Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials

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Lancet 2014; 383: 524–32

Published Online November 3, 2013 http://dx.doi.org/10.1016/S0140-6736(13)62218-7

This online publication has been corrected. The corrected version first appeared at thelancet.com on October 9, 2015

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Summary

Background In four randomised trials, human papillomavirus (HPV)-based screening for cervical cancer was compared with cytology-based cervical screening, and precursors of cancer were the endpoint in every trial. However, direct estimates are missing of the relative efficacy of HPV-based versus cytology-based screening for prevention of invasive cancer in women who undergo regular screening, of modifiers (eg, age) of this relative efficacy, and of the duration of protection. We did a follow-up study of the four randomised trials to investigate these outcomes.

Methods 176 464 women aged 20–64 years were randomly assigned to HPV-based (experimental arm) or cytology-based (control arm) screening in Sweden (Swedescreen), the Netherlands (POBASCAM), England (ARTISTIC), and Italy (NTCC). We followed up these women for a median of 6·5 years (1 214 415 person-years) and identified 107 invasive cervical carcinomas by linkage with screening, pathology, and cancer registries, by masked review of histological specimens, or from reports. Cumulative and study-adjusted rate ratios (experimental *vs* control) were calculated for incidence of invasive cervical carcinoma.

Findings The rate ratio for invasive cervical carcinoma among all women from recruitment to end of follow-up was 0·60 (95% CI 0·40–0·89), with no heterogeneity between studies (p=0·52). Detection of invasive cervical carcinoma was similar between screening methods during the first 2·5 years of follow-up (0·79, 0·46–1·36) but was significantly lower in the experimental arm thereafter (0·45, 0·25–0·81). In women with a negative screening test at entry, the rate ratio was 0·30 (0·15–0·60). The cumulative incidence of invasive cervical carcinoma in women with negative entry tests was 4·6 per 10^5 (1·1–12·1) and 8·7 per 10^5 (3·3–18·6) at 3·5 and 5·5 years, respectively, in the experimental arm, and 15·4 per 10^5 (7·9–27·0) and 36·0 per 10^5 (23·2–53·5), respectively, in the control arm. Rate ratios did not differ by cancer stage, but were lower for adenocarcinoma (0·31, 0·14–0·69) than for squamous-cell carcinoma (0·78, 0·49–1·25). The rate ratio was lowest in women aged 30–34 years (0·36, 0·14–0·94).

Interpretation HPV-based screening provides 60–70% greater protection against invasive cervical carcinomas compared with cytology. Data of large-scale randomised trials support initiation of HPV-based screening from age 30 years and extension of screening intervals to at least 5 years.

Funding European Union, Belgian Foundation Against Cancer, KCE-Centre d'Expertise, IARC, The Netherlands Organisation for Health Research and Development, the Italian Ministry of Health.

Introduction

Cervical screening aims to prevent invasive cervical carcinoma by detection and treatment of its precursors—cervical intraepithelial neoplasia grade 2 (CIN2) and, particularly, grade 3 (CIN3). In a cluster-randomised controlled trial from rural India,[1] women who had received little or no previous cervical screening either underwent one round of human papillomavirus (HPV) testing or had no screening, cytological analysis, or visual inspection. Cumulative incidence of advanced cancer (stage ≥2), but not of stage 1 invasive cancer, was lower in women who had one HPV screening round compared with those who had no intervention.[1] However, the effect of HPV testing—as an alternative to regular cytological screening—on incidence of invasive cancer has not been assessed adequately.

Four randomised controlled trials have been done—Swedescreen,[2] POBASCAM,[3,4] ARTISTIC,[5] and NTCC[6]—in which women from industrialised countries were followed up for at least two rounds of cervical screening. A lower CIN3 incidence was recorded after HPV testing compared with cytology. Despite different screening protocols, the relative incidence of CIN3 or worse histological findings after the first screening round was similar in all studies: rate ratios (HPV *vs* cytology) were 0·53 (95% CI 0·29–0·98) in Swedescreen, 0·52 (0·28–0·97) in ARTISTIC, 0·34 (0·15–0·75) in NTCC (in women aged 35 years or older), and 0·39 (0·27–0·53) in POBASCAM, with no evidence of heterogeneity (p=0·681).[7] These results show that HPV-based screening detects persistent high-grade CIN before cytology, thus increasing the probability of treatment before invasion.

Furthermore, the effect was similar with the different screening protocols applied, which suggests that efficacy in cancer prevention is dependent primarily on the screening test and not on the exact protocol used, providing a strong rationale for joint analysis of trials.

In the NTCC trial,[6] the overall incidence of invasive cancers was reduced significantly with HPV screening compared with cytology, and in POBASCAM,[4] incidence was diminished significantly at the second screening round. However, because none of the four randomised controlled trials was powered to show a reduction in cancer incidence, the numbers of cases in individual reports were small. Thus, precise direct estimates are absent for the relative efficacy of HPV-based versus cytology-based screening, of how efficacy changes according to age, cancer stage, and morphological features, and of the duration of protection against cancer. Such direct estimates are crucial to inform decisions about implementation of HPV-based screening as a routine activity and to define some important aspects of screening policies with HPV, such as the age at which to initiate screening and the optimum screening interval. Therefore, we pooled data from the four randomised trials and followed up the cohorts for analysis of invasive cervical carcinomas.

Methods

Study populations

Study populations and interventions used in the studies have been described elsewhere.[3–6,8–13] Women recruited to all four trials had not had a hysterectomy and were attending for routine screening within organised population-based programmes. Participants in Swedescreen were recruited from five Swedish regions between May, 1997, and November, 2000; those in NTCC were recruited from nine areas of Italy during two preplanned phases, between March, 2002, and December, 2004; women in ARTISTIC were recruited from the Greater Manchester region of the UK between July, 2001, and September, 2003; and individuals in POBASCAM were recruited from the Netherlands between January, 1999, and September, 2002. Women were excluded from NTCC if they were pregnant or treated for CIN in the previous 5 years, and participants in POBASCAM were excluded if they had CIN2 or higher or abnormal cytology detected in the previous 2 years. No exclusion criteria were used at recruitment in Swedescreen and ARTISTIC; however, women diagnosed with CIN2 or higher were usually followed up in gynaecological clinics and they did not attend routine screening for many years. Ethics approval was obtained in every study, and all women provided informed consent.

