

Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial



Guglielmo Ronco, Paolo Giorgi-Rossi, Francesca Carozzi, Massimo Confortini, Paolo Dalla Palma, Annarosa Del Mistro, Bruno Ghiringhello, Salvatore Girlando, Anna Gillio-Tos, Laura De Marco, Carlo Naldoni, Paola Pierotti, Raffaella Rizzolo, Patrizia Schincaglia, Manuel Zorzi, Marco Zappa, Nereo Segnan, Jack Cuzick, and the New Technologies for Cervical Cancer screening (NTCC) Working Group*

Summary

Background Human papillomavirus (HPV) testing is known to be more sensitive, but less specific than cytology for detecting cervical intraepithelial neoplasia (CIN). We assessed the efficacy of cervical-cancer screening policies that are based on HPV testing.

Methods Between March, 2004, and December, 2004, in two separate recruitment phases, women aged 25–60 years were randomly assigned to conventional cytology or to HPV testing in combination with liquid-based cytology (first phase) or alone (second phase). Randomisation was done by computer in two screening centres and by sequential opening of numbered sealed envelopes in the remaining seven centres. During phase one, women who were HPV-positive and aged 35–60 years were referred to colposcopy, whereas women aged 25–34 years were referred to colposcopy only if cytology was also abnormal or HPV testing was persistently positive. During phase two, women in the HPV group were referred for colposcopy if the HPV test was positive. Two rounds of screening occurred in each phase, and all women had cytology testing only at the second round. The primary endpoint was the detection of grade 2 and 3 CIN, and of invasive cervical cancers during the first and second screening rounds. Analysis was done by intention to screen. This trial is registered, number ISRCTN81678807.

Findings In total for both phases, 47 001 women were randomly assigned to the cytology group and 47 369 to HPV testing. 33 851 women from the cytology group and 32 998 from the HPV-testing group had a second round of screening. We also retrieved the histological diagnoses from screening done elsewhere. The detection of invasive cervical cancers was similar for the two groups in the first round of screening (nine in the cytology group vs seven in the HPV group, $p=0.62$); no cases were detected in the HPV group during round two, compared with nine in the cytology group ($p=0.004$). Overall, in the two rounds of screening, 18 invasive cancers were detected in the cytology group versus seven in the HPV group ($p=0.028$). Among women aged 35–60 years, at round one the relative detection (HPV vs cytology) was 2.00 (95% CI 1.44–2.77) for CIN2, 2.08 (1.47–2.95) for CIN3, and 2.03 (1.60–2.57) for CIN2 and 3 together. At round two the relative detection was 0.54 (0.23–1.28) for CIN2, 0.48 (0.21–1.11) for CIN3, and 0.51 (0.28–0.93) for CIN2 and 3 together. Among women aged 25–34 years, there was significant heterogeneity between phases in the relative detection of CIN3. At round one the relative detection was 0.93 (0.52–1.64) in phase one and 3.91 (2.02–7.57) in phase two. At round two the relative detection was 1.34 (0.46–3.84) in phase one and 0.20 (0.04–0.93) in phase two. Pooling both phases, the detection ratio of CIN2 for women aged 25–34 years was 4.09 (2.24–7.48) at round one and 0.64 (0.23–1.27) at round two.

Interpretation HPV-based screening is more effective than cytology in preventing invasive cervical cancer, by detecting persistent high-grade lesions earlier and providing a longer low-risk period. However, in younger women, HPV screening leads to over-diagnosis of regressive CIN2.

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Introduction

Several studies have shown that testing for the DNA of high-risk human papillomavirus (HPV) is more sensitive than cytology in detecting high-grade cervical intraepithelial neoplasia (CIN).^{1,2} Three randomised controlled trials, done in developed countries, showed decreased detection of high-grade lesions at the next screening round after screening with combined HPV testing and cytology^{3–5} compared with conventional^{3,4} or

liquid-based⁵ cytology (LBC) alone. Two of these trials enrolled women aged 30 years or older.^{3,4} However, none of the three trials found a significant decrease in the incidence of invasive cervical cancer in the second round of screening.

We did a large population-based randomised controlled trial, the New Technologies for Cervical Cancer (NTCC) screening study, in which women aged 25–60 years were randomly assigned to conventional cytology only or to

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Centre for Cancer Prevention, Turin, Italy (G Ronco MD, R Rizzolo MSc, N Segnan MD); Cancer Epidemiology CERMS, University of Turin, Italy (A Gillio-Tos PhD, L De Marco PhD); Institute for Cancer Study and Prevention, Florence, Italy

(F Carozzi PhD, M Confortini PhD, M Zappa MD); Veneto Oncology Institute

IRCCS, Padua, Italy (M Zorzi MSc, A Del Mistro MD); S Chiara Hospital, Trento, Italy (P Dalla Palma MD, S Girlando PhD); Agency for Public Health, Lazio Region, Rome, Italy (P Giorgi-Rossi PhD); S Anna Hospital, Turin, Italy (B Ghiringhello MD); Maggiore Hospital, Bologna, Italy (P Pierotti PhD); Emilia-Romagna Region, Bologna, Italy (C Naldoni MD); Centre for Cancer Prevention, Ravenna, Italy (P Schincaglia MD); and Wolfson Institute of Preventive Medicine, Queen Mary University of London, London, UK (Prof J Cuzick PhD)

Correspondence to:
Dr Guglielmo Ronco, Centro per la Prevenzione Oncologica (CPO), Via San Francesco da Paola 31, 10123 Torino, Italy
guglielmo.ronco@cpo.it

HPV-based screening. The trial was designed to assess the efficacy of different HPV-based screening policies and the most appropriate age of application. Two rounds of screening took place for each of two separate recruitment phases.

