Effect of Sequential Rounds of Cervical Cancer Screening on Management of HPV-positive Women: A 15-year Population-based Cohort Study from China

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ABSTRACT

Women are anticipated to go through more than two rounds of cervical screening in their lifetime. Human papillomavirus (HPV) testing is increasingly used as the primary cervical cancer screening test. However, triage strategies for HPV-positive women were usually evaluated at baseline screening. We assessed the effect of sequential rounds of cervical screening on several algorithms for HPV triage. A total of 1,997 women ages 35–45 years were enrolled in 1999 in Shanxi, P.R. China and followed up three times at approximately 5-year intervals. Cervical intraepithelial neoplasia (CIN) grade 2 or worse (CIN2+) prevalence by prior HPV results and performance of 12 triage algorithms with cytology, genotyping, and prior HPV were examined among 229 HPV-positive women at the fourth round. CIN2+ prevalence varied from 56.5% (95% confidence interval, 36.8%–74.4%) following 15 years HPV persistence to 3.5% (1.2%–9.9%) with an incident HPV within 15 years. Triage with cytology (with threshold of atypical squamous cells of undetermined significance) yielded positive predictive value (PPV) of 21.4% (13.8%–29.0%), entailing immediate colposcopic referral, and negative predictive value (NPV) of 97.4% (94.6%–100%), permitting retesting at short intervals. Triage with genotyping (16/18/31/33/45/52/58) or prior HPV results showed comparable performance with cytology. Among 11 triage algorithms with similar NPV to cytology, triage with prior HPV results and reflex genotyping (16/18) achieved highest PPV of 28.9% (18.8%–39.1%) and lowest colposcopy referral of 33.2% (27.4%–39.5%). HPV persistence across rounds is an effective risk stratifier in HPV-positive women. Mainstream cytology and genotyping, with or without consideration of prior HPV results, remain effective for HPV triage at fourth round.

Prevention Relevance: The study highlights the sustained effectiveness of mainstream HPV triage methods, such as cytology and genotyping, after sequential rounds of cervical screening. It also suggests that use of HPV persistence across rounds can improve management of HPV-positive women in cervical cancer screening.

Introduction

Cervical cancer screening is shifting from primary cytology screening to primary human papillomavirus (HPV) testing worldwide, with strong evidence from randomized controlled trials on the enhanced sensitivity and greater protection against high-grade cervical intraepithelial neoplasia (CIN; refs. 1–3). In the global strategy toward eliminating cervical cancer developed by the World Health Organization, a screening coverage target of 70% with HPV testing (or a similarly high sensitivity test) is proposed (4). One of the critical concerns in roll-out of an HPV-based screening program is the excessive colposcopy referral caused by high prevalence of transient HPV infections (5).

To mitigate the situation, several approaches potentially effective in triaging HPV-positive women have been evaluated, with cytology and HPV16/18 genotyping being the most routinely adopted methods in clinical practice (6). In most cases, triage evaluation was exclusively conducted in women attending their first round of screening. In fact, the prevalence of cervical lesions in subsequent rounds is expected to reduce because of early detection and intervention in previous rounds, especially in an HPV testing–based screening program (6, 7). It is likely that the subsequent rounds might also consist of a larger proportion of short-term HPV infections and minor abnormalities, which might impair the risk stratification capacity of triage tests (7, 8). Reevaluation of triage strategies is thus warranted to determine whether routinely adopted clinical actions for an abnormal test will be equally effective and efficient in managing HPV-positive women in later screening rounds.

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Furthermore, considering the fundamental role of persistent HPV infection, rather than transient infection in cervical carcinogenesis, the risk posed by a positive HPV test could vary according to how long the HPV infection has persisted (9, 10). This suggested the rationale of incorporating prior HPV results into consideration when triaging HPV-positive women in later rounds.

