

HPV negative cancers: how study design affects our ability to estimate clinical sensitivity and preventive efficacy

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Paolo Giorgi Rossi reports that as a principal investigator of an independent study funded by the Italian Ministry of Health (the data owner), he is making agreements to receive reagents and diagnostic tests at a reduced price or for free with the following companies: Roche Diagnostics, Hologic Gen-Probe, Abbott, and Qiagen.

Background

Comparison of Cervical Cancer Screening Results Among 256,648 Women in Multiple Clinical Practices

Amy J. Blatt, PhD¹; Ronald Kennedy, MD¹; Ronald D. Luff, MD, MPH²; R. Marshall Austin, MD, PhD³; and Douglas S. Rabin, MD¹

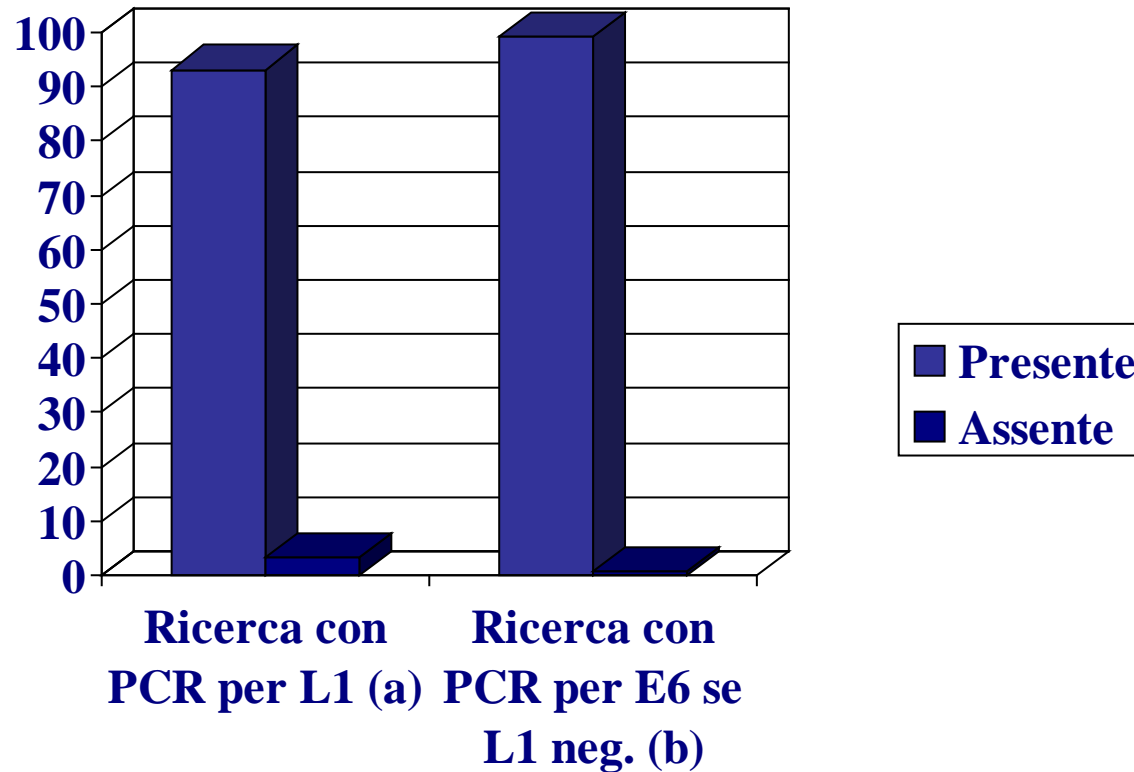
tive, and 29 (5.5%) were cotest negative. **CONCLUSIONS:** Compared with HPV-only testing, cotesting was more sensitive for the detection of \geq CIN3 in women ages 30 to 65 years. The current data suggest that approximately 19% of women with cervical cancer may be misdiagnosed by an HPV-only cervical screen. It is important to consider these data as the guidelines for cervical cancer screening undergo revision. *Cancer (Cancer Cytopathol)* 2015;000:000-000. © 2015

