

## ARTICLE

# Informed Cytology for Triaging HPV-Positive Women: Substudy Nested in the NTCC Randomized Controlled Trial

Christine Bergeron, Paolo Giorgi-Rossi, Frederic Cas, Maria Luisa Schiboni, Bruno Ghiringhello, Paolo Dalla Palma, Daria Minucci, Stefano Rosso, Manuel Zorzi, Carlo Naldoni, Nereo Segnan, Massimo Confortini, Guglielmo Ronco

**Affiliations of authors:** Department of Pathology, Laboratoire Cerba, Cergy Pontoise France (CB, FC); Interinstitutional Epidemiology Unit, AUSL Reggio Emilia, Italy, and Arcispedale S. Maria Nuova, IRCCS, Reggio Emilia, Italy; Department of Pathology, S. Giovanni Hospital, Rome, Italy (MLS); Laboratory Department University Hospital “Città della Salute e della Scienza di Torino,” Turin, Italy (BG); Department of Pathology, S. Chiara Hospital, Trento, Italy (PDP); Women’s Health Department, University Hospital, Padua, Italy (DM); Veneto Oncology Institute IRCCS, Padua, Italy (MZ); Emilia-Romagna Region, Bologna, Italy (CN); Laboratory of Preventive Oncology, Institute for Cancer Study and Prevention (ISPO), Florence (MC); Department of Cancer Screening, Center for Epidemiology and Prevention in Oncology (CPO), University Hospital “Città della Salute e della Scienza di Torino,” Turin, Italy (GR, NS, SR).

**Correspondence to:** Guglielmo Ronco, MD, PhD, Centro per la Prevenzione Oncologica (CPO), Via San Francesco da Paola 31, 10123 Torino, Italy (e-mail: [guglielmo.ronco@cpo.it](mailto:guglielmo.ronco@cpo.it)).

## Abstract

**Background:** Human papillomavirus (HPV)-based screening needs triage. In most randomized controlled trials (RCTs) on HPV testing with cytological triage, cytology interpretation has been blind to HPV status.

**Methods:** Women age 25 to 60 years enrolled in the New Technology in Cervical Cancer (NTCC) RCT comparing HPV testing with cytology were referred to colposcopy if HPV positive and, if no cervical intraepithelial neoplasia (CIN) was detected, followed up until HPV negativity. Cytological slides taken at the first colposcopy were retrieved and independently interpreted by an external laboratory, which was only aware of patients’ HPV positivity. Sensitivity, specificity, and positive (PPV) and negative (NPV) predictive values were computed for histologically proven CIN2+ with HPV status-informed cytology for women with a determination of atypical squamous cells of undetermined significance (ASCUS) or more severe. All statistical tests were two-sided.

**Results:** Among HPV-positive women, informed cytology had cross-sectional sensitivity, specificity, PPV and 1-NPV for CIN2+ of 85.6% (95% confidence interval [CI] = 76.6 to 92.1), 65.9% (95% CI = 63.1 to 68.6), 16.2% (95% CI = 13.0 to 19.8), and 1.7 (95% CI = 0.9 to 2.8), respectively. Cytology was also associated with subsequent risk of newly diagnosed CIN2+ and CIN3+. The cross-sectional relative sensitivity for CIN2+ vs blind cytology obtained by referring to colposcopy and following up only HPV positive women who had HPV status-informed cytology greater than or equal to ASCUS was 1.58 (95% CI = 1.22 to 2.01), while the corresponding relative referral to colposcopy was 0.95 (95% CI = 0.86 to 1.04).

**Conclusions:** Cytology informed of HPV positivity is more sensitive than blind cytology and could allow longer intervals before retesting HPV-positive, cytology-negative women.