Previous evidence from the POBASCAM (Population Based Screening Study Amsterdam) cohort suggested that HPV-positive women in the second HPV-based screening round can be suitably managed by cytology, HPV16/18 genotyping, and the HPV results at the previous screen (11). Currently, the screening interval adopted within HPV-based screening programs has varied from 3 to 10 years (6, 12, 13). It is possible that women would go through more than two screening rounds in their whole life. Here, we conducted a *post hoc* analysis within the Shanxi Province Cervical Cancer Screening Study I (SPOCCS I) in P.R. China, based on baseline exams and three subsequent rounds of screening at approximately 5 years apart, to determine the effect of sequential rounds of screening on test performance of triaging strategies to manage HPV-positive women and use of prior HPV results to allow optimized management. We used prevalent CIN2+ as the endpoint to reflect the sequential screening effect in a real-world context, noting that not all women were compliant to treatment of detected lesions in earlier rounds.

Materials and Methods

Study population

In 1999, 1,997 nonpregnant women ages 35–45 years with no history of cervical cancer or hysterectomy were enrolled in SPOCCS I via cluster sampling in Shanxi province. Three subsequent follow-up visits were conducted in 2005, 2010, and 2014. The study was approved by Human Subjects Review Board of the Cancer Institute/Hospital, Chinese Academy of Medical Sciences (CICAMS, Beijing, P.R. China) and conducted in accordance with the Ethical Principles for Biomedical Research Involving Human Subjects (Ministry of Health of the P.R. China) and the Declaration of Helsinki. All women provided written informed consent at both baseline and follow-up visits.

Screening and clinical management

Details regarding the study procedures in SPOCCS I and some other findings have been published previously (14–18). In brief, at enrollment, each woman underwent a cervical examination and testing, including high-risk HPV (hrHPV) DNA tests (hybrid capture 2, HC2), liquid-based cytology (LBC), visual inspection with acetic acid (VIA), fluorescence spectroscopy, and colposcopy examination. Final histologic diagnosis was based on four-quadrant biopsies and endocervical curettage for every woman. During the three subsequent visits, women were screened with HC2, LBC [with threshold of atypical squamous cells of undetermined significance (ASC-US)], and VIA (except VIA at fourth visit due to low sensitivity among older women; **Fig. 1**). Participants with any positive screening result were referred to colposcopy and underwent biopsies if necessary. All collected samples were transferred to the CICAMS (Beijing, P.R. China) central laboratory for test or diagnosis. Cervical biopsy slides were prepared and read by experienced histopathologists blinded to other screening results. For unavailable pathologic diagnoses due to nonattendance at colposcopy exams after positive screening results, a combination of screening tests was used to verify the final disease status (19). Residual cytologic specimens at baseline were not available. Residual cytologic specimens in the second, third, and fourth screening rounds were stored in the refrigerator (at −80°C). In 2016, a *post hoc* HPV genotyping test was performed in these specimens, which were positive by HC2, by using PCR-based Reverse Hybridization Line Probe Assay (INNO-LiPA Extra, Innogenetics) with a SPF10 Primers Set (DDL Diagnostic Laboratory; SPF10-LiPA; ref. 16). In this analysis, genotyping results only from the fourth screening round were used.

Statistical analyses

We first analyzed CIN grade 2 or worse (CIN2+) prevalence by screening rounds. The diagram of inclusions and exclusions is shown in **Fig. 1**. A total of 1,997 women ages 35–45 years old were recruited at baseline. After excluding nonattendance and hysterectomy, 1,742, 1,455, and 1,172 women, respectively, were eligible for inclusion in the analysis of the second, third, and fourth rounds. A total of 10, four, and 17 women were additionally excluded from the second, third, and fourth rounds' analysis temporarily, because of undetermined histology results at that year, but were eligible for inclusion in subsequent screening rounds (**Fig. 1**). The endpoint, prevalent CIN2+, was defined as both incident CIN2+ cases at corresponding round and previous CIN2+ cases which persisted because of no treatment applied. Given that four-quadrant biopsies were performed at baseline and to facilitate comparability across rounds, we simulated the same screening strategy at baseline as in the subsequent rounds, that is, cotesting using HPV and cytology (at threshold of ASC-US), with either positive tests referred to colposcopy. Because the sensitivity of cotesting was 100% at baseline, all 86 CIN2+ cases detected at baseline were included into analysis when comparing overall CIN2+ reduction and testing trend on lesion grades at diagnosis across rounds.