Methods

Patients

Two pre-planned recruitment phases were done between March, 2002, and December, 2004, as part of nine population-based cervical screening programmes in Italy. These programmes routinely invite all women aged 25–64 years for screening at 3-year intervals. Women aged 25–60 years who were not pregnant, had never undergone hysterectomy, had not been treated for CIN in the last 5 years, and who were attending for a new routine cervical screening episode, were eligible for enrolment in the study.^{6–8} All participants provided written informed consent, and the study was approved by the local ethics committees of participating centres.

Randomisation and masking

Women were randomly assigned to cytology or HPV-testing plus cytology (or HPV-testing alone in the second phase) in a 1:1 ratio. In Turin and Viterbo, two central computers provided randomisation after consent. In the other centres, sealed numbered envelopes containing the random allocation were prepared by the local coordinating centre, numbered, and provided to each unit. The envelopes were opened according to the centrally provided sequence, and the assignment was communicated to each woman. The results of all tests and diagnostic procedures were also communicated.

Procedures

Details on recruitment have been previously published.^{6–8} During both phases of recruitment, women in the cytology group had conventional cytology tests, classified according to the Bethesda 1991 system,⁹ and managed according to the standard protocol of each centre. In the HPV group, women had both liquid-based cytology (Thin-Prep system, Hologic, Marlborough, MA, USA) and HPV-DNA testing in phase one, and HPV-DNA testing only in phase two. HPV-DNA testing was done in seven laboratories using The Hybrid Capture 2 (HC2) hybridisation assay (Qiagen, Hilden, Germany) targeting HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. An HPV test was considered as positive at 1 relative light unit, as recommended by the manufacturer. High reproducibility of HPV testing between laboratories was documented.¹⁰

Management in the HPV group varied according to phase and age (figure 1). During phase one, women with a cytology result of atypical squamous cells of undetermined significance (ASCUS) or more severe were referred to colposcopy. Additionally, in phase one, all women who were HPV-positive and aged 35–60 years were referred to colposcopy,⁶ whereas women who were HPV-positive and aged 25–34 years were referred to colposcopy only if HC2 remained positive or if cytology was ASCUS or more severe after one year.⁷ During phase two, women were referred for colposcopy if the HPV test was positive.⁸

The same colposcopists examined women in the HPV and cytology groups, and had access to the results of the screening tests. All suspicious areas were biopsied. Results on cross-sectional accuracy, based only on the initial screening tests (and any short-term repeat screening before referral to colposcopy) have been published.^{6–8} Women with CIN2 or higher were given treatment whereas those with CIN1 were followed up colposcopically. Some women in both groups were recalled for a new colposcopy or short-interval cytology according to routine protocols, based on features of the colposcopy and on cytology. Women who were HPV positive were recalled yearly for repeat HPV testing and liquid-based cytology, until HPV tests were negative, and were referred to new colposcopy if cytology was ASCUS

Phase 1	
<p>Control group: conventional cytology</p> <ul style="list-style-type: none"> If cytology is ASCUS or more severe, refer to colposcopy (seven centres) If cytology is LSIL or more severe, refer to colposcopy; if ASCUS, repeat cytology (two centres) 	<p>Intervention group: thin-layer cytology and HPV test</p> <ul style="list-style-type: none"> If cytology is ASCUS or more severe, refer to colposcopy If normal cytology but HPV positive and 35 years or older, refer to colposcopy If normal cytology but HPV positive and younger than 35 years, retest for HPV and cytology: refer to colposcopy if still positive for HPV or if cytology becomes ASCUS
Phase 2	
<p>Control group: conventional cytology</p> <ul style="list-style-type: none"> If cytology is ASCUS or more severe, refer to colposcopy (seven centres) If cytology is LSIL or more severe, refer to colposcopy; if ASCUS, repeat cytology (two centres) 	<p>Intervention group: HPV test</p> <ul style="list-style-type: none"> Refer to colposcopy if positive at 1 pg/mL

Figure 1: Testing and intended management until first colposcopy, by screening group and phase (first round) ASCUS=atypical squamous cells of undetermined significance. LSIL=low-grade squamous intraepithelial lesion.

