MS. CLARE GILHAM (Orcid ID : 0000-0002-9477-6090)

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Triaging HPV positive women with normal cytology or low grade dyskaryosis: evidence from 10 year follow-up of the ARTISTIC trial cohort

Clare Gilham[1*], Alex Sargent[2], Julian Peto[1]

[1] London School of Hygiene & Tropical Medicine, London, UK
[2] Clinical Virology, Manchester University NHS Foundation Trust, Manchester UK

*corresponding author:
Clare Gilham
London School of Hygiene & Tropical Medicine
Keppel Street
London
WC1E 7HT
clare.gilham@lshtm.ac.uk
020 7927 2068

Running title (<60 chars): Triaging HPV+ women: evidence from the ARTISTIC cohort

Abstract

Objectives: To estimate long-term CIN3 risks associated with different triage strategies for HPV positive women with a view to reducing unnecessary referrals.

Design: The ARTISTIC trial cohort were recruited in Manchester in 2001-03 and were traced for CIN3 and cancer incidence through national registration until December 2015.

Results: The 10 year cumulative risk of CIN3+ was much higher for women with HPV16/18 infection (19.4%, 95%CI:15.8-23.8 with borderline/low-grade cytology and 10.7%, 95%CI:8.3-13.9 with normal cytology) than for those with other HPV types (7.3%, 95%CI:5.4-9.7 with borderline/low-grade cytology and 3.2%, 95%CI:2.2-4.5 with normal cytology). Among the 379 women with normal to-low-grade cytology and new HPV infection, the 10 year cumulative CIN3+ risk was 2.9% (95%CI:1.6-5.2).

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Conclusions: The CIN3 risk is confined to women with persistent type-specific HPV so partial genotyping test assays identifying HPV16/18 as a minimum are essential for efficient risk stratification. Immediate referral to colposcopy for HPV+ women with borderline or low-grade cytology and after a year if still HPV+ with normal cytology may be unnecessary. Low grade lesions can safely be retested to identify those with persistent HPV. Recall intervals of 1 year for HPV16/18 and 2 years for high risk HPV are justified for women with normal cytology and might also be considered for women with borderline/low-grade cytology. The minimal risk of invasive cancer that has progressed beyond stage 1A must be weighed against the advantages for patients and the NHS of reducing the number of referrals to colposcopy.

Funding: NIHR HTA and Cancer Research UK

Keywords: HPV, cervical screening, cervical cancer, CIN3, triage, cytology

Tweetable abstract (<280 chars): Cervical screening would be better for women and cheaper for the NHS if women with HPV and normal to low grade cytology were retested after a year or two when many infections will have cleared.

Introduction

Primary HPV testing with cytology triage of HPV positive women has been piloted at several sites in England following publication of the ARTISTIC Trial results over 3 rounds of HPV screening[1-3] and of the pooled results with other randomised trials showing a reduction in long-term cervical cancer risk.[4] (HPV refers throughout to the high-risk human papillomavirus types associated with cervical cancer.) The NHS Cervical Screening Programme (NHSCSP) is already one of the most successful in the world in preventing cervical cancer,[5, 6] and national roll out of primary HPV testing, due to be completed by the end of December 2019, should further increase sensitivity. However, colposcopy referrals will also increase without more efficient triage methods for HPV positive women. Unnecessary referral increases NHS costs and inconvenience to patients[7], and over-treatment of cervical intraepithelial neoplasia (CIN) can compromise later birth outcomes[8]. The majority of new HPV infections are transient and harmless, and about 70% disappear within a year with slower clearance thereafter[9]. Triage options to identify those whose CIN3+ risk justifies colposcopy referral include cytology, HPV genotyping and delayed retesting to identify the minority whose infection has persisted[10, 11]. Molecular triage tests such as viral or host DNA methylation are also being evaluated[12].

In England, women are invited for screening 3 yearly from age 25 to age 49 then 5 yearly from age 50 to 64. HPV positive women with borderline or worse cytology are referred to colposcopy[13]. Cytologically normal women who are HPV positive are recalled for annual cytology and HPV testing and referred to colposcopy if still HPV positive after 24 months.

The NHSCSP must strike an acceptable balance between cancer risk and the cost and patient inconvenience of excessive screening and unnecessary treatment. The current policy of reflex cytology for HPV+ samples and immediate colposcopy for high-grade cytology provides effective management for these women. Those with high-grade cytology at entry to ARTISTIC included all 10

prevalent cancers[14]. The contentious issue is how to triage HPV+ women with low grade or normal cytology. Acknowledging that there is no such thing as zero risk, Castle et al[15] proposed a triage policy based on CIN3 risk in which women are returned to routine screening with less than 2% risk, recalled earlier than the routine screening interval with 2-10% risk and referred to immediate colposcopy with over 10% risk. We have compared these thresholds with estimated CIN3 risks for alternative triage protocols including current NHSCSP policy.

Methods

Women were recruited to the ARTISTIC trial after attending routine cervical screening in Greater Manchester and randomly allocated in a ratio of 3:1 to have the HPV result revealed and acted upon, or concealed. Liquid based cytology (LBC) samples were collected for cytology and HPV testing, and followed for histopathology, between 2001 and 2009. Management of women with abnormal cytology was identical in both arms. Women in the revealed arm with normal cytology who tested HPV positive were invited for repeat HPV testing at 12 months and if still positive chose between immediate colposcopy or repeat HPV testing at 24 months. The study was extended to a third round where women on both arms were managed according to national guidelines. HPV testing was performed with Hybrid Capture 2 (Qiagen) with a cut-off of RLU/control of 1 pg/µl. Three HPV typing assays were used to genotype HC2 positive samples over various periods of the trial. Any high risk HPV type detected by any of the assays was included in the analysis[2, 3]. To recruit adequate numbers of older women, the minimum age was increased from 20 to 30 when the recruitment target for women aged 20–29 had been reached, then to 40 when there were enough aged 30–39, and so on.[1]

All participants in the study have been traced through the NHS Central Register mortality and cancer registration, including CIN3 to December 2015. The cohort was linked to the NHSCSP call-recall database to obtain lifetime cervical screening records, including reasons for ceasing screening (usually age or hysterectomy).