A total of 1,126 women who were included in the analysis of the fourth screening round with determined histology results were then analyzed for HPV prevalence by prior HPV results. In 229 HPV-positive women in the fourth round, the positive predictive value (PPV) to detect CIN2+ by prior HPV results and test performance of 12 different triage algorithms were evaluated (**Table 1**). HPV history across rounds was subdivided into six categories according to HPV results in the three prior rounds to assess differences in PPV to detect CIN2+ by categories. OR was estimated using logistic regression.

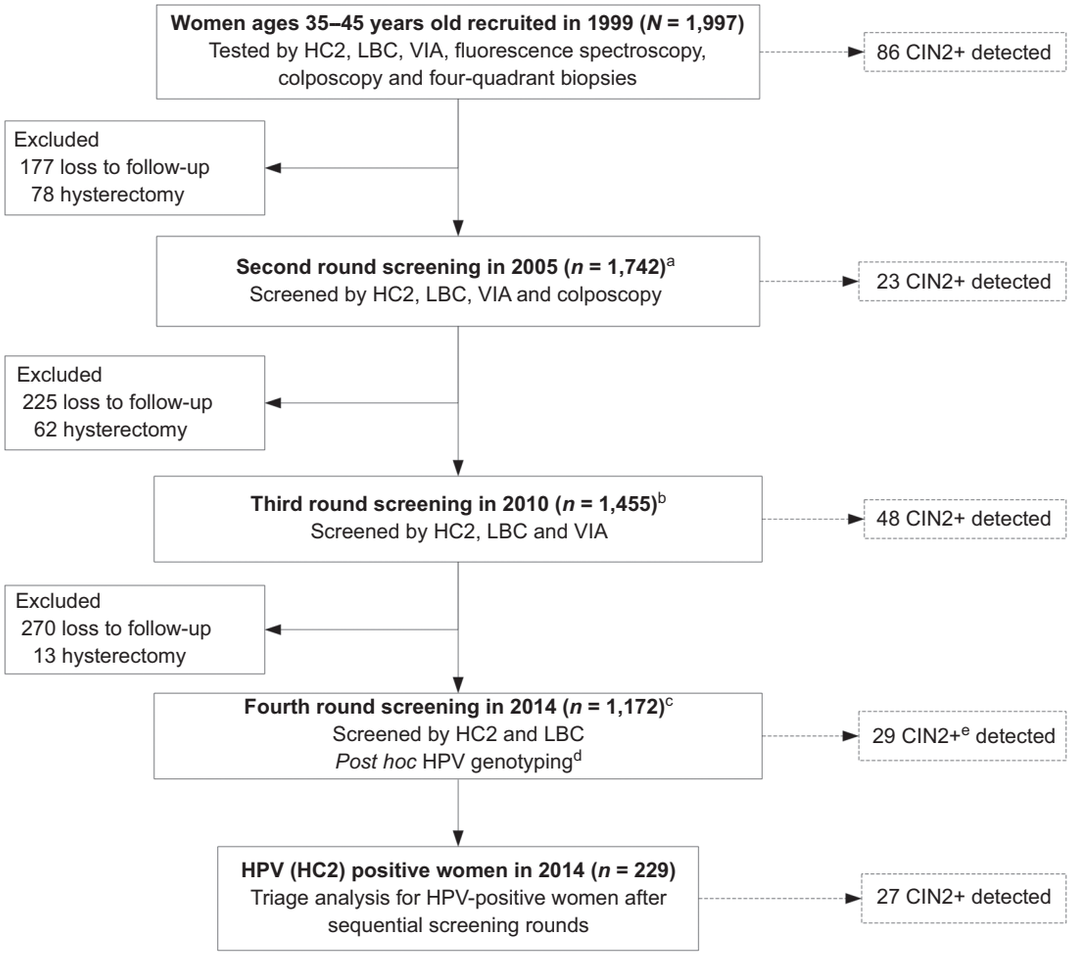

Figure 1.