	Number of eligible women randomised	Phase 1	Phase 2	End of follow-up*
Turin	28 114	March, 2002–Feb, 2003	July, 2003–Oct, 2004	Nov, 2008
Trento	7260	Feb, 2002–May, 2003	June, 2003–Dec, 2004	Nov, 2008
Padua	10 654	April, 2002–Jan, 2003	Sept, 2003–Nov, 2004	April, 2008
Verona (Soave)	7645	Aug, 2002–June, 2003	Oct, 2003–June, 2004	April, 2008
Bologna	4980	March, 2002–May, 2003	Oct, 2003–Dec, 2004	Nov, 2008
Imola	5831	March, 2002–April, 2003	Sept, 2003–Jul, 2004	Nov, 2008
Ravenna	7588	March, 2002–Nov, 2002	Sept, 2003–April, 2004	Oct, 2008
Florence	16 167	Feb, 2002–June, 2003	July, 2003–Dec, 2004	June, 2008
Viterbo	6131	March, 2002–March, 2003	Oct, 2003–Dec, 2004	April, 2008

*Follow-up was concluded for each woman 3.5 years after referral to the second round of screening or the date reported in this table, whichever came first.

Table 1: Number of participants and periods of randomisation and follow-up at each centre

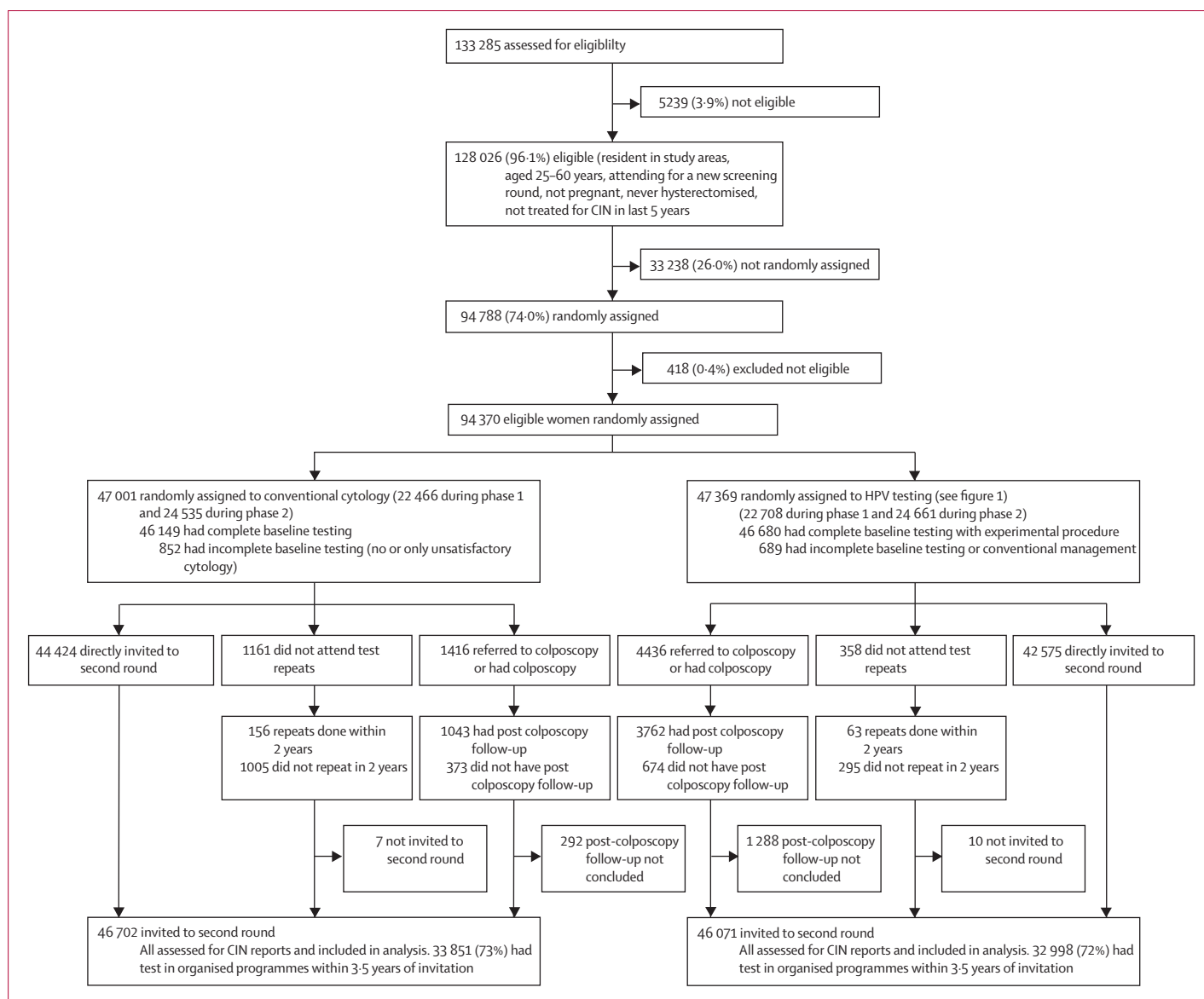


Figure 2: Trial profile

or more severe. Women who did not attend for any recommended test repeats within two years were invited for the new screening round. At the second round of screening, all women had conventional cytology testing and were managed according to the standard protocols of each centre.