History of High-Risk HPV and Pap Test Results in a Large Cohort of Patients With Invasive Cervical Carcinoma: Experience From the Largest Women's Hospital in China

Xiang Tao, MD, PhD^{1*}; Christopher C. Griffith, MD, PhD^{2*}; Xiangrong Zhou, MD¹; Zhiheng Wang, MD¹; Yabin Yan, MD¹; Zaibo Li, MD, PhD³; and Chengquan Zhao, MD²

rates of prior negative results with both hrHPV and Pap cytology. **CONCLUSIONS:** The results of the current study demonstrated prior results of hrHPV testing and Pap cytology in a population of women in China who had not undergone intensive prior screening. Both hrHPV testing and Pap cytology were found to have similar negative rates in this population and, not surprisingly, there were fewer women who had negative testing results using both testing modalities. *Cancer (Cancer Cytopathol)* 2015;123:421-7. © 2015 American Cancer Society.

Any HPV type in 932 invasive cancers of the cervix



(a) 93% Bosch et al - J.Natl Cancer Inst. 1995, 87:796-802

(b) >99% Walboomers et al. - J. Pathol. 1999; 189:12-9

Definitions

- Clinical sensitivity: (number of cancers positive to the tests)/(total number of cancers)
- Preventive efficacy: risk of developing a cancer in women undergoing screening/
risk of developing a cancer in women not undergoing screening

Definitions

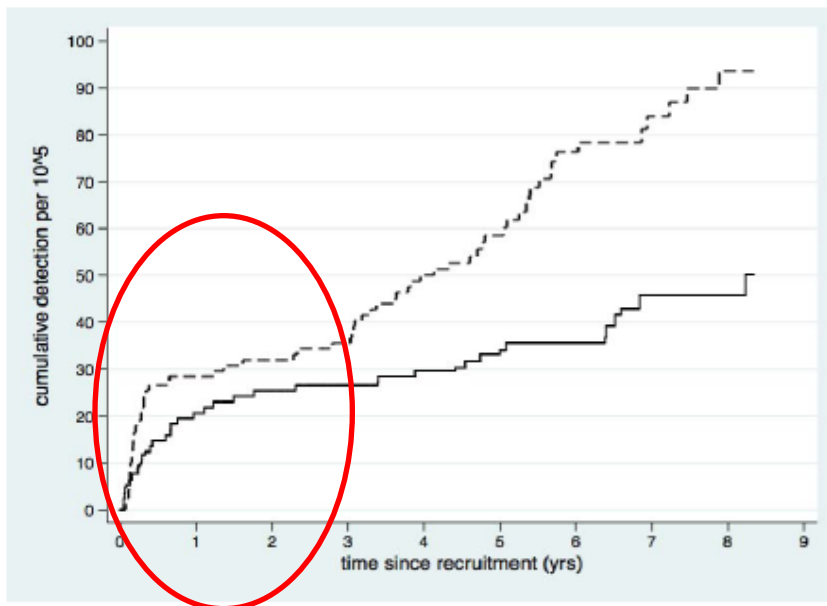
- Clinical (relative) sensitivity: (number of cancers positive to HPV test)/(total number of cancers positive to Pap or HPV)
- Relative Preventive efficacy: risk of developing a cancer in women undergoing HPV screening/ risk of developing a cancer in women undergoing screening with Pap test