Randomisation and masking

After enrolment, women were randomly assigned to either HPV-based or cytology-based screening in a 1:1 ratio, except in ARTISTIC (3:1 ratio). In POBASCAM, Swedescreen, ARTISTIC, and two centres of NTCC, central computers did the randomisation (not in blocks). In the remaining NTCC centres, sealed numbered envelopes containing the random allocation were prepared by the local coordinating centre and sent to every unit. The envelopes were opened according to the centrally provided sequence (done in blocks of eight in three centres, unblocked in the remaining). Women and clinical staff were not masked to randomisation, except in Swedescreen, in which participants and researchers were unaware of allocations during the first 6 years.

Interventions at first screening round

Women in the control arm had either liquid-based (ARTISTIC) or conventional (all other studies) cytological testing. Management essentially followed local routine guidelines. In most NTCC centres and in Stockholm (Swedescreen), women with a finding of ASC-US (atypical squamous cells of undetermined significance) or worse were referred directly for colposcopy, whereas in the other NTCC and Swedescreen centres, repeat cytology was an option. In POBASCAM and ARTISTIC, women with borderline or mild dyskaryosis—corresponding to ASC-US or low-grade squamous intraepithelial lesions in the Bethesda system[14]—were referred for repeat cytology.

Women in the experimental arm had either HPV testing alone (in phase 2 of NTCC) or both HPV testing and cytology (all other studies). In ARTISTIC and NTCC, DNA testing of high-risk HPV types was done with the hybrid capture 2 assay (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68), using the 1 pg/μL (1 μg/L) recommended cutoff. In POBASCAM and Swedescreen, PCR was done with GP5+ and GP6+ general primers, followed by enzyme immunoassay targeting the same HPV types as with the hybrid capture 2 assay, plus HPV66.[15] Interpretation of HPV testing and cytological analysis were masked reciprocally in all studies. In NTCC phase 1 (age 35–60 years) and phase 2 (any age), all HPV-positive women were referred directly for colposcopy. In the other studies, women were referred for immediate colposcopy on the basis of cytological findings, following the same rules as in the corresponding control arm. Cytology-negative HPV-positive women were referred for repeat HPV and then colposcopy if HPV infection persisted (we called this approach cytological triage). However, protocols differed slightly between studies with respect to retesting intervals, number of repeats, definition of persistence, and whether cytology was also repeated.[2–6] Women with CIN2 or more severe histological findings at colposcopy were referred for treatment. Almost all described interventions were concluded within 2·5 years of recruitment.

Interventions at subsequent screening rounds

After the first screening round was concluded, study participants were invited for further screening rounds

within the organised programmes, at the routine interval (5 years in the Netherlands and 3 years in Italy, Sweden, and the UK). In NTCC and Swedescreen, women from both the control and experimental arms had cytology-based screening—ie, no further HPV testing was done. In POBASCAM, all individuals had HPV screening and conventional cytology at the second round, according to the procedure in the experimental arm at round one (47% of women were tested for HPV, with no difference recorded between control and experimental arms);[4] thereafter, women underwent routine cytology-based screening. In ARTISTIC, at round two, women from each arm continued screening as in round one, and thereafter they had routine cytology-based screening.

Case ascertainment and validation

Our primary endpoint was invasive carcinoma of the cervix. We did not consider CIN, non-epithelial cervical cancers, and cancers at other sites. Potential cases of invasive cervical cancer arising during follow-up were identified in several ways, depending on the patient's location (appendix pp 1–4). Cervical carcinomas were classified by morphological features—if possible, as squamous-cell carcinoma or adenocarcinoma (including adenosquamous)[16]—and by FIGO (International Federation of Gynecology and Obstetrics) stage (1A *vs* >1A).

See Online for appendix

In Sweden (Swedescreen), cases were ascertained by linkage to the National Quality Registry for Cervical Screening, which contains a copy of cytology and histopathology reports from all laboratories in Sweden (both from organised screening and opportunistic testing), regional screening registries, and the National Cancer Registry. In the Netherlands (POBASCAM), cases of invasive cervical cancer were found by linkage to the PALGA archive, which contains a copy of all cytology and histopathology reports from organised screening and opportunistic testing. In Italy (NTCC), cases were identified from the computerised systems of participating screening centres and by linkage to local cancer and pathology registry databases. In the UK (ARTISTIC), cases of invasive cervical cancer were established by linkage to two local pathology units and the national cancer registration database.

In Swedescreen, original diagnostic slides and reports of potential cancer cases were reviewed by a pathologist who was unaware of the random allocation and HPV and cytology status. In NTCC, all histological slides from women with an original diagnosis of CIN1 or higher who were identified from screening registries were requested for review by a group of pathologists unaware of randomisation, HPV and cytology status, and the original histological diagnosis.[6,17] In POBASCAM, all histological slides from women with an original diagnosis of CIN1 or more, who were identified from PALGA, were requested for review by a group of pathologists who were masked to randomisation, HPV status, and cytology result.