The primary endpoint was the number of women with histologically confirmed pre-invasive lesions and invasive cervical cancer detected after randomisation. We recorded test results and histological findings from the computerised registration systems of the participating centres. To obtain histological diagnoses of high-grade CIN and invasive cervical cancer from screening done outside the trial, at the end of the second round of the second phase, we linked our database of recruited women to the

databases of the cancer registries (covering all centres except Viterbo) and of the pathology units in the catchment areas of NTCC (table 1). In the participating centres, completeness of population-based registration was considered high for CIN3 and invasive cervical cancer and adequate for CIN2. Pathology units registered all results for cervical histology. Migration outside the catchment areas for diagnostic procedures or for treatment of CIN was very limited.

Histology was initially read by local pathologists, who were not masked to screening test results. For women whose biopsy was locally determined to be CIN, all histological specimens were reviewed independently and blindly.¹¹ In addition to the already published results,⁶⁻⁸ we obtained tissue specimens for 699 of 962 women with

	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

*By Fisher's exact test. †p value for heterogeneity between age groups was 0.86 for round one and 0.87 over the first two rounds. ‡Five squamous-cell carcinomas (one stage T1A and four stage T1B) and two adenocarcinomas (one stage T1A and one TX). §Two adenocarcinomas, one stage T1A and one stage T1B.

Table 2: Total number of cases of invasive cervical cancer by screening group, age, and screening round

a diagnosis of CIN (231 of 329 for the cytology group and 468 of 633 for the HPV group; $p=0.21$). Each specimen was blindly reviewed by one pathologist randomly assigned from a group of nine participants. If the diagnosis differed from the original, a diagnosis was made by two further pathologists. Of the cases of CIN detected after the initial screening, 2% of CIN1 were upgraded to CIN2 or more severe, and 19% of CIN2 or higher were downgraded to CIN1 or no CIN. This reviewed diagnosis was used in the analysis.

Statistical analysis

We calculated the relative frequency (in the HPV vs cytology group) of histologically confirmed CIN2, CIN3 (including adenocarcinoma in situ), and invasive cervical cancer for the following three time periods, for both phases: trial screening round one (from recruitment until invitation to round two, including post-colposcopy follow-up and tests done within 2 years by women who missed repeats of the screening test at recruitment); trial screening round two (from invitation to the second round up to 3.5 years later, or until the end of follow-up, whichever came first—tests done by women who missed repeats at recruitment and were not retested within 2 years were also included); and the entire period encompassing the first two trial screening rounds.

Analysis was by intention to screen, and excluded 418 women who were ineligible at entry but were (erroneously) randomly assigned to a group. For round two, we only considered women invited for this round (figure 2). We tested for heterogeneity between phases in the group effect on detection of CIN in each time period, and if not significant, we computed pooled estimates. As in previous reports, we separately considered women aged 25–34 years and 35–60 years at enrolment. Differences in age and duration of follow-up were tested by the median score test. All p values are two-sided. SAS

version 8.2 was used for statistical analysis. This trial is registered, number ISRCTN81678807.

Role of the funding source

The funding source had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all data in the study. The corresponding author had the final responsibility for the decision to submit for publication.

Results

47 001 women were randomly assigned to the cytology group and 47 369 to the HPV group (figure 2). The median age at recruitment was 41 years in both groups. 23 680 women (50.4%) in the cytology group and 24 022 (50.7%) in the HPV group had a registered screening test in an organised screening programme within 4 years before enrolment. Randomisation periods and number of women recruited in each centre are reported in table 1.

1043 women from the cytology group had post-colposcopy follow-up and 156 had a repeat of the screening test done within 2 years of initial screening (figure 2). The corresponding values were 3762 and 63 in the HPV group. During the study period, 46 702 women (99.4%) from the cytology group were invited to the second screening round and 33 851 (73%) had at least one test in organised programmes within 3.5 years after invitation. In the HPV group, 46 071 women (97.3%) were invited to round two (slightly less than in the cytology group, because more women in the HPV group had not concluded post-colposcopy follow-up) and 32 998 (72%) attended. The median duration of follow-up was 1277 days in both groups ($p=0.13$). The median age at the start of the second round was 45 years in both groups ($p=0.19$). 695 women from the cytology group were referred to colposcopy during the second round and 625 (89.9%) attended, compared with 730 and 702 (89.7%), respectively, in the HPV group.

For both phases, during the first trial round, a similar number of invasive cervical cancers were detected in each group (table 2); however, in the second round, nine additional women in the cytology group had a diagnosis of invasive cervical cancer versus none in the HPV group ($p=0.004$). Five of these diagnoses were squamous-cell carcinomas (one stage T1A and four stage T1B) and four were adenocarcinomas (two stage T1A, one stage T1B, and one TX). Therefore, the proportion of invasive cervical cancers that were adenocarcinomas was 44% (95% CI 13.7–78.8), versus 11.6% observed nationwide by Italian cancer registries in 2005.¹² All women diagnosed with invasive carcinoma in the second round had normal cytology at round one. Overall, during the first two trial rounds, 18 invasive cancers of the cervix were detected in the cytology group versus seven in the HPV group ($p=0.028$).