Received: March 20, 2014; Revised: July 2, 2014; Accepted: November 25, 2014

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Screening based on human papillomavirus (HPV) testing allows earlier diagnosis of persistent high-grade cervical intraepithelial neoplasia (CIN 2+) than cytology-based screening (1) and is more effective in preventing invasive cervical cancer (2,3). However, the specificity of HPV testing for high-grade CIN is low (1). Therefore, methods are needed for selecting which HPV-positive women need colposcopy. Both the cross-sectional and the longitudinal accuracy of a candidate marker for triage are relevant, the latter in order to define at which interval women need retesting. However, it must be kept in mind that the probability of progression from HPV to cancer increases with increasing age of lesions (4,5), so that missing a prevalent lesion, which could be present over a long period, entails a greater risk of cancer than missing a newly arisen one, particularly a CIN2. Thus cross-sectional sensitivity is also crucial in order to determine retesting intervals.

Randomized controlled trials (RCTs) showed that referring to colposcopy only those HPV-positive women who also have abnormal cytology or show persistent HPV infection leads to an earlier diagnosis of persistent lesions than cytology (6–8) and increases efficacy without increasing the biopsy rate, compared with cytology based screening (3). In such studies, however, women had HPV and cytology cotesting, and cytology was interpreted without knowledge of the HPV status. Given the negligible difference in sensitivity between stand-alone HPV testing and cotesting (1), stand-alone HPV testing seems recommendable. Stand-alone HPV testing for primary screening was indeed recently approved by a committee appointed by the US Food and Drug Administration. In such a case, triage cytology would be interpreted with the knowledge that women are HPV positive.

We used slides from the New Technology in Cervical Cancer (NTCC) trial to study both the cross-sectional and longitudinal accuracy of “informed” cytology as a triage test in HPV-positive women, ie, interpreting the slides of HPV-positive women with knowledge of the HPV status.

## Methods

NTCC (9) was an RCT with two preplanned recruitment phases, which was conducted in nine population-based cervical screening programs in Italy. Women age 25 to 60 years who were not pregnant, had never undergone hysterectomy, had not been treated for CIN in the last five years, and who were attending for a new routine cervical screening episode were randomly assigned, after written informed consent, between February 2002 and December 2004, to conventional cytology (classified according to the Bethesda 1991 system and managed according to the standard protocol of each center) or to HPV-based screening, either in combination with liquid-based cytology (phase 1) (10,11) or alone (phase 2) (12). Details on random assignment and masking were previously provided (9–12).

HPV testing was done by Hybrid Capture 2 (HC2; Qiagen, Hilden, Germany). During phase 2, women in the HPV group were referred for colposcopy if the HPV test was positive (12). As a rule, women with CIN2+ were treated and those with CIN1 followed up colposcopically. If no CIN was detected, HPV-positive women were actively recalled after colposcopy for annual repeats of HC2 and liquid-based cytology (LBC), while HPV remained positive. They were referred to colposcopy if LBC was atypical squamous cells of undetermined significance (ASCUS) or more severe. In addition, recall for repeat colposcopy or repeat cytology was also possible in both arms on the basis of colposcopic and cytological findings, according to routine local protocols (9).

The women from both arms who were screen-negative at baseline were invited for the second screening round three years later, tested by conventional cytology, and managed according to the standard protocol of each center (9). NTCC is registered as an International Standard Randomized controlled trial, number ISRCTN81678807. We obtained multicenter and local research ethics approvals.

## Cytology Review

During phase 2 the large majority of HPV-positive women had cytology taken during subsequent colposcopies (Figure 1). Local interpretation of such cytology was never used in analyses because it was not blind to histology (13). We retrieved the cytological samples taken during the first colposcopy done after an HPV-positive test. Slides could not be retrieved in one center (Verona), representing 9.9% of all colposcopies done in phase 2 in the experimental arm (Figure 1). In addition, slides taken in Florence before July 29, 2004 could not be retrieved. Liquid-based cytology had been used in all centers except Viterbo.

Slides were reviewed, blind to histology results, in Laboratoire Cerba, Cergy Pontoise, France. The only information provided was woman's age and the fact that the woman was HPV positive. Previous dots were removed. The Bethesda 1991 system was used to be consistent with the original NTCC data in the control arm (12). Slides were first screened by a cytotechnician (FC). Those classified as abnormal (ASCUS+) were reviewed by an expert pathologist (CB).