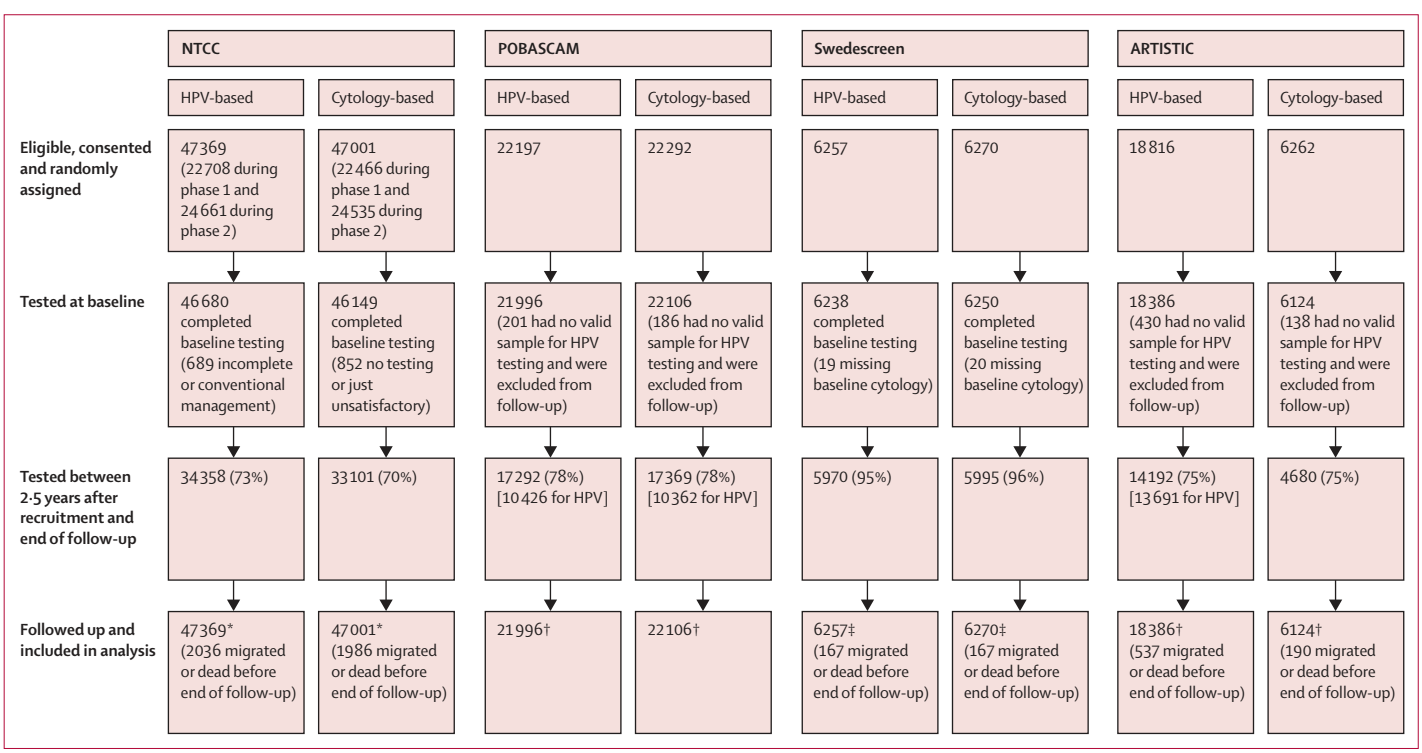

Figure 1: **Trial profiles**

*Follow-up ended in April, 2008, in Padua, Verona, and Viterbo; June, 2008, in Florence; October, 2008, in Ravenna; and November, 2008, in Turin, Trento, Bologna, and Imola. †Follow-up ended in December, 2009. ‡Follow-up ended in July, 2011, in Skåne; September, 2011, in Gothenburg; January, 2012, in Stockholm; and March, 2010, in the other regions.

In ARTISTIC, all pathology reports—and slides from cases with an equivocal report—were reviewed.

Statistical analysis

We analysed individual data by intention to screen. Every woman contributed years of observation from recruitment to end of follow-up, cancer detection, death, or migration, whichever occurred first. Dates for migration and death were not available for POBASCAM. In the other studies, 1·6% of years of observation were censored for these reasons. Further analyses restricted to women with a negative test at entry were censored 2·5 years after detection of CIN2 or CIN3, if any.

We calculated the cumulative incidence of invasive cervical cancer in each study arm using the Kaplan-Meier method, for all randomised women from enrolment to end of observation and for women who were HPV-negative at entry in the HPV arm and who were cytology-negative at entry in the cytology arm. Because of the 3:1 randomisation ratio used in the ARTISTIC trial, but not in the other studies, the crude pooled Kaplan-Meier estimate could be biased. Therefore, in the Kaplan-Meier analysis, we multiplied the numbers of cancers and women at risk in the ARTISTIC trial by 0·5 in the HPV arm and 1·5 in the cytology arm. We adjusted Greenwood's formula accordingly to calculate variance, and we calculated 95% CIs for a binomial proportion with the same value and variance.[18] Curves are plotted until the 75th percentile of the distribution of observation times. All p values are two-sided.

We calculated the overall study-adjusted (unweighted) invasive cervical cancer detection rate ratio in the experimental arm versus the control arm, considering studies as fixed effects.[19] We included all randomised women from enrolment to the end of observation and separately for the period from enrolment to 2·5 years thereafter (prevalence screen, roughly including the first primary test and related procedures, which could have led to detection of cancers prevalent at enrolment) and for the subsequent period. We also restricted analyses to women who were HPV-negative at entry in the experimental arm and those who were cytology-negative at entry in the control arm. When no invasive cervical cancer was recorded, 0·5 cases were added in the analysis. We assessed heterogeneity among studies with the χ^2 test[20] and I^2 statistic.[21] We also calculated rate ratios for the entire observation period for all randomised women according to cancer morphology, stage, and age group (<30, 30–34, 35–49, and ≥50 years) at recruitment. Finally, we calculated the rate ratio of the proportion of women who had at least one biopsy procedure, as a measure of extent of diagnostic procedures.