The detection of CIN2 and CIN3 during each study period, among women aged 35–60 years at enrolment, is shown in table 3. There was no significant difference in

the group effect between study phases (p for heterogeneity >0.15 in all comparisons). During the first screening round, the detection of CIN2 or CIN3 was significantly higher in the HPV group versus the cytology group, with a detection ratio of 2.03 (95% CI 1.60–2.57). Conversely, in the second round, the detection of CIN2–3 was significantly lower in the HPV group versus the cytology group, with a ratio of 0.51 (0.28–0.93). For the total detection of disease during the first two screening rounds, the ratio between the HPV group and the cytology group was 1.66 (1.34–2.06). Results were similar for detection of CIN2 and CIN3, separately. Among women 35 years and older, age (considered a continuous variable in logistic regression) was not a modifier of the group effect on detection of CIN2 and 3 in round one, or in the two rounds combined. However, the modification of group effect by age was of borderline significance (beta=−0.17; p=0.058) for the detection of CIN3 in round two, suggesting that the gain in protection obtained by HPV testing could increase with increasing age.

Among women aged 25–34 years at recruitment, there was a significant difference between the two recruitment phases in the relative detection of CIN3 by HPV testing (table 4). For phase one, the detection ratio between groups was close to 1, both during the first and the second trial rounds. In phase two, detection of CIN3 was much higher in the HPV group than in the cytology group in the first screening round, and lower in the second round. In phase two, the total detection ratio, for both screening rounds, of CIN3 in the HPV group versus the cytology group was 2.14 (95% CI 1.28–3.59). These effects were observed both among women aged 25–29 and 30–34 years at recruitment (table 5).

For ages 25–34 years, pooling both phases, the detection ratio of CIN2 in the HPV versus cytology group was 4.54 (95% CI 3.00–6.88) in the first round and 0.54 (0.23–1.27) in the second round, with no heterogeneity between phases. For the total detection of CIN2 over the first two screening rounds, the ratio between the HPV and cytology group was 3.11 (2.20–4.39). This ratio

	Phase one			Phase two			p value for heterogeneity between phases	Both phases pooled		
	HPV group	Cytology group	RD (95% CI)†	HPV group	Cytology group	RD (95% CI)†		HPV group	Cytology group	RD (95% CI)†‡
CIN2										
Women enrolled (invited to round two)	16706 (16332)	16658 (16561)	..	17724 (17401)	17747 (17658)	34430 (33733)	34405 (34202)	..
Screening round one, N (%)*	58 (0.35%)	28 (0.17%)	2.07 (1.32–3.24)	50 (0.28%)	26 (0.15%)	1.93 (1.20–3.09)	0.83	108 (70+38§) (0.31%)	54 (40+14§) (0.16%)	2.00 (1.44–2.77)
Screening round two, N (%)*	6 (0.04%)	8 (0.05%)	0.76 (0.26–2.19)	2 (0.01%)	7 (0.04%)	0.29 (0.06–1.40)	0.31	8 (0.02%)	15 (0.04%)	0.54 (0.23–1.28)
Total over both rounds, N (%)*	64 (0.38%)	36 (0.22%)	1.77 (1.18–2.67)	52 (0.29%)	33 (0.19%)	1.58 (1.02–2.44)	0.70	116 (0.34%)	69 (0.20%)	1.68 (1.25–2.26)
CIN3 or AIS										
Women enrolled (invited to round two)	16706 (16332)	16658 (16561)	..	17724 (17401)	17747 (17658)	34430 (33733)	34405 (34202)	..
Screening round one, N (%)*	50 (0.30%)	27 (0.16%)	1.85 (1.16–2.95)	48 (0.27%)	20 (0.11%)	2.40 (1.43–4.05)	0.46	98 (69+29§) (0.28%)	47 (43+4§) (0.14%)	2.08 (1.47–2.95)
Screening round two, N (%)*	5 (0.03%)	7 (0.04%)	0.72 (0.23–2.28)	3 (0.02%)	10 (0.06%)	0.30 (0.08–1.11)	0.32	8 (0.02%)	17 (0.05%)	0.48 (0.21–1.11)
Total over both rounds, N (%)*	55 (0.33%)	34 (0.20%)	1.61 (1.05–2.47)	51 (0.29%)	30 (0.17%)	1.70 (1.08–2.67)	0.87	106 (0.31%)	64 (0.19%)	1.65 (1.21–2.26)
CIN2, CIN3, or AIS										
Women enrolled (invited to round two)	16706 (16332)	16658 (16561)	..	17724 (17401)	17747 (17658)	34430 (33733)	34405 (34202)	..
Screening round one, N (%)*	107 (0.64%)	55 (0.33%)	1.94 (1.40–2.68)	98 (0.55%)	46 (0.26%)	2.13 (1.50–3.03)	0.70	206 (166+67§) (0.60%)	101 (83+18§) (0.29%)	2.03 (1.60–2.57)
Screening round two, N (%)*	11 (0.07%)	15 (0.09%)	0.74 (0.34–1.62)	5 (0.03%)	17 (0.10%)	0.30 (0.11–0.81)	0.15	16 (0.05%)	32 (0.09%)	0.51 (0.28–0.93)
Total over both rounds, N (%)*	118 (0.71%)	70 (0.42%)	1.68 (1.25–2.26)	103 (0.58%)	63 (0.35%)	1.64 (1.18–2.24)	0.90	221 (0.64%)	133 (0.39%)	1.66 (1.34–2.06)

N=number of cases. CIN=cervical intraepithelial neoplasia. RD=relative detection. AIS=adenocarcinoma in situ. *For round one and total over the two rounds, the number of randomised eligible women was the denominator. For round two, the number of women invited to round two was the denominator. †Ratio of detection in the HPV versus cytology group. ‡Adjusted by study phase. §Cases detected during recruitment+cases detected during post-colposcopy follow-up.

