Relative Performance of HPV DNA and E6/E7 mRNA Tests in Cervical Cancer Screening: Preliminary Results from a Multicentre Canadian Study

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Study Objective

- A multicentre prospective cohort study commenced in 2006
- Determine the application of HPV E6/E7 mRNA-based tests and MCM2/TOP2a in cervical cancer screening in comparison with the L1-based HC2 DNA test
- Presentation will focus on HPV E6/E7 mRNA and DNA tests

Study Rationale

- There is need to improve clinical specificity and PPV in cervical cancer screening and for the triage of borderline cytologic abnormalities
- Malignant transformation is characterized by over expression of E6/E7 oncogenes which can be detected by testing for E6/E7 mRNA
- Testing for E6/E7 mRNA can be more specific and accurate than DNA tests in identifying those truly at risk for cervical cancer with a greater prognostic value

Study Design

- 6 sites representing 6 of the 10 Canadian provinces participating in the study
- Study has both cross-sectional and longitudinal follow up components
- Cohort to be followed for at least 3 years
- Clinical endpoint: ≥ CIN 2
- ~3500 women, ≥19 years of age
- Two groups of patients, mostly colpo referral population, complemented by a cohort of routinely screened population

Methods

- Cervical specimens collected in ThinPrep PreservCyt
- ThinPrep LBC carried out on all cases at baseline
- Colposcopy/biopsy carried out as indicated as per standard of care
- Histology confirmed ≥ CIN 2 is the gold standard
- Adjudication of histology results underway

Tests Used in the Study

- APTIMA HPV Assay (Gen-Probe)
- Hybrid Capture 2 test (Qiagen)
- PreTect HPV-Proofer test (Norchip)
- AMPLICOR HPV test (Roche)

- Linear Array (Roche) used for genotyping
- All tests conducted blinded and independent of one another

Clinical Sensitivity of APTIMA

- Data analysis based on 1199 colpo referral cases
- 363 cases of ≥ CIN 2 identified (Disease prevalence 30.3%)
- This included
  - 12 cases of SCC
  - 4 AIS/ADC

- APTIMA was positive in 345 cases

Clinical Sensitivity

345/363 = 95.0% (95% CI: 92.8, 97.2)

Clinical Sensitivity: APTIMA vs HC2

≥ CIN2 = 363

		HC2		Total
		+	-	
APTIMA	+	335	10	345
	-	6	12	18
Total		341	22	363

APTIMA Sensitivity	345/363 = 95.0% (95% CI: 92.8, 97.3)
HC2 Sensitivity	341/363 = 93.9% (95% CI: 91.5, 96.4)
Agreement	347/363 = 95.6%

Cytology/Genotype Profile of 12 CIN2+ Cases Testing Negative by Both APTIMA & HC2

	LBC	LA Genotype
1.	Normal	Negative
2.	Normal	Negative
3.	ASCUS	Negative
4.	ASCUS	70
5.	LSIL	82
6.	ASCUS	61, 82
7.	Normal	16
8.	LSIL	16, 52, 70, 84
9.	Normal	56, 66
10.	Normal	42, 52, 53
11.	Normal	39, 62, 67, 89
12.	Normal	39, 42, 52, 53, 58, 61, 62, 73

Cytology/Genotype Profile of 10 CIN2+ Cases Testing Negative by HC2 and Positive by APTIMA

	LBC	LA Genotype
1.	ASCUS	16
2.	ASCUS	18, 52, 54
3.	LSIL	33,62
4.	Normal	16, 51, 52
5.	ASCUS	16, 45
6.	LSIL	16, 89
7.	HSIL	51, 53, 62, 81, 83
8.	ASCUS	51, 61
9.	LSIL	18
10.	Normal	18

Cytology/Genotype Profile of 6 CIN2+ Cases Testing Negative by APTIMA and Positive by HC2

	LBC	LA Genotype
1.	ASCUS	70
2.	HSIL	16, 18
3.	ASCUS	51, 61, 89
4.	LSIL	16, 25
5.	ASCUS-H	52, 81, 83
6.	ASCUS	16, 31, 51, 82

Clinical Specificity of APTIMA

Colpo Referral Cases, Total =1199; ≤ CIN 1, = 836 (69.7%)