Role of the funding source

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

Results

Figure 1 shows the trial profiles for the four randomised controlled trials, and table 1 summarises the main features of every study. Overall, 176 464 women were enrolled. Median age at recruitment was identical in both arms within every study (41 years for NTCC and

	Swedescreen (NCT00479375)	POBASCAM (ISRCTN20781131)	ARTISTIC (ISRCTN25417821)	NTCC (ISRCTN81678807)
Target age at recruitment (years)	32–38	29–61	20–64	25–60
Randomisation ratio (experimental *vs* control)	1:1	1:1	3:1	1:1
Primary test in the experimental arm	HPV (GP5+/GP6+ PCR) and conventional cytology	HPV (GP5+/GP6+ PCR) and conventional cytology	HPV (hybrid capture 2) and liquid-based cytology	Phase 1: HPV (hybrid capture 2) and liquid-based cytology Phase 2: stand-alone HPV (hybrid capture 2)
Primary test in the control arm	Conventional cytology	Conventional cytology	Liquid-based cytology	Conventional cytology
Tests in secondary and later screening rounds	In both arms: conventional cytology	At round 2 in both arms: HPV (GP5+/GP6+ PCR) and conventional cytology At round ≥3 in both arms: conventional cytology	At round 2 in both arms: corresponding with primary test At round ≥3 in both arms: cytology	In both arms: conventional cytology
Management of HPV-positive women	Cytological triage*	Cytological triage*	Cytological triage*	Colposcopy (in phase 2 and in women ≥35 years old in phase 1) Cytological triage* (in women aged 25–34 years in phase 1)
Screening interval for women with negative result (years)	3	5	3	3

*If cytology was negative, HPV-positive women were invited for repeat HPV testing, then colposcopy if infection persisted. If cytology was positive, women were referred immediately for colposcopy. This approach was denoted cytological triage.

Table 1: **Main features of the four randomised controlled trials**

	Invasive cell carcinomas (n)					Total person-years	Median follow-up (years)	Time from enrolment		Age at enrolment (years)				Women with a negative test at entry†
	Total	1A*	>1A*	SCC	AC			≤2·5 years	>2·5 years	<30	30–34	35–49	≥50	
NTCC														
Experimental	9	8	1	8	1	242 984	5·1	8/117 300	1/125 684	2/26 561	0/34 420	6/113 633	1/68 370	1/220 134
Control	24	13	11	14	10	241 025	5·1	11/116 429	13/124 597	1/25 842	2/33 361	14/113 065	7/68 757	14/224 766
POBASCAM														
Experimental	20	7	13	15	5	198 525	9·0	12/54 970	8/143 555	(0/381)‡	2/31 996	10/103 896	8/62 252	6/188 740
Control	28	13	15	16	12	199 340	9·0	9/55 248	19/144 092	(0/483)‡	11/31 897	10/105 260	7/61 692	17/192 679
Swedescreen														
Experimental	5	3	2	4	1	75 477	12·0	0/15 590	5/59 887	(0/14)‡	3/30 749	2/44 715	..	2/68 324
Control	7	3	4	5	2	75 465	12·0	3/15 606	4/59 858	(0/49)‡	2/30 448	5/44 967	..	4/68 360
ARTISTIC														
Experimental	10	3	3	8	2	136 223	7·5	5/45 849	5/90 374	1/28 106	0/20 180	7/56 373	2/31 564	3/114 862
Control	4	3	1	3	1	45 376	7·5	4/15 266	0/30 109	1/9321	0/6892	3/18 345	0/10 818	0/39 498
Pooled														
Experimental§	44	21	19	35	9	653 209	6·6	25/233 709	19/419 500	3/54 667	5/117 345	25/318 617	11/162 186	12/592 060
Control§	63	32	31	38	25	561 206	6·2	27/202 549	36/358 656	2/35 163	15/102 598	32/281 637	14/141 267	35/525 303
All	107	53	50	73	34	1 214 415	6·5	52/436 258	55/778 156	5/89 830	20/219 943	57/600 254	25/303 453	47/1 117 363

Data are number of cases/person-years, unless otherwise stated. AC=adenocarcinoma. CIN=cervical intraepithelial neoplasia. SCC=squamous-cell carcinoma. *In ARTISTIC, stage not available for four cases in HPV arm. †Observations are censored 2·5 years after CIN2 or CIN3, if any. ‡Women younger than 30 years at enrolment were excluded from the analysis. §Data not usable to compare arms because of different randomisation ratios in studies.

***Table 2*: Cases of invasive cervical carcinoma, number of person-years, and median duration of follow-up**

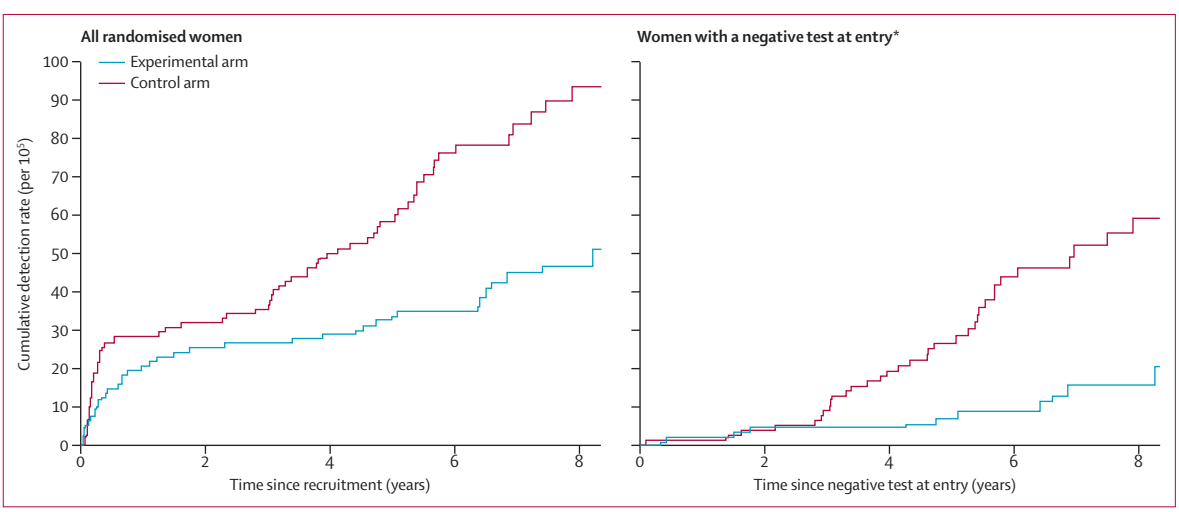

***Figure 2*: Cumulative detection of invasive cervical carcinoma**
*Observations are censored 2·5 years after CIN2 or CIN3 detection, if any.