No patients were involved in the development of the research. The work was funded by the NIHR-HTA and Cancer Research UK.

Statistical Analysis

Women were classified hierarchically into mutually exclusive groups: HPV16 or HPV18, any other HPV without HPV16 or HPV18 (i.e. HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 or 68), and HPV negative (HC2+ with untyped HPV or HC2 negative). We have shown that the 10 year CIN3+ risk in women with normal cytology is higher among those with HPV16 (12.4%, 95%CI:9.3-16.5) than those with HPV18 (6.9%, 95%CI:3.8-12.4) but these types were grouped because in the UK 80% of invasive cervical cancers are caused by them[16]. In the ARTISTIC population, HPV16 was about twice as common as HPV18.

Infections detected in the first HPV sample taken after entry were classified as new or persistent by comparing HPV genotypes with those identified at entry. Women with both new and persistent infections were classified as persistent. This analysis was stratified by time from entry to next test, which varied by cytology and randomisation arm: those with normal cytology were invited for a repeat HPV test after 1 year in the revealed arm, while in the concealed arm they were invited for routine follow-up cytology after 3 years. All women with borderline or low-grade cytology were invited for repeat cytology after 6 months.

Cumulative CIN3+ risks were estimated by Kaplan-Meier methods modified to allow for interval-censoring. Cumulative risks were calculated from entry (round 1) and also from round 2 (defined as

the first HPV test taken 30-48 months after entry). Women were censored at date of last smear prior to hysterectomy except 608 women who were censored at last smear within the trial as they were not successfully linked to call-recall data. All analyses were censored on 30 April 2015 to allow for late cancer registration. CIN3+ histology was backdated to the beginning of follow-up (round 1 or round 2) where the histology/registration occurred within a year of the beginning of follow-up. These cases at time zero give an initial step in the K-M curve showing prevalence. Later CIN3+ histology/registration dates were backdated to the first test in the preceding year, then further backdated to the midpoint of the interval between that test and the preceding test to estimate the approximate date when they became screen-detectable. The cumulative risks therefore include CIN3s which would be diagnosed if screened at that point in time.. Cervical cancers are shown in brackets in the tables by time to cancer registration, including those preceded by CIN3 diagnosis. All analyses were programmed in STATA 15.1 (Stata Corp 2017).

Results

The analysis includes the 24,496 women who were successfully flagged for cancer incidence and mortality. From enrolment to the end of follow-up 482 CIN3 and 23 invasive cervical cancers were diagnosed from national registrations or via local histopathology data. Of the 13,591 women who had an HPV test at the follow-up round approximately 3 years after entry, 75 were diagnosed with CIN3 and 7 with invasive cervical cancer before April 2015. Average follow-up was 12 years.

There were 2,383 HR-HPV+ women with normal, borderline or low-grade cytology at entry (table 1). The majority of this triage population had normal cytology (60% in round 1, 71% in round 2), and roughly equal proportions of the remainder had borderline or low-grade cytology. The 10 year cumulative risk of CIN3+ was much higher with HPV16/HPV18 infection (table 1 and figure 1a: 19.4%, 95%CI:15.8-23.8 with borderline/low-grade cytology and 10.7%, 95% CI:8.3-13.9 with normal cytology) than with other HPV types (7.3%, 95%CI:5.4-9.7 with borderline/low-grade cytology and 3.2%, 95%CI:2.2-4.5 with normal cytology). The higher risk at round 1 in ARTISTIC was largely accounted for by higher prevalence of high-grade cytology, as the CIN3 risks stratified by cytology were similar in both rounds (Table S1). The proportion of HPV positive women who had high grade dyskaryosis was 15% aged <45 years and 11% aged 45-64 in round 1, and only 5% aged <45 and 2% aged 45-64 in round 2. Among HPV positive women with normal to low-grade cytology figures 1b and 1c show little effect of age on cumulative CIN3+ risk below age 45 ($p=0.2$) but a much lower 10 year CIN3+ rate (1.6%:4/243) at age 45 or over ($p<0.0001$ compared to women aged under 45). Among women with high grade cytology at entry there was no age difference (cumulative risks of 55.1% and 53.6% in women aged under and over age 45 respectively, $p=0.9$). In women who were HPV+ at entry the cumulative CIN3 and cervical cancer risks showed opposite effects in relation to age. Respective cumulative risks (average follow-up 12 years) for age at entry 20-29, 30-44 and 45-64 were 16.0% (236/1475), 16.0% (165/1032) and 7.0% (19/273) for CIN3 and 0.2% (3/1475), 1.0% (10/1032) and 1.5% (4/273) for cervical cancer. The 10 prevalent invasive cancers were all diagnosed within 7 months of entry, and all presented with high-grade cytology. In contrast, only 2 of the 13 incident cervical cancers diagnosed 4 or more years later had abnormal cytology at entry (1 borderline, 1 moderate).