Sensitivity for cancer \leftrightarrow preventive efficacy

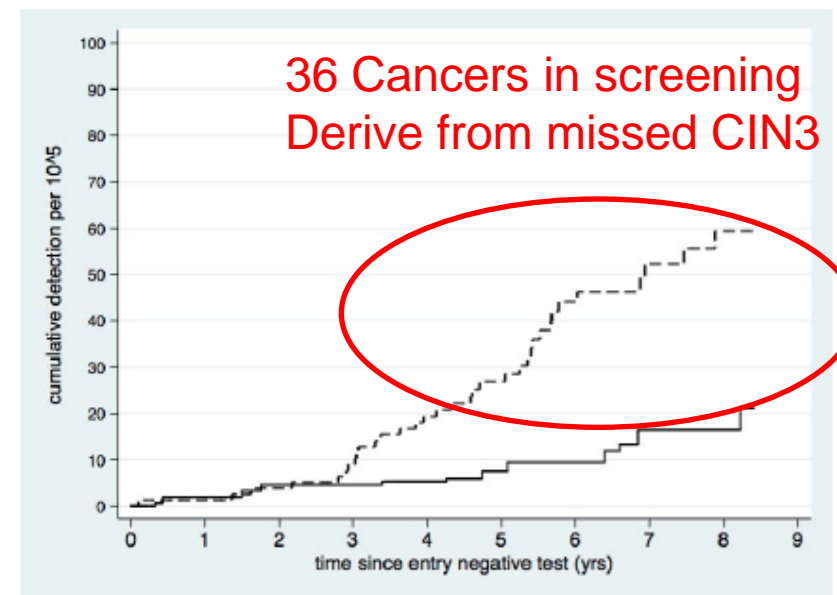
- Cancer is a very rare event in screened women
- CIN3 takes long time to progress to cancer
- In theory a test with 100% sensitivity for CIN3 and 0% sensitivity for cancer may have a 100% preventive efficacy

Preventive efficacy: HPV test is more effective than Pap test in prevent cancer incidence (higher sensitivity for CIN2 and CIN3)

A) All randomized women



B) Women negative at entry test



Solid lines: HPV group. Dotted lines: cytology group
In panel (B) observations are censored 6 months after CIN2 or CIN3 detection, if any.

1,214,415 person/years

Prevalent cancer: the two test identify almost the same number. Similar sensitivity. Not higher for HPV.

In absolute terms very high: few cancers in HPV in the

Screening history of the 19 cancers diagnosed in the HPV arm

	N	%
HPV-	8	42,1
- pap test-	8	42,1
- a: No improvement if co-testing is adopted		21,1
- microinvasive	5	26,3
HPV+	11	57,9
- HPV- at 1 year, cancer after 10 years	1	5,3
- did not repeat HPV at 1 year	5	26,3
- colposcopy without biopsy	4	21,1
TOTAL	19	100

Sensitivity for cancer is not the main issue for efficacy of screening

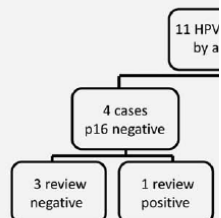
How to estimate sensitivity for cancer?

- With robust studies within screening programs it is very difficult to estimate sensitivity for cancer:
- We cannot have systematic assessment for all negative women
- Colposcopy is not enough sensitive
- Cancer is too rare to make random sample of negative
- Only relative sensitivity can be estimated, but screen detected cancers are rare and prospective studies do not have enough power

NTCC 7 and 9 cancers at baseline

	HPV group	Cytology group	p value*
All ages pooled			
Screening round one	7	9	0.62
Screening round two	0	9	0.004
Total over first two rounds	7	18	0.028
Women aged 35-60 years at recruitment†			
Screening round one	6	8	0.61
Screening round two	0	7‡	0.016
Total over first two rounds	6	15	0.052
Women aged 25-34 years at recruitment†			
Screening round one	1	1	1.00
Screening round two	0	2§	0.50
Total over first two rounds	1	3	0.37

Athena no cancers



‡ 1 case with
 † Including
 § Not confi

Figure 2. Overview of cobas-negative CIN3/ACIS cases on re-review.