## Endpoint Assessment

The primary endpoint was histology-proven CIN2 or -3 or invasive cervical cancer (CIN2+). We recorded test results and histological findings from the computerized registration systems of participating screening centers. At the end of the recruitment phase, for women who had a biopsy locally diagnosed as CIN1 or more, all histological specimens taken within one year of referral to colposcopy were reviewed by a group of pathologists who were unaware of the original diagnosis and random assignment (14). The same exercise was repeated at the time of the second screening round (9). Adenocarcinomas in situ were considered with CIN3. In addition, to obtain histological diagnoses performed outside the trial, after the end of the second round we linked the database of recruited women to those of the cancer registries (covering all centers except Viterbo) and of the pathology units present in the catchment areas of NTCC (9).

## Statistical Methods

### Cross-Sectional Analyses

The most severe histological diagnosis performed at recruitment (within one year from referral to colposcopy) was considered. We computed the sensitivity and specificity of atypical cells of undetermined significance or more severe (ASCUS+) informed cytology for histologically determined CIN2+ and CIN3+ among HPV-positive women. We also computed the absolute risk of CIN2+ and of CIN3+ in HPV-positive women with ASCUS+ cytology (ie, the positive predictive value [PPV]) and in HPV-positive women with cytology less than ASCUS (ie, 1 minus the negative predictive value [1-NPV]) and the ratio of such risks (RR). Confidence intervals (CIs) were computed on the basis of the exact binomial distribution.

