and consistent correlation with cytological grades found at enrolment. Cytology was performed in the colposcopy clinic an average of 7.9 months after the index referral LSIL cytology, and during this interval LSILs regressed in 48.9% and progressed to HSIL in 8.6% in all ages (Table 6). These rates were consistent with a regression of 41.9% and progression of 7% reported after an average of 2-month interval in the ALTS trial of ASCUS population [29], and 56.4% and 7.8%, respectively, found in a Norwegian study of ASCUS/LSIL populations after a median of 7 months [30]. The above observation is particularly important in considering the usefulness of HPV testing for triaging women referred to colposcopy

with a history of LSIL cytology as lesion regression would directly influence HPV positivity rates. Since most LSILs regress spontaneously, a large proportion of women with LSIL cytology referred to colposcopy no longer have LSIL by the time they are seen in colposcopy clinics as observed in our study, and this reduces the overall HPV positivity rates, consequently making HPV testing cost-effective in this setting. It is important to note our study was conducted in a routine colposcopy clinic in a practical setting. Our study showed overall HPV positivity rates of 55.2% in all ages and 50.8% in women ≥30 years (Table 1). These were similar to an overall HPV positivity rate of 50.6% found in the ALTS-ASCUS trial which led to an ASCUS-HPV triage recommendation [29], but substantially lower than the pooled HPV positivity rate of 76% reported in populations with concurrent LSIL [31]. We could also surmise that the reduced HPV positivity in our study population as described above was the reason for the failure to demonstrate a significantly higher specificity of CINtec PLUS than HPV test that has been shown in many studies [4,6,10,11,13]. It is apparent the reduced HPV positivity conferred increased specificity, thus narrowing the difference in specificity rates between CINtec PLUS and HPV tests in our study population albeit showing a non-significantly higher specificity for CINtec PLUS compared to HPV test. Regardless, based our study, it may be concluded that HPV testing, especially with partial genotyping, could be effective for triaging women with a history of LSIL cytology referred to colposcopy in routine clinical settings.

HPV primary screening and ASCUS-HPV triage are recommended for women ≥30 years in many countries. As part of routine cervical screening guidelines, ASCUS-HPV triage was implemented over a decade ago for women ≥30 years in Newfoundland and Labrador. Our records based on cobas HPV testing show an average HPV positivity rate of 30% in this population, thus helping to reduce the number of women requiring immediate colposcopy referral by about 70%; the reduction could be as high as 85% if using genotype 16/18-specific risk threshold for referral (Paper in preparation). Further CINtec PLUS has been approved by Health Canada as an adjunct test for risk stratification to colposcopy referral. These, together with a poor efficacy of ProEx C immunoassay for ASCUS and LSIL triage that we found in our previous study [3], provided the basis and impetus to our present study.

Our study was conducted in a routine colposcopy referral setting with a study cohort representative of Canadian LSIL referral populations without any intervention for the purpose of the study, especially regarding the time interval between referral LSIL cytology and initial colposcopy clinic visit. The average interval of about 8 months observed in our study is likely representative of colposcopy waiting time in Canadian settings and may be reflective of prevailing colposcopy backlogs and workload. This underscores the importance of using effective triage strategies to reduce unnecessary accumulation of referral women in colposcopy clinics who are not at immediate risk. Our report is preliminary based on baseline findings and a reduced CIN2+ biopsy-confirmed sample size because not all patients in the study underwent biopsy for verification of disease outcome since biopsy was obtained from only those found to have colposcopy-detected lesions per routine clinical practice. Plotting risks for pre-cancer on pre- and post-test probability plots should shed more light in assessing the efficacy of triage tests including testing for genotypes 16/18 as indicated by Arbyn et al. [28]. The study is ongoing with follow up via medical records review which may provide further data on predictive values of CINtec PLUS and cobas HPV tests for triaging women referred to colposcopy with a history of LSIL cytology.

5. Conclusions

Based on our results, it may be concluded either CINtec PLUS or cobas HPV test could serve as a predictor of CIN2+ with an equally high sensitivity in women ≥30 years referred to colposcopy with a history of LSIL cytology. In women <30 years, CINtec PLUS showed lower sensitivity than HPV test, and this needs to be considered in weighing post-test pre-cancer risk if this test is used for LSIL triage. Regardless CINtec PLUS or cobas HPV test can significantly reduce the number of women requiring further investigations and follow up in colposcopy clinics.

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CRediT authorship contribution statement

Sam Ratnam: Conceptualization, Funding acquisition, Methodology, Supervision, Data analysis, Writing-original draft, Writing- review and editing. **Dan Jang:** Project administration, Supervision, Data curation, Resources, Laboratory technical support, Writing-review. **Laura Gilbert:** Project administration, Investigation, Methodology, Laboratory technical support, Resources, Data curation, Formal analysis, Writing- review and editing. **Reza Alaghehbandan:** Investigation, Formal analysis, Validation, Writing- review and editing. **Miranda Schell:** Investigation, Formal analysis, Validation, Writing- review and editing. **Robert Needle:** Investigation, Data curation, Laboratory technical support, Writing-review. **Anne Ecobichon-Morris:** Study coordination, Technical support, Writing-review. **Peizhong Peter Wang**: Formal analysis, Writing-review and editing. **Mozibur Rahman:** Resources, Writing-review. **Dustin Costescu:** Resources, Writing-review and editing. **Laurie Elit:** Resources, Writing-review. **George Zahariadis:** Resources, Writing-review. **Max Chernesky:** Resources, Methodology, Supervision, Writing-review and editing.All authors attest they meet the ICMJE criteria for authorship and approved the final version for submission.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pvr.2020.100206.

Appendix

Table 4a
Comparison of CINtec PLUS and HPV tests by biopsy result: Women <30 years of age (n = 89).

		HPV test result					
		≤ CIN1, n = 59			CIN2+, n = 30		
		Positive	Negative	Total	Positive	Negative	Total
CINtec PLUS result	Positive	26	5	31	20	1	21
	Negative	14	14	28	8	1	9
Total		40	19	59	28	2	30
McNemar		p = 0.064			p = 0.039		

Table 4b
Comparison of CINtec PLUS and HPV tests by biopsy result: Women ≥30 years of age (n = 135).

		HPV test result					
		≤ CIN1, n = 111			CIN2+, n = 24		
		Positive	Negative	Total	Positive	Negative	Total
CINtec PLUS result	Positive	38	12	50	22	1	23
	Negative	17	44	61	1	0	1
Total		55	56	111	23	1	24
McNemar		p = 0.458			p = 1.000		

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