Table 2 shows the result of the first HPV test after entry in women who were HPV positive with normal to low-grade cytology at entry. The 10 year CIN3+ risk was much lower in women with normal cytology at entry who had cleared their initial infection than in those with persisting infections. The proportion of women with normal cytology who had cleared their initial infection increased from 57% after 1 year to 74% after 3 years and 79% after 5 years, but the proportion acquiring new infections also increased (6% at 1 year, 8% at 3 years, 10% at 5 years). The majority of

those with borderline or low-grade cytology at entry returned for a repeat test after approximately 6 months, when 32% had cleared their initial infection (10 year CIN3+ risk 2.3% (95%CI:0.9-5.9) cleared, 17.7% (95%CI:14.2-21.9) persisting).

HPV16 was more likely to persist than other high risk HPV types. In HPV+ women with normal cytology at entry retested at 1 year 55% (74/135) of HPV16 infections persisted compared to 40% (196/495) of non-16 infections (p=0.002). Among women with borderline cytology at entry, 80% (140/174) of HPV16 infections persisted until the next test 6 months later compared to 63% (239/382) of non-16 infections (p<0.001).

CIN3+ risks following round 2 among women who tested HPV negative at entry (table 3) emulate a second round of HPV primary screening. The new infection rate was low: 11.9% in women aged 25-29, 4.6% in women aged 30-39 and 1.6% in women aged 40-64. Among the 387 women with a new infection, 70% had normal cytology, 28% had borderline/low-grade cytology and just 2% had high-grade cytology. Among the 269 women with normal cytology and the 110 women with borderline/low-grade cytology, the 10 year CIN3+ risks were 1.5% (95%CI:0.6-3.9) and 6.4% (95%CI:3.1-12.9) respectively. Fewer of these infections persisted compared to those detected at baseline (shown in table 2): in women with normal cytology, the 12 month persistence rate was 26% following a new infection at round 2 compared to 43% from any infection detected at baseline; and in women with borderline/low-grade cytology, the 6 month persistence rate was 40% following a new infection at round 2 compared to 68% from any infection detected at baseline.

Discussion

Main Findings

The convention that a CIN3 risk of about 10% is a reasonable threshold for immediate colposcopy[12, 15] would support the current triage strategies adopted by the NHSCSP (table 4). However partial genotyping allows further stratification implying rapid referral only of women with HPV16/18 infection and borderline/low-grade cytology (5 year risk 18%). These women constitute 16% of the HPV+ population who do not have high grade dyskaryosis (table 1, table 4). Over half of the women with borderline/low-grade cytology had non 16/18 HPV infections, of which 40% cleared after 6 months. The 5 year cumulative risk in those persisting beyond 6 months was 11.0% (95%CI:7.4%-16.2%) (table 2, table 4). This indicates that the triage protocol for those with borderline/low-grade cytology being rolled out in England is too conservative. There would be a 23% reduction in referrals if those with non-16/18 HPV types were recalled after 6 months and referred only if persistent, and extending the recall interval to 1 year would further reduce the number of referrals.

Women with normal cytology and HPV16/18 infections persisting for 1 year have a substantial CIN3+ risk and delaying recall for 1 year reduces referrals by around half (table 4). The cumulative 5 year risk in women with non 16/18 infections (2.2%) is near the threshold for routine recall recommended by Castle[15] so a 2 year delay in recalling them appears justified. A delay in retesting of a year for HPV16/18 and 2 years for other HPV types for HPV+ women with normal cytology, was adopted by some sites during the pilot but will not continue after the national roll-out.

Reflex cytology provides a good triage strategy for women undergoing their first HPV test as well as for previously screened women. However, CIN3+ rates were significantly lower in women with a previous negative HPV test (table 3). After the first round of HPV screening the HPV negative majority (89% in ARTISTIC) have a 10 year CIN3+ risk of only 0.4%. At the second round of primary HPV testing the great majority of HPV+ women who were HPV- at their previous test will have

normal cytology or the low-grade dyskaryosis associated with a new HPV infection (table 3). Their 10 year CIN3+ risk is only 1.5% with normal cytology and 6.4% with borderline or low-grade cytology, and these women could safely be recalled for repeat testing after at least a year. The HPV positive minority at round 1 have a 10 year CIN3+ risk of 14.9%, account for 83% of subsequent CIN3+ cases, and remain at much higher risk for invasive cancer beyond 10 years (Table 1: 5/2780 vs. 0/21716 cancers beyond 10 years, $p<0.0001$). The continuing clinical burden of repeated HPV testing, cytology and colposcopy will thus be largely confined to women who are HPV+ at their first test, some of whom will have long-standing precancer missed by previous cytology[14].

Strengths and limitations

We have individually linked HPV, screening and cancer registration data for this large cohort with almost complete follow-up over 14 years. The cohort included women being screened at age 20-24 as well as a large number of women aged 40-64. Results at rounds 1 and 2 reflect both the introduction of HPV testing and the much lower CIN3 rate at subsequent rounds. The main limitations are that the HC2 HPV assay was the only commercially available test in 2001 but is no longer used by the NHSCSP, and that colposcopy was rarely done without abnormal cytology and biopsies were not taken without atypical colposcopic appearance. Further evidence is needed on long-term cancer risks following negative HPV tests using modern assays to determine whether their sensitivity is adequate (see table 1 footnotes), and on biopsy of persistent HPV with normal colposcopic appearance.

Interpretation

Genotyping

Some of the approved NHSCSP assays can identify genotypes HPV16/18, but triage depends only on a positive/negative result. Partial genotyping allowing further stratification is beneficial for two reasons. First, the risk of CIN3+ is substantially higher for HPV16/18 than for other HPV types irrespective of cytology (table 1). The latter constitute the majority of women, their clearance rates are higher and delaying their repeat test will have the greatest impact on the number of colposcopy referrals. The second advantage is that persistent HPV16 or HPV18 infections can be distinguished from new infections. As the recall interval increases, so does the proportion of double positive infections which are in fact one infection clearing followed by new infection. Such type swaps occurred in 12% (37/307) of women with normal cytology after 1 year, 22% (22/98) after 3 years and 31% (14/45) at 5 years (table 2). The 5-year CIN3 risk for those with type-specific persistence is 4-5 times greater than for those with new infections, whose risk is independent of previous HPV infection that has disappeared[14].