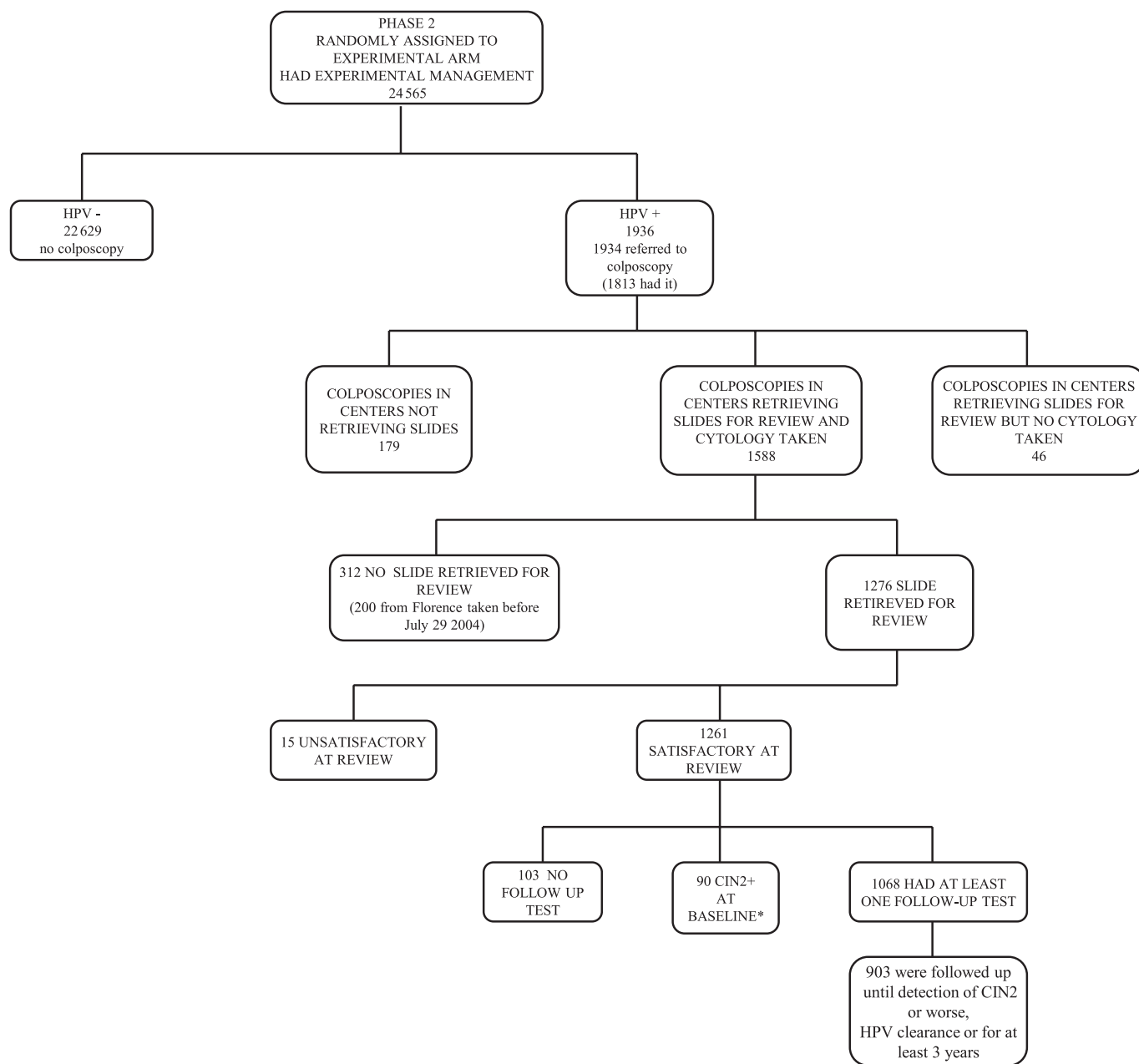


Figure 1. Study profile.

Finally, we estimated the relative—compared with the conventional arm—sensitivity and immediate referral that would have been obtained by referring to colposcopy only the women who were HPV positive and had ASCUS+ cytology interpreted with knowledge of HPV positivity. Following the same approach we had used with p16 immunostaining (13), the relative sensitivity was estimated by multiplying the previously estimated (12) relative sensitivity of stand-alone HPV testing vs blind conventional cytology for the sensitivity of “informed” ASCUS+ cytology among HPV-positive women obtained in this study. The relative immediate referral was estimated by multiplying the relative referral of stand-alone HPV for the proportion of women who were ASCUS+ at informed cytology among all who were HPV positive. Confidence intervals were computed applying the Monte Carlo Markov Chain (MCMC) method (15) using the WinBUGS 1.4.3 software (16) (Supplementary Methods, available online).

#### Longitudinal Analyses

The same analyses described above were repeated, always considering cytology at the first colposcopy: 1) for lesions diagnosed during follow-up, within three years after recruitment, for whom follow-up is very complete, among HPV-positive women who had not had CIN2+ diagnosed at baseline and 2) for lesions detected either at recruitment or follow-up.

For comparison, the absolute probabilities of CIN3+ detection at the second screening round among the women (all ages) recruited in phase 2 who were HPV negative (experimental arm) or cytologically normal (conventional arm) at baseline were obtained. As these women had no colposcopy at recruitment or postcolposcopy follow-up, detection at round 2 provides an estimate of the cumulative incidence up to round 2 of lesions persistent and subsequently detectable by cytology.

The relative sensitivity vs blind conventional cytology of referring to colposcopy and having postcolposcopy follow-up just in women positive to HPV and with ASCUS+ cytology interpreted with knowledge of HPV positivity was estimated following the approach we previously used for the analysis of longitudinal data on p16 triage (17). We multiplied the relative detection rate obtained within three years of recruitment between study arms for the sensitivity of informed cytology for lesions detected at recruitment or follow-up (Supplementary Methods, available online).

All statistical tests were two-sided.

## Results

### Cross-Sectional Analyses

During phase 2, 1588 women had a positive HPV test and a colposcopy with concurrent cytology in one of the centers where slides could be retrieved for review. We retrieved slides for 1276 (80.4%) of such women. In Florence, because of a problem with the archive, none of the 200 slides taken before July 29, 2004 could be retrieved; after excluding those, 91.9% of relevant slides could be retrieved (Figure 1). Of the retrieved slides, 15 (1.2%) were judged as unsatisfactory by the reviewer, leaving 1261 for the interpretation, 1205 (95.6%) of which were liquid-based preparations.