POBASCAM, 39 years for ARTISTIC, and 35 years for Swedescreen). The proportion of women with further screening beyond 2·5 years after recruitment was similar in both arms within every study, ranging from 71% in NTCC to 95% in Swedescreen. Of women whose first cervical screening test was negative (and who were expected to follow the same protocol thereafter), the average number of subsequent tests was similar between arms in POBASCAM (1·13 in both) and ARTISTIC (1·20 in both), but it was slightly higher in the cytology arm in NTCC (1·05 *vs* 0·71) and Swedescreen (2·93 *vs* 2·81).

Women were followed up for a total of 1 214 415 person-years (median 6·5 years). In total, 107 invasive cervical carcinomas were detected (table 2). In Swedescreen, of 20 potential cases reviewed, 12 were confirmed as invasive cervical carcinoma (appendix p 1). One case in the cancer registry with no diagnostic slide and two cases with diagnostic slides but not accepted as cases by the cancer registry were excluded. In NTCC, of 43 potential invasive

cases initially identified, 23 had the original slide reviewed (17 confirmed); the original reports of the remaining 20 cases were obtained (13 confirmed). Three cases originally diagnosed as CIN were reclassified as invasive cervical cancer during masked review of slides (appendix p 2). In POBASCAM, of 40 potential cases of invasive cervical cancer, the slide was reviewed for 36 and the original report for four. One case was downgraded to CIN3, but one CIN3, seven in-situ adenocarcinomas, and one endometrioid carcinoma were reclassified as invasive carcinoma of the cervix (appendix p 3). In ARTISTIC, 18 potential invasive cases were identified. Slides were examined for four cases with equivocal reports. Overall, 14 were confirmed as invasive cervical cancer (appendix p 4).

When considering all randomised women, cumulative detection of invasive cervical carcinoma was similar in both arms up to about 2 years from enrolment, but diverged thereafter, reaching 46·7 per 10^5 (95% CI 32·1–65·5) in the experimental arm and 93·6 per 10^5 (70·5–121·8) in the control arm 8 years after enrolment (figure 2). The corresponding overall rate ratio was 0·60 (95% CI 0·40–0·89; table 3). No evidence of heterogeneity was noted between studies (p=0·52), and a random-effects model[20] gave an almost identical estimate (0·61, 0·41–0·91). Detection of invasive cancers of the cervix did not differ significantly between the two arms during the prevalence screen up to 2·5 years from enrolment (0·79, 0·46–1·36), but was significantly lower in the experimental arm thereafter (0·45, 0·25–0·81; table 3). 11 of 19 cancers detected in the experimental arm during follow-up were HPV-positive at baseline. Within 2·5 years of recruitment, ten of them had not undergone biopsy and one biopsy showed CIN1.

The study-adjusted rate ratio after a negative test on entry (cytology-negative in the control arm and HPV-negative in the experimental arm) was 0·30 (0·15–0·60; table 3). No heterogeneity was noted between studies (p=0·23), and the random-effects model[20] estimate was almost identical (0·34, 0·14–0·86). Cumulative incidence of invasive cervical cancer was 15·4 per 10^5 (95% CI 7·9–27·0) and 36·0 per 10^5 (23·2–53·5), respectively, 3·5 and 5·5 years after a negative cytology test on entry versus 4·6 per 10^5 (1·1–12·1) and 8·7 per 10^5 (3·3–18·6), respectively, 3·5 and 5·5 years after a negative HPV test on entry (figure 2).

The proportion of adenocarcinomas fell by age: 40% in women younger than 30 years, 35% in those aged 30–34 years, 30% in women age 35–49 years, and 23% in those 50 years or older. When we pooled data for women in the overall study period, we recorded a lower study-adjusted rate ratio for adenocarcinoma than for squamous-cell carcinoma, whereas rate ratios were similar for cancers of all stages (table 4). Considering age at enrolment, the lowest rate ratio (0·36, 95% CI 0·14–0·94) was noted in women aged 30–34 years

	All randomised women			Women with negative test at entry*
	Overall	≤2·5 years from enrolment	>2·5 years from enrolment†	
NTCC	0·37 (0·17–0·80)	0·72 (0·29–1·80)	0·08 (0·01–0·58)	0·07 (0·01–0·56)
POBASCAM	0·72 (0·40–1·27)	1·34 (0·57–3·18)	0·42 (0·18–0·96)	0·36 (0·14–0·91)
Swedescreen	0·71 (0·23–2·25)	0·17 (0·01–3·33)	1·25 (0·34–4·65)	0·50 (0·09–2·73)
ARTISTIC	0·83 (0·26–2·66)	0·42 (0·11–1·55)	3·33 (0·18–60·98)	2·06 (0·10–41·19)
Pooled rate ratio (fixed effects)	0·60 (0·40–0·89)	0·79 (0·46–1·36)	0·45 (0·25–0·81)	0·30 (0·15–0·60)
I^2 (p for heterogeneity between studies)	0·0% (0·52)	12·3% (0·33)	56·8% (0·074)	21·4% (0·23)

Data are rate ratio (95% CI), unless otherwise stated. Rate ratio is the cancer detection rate in the experimental *vs* control arm. *Observations are censored 2·5 years after CIN2 or CIN3, if any. †Of cases in the experimental arm, 0/1 in NTCC, 5/8 in POBASCAM, 3/5 in Swedescreen, and 3/5 in ARTISTIC were HPV-positive at baseline.