Implications of delayed recall

Screening policy is inevitably based on CIN3 diagnosis because of the extreme rarity of invasive cancer in screened women. The primary aim of screening is to prevent cancer by detecting and treating CIN3, but a major additional benefit is early cancer diagnosis. Most cervical cancers in regularly screened women are diagnosed at FIGO stage 1[6]. The virtual absence of cancer in women aged under 20 despite the large number of HPV infections from age 14 to 19[17] suggests that a delay of 6-12 months in diagnosing CIN3 will not cause a detectable increase in cancer risk. Prevalent cancers, including all 10 in ARTISTIC, almost always present with high grade cytology. In 8,056 colposcopy referrals during the first round of the UK pilot, invasive cancer was diagnosed in two (0.035%) women following borderline/low-grade cytology and 56 (2.35%) following high-grade cytology[18]. This indicates that CIN3 is usually incipient following borderline/low-grade cytology but is

often prevalent in women with high-grade cytology. The respective CIN3 and cancer risks beyond 2.5 years from entry to ARTISTIC were 3.3% (47/1407) and 0.4% (6/1407) in HPV+ women with normal cytology and 2.7% (23/852) and 0.1% (1/852) in HPV+ women with borderline/low-grade cytology at entry (table 1). A delay in retesting of a year for HPV16/18 and 2 years for other HPV types, might therefore be considered for borderline/low-grade cytology. This would delay CIN3 diagnosis but will rarely delay cancer diagnosis. The minimal increase in the risk of developing cancer was accepted before HPV testing was introduced as a triage test for primary cytology screening around 10 years ago, and it is not clear that this risk/benefit balance should be abandoned for non 16/18 HPV infection with low-grade cytology, which is the normal manifestation of an active HPV infection, particularly in younger women[19].

Colposcopy guidelines

Part of the continuing long-term increase in cumulative incidence of CIN3+ in HPV+ women with normal cytology (figure 1a) is due to occult CIN3 undetected by cytology. In a prospective study in the Netherlands 6 of the 8 women with untreated CIN who regressed to normal cytology and colposcopy but remained HPV positive over several years had occult CIN3 detected by random biopsy[20]. Current colposcopy guidelines[21] do not recommend taking biopsies from women with normal, borderline or low-grade cytology without an atypical transformation zone. This was based on the 8% CIN2+ risk in women attending colposcopy but not eligible for biopsy whose HPV status was not known[22], which must be an underestimate for persistently HPV+ women. HPV+ women with normal cytology in the revealed arm of ARTISTIC were offered colposcopy if they were still HC2+ a year later. Among the 169 women who attended colposcopy, only half (81) of whom were biopsied, 9 CIN3s (5.3%) were diagnosed immediately and 15 CIN3s were diagnosed after further biopsies, a cumulative CIN3+ risk of 14.2%. It is not known how many more would have been diagnosed with random biopsy. A US trial reported an 8.5% prevalence of CIN3 in women with normal cytology referred to colposcopy and biopsy (directed or random) after a single positive test for HPV 16/18.[23] Women with HPV infection and normal cytology form the majority of the triage population, and there is an urgent need for better evidence to inform colposcopy management guidelines for these women.

Triage in older women

Among women aged <45 who were HPV+ at entry the long-term CIN3 risk (16.0%) was unrelated to age but was lower (7.0%) in HPV+ women aged ≥45. These findings are similar to those reported from the UK pilot (4.8% in HPV+ women aged ≥50 vs 9.1% in HPV+ women aged 30-49)[13]. This reduction could be due to physical difficulties in collecting adequate pathology[24] or under-diagnosis of occult CIN3[20] in older women. Invasive cervical cancer risk increased from 0.2% at age 20-29 to 1.0% at 30-44 and 1.5% above age 45 so a longer delay before retesting would not be safe. Irrespective of cytology post-menopausal women with persistent HPV might choose therapeutic LEEP rather than repeated screening. [13]

Conclusions

Immediate referral of all HPV+ women with borderline or low-grade cytology as recommended for the roll-out of primary HPV screening will not be cost-effective, particularly in women who have tested HPV negative in the previous round of screening. We suggest that low grade lesions can safely be retested to identify those with persistent HPV. Recall intervals of 1 year for HPV 16/18 and 2 years for the remainder have been piloted in some regions for women with normal cytology and might also be considered for women with borderline/low-grade cytology. The risk is confined to

women with a persisting type-specific HPV so partial genotyping test assays identifying HPV 16/18 as a minimum provide more efficient risk stratification. The minimal risk of invasive cancer that has progressed beyond stage 1A must be weighed against the advantages for patients and the NHS of reducing the number of referrals to colposcopy.

The major unresolved issue which will become increasing important is management of women with persistent HPV infection and normal cytology after two rounds of triage. A study should be conducted to evaluate whether random biopsy (or LEEP in post-menopausal women) is substantially more sensitive than colposcopically directed biopsy for diagnosing disease in women with type-specific HPV persistence and normal or low grade cytology. This could reduce the number of repeat tests and colposcopies that these women have to endure.

Statements

Ethical approval: Ethical approval of the study was obtained from South East Coast NRES Committee (14/LO/0627) on 13/05/2014 and Section 251 support to process confidential patient information without consent was given by UK Health Research Authority Confidentiality Advisory Group.