Table 1 shows the cross-sectional accuracy of unblinded cytology among HC2-positive women. The sensitivity of ASCUS+ cytology was 85.6% (95% CI = 76.6 to 92.1) for CIN2+ and 88.1% for

CIN3+ and the specificity of cytology less than ASCUS was 65.9% (95% CI = 63.1 to 68.6) for histology less than CIN2+ and 64.0% for histology less than CIN3. ASCUS+ cytology was associated with risk of carrying a CIN2+ (RR = 9.77) and CIN3+ (RR = 12.20): 16.2% (95% CI = 13.0 to 19.8) of women with ASCUS+ cytology carried a CIN2+ and 7.8% a CIN3+, while 1.7% (95% CI = 0.9 to 2.8) of women with cytology less than ASCUS carried a CIN2+ and 0.6% a CIN3+ (including one cancer).

The relative sensitivity of HPV testing with triage of positive women by informed cytology vs stand-alone blind cytology was 1.58 (95% CI = 1.22 to 2.01) for CIN2+ and 1.41 (95% CI = 0.99 to 1.96) for CIN3+. If just HPV+ women with ASCUS+ cytology were referred for colposcopy, immediate referral would have been similar to that observed in the control arm (relative referral = 0.95; 95% CI = 0.86 to 1.04) (Table 2).

### Longitudinal Analyses

Of the 1171 women who had no CIN 2+ detected at enrollment, 1068 (91%) received further tests as part of the postcolposcopy follow-up. Median duration of their follow-up was 1099 days (interquartile range = 752 to 1344). Follow-up was complete (followed until detection of CIN2+ or until a negative HPV test or for at least three years) in 903 (85%) of the 1068 women who had any active follow-up, while 165 (15%) had a shorter follow-up without disease or resolution of HPV infection.

The longitudinal accuracy of baseline-informed cytology among women HPV positive at baseline is reported in Table 1. Baseline ASCUS+ cytology was associated with the risk of

Table 1. Accuracy of ASCUS or more severe cytology unblinded to HPV status among HC2-positive women\*

Histological endpoint	Sensitivity, n, % (95% CI)	Specificity, n, % (95% CI)	PPV, n,% (95% CI)	1-NPV, n, % (95% CI)	RR (95% CI)
Cross-sectional					
CIN2+	77/90 85.6% (76.6 to 92.1)	772/1171 65.9% (63.1 to 68.6)	77/476 16.2% (13.0 to 19.8)	13/785 1.7% (0.9 to 2.8)	9.77 (5.49 to 17.39)
CIN3+	37/42 88.1% (74.4 to 96.0)	780/1219 64.0% (61.2 to 66.7)	37†/476 7.8% (5.5 to 10.6)	5‡/785 0.6% (0.2 to 1.5)	12.20 (4.83 to 30.84)
Longitudinal					
CIN2+	33/49 67.4% (52.5 to 80.1)	639/1019 68.0% (65.1 to 70.9)	33/359 9.2% (6.4 to 12.2)	16/709 2.3% (1.3 to 3.6)	4.07 (2.27 to 7.30)
CIN3+	16/26 61.5% (40.6 to 79.8)	699/1042 67.1% (64.1 to 69.9)	16§/359 4.5% (2.6 to 7.1)	10  /709 1.4% (0.7 to 2.6)	3.16 (1.45 to 6.89)
During recruitment and follow-up					
CIN2+	110/139 79.1% (71.4 to 85.6)	756/1122 67.4% (64.6 to 70.1)	110/476 23.1% (19.4 to 27.2)	29/785 3.7% (2.5 to 5.3)	6.26 (4.22 to 9.27)
CIN3+	53/68 77.9% (66.2 to 87.1)	770/1193 64.5% (61.8 to 67.3)	53/476 11.1% (8.5 to 14.3)	15/785 1.9% (1.1 to 3.1)	5.83 (3.32 to 10.22)

\* Numbers in parentheses are 95% confidence intervals. ASCUS = atypical squamous cells of undetermined significance; CIN2+ (CIN3+) = cervical intraepithelial neoplasia grade 2 (grade 3) or more severe; HC2 = hybrid capture 2; HPV = human papillomavirus; NPV = negative predictive value; PPV = positive predictive value; RR = relative risk, ratio between PPV and (1-NPV).

† Including two invasive cancers.

‡ Including one invasive cancer.

§ Including one invasive cancer.

|| No invasive cancer.