Table 3: Detection of histologically confirmed and reviewed CIN2 and CIN3 by study group, recruitment phase, and screening round in women aged 35–60 years at recruitment

	Phase one			Phase two			p value for heterogeneity between phases	Both phases pooled		
	HPV group	Cytology group	RD (95% CI)†	HPV group	Cytology group	RD (95% CI)†		HPV group	Cytology group	RD (95% CI)†‡
CIN2										
Women enrolled (invited to round two)	6602 (5761)	5808 (5769)	..	6937 (6577)	6788 (6714)	..		12939 (12338)	12596 (12483)	
Screening round one, N (%)*	55 (0.92%)	13 (0.22%)	4.09 (2.24–7.48)	71 (1.02%)	14 (0.21%)	4.96 (2.80–8.79)	0.65	126 (87+39§) (0.97%)	27 (21+6§) (0.21%)	4.54 (3.00–6.88)
Screening round two, N (%)*	3 (0.05%)	7 (0.12%)	0.43 (0.11–1.66)	5 (0.08%)	8 (0.12%)	0.64 (0.21–1.95)	0.66	8 (0.06%)	15 (0.12%)	0.54 (0.23–1.27)
Total over both rounds, N (%)*	58 (0.97%)	20 (0.34%)	2.81 (1.69–4.66)	76 (1.10%)	22 (0.32%)	3.38 (2.11–5.43)	0.60	134 (1.04%)	42 (0.33%)	3.11 (2.20–4.39)
CIN3 or AIS										
Women enrolled (invited to round two)	6602 (5761)	5808 (5769)	..	6937 (6577)	6788 (6714)
Screening round one, N (%)*	23 (0.38%)	24 (0.41%)	0.93 (0.52–1.64)	44 (0.63%)	11 (0.16%)	3.91 (2.02–7.57)	0.0009
Screening round two, N (%)*	8 (0.14%)	6 (0.10%)	1.34 (0.46–3.84)	2 (0.03%)	10 (0.15%)	0.20 (0.04–0.93)	0.036
Total over both rounds, N (%)*	31 (0.52%)	30 (0.53%)	0.99 (0.61–1.65)	46 (0.66%)	21 (0.31%)	2.14 (1.28–3.59)	0.036
CIN2, CIN3, or AIS										
Women enrolled (invited to round two)	6602 (5761)	5808 (5769)	..	6937 (6577)	6788 (6714)	12939 (12338)	12596 (12483)	
Screening round one, N (%)*	78 (1.30%)	37 (0.64%)	2.00 (1.38–3.01)	115 (1.66%)	25 (0.37%)	4.50 (2.92–6.93)	0.007	193 (127+66§) (1.49%)	62 (52+10§) (0.49%)	3.03 (2.28–4.03)
Screening round two, N (%)*	11 (0.19%)	13 (0.23%)	0.85 (0.38–1.89)	7 (0.11%)	18 (0.27%)	0.40 (0.17–0.95)	0.21	18 (0.15%)	31 (0.25%)	0.59 (0.33–1.05)
Total over both rounds, N (%)*	89 (1.48%)	50 (0.86%)	1.72 (1.22–2.43)	122 (1.76%)	43 (0.63%)	2.78 (1.96–3.92)	0.054	211 (0.63%)	93 (0.74%)	2.21 (1.73–2.81)

N=number of cases. CIN=cervical intraepithelial neoplasia. RD=relative detection. AIS=adenocarcinoma in situ. *For round one and total over the two rounds, the number of randomised eligible women was the denominator. For round two, the number of women invited to round two was the denominator. †Ratio of detection in the HPV versus cytology group. ‡Adjusted by study phase. §Cases detected during recruitment+cases detected during post-colposcopy follow-up.

Table 4: Detection of histologically confirmed and reviewed CIN2 and CIN3 by study group, recruitment phase, and screening round in women aged 25–34 years at recruitment

was 3.84 (2.26–6.52) among women aged 25–29 years at recruitment and 2.61 (1.65–4.13) among women aged 30–34 years, with no evidence of heterogeneity between these two subgroups (table 5).

For all ages combined, no evidence of heterogeneity between centres was found for the effect of HPV screening on the detection of high-grade CIN during round one, round two, or both combined.