Availability of data: No additional data are available, in compliance with ethical and governance regulations under which this research was undertaken.

Conflicts of interest: CG received speaker fees from Roche paid to employer and a grant from NIHR-HTA to fund this project. AS attended meetings with HPV assay manufacturers; speaker fees from Roche; travel and accommodation from Roche and Abbott for training and user group meetings; Roche, Abbott, Hologic, Becton Dickinson and Cepheid provided kits for assay validation purposes; PHE provided funding to support the NHS screening laboratory activity for the pilot. JP received a grant from NIHR-HTA to fund this project.
Completed disclosure of interest forms are available to view online as supporting information.

Funding: The work was funded by the NIHR-HTA and Cancer Research UK.

Authorship: CG conducted all statistical analysis and drafted the report. AS conducted all HPV testing for the ARTISTIC trial. JP was the epidemiological PI of the ARTISTIC trial and contributed to the design of the trial, initiated the follow-up study, contributed to the statistical analysis and writing of the report.

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Table 1 – Cumulative CIN3+ risks from entry by HPV partial genotype and cytology. Invasive cervical cancers (ICC) are also shown in brackets.

			Follow-up from entry								
HPV/cytology at entry		**To Dec 2015[2]**	**2.5 year risk from entry**			**5 year risk from entry**			**10 year risk from entry**		
	n (%[1]) at baseline	**n CIN3+ (ICC)**	**n CIN3+ (ICC)**	**%**	**95% CI**	**n CIN3+ (ICC)**	**%**	**95% CI**	**n CIN3+ (ICC)**	**%**	**95% CI**
HR-HPV negative[3]	21716 (88.7%)	86 (6)[4]	29 (1)	0.13%	(0.09%, 0.19%)	50 (2)	0.23%	(0.18%, 0.30%)	80 (6)	0.37%	(0.30%, 0.47%)
HR-HPV positive	2780 (11.3%)	419 (17)	337 (9)	12.1%	(11.0%, 13.4%)	385 (9)	13.9%	(12.6%, 15.2%)	413 (12)	14.9%	(13.6%, 16.3%)
Normal cytology											
HPV16/HPV18	478 (17.2%)	51 (2)	23	4.8%	(3.2%, 7.2%)	42	8.8%	(6.6%, 11.7%)	51	10.7%	(8.3%, 13.9%)
Other HR-HPV	961 (34.6%)	34 (4)	9	0.9%	(0.5%, 1.8%)	21 (2)	2.2%	(1.4%, 3.4%)	30 (2)	3.2%	(2.2%, 4.5%)
All HR-HPV+	1439 (51.8%)	85 (6)	32	2.2%	(1.6%, 3.1%)	63 (2)	4.4%	(3.5%, 5.6%)	81 (4)	5.7%	(4.6%, 7.0%)
Borderline/low-grade cytology											
HPV16/HPV18	376 (13.5%)	75 (1)	61	16.2%	(12.9%, 20.4%)	69	18.4%	(14.8%, 22.7%)	73	19.4%	(15.8%, 23.8%)
Other HR-HPV	568 (20.4%)	41	31	5.5%	(3.9%, 7.7%)	38	6.7%	(4.9%, 9.1%)	41	7.3%	(5.4%, 9.7%)
All HR-HPV+	944 (34.0%)	116 (1)	92	9.8%	(8.0%, 11.8%)	107	11.4%	(9.5%, 13.6%)	114	12.1%	(10.2%, 14.4%)
Moderate/severe cytology											
HPV16/HPV18	250 (9.0%)	159 (5)	158 (4)	63.2%	(57.3%, 69.2%)	159 (4)	63.6%	(57.7%, 69.5%)	159 (5)	63.6%	(57.7%, 69.5%)
Other HR-HPV	147 (5.3%)	59 (4)	55 (4)	37.4%	(30.2%, 45.8%)	56 (4)	38.1%	(30.8%, 46.5%)	59 (4)	40.2%	(32.8%, 48.6%)
All HR-HPV+	397 (14.3%)	218 (10)[5]	213 (9)[5]	53.7%	(48.8%, 58.6%)	215 (9)[5]	54.2%	(49.4%, 59.1%)	218 (10)[5]	54.9%	(50.1%, 59.9%)
All women from entry	24496	505 (23)	366 (10)			435 (11)			493 (18)		

[1] percentages by cytology are given out of 2780 total HR-HPV+ women

[2]In 12 to 14 years of follow-up

[3]HC2 negative or HC2 positive with no HR-HPV detected.

[4] One invasive cancer diagnosed 4 months after entry and 4 cancers diagnosed more than 2.5 years after entry tested HC2 negative, but HR-HPV was detected on later retesting of the entry sample by PCR, so 5 of the 6 cancers shown as HR-HPV negative might have been HPV positive with a modern HPV assay.[14]

[5] One invasive cancer diagnosed 4 months after severe cytology at entry which tested HC2 positive with insufficient sample for typing is shown in the table as HR-HPV+

Table 2 – Cumulative CIN3+ risks following next HPV test in women who were HR-HPV positive with normal, borderline or low-grade cytology at entry. Invasive cervical cancer (ICC) cases are also shown in brackets.