**Table 2.** Relative sensitivity and referral to colposcopy vs cytology as primary test\*

Period considered	Criterion for referral	Relative sensitivity CIN2+ (95% CI)	Relative sensitivity CIN3+ (95% CI)	Relative referral to col- poscopy (95% CI)
Enrollment	ASCUS+ cytology aware of HPV status	1.58 (1.22 to 2.01)	1.41 (0.99 to 1.96)	0.95 (0.86 to 1.04)
	All HPV+	1.91 (1.52 to 2.41)	1.62 (1.17 to 2.24)	2.65 (2.48 to 2.83)
Enrollment and follow-up	ASCUS+ cytology aware of HPV status	2.20 (1.52 to 3.17)	2.36 (1.58 to 3.51)	- †
	All HPV+	2.86 (1.99 to 4.11)	2.89 (1.94 to 4.32)	- †

\* ASCUS = atypical squamous cells of undetermined significance; CIN2+ (CIN3+) = cervical intraepithelial neoplasia grade 2 (grade 3) or more severe; HPV = human papillomavirus.

† Only women referred at enrollment could be referred during follow-up.

developing (or having detected) a new CIN2+ (RR = 4.07) and CIN3+ (RR = 3.16) in the next three years. The risk of new CIN2+ was 2.3% and that of new CIN3+ was 1.4% (no invasive cancer) if baseline-informed cytology was less than ASCUS, while it was 9.2% and 4.5% (including one invasive cancer), respectively, if baseline-informed cytology was ASCUS+. Longitudinal sensitivity was 67.4% for CIN2+ and 61.5% for CIN3+.

The overall risk of having a lesion detected at recruitment or during follow-up in HPV-positive women with baseline cytology less than ASCUS was 3.7% for CIN2+ and 1.9% for CIN3+. The corresponding risks among women with ASCUS+ baseline cytology were 23.1% for CIN2+ and 11.1% for CIN3+ (Table 1). By comparison, the detection rate of CIN3+ at round 2 was 4 of 22 629 (0.02%, 95% CI = 0.000 to 0.05), including no invasive cancer among women HPV negative at baseline, and 18 of 23 268 (0.08%, 95% CI = 0.044 to 0.11), including three cancers among women in the cytology arm who had baseline blind cytology less than ASCUS.

The relative sensitivity vs stand-alone blind cytology obtained if only HPV-positive women with ASCUS+ informed cytology were referred to colposcopy and had postcolposcopy follow-up was 2.20 (1.52 to 3.17) for CIN2+ and 2.36 (1.58 to 3.51) for CIN3+ (Table 2).

There was no evidence that cross-sectional or longitudinal sensitivity, specificity, PPV, or NPV of informed cytology within HPV-positive women depended on age or that age was an effect modifier of the association between informed cytology and CIN2+ or CIN3+.

## Discussion

Our data suggest that cytology interpreted with knowledge of the HPV status is more sensitive than cytology read without knowledge of the HPV status. This increase could be the result of greater attention in reading slides from women known to be HPV positive and of the use of this knowledge for interpreting cell abnormalities that would otherwise be considered as irrelevant. In the Finnish trial, the detection rate in the experimental arm (stand-alone HPV with cytological triage of HPV+ women) was increased, compared with cytology alone, even when just considering women with abnormal baseline cytology (18); Therefore, in that study cytology read with knowledge of HPV positivity had higher sensitivity than “blind” cytology, even though published data do not allow computation of its absolute value. In addition, in that study (18), immediate referral to colposcopy was also increased in the experimental compared with the conventional arm, while the present results suggest similar or slightly reduced immediate referral. Increased sensitivity with minimal reduction in specificity was also observed in

a small nonrandomized study where all slides were read both with and without knowledge of HPV status (19).

Most of the remaining available studies evaluated “blind” cytology in cotesting used also to triage HPV-positive women. Table 3 reports the sensitivity of cytology greater than or equal to ASCUS among HPV-positive women and the corresponding immediate referral calculated from published data. Sensitivity was always lower than that observed in the present study, except in ARTISTIC, which was the only RCT where no increase in sensitivity with HPV-based compared with cytology-based screening was observed. In the other studies, sensitivity for CIN2+ ranged from 52.6% in ATHENA to 74.3% in POBASCAM. Conversely, in such studies, the immediate referral to colposcopy was about half of what would have resulted when referring to colposcopy all women with baseline ASCUS+ cytology, while in the present study informed cytology would not have resulted in any reduction of immediate referral. It must be kept in mind that in our study cytology interpretation had no practical effect on women’s management. Thus, interpretation criteria could be broader in real life.