Discussion

We observed a significant decrease in cases of invasive cancer detected during the second round in the HPV group compared with the cytology group. Also, the incidence of invasive cancer was similar between screening groups in the first round, and there was a significantly lower number of cases in the HPV group versus the cytology group over the two screening rounds, indicating that HPV-based screening is more effective

than cytology in preventing invasive cervical cancer. A likely explanation for the better efficacy with HPV-testing was the earlier detection of clinically relevant lesions and treatment of precancers before invasion, whereas the lead time with cytology was not always sufficient for this purpose. Notably, a high proportion of invasive cancers detected in the cytology group at the second round were adenocarcinomas. This is consistent with previous studies^{13,14} reporting that cytology is less effective in preventing adenocarcinomas than squamous-cell carcinomas. A high occurrence of adenocarcinomas after cytology testing was previously observed in one of the participating centres in the current study.¹⁵

A study in rural India,¹⁶ which mainly included women who had not had previous screening, reported a significant decrease in the incidence of advanced cervical cancers, and in deaths from cervical cancer, with HPV-testing versus cytology or no intervention. However, the

study did not show a decrease in the incidence of stage 1 cancers or total incidence of cancers. Also, by contrast with studies in developed countries, an increased sensitivity of HPV testing versus cytology for high-grade CIN was not found. Our study is the first, to our knowledge, to show a greater efficacy for HPV testing versus cytology for preventing invasive cancers in a developed country, where cytological screening has been in place for years and advanced cervical cancers are extremely rare among screened women.

For women aged 35 years or older, in the HPV group versus cytology group, the decreased incidence of both CIN2 and CIN3 in the second round, preceded by increased detection in the first round, indicates that HPV-based cervical screening provides more sensitive and earlier detection of persistent high-grade CIN than cytology alone. The decrease in incidence of CIN2 and CIN3 in the second round during phase two (in the HPV vs cytology group), in which only HPV testing was used, was as great as the decrease in CIN2 and CIN3 for phase one, where both HPV testing and liquid-based cytology (LBC) were used. Therefore, combining HPV testing with cytology does not seem to increase the detection of persistent lesions versus HPV testing alone. Similar decreases in the detection of CIN2 and CIN3 at the second round (ratios of detection in the HPV vs cytology group of about 0.5) have been noted in other randomised trials comparing HPV plus cytology with cytology alone.³⁻⁵ We have previously shown that adding LBC to HPV testing led to a much lower positive predictive value for CIN2-3,⁶⁻⁸ without any gain in sensitivity. Our data support the use of stand-alone HPV testing as the primary screening test.

The extremely low detection of CIN3 at round two in the HPV group (2 per 10 000) indicates that HPV-based screening at extended intervals is safe. Observational studies also suggest a long duration of low risk for CIN3 after a single negative HPV test.¹⁷⁻¹⁹ Similar to the current study, two previously published trials^{3,4} found an increased detection of CIN3 in the HPV group compared with the cytology group at round one. No such difference was observed in a third trial (ARTISTIC)⁵ that compared LBC alone with LBC plus HPV testing, although decreased detection of CIN3, in the HPV group relative to cytology, at round two was noted. The lack of increased detection of CIN3 with HPV at round one in the ARTISTIC trial could be due to overdiagnosis of regressive lesions with LBC.²⁰

More CIN2 and CIN3 were detected in the HPV group than in the cytology group in the two rounds in our study; however, this was not observed in two other randomised trials^{4,5} (one of which used HPV testing in both groups at round two⁴). Because we used only cytology in both groups at round two, it is possible that some of the lesions detected by HPV at round one will be detected by cytology at round three or later, reflecting a substantial lead-time gain for HPV.²¹ Another possible explanation is that some

	Screening round one	Screening round two	Total over both rounds
CIN2			
25-29 years at recruitment	8.81 (3.39-13.68)	0.50 (0.15-1.68)	3.84 (2.26-6.52)
30-34 years at recruitment	3.41 (2.02-5.75)	0.58 (0.17-1.97)	2.61 (1.65-4.13)
p value for heterogeneity between age groups	0.11	0.88	0.28
CIN3			
25-29 years at recruitment			
Phase one	0.61 (0.24-1.58)	1.00 (0.25-4.00)	0.71 (0.32-1.53)
Phase two	3.72 (1.39-9.96)	0.51 (0.09-2.79)	2.29 (1.05-4.99)
p value for heterogeneity between phases	0.0071	0.55	0.033
30-34 years at recruitment			
Phase one	1.20 (0.58-2.48)	2.00 (0.37-10.91)	1.30 (0.66-2.53)
Phase two	4.07 (1.67-9.91)	0.00 (p=0.015)	2.04 (1.02-4.04)
p value for heterogeneity between phases	0.0329	0.015	0.35
p value for heterogeneity between age groups within phase one	0.27	0.53	0.24
p value for heterogeneity between age groups within phase two	0.89	0.12	0.83
CIN2-3			
25-29 years at recruitment	3.46 (2.23-5.38)	0.63 (0.29-1.39)	2.35 (1.63-3.37)
30-34 years at recruitment	2.74 (1.88-3.98)	0.54 (0.23-1.27)	2.10 (1.51-2.91)
p value for heterogeneity between age groups	0.43	0.78	0.81

CIN=cervical intraepithelial neoplasia.