	Intervention group					Control group				
	Total	CIN0/1	CIN2	CIN3	Cervical cancer	Total	CIN0/1	CIN2	CIN3	Cervical cancer
Baseline screen										
Inadequate cytology										
HPV DNA positive	2	0	1	0	0	0	0	0	0	0
HPV DNA negative	31	1	0	0	0	27	2	0	0	0
Normal cytology										
HPV DNA positive	724	48	31	29	2	766	9	6	9	1
HPV DNA negative	18562	218	7	3	0	18607	233	4	2	0
Borderline or mild dyskaryosis										
HPV DNA positive	185	29	24	34	3	192	30	25	29	1
HPV DNA negative	330	28	3	3	1	335	22	2	5	0
Moderate dyskaryosis or worse										
HPV DNA positive	146	11	26	87	5	160	20	24	93	4
HPV DNA negative	19	6	4	3	1	19	4	4	6	0
Total	19999	341	96	159	12	20106	320	65	144	6

Pobascam 12 and 6 cancers at baseline

Follow-back studies may be useful...

Original Article

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Screening false negatives

TABLE 1. Results of Human Papillomavirus-Only Testing, Papanicolaou-Only Testing, and Cotesting in Women Ages 30 to 65 Years

Test	No. Wor	No. of Test Results					iCC	Adenocarcinoma	Total Cancers ^a
		ASC	LSIL	HSIL	ASC+LSIL	ASC+HSIL			
Total	256,040	241,002	10,030	3303	199	310	169	526	
Baseline HPV test									
Indeterminate	2	2	0	0	0	0	0	0	
HPV negative	64,870	64,172	453	140	7	34	45	98	
HPV positive	191,776	177,488	10,443	3225	192	276	124	428	
Baseline Pap test									
UNSAT	866	825	18	11	0	6	3	12	
Pap negative	66,478	65,173	972	230	39	24	35	64	
Total Pap positive	189,304	175,664	9906	3124	160	280	131	450	
Baseline HPV/Pap cotest ^b									
HPV negative/Pap negative	27,123	27,017	62	19	1	7	14	24	
HPV negative/Pap positive	37,243	36,658	390	120	6	26	29	69	
HPV positive/Pap negative	39,290	38,093	909	211	37	17	21	40	
HPV positive/Pap positive	152,124	139,067	9517	3004	155	254	102	381	
HPV negative/Pap UNSAT	504	497	1	1	0	1	2	5	
HPV positive/Pap UNSAT	362	328	17	10	0	5	1	7	
HPV indeterminate/Pap negative	1	1	0	0	0	0	0	0	
HPV indeterminate/Pap positive	1	1	0	0	0	0	0	0	
HPV indeterminate/Pap UNSAT	0	0	0	0	0	0	0	0	

Abbreviations: AIS, adenocarcinoma in situ; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; Pap, Papanicolaou; SCC, UNSAT, unsatisfactory.

^a SCC, adenocarcinoma, adenosquamous carcinoma, and cervical cancer of unknown histology are included under "Total Cancers."

^b The cotest results include unsatisfactory and indeterminate results in which HPV-only or Pap-only data were used for total biopsy counts.

Screening false negatives

TABLE 2. Sensitivity and Specificity With 95% Confidence Limits and Positive and Negative Predictive Values of Human Papillomavirus-Only Testing, Papanicolaou-Only Testing, and Cotesting for Cervical Intraepithelial Neoplasia Grade 3 or More Severe Results^a

Test	Sensitivity (95% CI), %	Specificity (95% CI), %	PPV, %	NPV, %
Pap only	91.3 (91-92.6)	26.3 (26.1-26.4)	1.97	99.50
HPV only	94 (93.3-94.7)	25.6 (25.4-25.8)	2	99.62
Cotesting	98.8 (98.6-99.2)	10.9 (10.8-11)	1.76	99.83

Abbreviations: CI, confidence interval; HPV, human papillomavirus; NPV, negative predictive value; Pap, Papanicolaou; PPV, positive predictive value.

^aOf 256,648 women, 4090 (1.6%) had cervical intraepithelial neoplasia grade 3 or more severe biopsy results. The distributions of positive and negative screening results by biopsy finding are summarized in Table 1.