Flowchart of the analytic population from SPOCCS I in four screening rounds, in P.R. China. [a]A total of 10 women were excluded from second round of analysis because of undetermined histology results in that year, but were eligible for inclusion in subsequent screening rounds. [b]A total of four women were excluded from third round of analysis because of undetermined histology results in that year, but were eligible for inclusion in subsequent screening rounds. [c]A total of 17 women were excluded from fourth round of analysis because of undetermined histology results in that year, but were eligible for inclusion in subsequent screening round. [d]HPV genotyping was performed using residual cytologic samples tested positive by HC2 in second, third, and fourth rounds of screening. In this analysis, only results from fourth screening round were used. [e]Two CIN2+ cases were HPV negative.

The test performance of multiple triage strategies among HPV-positive women was evaluated by calculating the sensitivity, specificity, PPV, and the negative predictive value (NPV) for CIN2+, with respective 95% confidence intervals (CI). In determining the test performance of triage algorithms and to make scientific decisions according to posttest result, a benchmarking concept of equal management to equal risk has been developed, with different thresholds and management options proposed in the United States and European countries (20–25). In this study, we adopted the intermediate benchmark risk levels proposed by Arbyn and colleagues, that is, a threshold of 20% for PPV to CIN2+, which is actually the absolute CIN2+ prevalence after positive triage test(s) in HPV-positive women entailing immediate referral to colposcopy, and 98% for NPV to CIN2+, which is one minus the absolute CIN2+ prevalence after negative triage test(s) in HPV-positive women ensuring return at routine screening intervals (24, 25). Relative sensitivity, specificity, PPV, and NPV in HPV-positive women were calculated using cytology with a threshold of ASC-US to triage HPV-positive women as the comparator for all other triage strategies. Colposcopy referral rate was evaluated among HPV-positive women, with women who are HPV positive and then positive by corresponding triage strategies as the numerator.

Relative sensitivity and specificity were calculated using Stata (version 14.0, StataCorp). The remaining statistical analyses were performed using R (version 3.6.0). $P < 0.05$ (two-sided) was considered to be statistically significant.

Results

Table 2 shows the CIN2+ prevalence and distribution of lesion grades at the baseline and three subsequent rounds of

Table 1. Twelve triage algorithms analyzed at fourth screening round.

	HPV triage algorithms	First triage test	Test result	Simulative clinical action
1	Cytology (with ASC-US+ threshold)	Cytology	ASC-US+	Colposcopy referral
			Normal	—
2	Genotyping (16/18)	Genotyping	HPV 16/18 positive	Colposcopy referral
			HPV 16/18 negative	—
3	Genotyping (16/18/31/33/45/52/58)	Genotyping	HPV 16/18/31/33/45/52/58 positive	Colposcopy referral
			HPV 16/18/31/33/45/52/58 negative	—
4	Genotyping (16/18) with reflex cytology	Genotyping	HPV 16/18 positive	Colposcopy referral
			HPV 16/18 negative	Cytology reflex (with ASC-US+ threshold)
5	Genotyping (16/18/31/33/45/52/58) with reflex cytology	Genotyping	HPV 16/18/31/33/45/52/58 positive	Colposcopy referral
			HPV 16/18/31/33/45/52/58 negative	Cytology reflex (with ASC-US+ threshold)
6	HPV results across rounds-a	HPV results across rounds	Persistent HPV within 4–15 years	Colposcopy referral
			Incident HPV within 4–15 years	—
7	HPV results across rounds-a with cytology reflex	HPV results across rounds	Persistent HPV within 4–15 years	Colposcopy referral
			Incident HPV within 4–15 years	Cytology reflex (with ASC-US+ threshold)
8	HPV results across rounds-a with genotyping (16/18) reflex	HPV results across rounds	Persistent HPV within 4–15 years	Colposcopy referral
			Incident HPV within 4–15 years	Genotyping (16/18) reflex
9	HPV results across rounds-a with genotyping (16/18/31/33/45/52/58) reflex	HPV results across rounds	Persistent HPV within 4–15 years	Colposcopy referral
			Incident HPV within 4–15 years	Genotyping (16/18/31/33/45/52/58) reflex
10	HPV results across rounds-b with cytology reflex	HPV results across rounds	Persistent HPV within 9–15 years	Colposcopy referral
			Persistent HPV within 4 years or Incident HPV within 4–15 years	Cytology reflex (with ASC-US+ threshold)
11	HPV results across rounds-b with Genotyping (16/18) reflex	HPV results across rounds	Persistent HPV within 9–15 years	Colposcopy referral
			Persistent HPV within 4 years or Incident HPV within 4–15 years	Genotyping (16/18) reflex
12	HPV results across rounds-b with Genotyping (16/18/31/33/45/52/58) reflex	HPV results across rounds	Persistent HPV within 9–15 years	Colposcopy referral
			Persistent HPV within 4 years or Incident HPV within 4–15 years	Genotyping (16/18/31/33/45/52/58) reflex