Median time to next HPV test and HPV status	n women	n CIN3+ (ICC)	2.5 year risk from next test			5 year risk from next test			10 year risk from next test		
			n CIN3+ (ICC)	%	95% CI	n CIN3+ (ICC)	%	95% CI	n CIN3+ (ICC)	%	95% CI
Normal cytology[2]											
1 year[1]											
HPV cleared	323 (51.4%)	7[3]	3	0.9%	(0.3%, 2.9%)	4	1.2%	(0.5%, 3.3%)	7	2.2%	(1.1%, 4.6%)
HPV new infection	37 (5.9%)	0	0	0%		0	0%		0	0%	
HPV persisting	269 (42.8%)	37 (2)	25	9.3%	(6.4%, 13.4%)	32(1)	11.9%	(8.6%, 16.4%)	37(2)	13.8%	(10.2%, 18.5%)
HPV 16/18 persisting	103 (52.3%)	25 (1)	18	17.5%	(11.4%, 26.3%)	23	22.3%	(15.4%, 31.7%)	25 (1)	24.3%	(17.1%, 33.8%)
Other HRHPV persisting	166 (38.4%)	12 (1)	7	4.2%	(2.0%, 8.6%)	9 (1)	5.4%	(2.9%, 10.2%)	12 (1)	7.3%	(4.2%, 12.4%)
3 years[1]											
HPV cleared	193 (66.3%)	1[4]	1	0.5%	(0.1%, 3.6%)	1	0.5%	(0.07%, 3.6%)	1	0.5%	(0.07%, 3.6%)
HPV new infection	22 (7.6%)	1	0	0%		0	0%		1	4.6%	(0.7%, 28.1%)
HPV persisting	76 (26.1%)	17 (2)	14 (1)	18.4%	(11.4%, 29.1%)	15 (1)	19.7%	(12.4%, 30.6%)	17 (2)	22.4%	(14.6%, 33.5%)
HPV 16/18 persisting	31 (27.9%)	6	6	19.4%	(9.2%, 38.1%)	6	19.4%	(9.2%, 38.1%)	6	19.4%	(9.2%, 38.1%)
Other HRHPV persisting	45 (25.0%)	11 (2)	8 (1)	17.8%	(9.3%, 32.4%)	9 (1)	20.0%	(11.0%, 34.9%)	11 (2)	24.4%	(14.4%, 39.8%)
5 years[1]											
HPV cleared	101 (69.2%)	1[5]	1	1.0%	(0.1%, 6.8%)	1	1.0%	(0.1%, 6.8%)			
HPV new infection	14 (9.6%)	1	1	7.1%	(1.0%, 40.9%)	1	7.1%	(1.0%, 40.9%)			
HPV persisting	31 (21.2%)	11	9	29.0%	(16.3%, 48.4%)	11	35.5%	(21.5%, 54.9%)			
HPV 16/18 persisting	20 (32.8%)	9	8	40.0%	(22.4%, 64.3%)	9	45.0%	(26.5%, 68.7%)			
Other HRHPV persisting	11 (12.9%)	2	1	9.1%	(1.3%, 49.2%)	2	18.2%	(4.9%, 55.3%)			
Borderline/low-grade cytology[6]											
6 months[1]											
HPV cleared	153 (27.5%)	4	3	2.0%	(0.6%, 6.0%)	4	2.6%	(1.0%, 6.8%)	4	2.6%	(1.0%, 6.8%)
HPV new infection	24 (4.3%)	0	0			0			0		
HPV persisting	379 (68.2%)	67	59	15.6%	(12.3%, 19.6%)	65	17.2%	(13.7%, 21.3%)	67	17.7%	(14.2%, 21.9%)
HPV 16/18 persisting	178 (78.8%)	45	39	21.9%	(16.5%, 28.7%)	43	24.2%	(18.5%, 31.2%)	45	25.3%	(19.6%, 21.4%)
Other HRHPV persisting	201 (60.9%)	22	20	10.0%	(6.5%, 15.0%)	22	11.0%	(7.4%, 16.2%)	22	11.0%	(7.4%, 16.2%)

[1] Median follow up times are shown, the time range for each are for normal cytology: 1.13 years (<2 years), 3.12 years (2-4 years), 5.19 years (≥4 years) and 6.4 months (<2 years) for borderline or low-grade cytology.

[2]317 women with cytology and no HPV at follow-up (9 CIN3+) and 56 women with no follow-up recorded (no CIN3+) are excluded

[3] 1 had same type after a negative HPV test, 2 cleared and were infected with new HR HPV types and 4 had no subsequent HPV tests prior to diagnosis (sometimes several years later)

[4] HR-HPV NK as sample insufficient for typing

[5] Assumed cleared (HC2 neg) but same type at diagnosis as at entry

[6] 350 with cytology and no HPV at follow-up (39 CIN3+), 27 women with no follow-up within 2 years (6 CIN3+) and 11 women with no follow-up recorded (no CIN3+) are excluded

Table 3 – Cumulative CIN3+ risks following round 2 in women who tested HR-HPV negative[3] at entry. Invasive cervical cancer (ICC) cases are also shown in brackets.