PPV per cancro di inadeguato:

HPV+ $7/362 = 2\%$

HPV- $5/504 = 1\%$

Why follow back studies should be interpreted cautiously

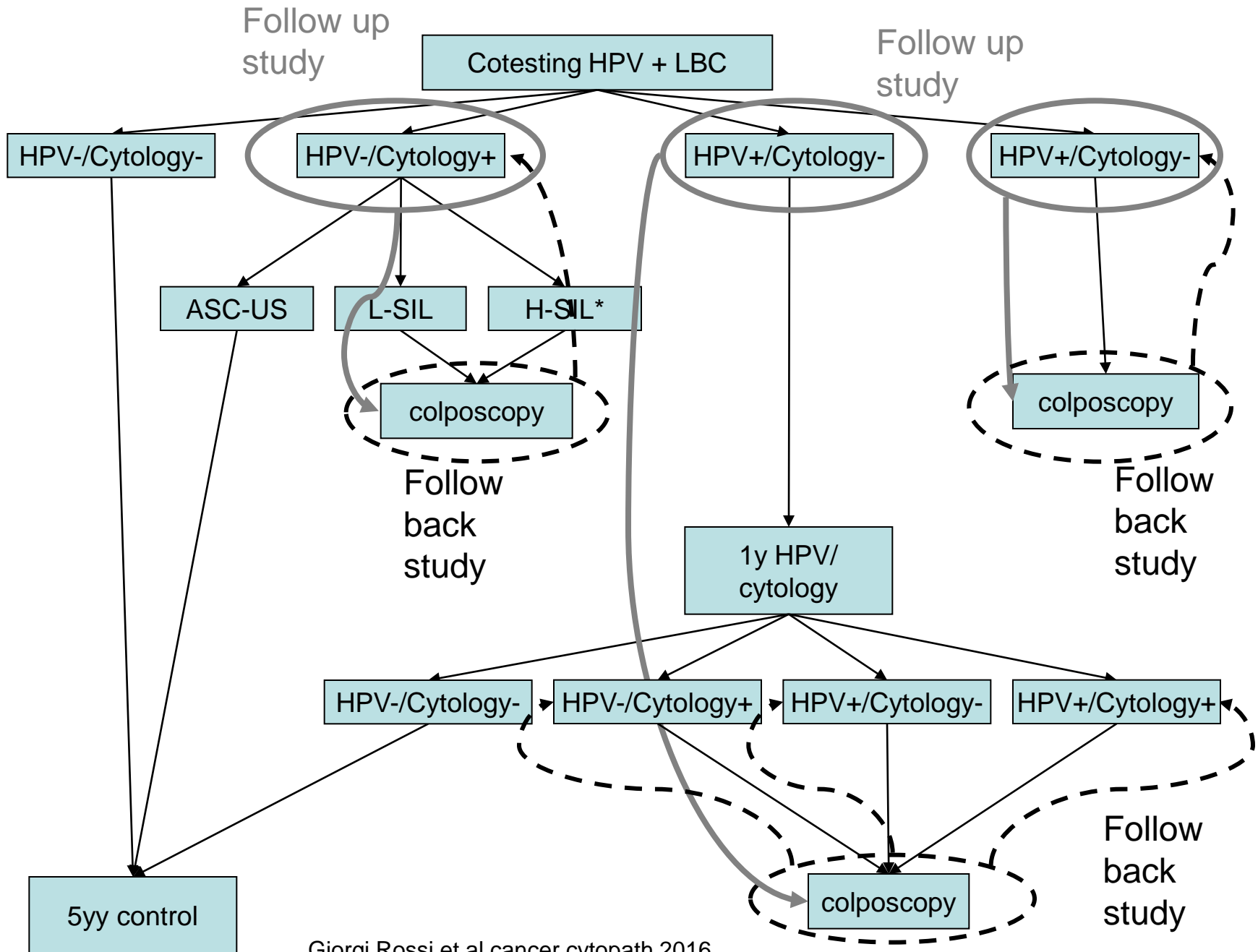


TABLE 2. hrHPV Testing Results for Different Histological Types of Cervical Cancer Within 1 Year Prior to the Histologic Diagnosis