Abbreviation: ASC-US+, ASC-US or higher.

screening. CIN2+ prevalence was 4.3% (95% CI, 3.5%–5.3%) in women at baseline and reduced to 1.3% (95% CI, 0.9%–2.0%), 3.3% (95% CI 2.5%–4.4%), and 2.5% (95% CI, 1.8%–3.6%), respectively, in women attending their second, third, and fourth round, which were 70%, 24%, and 43% reduction, respectively, compared with that at baseline. Cases were detected at an earlier grade at later screening rounds ($P = 0.029$). Among 86 CIN2+ cases detected at baseline, half of the cases were diagnosed at CIN2 and 14% were cervical cancer. In the fourth round of screening, the proportion of CIN2 increased to 72%, while proportion of cervical cancer decreased to 3.4% (**Table 2**).

In 1,155 women attending their fourth screening round with determined histology results in 2014, HPV prevalence correlated well with HPV results in previous rounds, as shown in **Table 3**. Among women with consecutive positive HPV tests over 1–3 rounds, HPV prevalence was 34.9% (95% CI, 28.1%–42.5%) for women first positive 4 years ago, 50% (95% CI, 36.4%–63.6%) for women first positive 9 years ago, and 56.1% (95% CI, 41.0%–70.1%) for women first positive 15 years ago. These prevalence rates were 3.8, 7.1, and 9.1 times higher for women positive once, twice, or thrice through screening rounds compared with women with three consecutive negative HPV tests over 15 years. In 229 HPV-positive women in fourth round, PPV to detect CIN2+ by HPV history across rounds significantly increased from 3.5% (95% CI, 1.2%–9.9%) following an incident HPV within 15 years to 56.5% (95% CI, 36.8%–74.4%) following 15 years HPV persistence (**Table 3**).

Twelve different algorithms, with or without consideration of HPV history across rounds, were evaluated to identify the test performance in managing HPV-positive women at fourth screening round (**Table 4**). PPV estimate of HPV primary screening alone was 11.8% (95% CI, 8.2%–16.6%), supporting the need for a secondary triage strategy.

Table 2. CIN2+ prevalence after sequential rounds of cervical cancer screening.

Calendar year	Round	*N*	CIN2+ prevalence			Lesion grade distribution at diagnosis			
			n	% (95% CI)	OR (95% CI)	CIN2, *n* % (95% CI)	CIN3, *n* % (95% CI)	Cervical cancer, *n* % (95% CI)	Overall *P*[a]
1999	Baseline	1,997	86[b]	4.3 (3.5–5.3)	Reference	43 50.0 (39.5–61.3)	31 36.0 (25.6–47.3)	12 14.0 (3.5–25.2)	0.029
2005	Second	1,732	23	1.3 (0.9–2.0)	0.30 (0.18–0.48)	16 69.6 (56.5–90.8)	7 30.4 (17.4–51.7)	0 0.0 (0.0–21.3)	
2010	Third	1,451	48	3.3 (2.5–4.4)	0.76 (0.52–1.10)	26 54.2 (41.7–69.7)	19 39.6 (27.1–55.1)	3 6.2 (0.0–21.8)	
2014	Fourth	1,155	29	2.5 (1.8–3.6)	0.57 (0.37–0.88)	21 72.4 (58.6–88.6)	7 24.1 (10.3–40.3)	1 3.4 (0.0–19.6)	