HPV and cytology test result at round 2 in women who tested HR-HPV negative at baseline	n (%[1]) at round 2	n CIN3+ (ICC)[2]	2.5 year risk from round 2			5 year risk from round 2			10 year risk from round 2		
			n CIN3+	%	95% CI	n CIN3+ (ICC)	%	95% CI	n CIN3+ (ICC)	%	95% CI
HR-HPV negative[3]	11,945 (96.9%)	16 (2)	8	0.07%	(0.03%, 0.13%)	11 (1)	0.09%	(0.05%, 0.17%)	16 (2)	0.14%	(0.08%, 0.22%)
HR-HPV positive	387 (3.1%)	14 (1)[4]	13 (1)	3.4%	(2.0%, 5.7%)	14 (1)	3.6%	(2.2%, 6.0%)	14 (1)	3.6%	(2.2%, 6.0%)
Normal cytology											
HPV16/HPV18	75 (19.4%)	2 (1)[4]	1 (1)	1.3%	(0.2%, 9.1%)	2 (1)	2.7%	(0.7%, 10.2%)	2 (1)	2.7%	(0.7%, 10.2%)
Other HR-HPV	194 (50.1%)	2	2	1.0%	(0.3%, 4.1%)	2	1.0%	(0.3%, 4.1%)	2	1.0%	(0.3%, 4.1%)
All HR-HPV+	269 (69.5%)	4	3	1.1%	(0.4%, 3.4%)	4	1.5%	(0.6%, 3.9%)	4	1.5%	(0.6%, 3.9%)
Borderline/low-grade cytology											
HPV16/HPV18	49 (12.7%)	4	4	8.2%	(3.1%, 20.3%)	4	8.2%	(3.1%, 20.3%)	4	8.2%	(3.1%, 20.3%)
Other HR-HPV	61 (15.8%)	3	3	4.9%	(1.6%, 14.5%)	3	4.9%	(1.6%, 14.5%)	3	4.9%	(1.6%, 14.5%)
All HR-HPV+	110 (28.4%)	7	7	6.4%	(3.1%, 12.9%)	7	6.4%	(3.1%, 12.9%)	7	6.4%	(3.1%, 12.9%)
Moderate/Severe cytology											
HPV16/HPV18	4 (1.0%)	1	1	25.0%	(4.0%, 87.2%)	1	25.0%	(4.0%, 87.2%)	1	25.0%	(4.0%, 87.2%)
Other HR-HPV	4 (1.0%)	2	2	50.0%	(15.5%, 94.2%)	2	50.0%	(15.5%, 94.2%)	2	50.0%	(15.5%, 94.2%)
All HR-HPV+	8 (2.1%)	3	3	37.5%	(13.9%, 77.1%)	3	37.5%	(13.9%, 77.1%)	3	37.5%	(13.9%, 77.1%)
Total women	12,332[5]	30 (3)	21 (1)			25 (2)			30 (3)		

[1] percentages by cytology are given out of 387 HR-HPV+ women

[2] In 12 years of follow-up

[3] HC2 negative or HC2 positive with no HR-HPV detected

[4] HPV16 was detected at round 2 in this prevalent invasive cancer. This woman tested HC2 negative at entry but HPV16 was detected on later retesting of the entry sample by PCR, so it might have been HPV positive with a modern HPV assay.[14]

[5] 54 women with no cytology taken at round 2 are excluded from the table

Table 4 – Clinical outcomes of different triage strategies of HR-HPV positive women by cytology and HPV genotype

Strata	Action	Estimated proportion of referrals % (n)[1]	5 year cumulative CIN3 risk
Current policy:			
Normal cytology			
	Repeat 12 months, referral if abnormal cytology	NK	NK
	Repeat 12 months, repeat 12 months if still HPV positive[2]	49% (306/629)	10.5% (7.5%, 14.5%)
	Repeat 24 months, referral if still HPV positive	33% (97/290)[3]	15.5% (9.6%, 24.3%)
Borderline/low-grade cytology			
	Immediate referral	100%	11.4% (9.5%, 13.6%)
Alternative strategies:			
Normal HPV16/18			
(n=478, 20%)	Immediate referral	100%	8.8% (6.6%, 11.7%)
	Repeat 12 months, referral if persistent	52% (103/197)	22.3% (15.4%, 31.7%)
	Repeat 36 months, referral if persistent	28% (31/111)	19.4% (9.2%, 38.1%)
Normal Other HR-HPV			
(n= 961, 40%)	Immediate referral	100%	2.2% (1.4%, 3.4%)
	Repeat 12 months, referral if persistent	38% (166/432)	5.4% (2.9%, 10.2%)
	Repeat 36 months, referral if persistent	25% (45/180)	20.0% (11.0%, 34.9%)
Borderline/ low-grade HPV16/18			
(n=376, 16%)	Immediate referral	100%	18.4% (14.8%, 22.7%)
	Repeat 6 months, referral if persistent	79% (178/226)	24.2% (18.5%, 31.2%)
Borderline/low-grade Other HR-HPV			
(n=568, 24%)	Immediate referral	100%	6.7% (4.9%, 9.1%)
	Repeat 6 months, referral if persistent	61% (201/330)	11.0% (7.4%, 16.2%)

[1] n gives the number used to calculate the estimated proportion of referrals

[2] and normal cytology

[3] based on average 36 month recall interval (24-48 months)