Table 3: **Study-adjusted pooled relative detection rate of invasive cervical carcinoma**

	Pooled rate ratio* (95% CI)	I^2 (p for heterogeneity between studies)
Morphology		
Squamous-cell carcinoma	0·78 (0·49–1·25)	0·0% (0·84)
Adenocarcinoma	0·31 (0·14–0·69)	0·0% (0·59)
Adenocarcinoma *vs* squamous-cell carcinoma	0·34 (0·12–0·90)	..
Stage		
1A	0·58 (0·34–1·01)	0·0% (0·82)
>1A	0·56 (0·31–1·00)	31·8% (0·22)
>1A *vs* 1A	0·86 (0·35–2·13)	..
Age at enrolment (years)		
<30†	0·98 (0·19–5·20)	0·0% (0·34)
30–34	0·36 (0·14–0·94)	7·2% (0·36)
35–49	0·64 (0·37–1·10)	0·0% (0·55)
≥50	0·68 (0·30–1·52)	36·5% (0·21)

All randomised women are included, for the overall study period. *Estimates (experimental *vs* control arm) obtained by a study-adjusted fixed effects model.[19] †Women from Swedescreen and POBASCAM excluded.

Table 4: **Study-adjusted pooled relative detection rate of invasive cervical carcinoma, by morphology, stage, and age at enrolment**

	Number of women (%) who had biopsy*		Rate ratio† (95% CI)	I^2 (p for heterogeneity between studies)
	Experimental arm	Control arm		
NTCC	2538 (5%)	1127 (2%)	2·24 (2·09–2·39)	..
POBASCAM	1535 (7%)	1533 (7%)	1·01 (0·94–1·08)	..
Swedescreen	675 (11%)	701 (11%)	0·97 (0·87–1·07)	..
ARTISTIC	1716 (9%)	528 (9%)	1·08 (0·97–1·19)	..
Pooled rate ratio (fixed effects)	..	..	1·35 (1·30–1·40)	99·1% (<0·0001)
Pooled rate ratio (fixed effects, NTCC excluded)	..	..	1·02 (0·97–1·07)	30·7% (0·236)

*During the entire period of observation. †Experimental *vs* control arm.

Table 5: **Biopsy procedures undertaken in the four randomised trials, individual and pooled effects**

(table 4). However, the efficacy of HPV testing did not differ significantly between women aged 30–34 years and 35 years and older (p=0·13).

The rate ratio for women who had a biopsy in the experimental and control arms differed significantly between studies (p<0·0001, table 5). When we excluded data for NTCC, which had a ratio of 2·24 (95% CI 2·09–2·39), no heterogeneity was detected between the remaining studies (p=0·236) and no increase was seen in the experimental arm (pooled ratio 1·02, 95% CI 0·97–1·07).

Discussion

Our pooled analysis of four randomised controlled trials of HPV-based cervical screening versus conventional cytology showed a significant reduction in invasive cervical cancers in women who had HPV-based screening. When all randomised women and all cancers diagnosed from enrolment—including cases already present (prevalent)—were considered, detection of invasive cervical carcinomas was significantly lower with HPV-based testing.

Panel: Research in context

Systematic review

We searched Medline and Embase with the keywords: "screening", "sensitivity and specificity", "detection rate", "cervical intraepithelial neoplasia", "uterine cervical neoplasms", "papillomavirus", and "HPV". From this search we identified 22 original reports of randomised clinical trials comparing HPV-based with cytology-based screening in the general population. In one cluster-randomised study from rural India, once-in-lifetime screening with HPV was compared with once-in-lifetime cytology, visual inspection, or no screening. The results showed reduced mortality from cervical cancer and lower incidence of stage 2 or higher (but not stage 1) cancer.[1] However, an estimate of the effect of HPV screening on cancer incidence among regularly screened women was not given. In the remaining reports, only results at baseline or during the first trial screening round and for cancer precursors (as a predefined endpoint) were given. In one randomised controlled trial,[22] double-testing was done of all women, and only the order of tests was random. In two other studies (FOCAL[23] and a Finnish randomised trial[24]), data were reported for only the first screening round. Four trials[2–6] reported data for two screening rounds. In a meta-analysis[7] of aggregated data from three of these studies,[2,4,6] the occurrence of invasive cancer was reduced significantly at the second study screen, but the cumulative incidence from recruitment was not considered. Thus, the decrease might have been attributable to earlier detection of cancer by HPV testing compared with cytology. In none of the randomised trials identified by our search was the efficacy of HPV testing versus cytology looked at according to age, cancer stage, and morphology, nor was direct evidence reported for the duration of protection from invasive cancer with HPV-based screening.

Interpretation

Our extended follow-up of the four randomised controlled trials with data for two screening rounds enabled large-scale estimation of the effect of HPV screening on invasive cervical carcinoma in women who have regular screening. HPV-based screening prevented more invasive cervical cancers than did cytology. Different screening protocols used in the four studies did not affect efficacy of HPV testing. Increased protection against invasive cervical cancer was noted in women aged 30–35 years, and HPV screening every 5 years was most protective against invasive cancers of the cervix, compared with cytology done every 3 years. We recommend implementation of HPV-based cervical screening with triage from age 30 years at intervals of at least 5 years.

Data obtained at enrolment are essential to prove that HPV-based screening provides greater protection for prevention of cervical carcinoma. A reduction in the number of invasive cancers at second or later screens could simply be attributable to earlier diagnosis of invasive cervical cancer by HPV testing at the first (prevalence) screen (panel). In such a case, we would expect cancer detection to be higher in the experimental arm in the first 2·5 years, because this period mainly includes prevalent cases. In our analysis, however, rates noted in the first 2·5 years were similar in the experimental and control arms. Therefore, the best estimate for the gain in reducing incidence of invasive cervical cancers—ie, the true gain in efficacy—by HPV-based screening is provided by the rate ratio recorded after 2·5 years (0·45), particularly among women with a negative screening test at baseline (0·30), which essentially excludes prevalent cases.

Asymptomatic prevalent cancers were identified by different tests in the two arms. However, because cancer detection at the first (prevalence) screen was similar in the two arms, this factor could not have affected our estimate of the true gain with HPV screening. If anything, a non-significant, slightly lower rate of detection of prevalent cancers was recorded in the experimental arm, which could have underestimated the true reduction in incidence with HPV screening. Moreover, the gain could be larger in settings in which the quality of cytology is lower.