[a]Asymptotic linear-by-linear association test.
[b]Given that four-quadrant biopsies were performed at baseline, the same screening strategy as in the subsequent rounds was simulated at baseline, that is, cotesting using HPV and cytology (at threshold of ASC-US), with either positive tests referred to colposcopy. Because the sensitivity of cotesting was 100% at baseline, all 86 CIN2+ cases detected in 1,997 were included into analysis when comparing overall CIN2+ reduction and testing trend on lesion grades at diagnosis across rounds.

Among the first five traditional strategies that did not include HPV information in previous screening rounds (strategy 1–5), cytology (strategy 1), as the reference test, demonstrated a PPV of 21.4% (95% CI, 13.8%–29.0%) for CIN2+, with the estimate higher than the colposcopy threshold of 20%, and NPV at 97.4% (95% CI, 94.6%–100%), with the estimate slightly below the routine screening threshold of 98% (**Table 4**). Genotyping (16/18) (strategy 2) showed a significantly higher PPV at the cost of lower NPV than cytology (relative PPV at 1.49; 95% CI, 1.06–2.10 and relative NPV at 0.96; 95% CI, 0.92–1.00). Triage with expanded genotyping (16/18/31/33/45/52/58) (strategy 3) achieved comparable performance with cytology (**Table 4**). Combining cytology with genotyping (16/18/31/33/45/52/58) (strategy 5) detected all CIN2+ cases identified by HPV tests, but dramatically reduced PPV (relative PPV at 0.70; 95% CI, 0.60–0.82).

Triage with HPV history alone (strategy 6) achieved comparable PPV and NPV with cytology (relative PPV at 1.07; 95% CI, 0.84–1.35 and relative NPV at 1.00; 95% CI, 0.96–1.04; **Table 4**). Adding reflex cytology to triage women with incident HPV (strategy 7) achieved NPV at 100%, but was also accompanied by a significantly reduced PPV (relative PPV at 0.80; 95% CI, 0.69–0.92). Adding reflex HPV16/18 genotyping (strategy 8) or reflex HPV16/18/31/33/45/52/58 genotyping (strategy 9) to triage women with incident HPV did not improve NPV and showed similar or reduced PPV compared with triage with HPV history alone (**Table 4**). Further triaging women with 4 years of HPV persistence (strategy 10–12) and only referring women with 9–15 years of HPV persistence to immediate colposcopy improved PPV, without significant loss on NPV (**Table 4**).

Colposcopy referral rates among HPV-positive women at fourth screening round are presented in **Table 4**. Among all strategies with comparable NPV estimates with cytology, triage algorithm which combined prior HPV results and reflex genotyping (16/18) in women following an incident HPV or persistent HPV within 4 years (strategy 11) achieved the lowest colposcopy referral rate at 33.2% (95% CI, 27.4%–39.5%). In contrast, expanded genotyping (16/18/31/33/45/52/58), either combined with cytology or prior HPV results, imposed the highest colposcopy referral rate (strategy 5, 78.2%; 95% CI, 72.4%–83.0% and strategy 9, 72.1%; 95% CI, 65.9%–77.5%).

Discussion

On the basis of a longitudinal cohort, our study reported the effect of four sequential rounds of screening on CIN2+ prevalence and the test performance of different triage algorithms based on cytology, genotyping, and HPV history across rounds. At fourth screening round, we found a 43% CIN2+ reduction

Table 3. HPV prevalence and PPV to detect CIN2+ in fourth screening round, stratified by HPV results across rounds.