In the conventional arm of NTCC, a few centers referred only women with “low-grade squamous intraepithelial lesions” or more cytology immediately to colposcopy. When excluding such centers, the relative sensitivity remained almost unchanged (1.57) for CIN2+ and higher (1.63) for CIN3+. Some 96% of reviewed slides were liquid-based, while all cytology in the control arm was conventional. In a previous study, we observed a 1.17 (0.87 to 1.56) relative sensitivity of LBC vs conventional cytology for CIN2+ but a 0.84 (0.56 to 1.25) relative sensitivity for CIN3+ (23). No increase in sensitivity with LBC was observed in another randomized controlled trial (24). During phase 1 of NTCC, in which LBC was also used in the HPV arm but interpretation was blind to HPV, both the sensitivity of ASCUS+ cytology in HPV+ women and the corresponding referral were lower than those observed here (Table 3). Finally, in a previous study (25), the laboratory that reviewed cytology in the present study had shown sensitivity similar to that of the NTCC centers.

Randomized controlled trials with blind interpretation of cytology showed that HPV testing with cytological triage and test repeat in HPV-positive, cytologically normal women allowed earlier diagnosis of persistent lesions than cytology (6–8). The present data suggest that even earlier diagnosis of high-grade CIN (HGCIN), therefore even higher protection, could be achieved if cytologists were aware of HPV status. This finding gives further support to triage strategies instead of cotesting strategies. If cotesting is performed, cytology should be interpreted after HPV results are obtained. There is, however, the risk that such knowledge leads to overinterpretation and increases the overall referral. Training and strict monitoring are therefore needed.

**Table 3.** Sensitivity and immediate referral to colposcopy in studies applying cytological triage, according to knowledge of HPV status\*

Study	Cross-sectional sensitivity†		Immediate referral to colposcopy ‡	
	Cin2+	Cin3+	Absolute	Relative to stand alone “blind” cytology
HPV status not known				
ATHENA (20)§	52.6% (200/380)	52.8% (133/252)	2.7%	0.42
CCCaST (21)  §	59.9%	NA	1.1%	0.38
Swedescreen (6)	69.9% (58/83)	72.9% (35/48)	1.7%	NA
POBASCAM (7)	74.3% (179/241)	74.5% (187/251)	1.7%	0.47
NTCC Phase 1(10)¶	76.8% (96/125)	82.7% (43/52)	3.2%	0.83
ARTISTIC (7)#	92.4% (391/423)	95.6% (216/226)	6.4%	0.50
HPV status known				
This study	85.6% (77/90)	88.1% (37/42)	2.8%	0.99
Pilot Veneto Italy (22)**	77.4% (41/53)	NA	2.8%	1.07

\* It was not possible to obtain the relevant estimates for the Finnish trial from published data, because results were not shown by initial cytology. Sensitivity and referral were computable only for Papanicolaou class III to V, which entailed immediate referral. CIN2+ (CIN3+) = cervical intraepithelial neoplasia grade 2 (grade 3) or more severe; HPV = human papillomavirus; LBC = liquid-based cytology; NA = not available; NTCC = New Technology in Cervical Cancer.

† Sensitivity is computed as the proportion of CIN2+ (CIN3+) detected in women HPV positive at baseline that also had ASCUS+ cytology at baseline.

‡ Referral is computed assuming that all women HPV positive and with ASCUS+ cytology are directly referred to colposcopy. Relative referral is computed assuming that all women from the conventional arm with ASCUS+ cytology are directly referred to colposcopy.

§ Relative referral to colposcopy computed as (women positive to both HPV and cytology)/(women positive to cytology only).

|| Sensitivity is computed as (value for HPV screening followed by Pap triage)/(value for HPV only).

¶ LBC was used in the experimental arm, conventional cytology in the control arm.