Types	No. of Cases	hrHPV Positive	Positivity Rate, %	Negative Rate, %
Squamous cell carcinoma	449	383	85.3	14.7
Adenocarcinoma	21	14	66.7	33.3
Usual type	15	10	66.7	33.3
Serous type	1	1	100	0
Clear cell type	1	1	100	0
Minimal deviation	2	0	0	100
Adenoid basal cell type	2	2	100	0
Adenosquamous carcinoma	7	6	85.7	14.3
Total	477	403	84.5	15.5

Abbreviation: hrHPV indicates high-risk human papillomavirus.

TABLE 5. Pap Testing Results Within 1 Year Before the Histological Diagnosis of Invasive Cervical Cancer

Pap Test	SCC, No. (%)	AdC, No. (%)	ADSQ, No. (%)	Total, No. (%)
Malignant	84 (37.3)	2 (22.2)	1 (25.0)	87 (36.6)
HSIL	72 (32.0)	1 (11.1)	1 (25.0)	74 (31.1)
ASC-H	10 (4.4)	0	1 (25.0)	11 (4.6)
AGC	4 (1.8)	3 (33.3)	0	7 (2.9)
LSIL	6 (2.7)	0	0	6 (2.5)
ASC-US	15 (6.7)	1 (11.1)	0	16 (6.7)
NILM	34 (15.1)	2 (22.2)	1 (25.0)	37 (15.5)
Total	225	9	4	238

Abbreviations: AdC, adenocarcinoma; ADSQ, adenosquamous carcinoma; AGC, atypical glandular cells; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy; Pap, Papanicolaou; SCC, squamous cell carcinoma.

recorded. **RESULTS:** Of 3714 patients with invasive cervical carcinoma, over a 46-month period, 525 had prior hrHPV testing using Hybrid Capture 2 within 3 years and 238 patients had Pap cytology testing within 1 year before the histological

TABLE 6. Pap and HPV Testing Results Within 1 Year Before the Histological Diagnosis of Cervical Cancer

Pap Test		HPV Positive		HPV Negative	
		No. of Cases	%	No. of Cases	%
Abnormal	Malignant	78	37.9	6	24.0
	HSIL	67	32.5	6	24.0
	ASC-H	10	4.9	1	4.0
	AGC	6	2.9	1	4.0
	LSIL	3	1.5	1	4.0
	ASC-US	15	7.3	1	4.0
NILM		27	13.1	9	36.0
	Total	206	100	25	100

Abbreviations: AGC, atypical glandular cells; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy; Pap, Papanicolaou.

3714 casi.

525 with previous HPV

238 with previous Pap

206 with both

3157 no previous test

Why a Pap test has been done after a negative HPV test?