A similar gain in efficacy was recorded with HPV testing for prevention of microinvasive and frankly invasive cancers, which have worse prognosis and greater effect on quality of life. The larger gain noted for adenocarcinoma compared with squamous-cell carcinoma accords with the known lower efficacy for detection of adenocarcinoma by cytological screening.[25,26]

Identification of invasive cervical cancers was based on linkage with population-based registries, to also include symptomatic invasive cancers of the cervix and those detected by opportunistic screening. Reports and, in most cases, histological specimens were reviewed by pathologists who were unaware of the random allocation and screening test status. The intensity of screening could have affected cancer prevention in women with a negative screening result at entry, but subsequent testing was most intense in the control arm. In POBASCAM, all women were invited for HPV-based screening at the second round, and almost half attended, so a reduced difference between arms was expected thereafter. Indeed, nine of ten invasive cervical cancers diagnosed over 6 years after recruitment had not been tested for HPV at round two.

We studied all women recruited in the four population-based randomised controlled trials for whom information was available from at least two screening rounds. The studies used different screening protocols, in particular for directly referring HPV-positive women to colposcopy or triaging them by cytology and for using stand-alone HPV or cotesting (HPV and cytology). The pooled efficacy

of HPV-based screening that we report represents an average (weighted by precision) of the effects of such procedures. We previously noted that such procedures resulted in a reduction of CIN3 or higher grades at the second screening round in women undergoing HPV-based screening compared with cytology, which was similar in all four trials,[7] suggesting comparable lead-time gain. Therefore, we had strong reason to expect comparable efficacy. We recorded no differences in efficacy between studies, with consistently lower overall detection rates of invasive cervical cancer in the experimental arm. These findings suggest that the gain over cytology of using or not using primary HPV testing is much larger than the variability in efficacy, if any, between different HPV-based screening protocols.

The dissimilar protocols resulted, however, in very different costs. In particular, in the studies that used cytological triage, the biopsy rate was not increased in the experimental arm whereas it was doubled with direct referral of all HPV-positive women for colposcopy (in NTCC). This finding supports the use of triage. Because cotesting leads to many unnecessary colposcopy procedures,[11,13] stand-alone HPV testing also seems recommendable.

Findings of a randomised controlled trial from Finland showed no reduction in detection of invasive cervical cancer in the experimental arm during the first screening round.[24] The researchers suggested that their follow-up period (average 3·6 years when 5-year screening intervals were used) might have been too short, because many invasive cancers of the cervix are screen-detected at subsequent rounds, which was indeed the case in our study. Since the Finnish study had augmented detection rates for CIN3 and higher grades with HPV testing versus cytology in the first screening round, similar to other studies,[7] a comparable effect on cancer incidence with long-term follow-up is expected.

Our results show that at age 30–34 years, the gain in efficacy with HPV testing is at least similar to, and possibly larger than, that achieved in older women. Possible explanations for this finding are the increased proportion of adenocarcinomas in younger age groups in our pooled data or faster progression to cancer from CIN undetectable by cytology in younger women compared with older women. Moreover, low efficacy of cytology has been noted in young women.[27] Data from NTCC suggested overdiagnosis of regressive CIN with HPV screening at age 25–34 years,[6] implying that we should be cautious when screening young women in this way. Overdiagnosis was not noted in POBASCAM at age 30–33 years.[4] Independent of the reasons for such a discrepancy, our pooled data suggest a relevant gain in efficacy with HPV testing, starting from age 30 years (data at younger ages are too sparse to draw conclusions).

The recorded cumulative incidence of cervical cancer was lower 5·5 years after a negative HPV test than 3·5 years after a negative cytology result, indicating that 5-year intervals for HPV screening are safer than 3-year intervals for cytology. With HPV testing, short screening intervals are expected to result in low specificity, because recently acquired infections are mostly transient,[28] and possibly in overdiagnosis of regressive CIN. These situations can be avoided by extending the interval between screens. HPV screening every 5 years could reduce the number of unnecessary colposcopy and biopsy procedures compared with more frequent cytology, possibly also cutting costs.

In conclusion, data from follow-up analysis of four large randomised cohorts show that HPV-based cervical screening provides 60–70% greater protection against invasive cancer compared with cytology-based screening. Prevention of cancer in young women is a priority; our findings support HPV-based screening with triage at prolonged intervals, starting at age 30 years.

Contributors

GR, JD, JP, JB, CJLMM, and PG-R had the idea for and designed the study. KME, CG, JB, and ST updated follow-up data and provided them in a standardised format. ST did statistical analyses under the guidance of GR, JP, JD, and JB. GR, CJLMM, JD, PJFS, JB, HK, MA, NS, PG-R, and JP contributed to interpretation of data. GR and JD wrote the report. All authors critically revised the report.

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Conflicts of interest

CJLMM has been a member of the scientific advisory board of Qiagen, has received speaker's fees from GlaxoSmithKline, Merck, and Roche, is a shareholder of Self-Screen, a spin-off company from the VU University Medical Centre, and owns equity in Delphi Biosciences (which made the lavage self-sampling device) and Diassay (which markets a CE-labelled GP5+/6+ PCR test). His institution has received consultancy fees from Qiagen. PJFS has been an advisory board member for Gen-Probe, Roche, and GlaxoSmithKline and is a shareholder of Self-Screen. JB has received speaker's fees from Qiagen and has received an advisory fee from GlaxoSmithKline. All other authors declare that they have no conflicts of interest.

Acknowledgments

This study was funded by the European Union (PREDHICT project, FP7 grant agreement no 242061), Belgian Foundation Against Cancer, KCE-Centre d'Expertise (Brussels, Belgium), IARC (Lyon, France), The Netherlands Organisation for Health Research and Development (The Hague), and the Italian Ministry of Health. We thank all the staff for assistance in running the four randomised trials and the women who participated.

References

1 Sankaranarayanan R, Nene BM, Shastri SS, et al. HPV screening for cervical cancer in rural India. *N Engl J Med* 2009; **360:** 1385–94.