# No gain in sensitivity was observed at round 1 with HPV vs cytology.

\*\* Only historical, nonrandomized control was available.

HPV-positive women with normal cytology are usually retested after one year. Longer intervals after a normal cytology interpreted with information of HPV status could reduce the overall referral. The three-year cumulative detection of CIN3+ in HPV+ women with normal baseline cytology was much higher than the detection of CIN3+ at round 2 among women who were HPV negative at baseline. It was also higher than that among women who had normal cytology at baseline in the conventional arm. In this group, however, the large majority of women were HPV negative, therefore at low prior risk. In addition 3 of 23 268 of these women had cancer detected at the second screening round, thus showing incomplete protection. Therefore, neither the five-year interval suggested for HPV negative women (3) nor the intervals applied for women with stand-alone normal cytology (three years in Europe) can be applied for HPV+ women negative to informed cytology. However, the longitudinal sensitivity and the risk of new CIN3 in HPV-positive women with normal cytology were not so different from the values observed with p16 triage (66.9% sensitivity for CIN2+ and 77.8% for CIN3+), for which we suggested a two- to three-year interval (17). More relevant, the cross-sectional sensitivity of ASCUS+ cytology among HPV-positive women was not very far from that of p16 (88% for CIN2+ and 91% for CIN3+) (13) and higher than the cross-sectional sensitivity of HPV16/18 genotyping reported by the ATHENA study (51.8% for CIN2+ and 59.5% for CIN3+) (20). As discussed in the introduction, cross-sectional sensitivity is the most relevant parameter for choosing retesting intervals in order to prevent cancer, especially at the first screening round. Thus, a two-year interval could be suggested. HPV testing with informed cytological triage would allow lower immediate referral to colposcopy than p16 triage (13). The overall referral (immediate + retesting) could not be directly observed in our study and largely depends on HPV clearance. Persistence of HPV infection after two years is less than 35% (26).

NTCC was population-based and nested in routine organized screening in a low-risk population. Over 70% of eligible women were enrolled (9), suggesting that results are applicable to routine practice. Cytological slides were interpreted without knowledge of the original interpretation and of histology. They could not be retrieved at all in one center and for a defined time period in another. It is difficult to imagine that these slides and corresponding women had peculiar features that would result in selection bias. Slide retrieval was almost complete in the remaining centers.

All HPV-positive women were initially referred to colposcopy, with high compliance, thus avoiding verification bias. Completeness of clinical postcolposcopy follow-up was also high. The endpoint was mainly determined by a review of histology blinded to HPV testing and cytology results. In addition, we searched cancer registries and pathology units for lesions detected outside participating programs. If a histological diagnosis of HGGIN was made, it would likely be registered in one of these sites (9).

One limitation of the present study is that we could not directly observe the complete process of HPV testing, cytology, and HPV retesting in cytologically normal women. Pilot projects applying such a protocol are being conducted in Italy. They will provide direct evidence on the actual overall referral and on the risk of CIN3+ at one-year repeat.

In conclusion, cytology informed of HPV positivity is more sensitive than blind cytology. Screening programs with informed cytology triage are expected to perform better than predicted by trials and could possibly allow longer intervals before retesting HPV-positive women with normal cytology.

## Funding

The New Technology in Cervical Cancer (NTCC) main trial was financially supported by the European Union (Europe against

cancer contracts S1.2.327046 and SPC2002473), by the Italian Ministry of Health (special project “Valutazione di nuove tecnologie per lo screening del cervicocarcinoma” and L138/2004 project “NTCC- Nuove tecnologie per lo screening del cervicocarcinoma”) and the Regional Health administrations of participating Regions. The study on cytology review and follow-up was supported by the Italian Ministry of Health (Special Project “Valutazione di nuove tecnologie per lo screening del cervicocarcinoma – follow up”), by the European Union (CoHear project, FP7 grant agreement n° HEALTH-F3-2013-603019) and by the Associazione Italiana per la Ricerca sul Cancro (AIRC, Project IG14119).

## Notes

The funders had no role in study design, data collection, data analysis, data interpretation or writing of the report, nor in the decision to submit for publication.

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