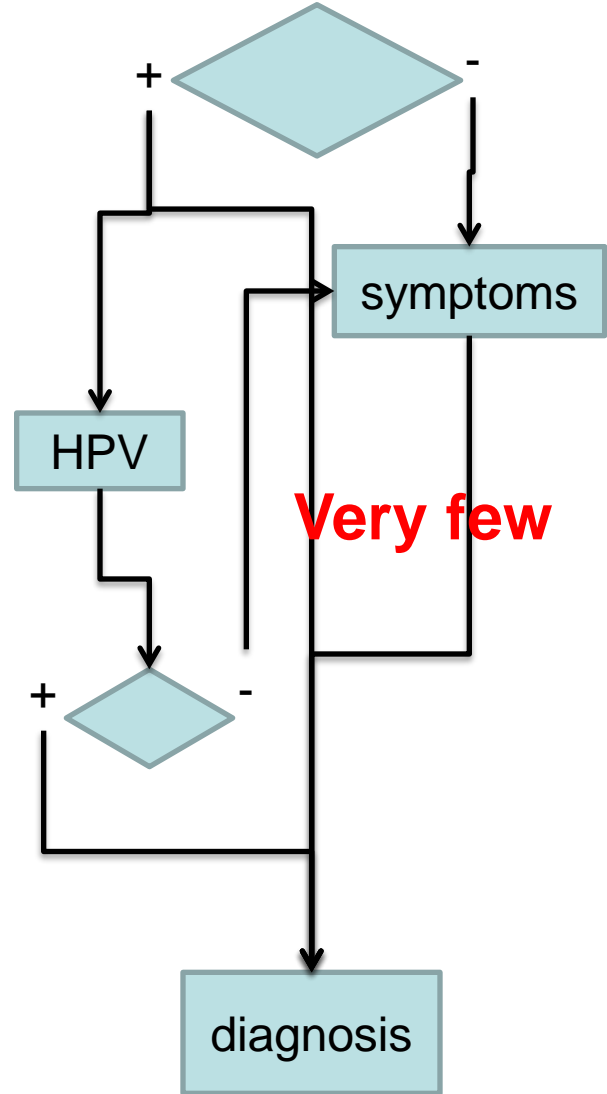
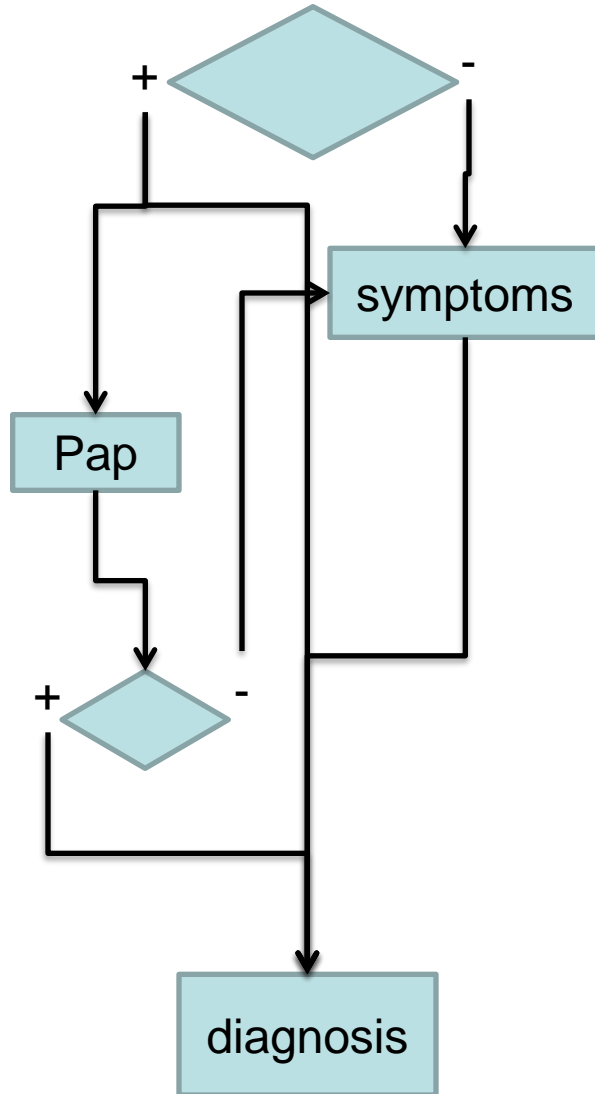
Was the woman symptomatic?