Table 5: Relative detection (95% CIs) of CIN2 and CIN3 with HPV testing versus cytology by age at recruitment for women 25-34 years

of the excess lesions detected at recruitment by HPV testing would have regressed before the second round if left untreated. The relative incidence of CIN3 during further follow-up will be required to fully assess the extent to which the higher detection rate with HPV testing reflects diagnosis of persistent lesions. In the other trials, only HPV-positive women with abnormal cytology or with persistent infection were referred to colposcopy, whereas we directly referred all HPV-positive women aged 35 years or older. It is possible that immediate referral leads to detection of a higher proportion of regressive lesions. We previously showed^{6,8} that direct referral is associated with a substantial increase in colposcopies, whereas the number of colposcopies was less with cytological triage.^{3-5,7} Therefore, HPV-positive women aged 35 years or older should be triaged by cytology (or molecular markers such as P16²²) before referral to colposcopy.

Among younger women, for whom different protocols were applied during the two phases of recruitment, the effect of HPV testing on the detection of CIN3 was also different between phases. In particular, for women recruited during phase one, who were referred to colposcopy only if cytological abnormalities were present or if HPV infection persisted for at least 1 year, no decrease in the detection of CIN3 was observed for the HPV versus cytology group in the second round. In the second phase, direct referral of all HPV-positive women

led to a large decrease in detection of CIN3 (ratio 0.2 vs the cytology group) in the second round, but still resulted in a doubling of lesions detected over the entire follow-up period. As with women age 35–60 years, both a lead-time gain of more than 3 years with HPV-testing versus cytology, and overdiagnosis of regressive lesions, could contribute to this finding and further follow-up is needed. The high early occurrence of CIN3 after HPV infection in young women²³ is consistent with large lead-time gains with HPV testing in this age group. However, the clinical relevance of these lesions is unclear.²⁴ In any case, for young women, both with cytological triage (phase one) and with direct referral (phase two) the detection of CIN2 was much higher in the HPV than cytology group at round one, but only slightly lower at round two, suggesting that a large number of regressive CIN2 lesions were identified and treated. Indeed, CIN2 is a less defined and reproducible diagnosis than CIN3^{11,25} and is more regressive.²⁶ Overtreatment of regressive lesions is a problem because excisional treatment of cervical lesions is associated with increased risk of pregnancy-related morbidity.^{27,28}

Our study was done within organised screening programmes with more than 70% of eligible women enrolled, suggesting that results are applicable to routine practice. We applied the 1991 Bethesda system for classification of cytology results to avoid problems due to a switch to the 2001 classification, which was introduced just before the start of the study. Since most women with ASCUS cytology were referred to colposcopy, it is unlikely that application of the 2001 classification would have led to substantially different results. However, because the 2001 definitions were somewhat more restrictive than the 1991 definitions, specificity of cytology in our study could have been slightly lower and sensitivity slightly higher than with the 2001 classification.

The women enrolled in our study were aware of the screening method and of results of tests and diagnostic procedures, because we expected very low compliance with referral to colposcopy if the reason was not explained. However, the endpoints of our study are asymptomatic screen-detected lesions. The practitioners who did colposcopy and the local interpretation of histology were not masked to screening test results, but histology was reviewed blindly. The biopsy rate at colposcopy was similar in both groups. Therefore, direct bias in endpoint assessment is unlikely. Finally, attendance to the second screening round in an organised programme and compliance to a recommendation for colposcopy was similar in both study groups at recruitment and during follow-up. Therefore, non-blinding of the women in the study had no effect on their attendance to the procedures leading to endpoint assessment.

Over 70% of the women invited to round two actually attended within the organised programmes. It is plausible that most of the remaining women had cytology elsewhere, of their own initiative. Although we could not

retrieve these tests, we searched cancer registries and pathology services for lesions detected outside participating programmes. If a histological diagnosis of CIN was made, it would likely be registered in one of these sites. Indeed, the proportion of women with a CIN2, CIN3, or invasive cancer at round two was similar among women who did and did not attend organised programmes. Therefore, lack of completeness of follow-up is unlikely to explain our findings.

In conclusion, HPV testing in younger women results in overdiagnosis of regressive CIN2. For women aged 35 years or more, our results support the use of HPV-DNA testing for primary screening at prolonged intervals, with cytology reserved for triage of HPV-positive women. Further follow-up is needed to define how long screening intervals can be safely extended. Research is needed to define the optimum management of HPV-positive women, both in terms of criteria for referral to colposcopy and of type and frequency of follow-up, to minimise the costs related to increased referral to colposcopy and overdiagnosis of regressive lesions. A full cost-benefit analysis is underway.

Contributors

GR was the project leader and designed the study with NS and JC. GR, PG-R, and JC drafted the manuscript. AG-T, LDM, ADM, SG, PP, and FC were responsible for local HC2 testing. FC supervised the interlaboratory quality assurance for HC2. MC supervised the quality assurance for cytology interpretation. PDP and BG coordinated histology review. GR and RR were responsible for statistical analysis. MZ, PDP, CN, PS, MC, MZ, and PG-R organised the local fieldwork. All authors critically reviewed the manuscript.

Conflicts of interest

JC is an occasional adviser to Qiagen and Roche. GR, JC, and FC are occasional advisers to Gen-Probe, JC and FC to Abbot, and FC to Sanofi and GlaxoSmithKline.

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