		Disease		Total
		+	-	
APTIMA	+	345	459	804
	-	18	377	395
Total		363	836	1199

Clinical Specificity	377/836 = 45.1% (95% CI: 41.7, 48.5)
Clinical Sensitivity	345/363 = 95.0% (95% CI: 92.8, 97.3)
PPV	345/804 = 42.9% (95% CI: 39.5, 46.3)
NPV	377/395 = 95.4% (95% CI: 93.4, 97.5)
Accuracy	722/1199 = 60.2% (95% CI: 57.4, 63.0)

Clinical Specificity: APTIMA vs HC2

Colpo Referral Cases, Total =1199; ≤ CIN 1, N = 836 (69.7%)

		HC2		Total
		+	-	
APTIMA	+	430	29	459
	-	74	303	377
Total		504	332	836

HC2 Specificity	332/836 = 39.7% (95% CI: 36.4, 43.0)
APTIMA Specificity	377/836 = 45.1% (95% CI: 41.7, 48.5)
Agreement	733/836 = 87.7%

Comparative Performance of APTIMA and HC2

Based on Colpo Referral Cases, N=1199

	Sensitivity	Specificity	PPV	NPV	Accuracy
APTIMA	95.0%	45.1%	42.9%	95.4%	60.2%
HC2	93.9%	39.7%	40.4%	93.8%	56.1%

Correlation of Cytology/Histology with APTIMA and HC2

Based on Colpo Referral Cases, N = 1154

LBC	Histology	N	APTIMA		HC2	
			+	-	+	-
Normal n = 363	≥ CIN 2	58	50 (86.2%)	8	48 (82.8%)	10
	≤ CIN 1	305	138	167 (54.8%)	150	155 (50.8%)
ASCUS n = 340	≥ CIN 2	82	77 (93.9%)	5	76 (92.7%)	6
	≤ CIN 1	258	136	122 (47.3%)	152	106 (41.1%)