2 Naucler P, Ryd W, Törnberg S, et al. Human papillomavirus and Papanicolaou tests to screen for cervical cancer. *N Engl J Med* 2007; **357:** 1589–97.

3 Bulkmans NW, Berkhof J, Rozendaal L, et al. Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. *Lancet* 2007; **370:** 1764–72.

4 Rijkaart DC, Berkhof J, Rozendaal L, et al. Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial. *Lancet Oncol* 2012; **13:** 78–88.

5 Kitchener HC, Almonte M, Thomson C, et al. HPV testing in combination with liquid-based cytology in primary cervical screening (ARTISTIC): a randomised controlled trial. *Lancet Oncol* 2009; **10:** 672–82.

6 Ronco G, Giorgi-Rossi P, Carozzi F, et al, and the New Technologies for Cervical Cancer screening (NTCC) Working Group. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. *Lancet Oncol* 2010; **11:** 249–57.

7 Arbyn M, Ronco G, Anttila A, et al. Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. *Vaccine* 2012; **30** (suppl 5)**:** F88–99.

8 Elfgren K, Rylander E, Rådberg T, et al, and the Swedescreen Study Group. Colposcopic and histopathologic evaluation of women participating in population-based screening for human papillomavirus deoxyribonucleic acid persistence. *Am J Obstet Gynecol* 2005; **193:** 650–57.

9 Bulkmans NW, Rozendaal L, Snijders PJ, et al. POBASCAM, a population-based randomized controlled trial for implementation of high-risk HPV testing in cervical screening: design, methods and baseline data of 44,102 women. *Int J Cancer* 2004; **110:** 94–101.

10 Kitchener HC, Almonte M, Wheeler P, et al, and the ARTISTIC Trial Study Group. HPV testing in routine cervical screening: cross sectional data from the ARTISTIC trial. *Br J Cancer* 2006; **95:** 56–61.

11 Ronco G, Segnan N, Giorgi-Rossi P, et al, and the New Technologies for Cervical Cancer Working Group. Human papillomavirus testing and liquid-based cytology: results at recruitment from the new technologies for cervical cancer randomized controlled trial. *J Natl Cancer Inst* 2006; **98:** 765–74.

12 Ronco G, Giorgi-Rossi P, Carozzi F, et al, and the New Technologies for Cervical Cancer screening Working Group. Human papillomavirus testing and liquid-based cytology in primary screening of women younger than 35 years: results at recruitment for a randomised controlled trial. *Lancet Oncol* 2006; **7:** 547–55.

13 Ronco G, Giorgi-Rossi P, Carozzi F, et al, and the New Technologies for Cervical Cancer Screening Working Group. Results at recruitment from a randomized controlled trial comparing human papillomavirus testing alone with conventional cytology as the primary cervical cancer screening test. *J Natl Cancer Inst* 2008; **100:** 492–501.

14 Solomon D, Davey D, Kurman R, et al, and the Forum Group Members, and the Bethesda 2001 Workshop. The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA* 2002; **287:** 2114–19.

15 Jacobs MV, Snijders PJ, van den Brule AJ, Helmerhorst TJ, Meijer CJ, Walboomers JM. A general primer GP5+/GP6(+)-mediated PCR-enzyme immunoassay method for rapid detection of 14 high-risk and 6 low-risk human papillomavirus genotypes in cervical scrapings. *J Clin Microbiol* 1997; **35:** 791–95.

16 Parkin DM, Shanmugaratnam K, Sobin L, Ferlay J, Whelan SL. Histological groups for comparative studies: technical report no 31. Lyon: IARC, 1998.

17 Dalla Palma P, Giorgi Rossi P, Collina G, et al, and the NTCC Pathology Group. The reproducibility of CIN diagnoses among different pathologists: data from histology reviews from a multicenter randomized study. *Am J Clin Pathol* 2009; **132:** 125–32.

18 Rothman KJ. Estimation of confidence limits for the cumulative probability of survival in life table analysis. *J Chronic Dis* 1978; **31:** 557–60.

19 Breslow NE, Day NE. Statistical methods in cancer research: vol II—the design and analysis of cohort studies. Lyon: IARC Scientific Publications, 1987.

20 Greenland S. Quantitative methods in the review of epidemiologic literature. *Epidemiol Rev* 1987; **9:** 1–30.

21 Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; **21:** 1539–58.

22 Mayrand MH, Duarte-Franco E, Rodrigues I, et al, and the Canadian Cervical Cancer Screening Trial Study Group. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. *N Engl J Med* 2007; **357:** 1579–88.

23 Ogilvie GS, Krajden M, van Niekerk DJ, et al. Primary cervical cancer screening with HPV testing compared with liquid-based cytology: results of round 1 of a randomised controlled trial—the HPV FOCAL Study. *Br J Cancer* 2012; **107:** 1917–24.

24 Leinonen MK, Nieminen P, Lönnberg S, et al. Detection rates of precancerous and cancerous cervical lesions within one screening round of primary human papillomavirus DNA testing: prospective randomised trial in Finland. *BMJ* 2012; **345:** e7789.

25 Zappa M, Ciatto S. Cervix cancer: case-control studies on screening. In: Sankila R, Demaret E, Hakama M, Lynge E, Schouten LJ, Parkin DM, eds. Evaluation and monitoring of screening programmes. Brussels: Europe Against Cancer Programme, 2000: 99–118.

26 Andrae B, Kemetli L, Sparén P, et al. Screening-preventable cervical cancer risks: evidence from a nationwide audit in Sweden. *J Natl Cancer Inst* 2008; **100:** 622–29.

27 Sasieni P, Castanòn A, Cuzick J. Effectiveness of cervical screening with age: population based case-control study of prospectively recorded data. *BMJ* 2009; **339:** b2968.

28 Rodríguez AC, Schiffman M, Herrero R, et al. Longitudinal study of human papillomavirus persistence and cervical intraepithelial neoplasia grade 2/3: critical role of duration of infection. *J Natl Cancer Inst* 2010; **102:** 315–24.