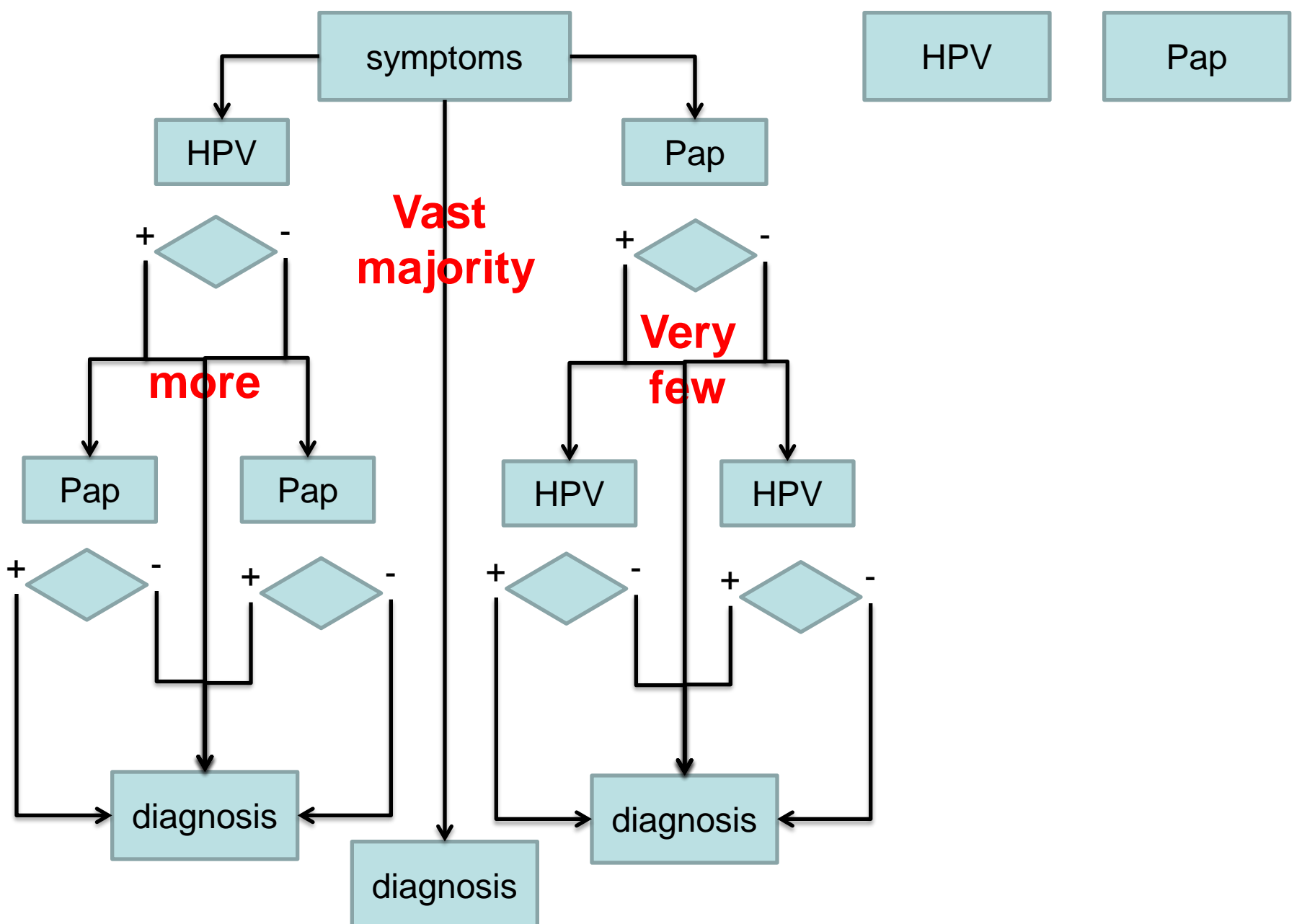
More?

symptoms

HPV

Pap





Synthesis

- The study by Blatt and coll. systematically underestimates relative sensitivity of HPV vs Pap
- The study by Tao and coll. may under-or over-estimate the sensitivity of both tests. Difficult to establish if the errors are differential for HPV and Pap not knowing diagnostic protocols.

Conclusions

- It is very difficult to accurately estimate absolute and relative sensitivity of HPV and Pap test for cancer
- Sensitivity of HPV is higher than Pap for CIN, for cancer sensitivity may be comparable.
- HPV negative cancers do exist (0.1%-15% depending on the type of test), but their relevance in screening programs is surely very low
- Monitoring should assess their occurrence.