Correlation of Cytology/Histology with APTIMA and HC2

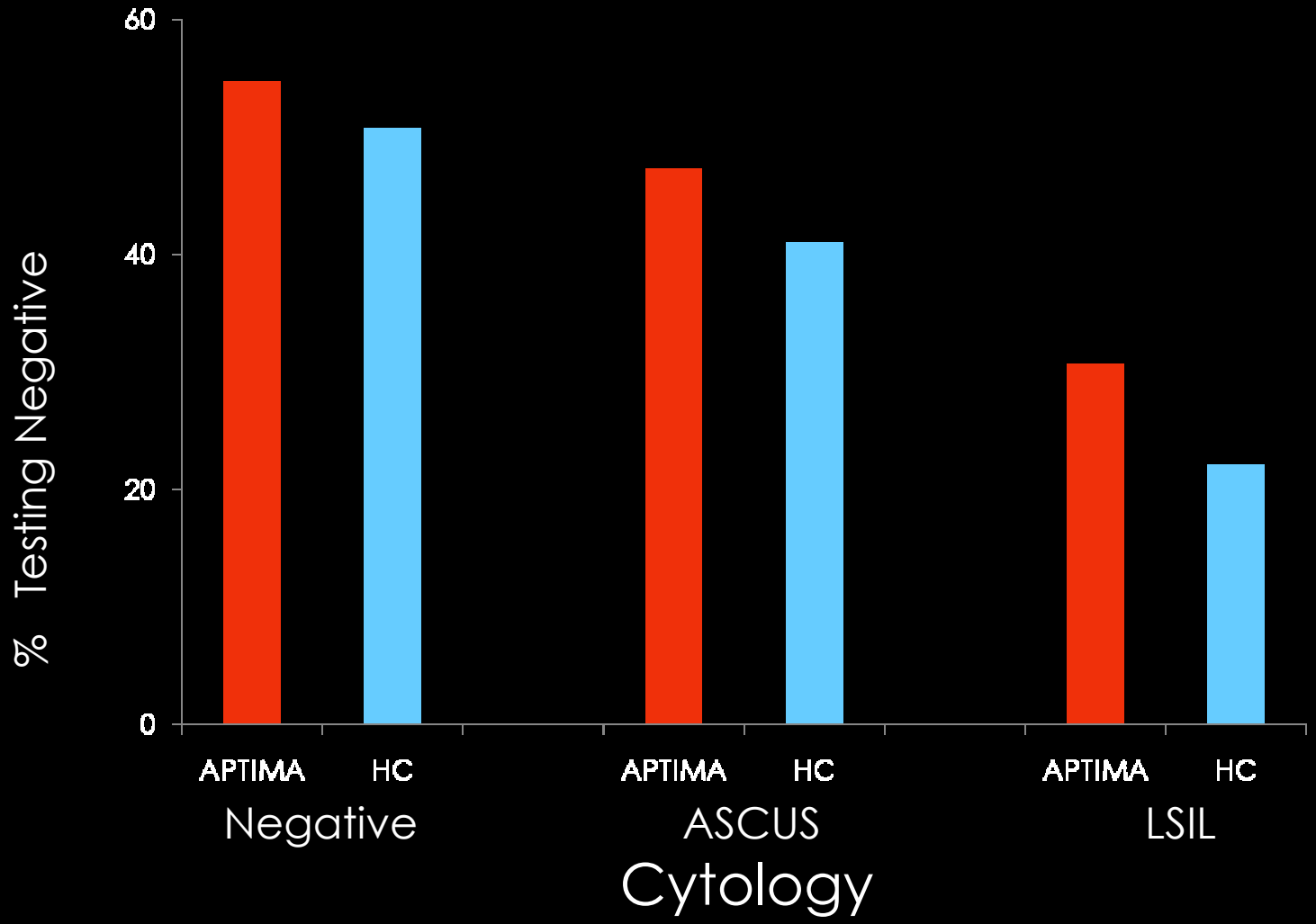
Based on Colpo Referral Cases, N = 1154

LBC	Histology	N	APTIMA		HC 2	
			+	-	+	-
LSIL n= 308	≥ CIN 2	100	97 (97%)	3	95 (95%)	5
	≤ CIN 1	208	144	64 (30.8%)	162	46 (22.1%)
HSIL n = 143	≥ CIN 2	116	110	6	112	4
	≤ CIN 1	27	27	0	27	0

Specificity of APTIMA and HC2

≤ CIN 1 cases stratified by cytology

Colpo Referral Cases, N = 1154



Clinical Specificity: APTIMA vs HC2 Routine Screen Population

N= 1120

≥ CIN 2, =7 cases (Disease prevalence, 0.6%); ≤ CIN1 = 1113

		Disease	
		+	-
APTIMA	+	7	138
HC2	+	7	174

Among those with no disease and having mostly normal cytology, proportions testing positive:

APTIMA 138/1113 = 12.4%

HC2 174/1113 = 15.6%*

*Statistically significant

Reactivity in the Absence of Genotypes Included in the Assay as Determined by Linear Array

N =1376

Test	Number of Cases Testing +ve	Histology		
		Negative	CIN I	≥ CIN 2
APTIMA	26 (1.9%)	21	5	0
HC2	79 (5.7%)*	62	16	1

*Statistically significant

Genotypes Detected by Linear Array in the Absence of Those Included in the Assays

N =1376

APTIMA	54, 70, 53, 67, 6, 11, 81, 91, 90
HC2	53, 42, 54, 66, 70, 6, 40, 55, 6, 61, 62, 67, 72, 81, 83, 84, 89, 73, 90

Mostly co-infections

PreTect HPV-Proofer Test

- Genotype specific test based on real-time NASBA technology
- Targets E6/E7 mRNA of types 16, 18, 31, 33, and 45
- There is indication of greater specificity and accuracy compared with DNA tests

Performance of PreTect HPV-Proofer

Colpo Referral Cases, N = 836

≥ CIN 2, = 238 cases (Disease prevalence, 28.5%); ≤ CIN 1, = 598

		Disease		Total
		+	-	
PreTect	+	183	146	329
Proofer	-	55	452	507
Total		238	598	836

Sensitivity	183/238 = 76.9% (95% CI: 71.5, 82.2)
Specificity	452/598 = 75.6% (95% CI: 72.1, 79.0)
PPV	188/329 = 55.6% (95% CI: 50.3, 61.0)
NPV	452/507 = 89.2% (95% CI: 86.4, 91.9)
Accuracy	635/836 = 76.0% (95% CI: 73.1, 78.9)

Performance of APTIMA

Colpo Referral Cases, N = 836

≥ CIN 2, = 238 cases (Disease prevalence, 28.5%); ≤ CIN 1, = 598

		Disease		Total
		+	-	
APTIMA	+	225	317	542
	-	13	281	294
Total		238	598	836

Sensitivity	225/238 = 94.5% (95% CI: 91.7, 97.4)
Specificity	281/598 = 47.0% (95% CI: 43.0, 51.0)
PPV	225/542 = 41.5% (95% CI: 37.4, 45.7)
NPV	281/294 = 95.6% (95% CI: 93.2, 97.9)
Accuracy	506/836 = 60.5% (95% CI: 57.2, 63.8)