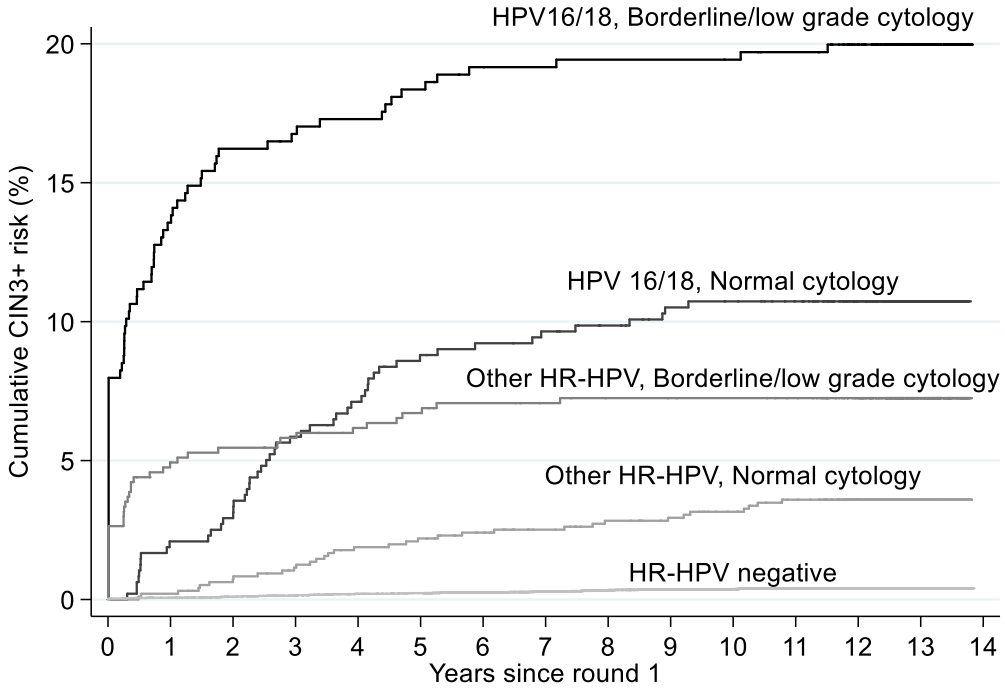

Figure 1a – Cumulative CIN3+ risk by HPV type and cytology

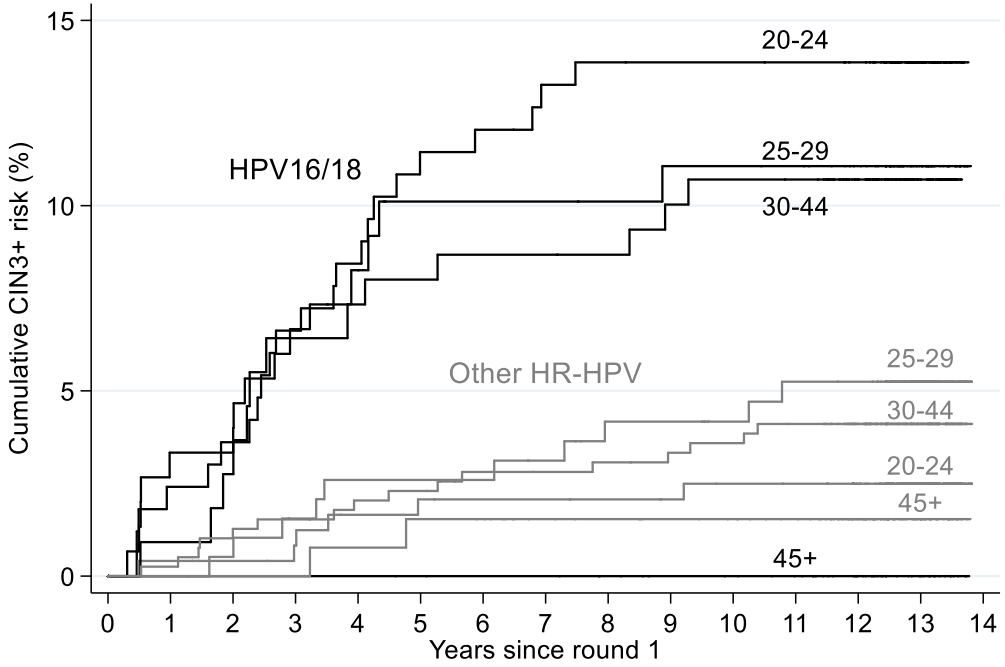

Figure 1b – Cumulative CIN3+ risk by age and HPV type in women with normal cytology at entry

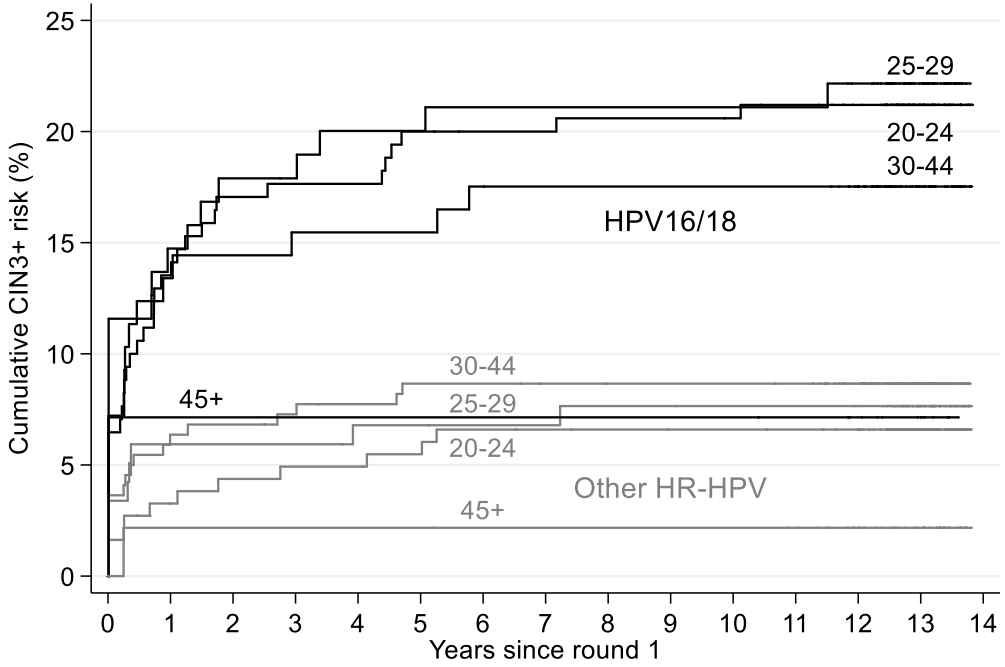

Figure 1c – Cumulative CIN3+ risk by age and HPV type in women with borderline/low-grade cytology at entry