Comparative Performance of APTIMA and PreTect Proofer

Based on Colpo Referral Cases, N = 836

	Sensitivity	Specificity	PPV	NPV	Accuracy
APTIMA	94.5%	47.0%	41.5%	95.6%	60.5%
PreTect Proofer	76.9%*	75.6%*	55.6%	89.2%	76.0%*

*Statistically significant

Sensitivity:
APTIMA vs PreTect HPV-Proofer

Excluding cases containing genotypes not included in PreTect
≥ CIN 2, N = 238 - 27 = 211

		PreTect Proofer		Total
		+	-	
APTIMA	+	183	24	207
	-	0	4	4
Total		183	28	211

PreTect sensitivity	183/211 = 86.7% (95% CI: 82.1, 91.3)
APTIMA sensitivity	207/211 = 98.1% (95% CI: 96.3, 99.9)
Agreement	187/211 = 88.6% (Kappa = 0.224)

AMPLICOR Test

- Based on target DNA amplification
- Has high sensitivity
- Specificity is likely to be lower

Performance of AMPLICOR

Colpo Referral Cases, N = 488

≥CIN2, N = 106 cases (Disease prevalence, 21.7%); ≤CIN1, 382

		Disease		Total
		+	-	
AMPLICOR	+	102	218	320
	-	4	164	168
Total		106	382	488

Sensitivity	102/106 = 96.2% (95% CI: 92.6, 99.9)
Specificity	164/382 = 42.9% (95% CI: 37.9, 47.9)
PPV	102/320 = 31.9% (95% CI: 26.8, 37.0)
NPV	164/168 = 97.6% (95% CI: 95.3, 99.9)
Accuracy	266/488 = 54.5% (95% CI: 50.1, 58.9)

Performance of APTIMA

Colpo Referral Cases, N= 488

≥CIN2, = 106 cases (Disease prevalence, 21.7%); ≤ CIN1, = 382

		Disease		Total
		+	-	
APTIMA	+	97	154	251
	-	9	228	237
Total		106	382	488

Sensitivity	97/106 = 91.5%	(95% CI: 86.2, 96.8)
Specificity	228/382 = 59.7%	(95% CI: 54.8, 64.6)
PPV	97/251 = 38.6%	(95% CI: 32.6, 44.6)
NPV	228/237 = 96.2%	(95% CI: 93.8, 98.6)
Accuracy	325/488 = 66.9%	(95% CI: 62.4, 70.8)

Comparative Performance of APTIMA and AMPLICOR

Based on Colpo Referral Cases, N = 488

	Sensitivity	Specificity	PPV	NPV	Accuracy
APTIMA	91.5%	59.7%	38.6%	96.2%	66.9%
AMPLICOR	96.2%	42.9%*	31.9%	97.6%	54.5%*

*Statistically significant

Reactivity in the Absence of Genotypes Included in the Assay as Determined by Linear Array

N = 488

Test	Number of Cases Testing +ve	Histology		
		Negative	CIN I	≥ CIN 2
APTIMA	7 (1.4%)	7	0	0
AMPLICOR	18 (3.7%)	17	0	1

Genotypes Detected by Linear Array in the Absence of Those Included in the Assays

N = 488

APTIMA	67, 70, 82, 42, 53, 55, 61, 62, 89
AMPLICOR	66, 89, 84, 62, 6, 42, 53, 55, 62, 61, 40, 70, 72, 73, 82

Mostly co-infections

Conclusions

- Study data mostly based on colpo referral population and the tests showed different performance characteristics
- A range of bias between clinical sensitivity and specificity was observed
- APTIMA showed equal clinical sensitivity compared with HC2 , 95% vs 94%
- APTIMA showed slightly lower clinical sensitivity compared with AMPLICOR, 92% vs 96%
- APTIMA showed a significantly higher clinical sensitivity compared with PreTect HPV-Proofer, 95% vs 77%

Conclusions

- APTIMA showed a trend toward higher clinical specificity compared with HC2, 45% vs 40%, in referral population, and the difference was significant in screen population.
- APTIMA showed a significantly higher clinical specificity compared with AMPLICOR, 60% vs 43%
- PreTect HPV-Proofer showed a significantly higher clinical specificity over APTIMA, 76% vs 46%, with a greater accuracy
- APTIMA can serve as a reliable test in cervical screening, and has the potential to reduce colpo referral rate in ASCUS triage while maintaining a high clinical sensitivity

Study Team

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