HPV results in previous rounds			*N*	Positive HPV in fourth round		Category of HPV results across rounds[a]	PPV to detect CIN2+[b]		
2010	2005	1999	total	*n*	Prevalence (95% CI)	OR (95% CI)		*n*	PPV (95% CI)	OR (95% CI)
Pos	Pos	Pos	41	23	56.1 (41.0–70.1)	9.09 (4.73–17.76)	Persistent HPV within 15 years	13	56.5 (36.8–74.4)	35.53 (9.63–176.23)
Pos	Pos	Neg	48	24	50.0 (36.4–63.6)	7.12 (3.86–13.15)	Persistent HPV within 9 years	4	16.7 (6.7–35.9)	5.47 (1.12–29.65)
Pos	Neg	Neg	166	58	34.9 (28.1–42.5)	3.82 (2.58–5.65)	Persistent HPV within 4 years	7	12.1 (6.0–22.9)	3.75 (0.99–18.01)
Neg	Pos	Pos	95	18	18.9 (12.3–28.0)	1.66 (0.93–2.86)	Incident HPV within 4 years	0	0.0 (0.0–17.6)	0.00 (0.00–12.14)
Neg	Neg	Pos	115	21	18.3 (12.3–26.3)	1.59 (0.92–2.65)	Incident HPV within 9 years	0	0.0 (0.0–15.5)	0.00 (0.00–10.34)
Neg	Neg	Neg	690	85	12.3 (10.1–15.0)	Reference	Incident HPV within 15 years	3	3.5 (1.2–9.9)	Reference

Abbreviations: Neg, negative; pos, positive.
[a]In women HPV positive at fourth round of screening.
[b]PPV of HPV primary screening at fourth round of screening.

Table 4. Clinical performance of different triage strategies for detection of CIN2+ in HPV-positive women attending fourth round of screening ($N = 229$).

	Triage algorithm	Sensitivity % (95% CI)	Relative sensitivity (95% CI)	Specificity % (95% CI)	Relative specificity (95% CI)	PPV % (95% CI)	Relative PPV (95% CI)	NPV % (95% CI)	Relative NPV (95% CI)	Colposcopy referral % (95% CI)
1	Cytology (with ASC-US+ threshold)	88.9 (77.0–100)	Reference	56.4 (49.6–63.2)	Reference	21.4 (13.8–29.0)	Reference	97.4 (94.6–100.0)	Reference	48.9 (42.5–55.3)
2	Genotyping (16/18)	55.6 (36.8–74.3)	0.63 (0.44–0.88)	84.2 (79.1–89.2)	1.49 (1.31–1.70)	31.9 (18.6–45.2)	1.49 (1.06–2.10)	93.4 (89.8–97.0)	0.96 (0.92–1.00)	20.5 (15.8–26.2)
3	Genotyping (16/18/31/33/45/52/58)	88.9 (77–100.0)	1.00 (0.82–1.22)	48.0 (41.1–54.9)	0.85 (0.70–1.04)	18.6 (11.9–25.3)	0.87 (0.69–1.10)	97.0 (93.7–100.0)	1.00 (0.95–1.04)	56.3 (49.9–62.6)
4	Genotyping (16/18) and cytology	92.6 (82.7–100.0)	1.04 (0.96–1.13)	48.5 (41.6–55.4)	0.86 (0.80–0.93)	19.4 (12.6–26.2)	0.9 (0.83–0.99)	98.0 (95.3–100.0)	1.01 (0.99–1.02)	56.3 (49.9–62.6)
5	Genotyping (16/18/31/33/45/52/58) and cytology	100.0 (100.0–100.0)	1.13 (0.98–1.29)	24.8 (18.8–30.7)	0.44 (0.36–0.54)	15.1 (9.8–20.3)	0.70 (0.60–0.82)	100.0 (100.0–100.0)	1.03 (1.00–1.06)	78.2 (72.4–83.0)
6	HPV results across rounds-a[a]	88.9 (77–100.0)	1.00 (0.82–1.22)	59.9 (53.1–66.7)	1.06 (0.90–1.25)	22.9 (14.8–30.9)	1.07 (0.84–1.35)	97.6 (94.9–100.0)	1.00 (0.96–1.04)	45.9 (39.5–52.3)
7	HPV results across rounds-a[a] and cytology	100.0 (100.0–100.0)	1.13 (0.98–1.29)	35.1 (28.6–41.7)	0.62 (0.54–0.72)	17.1 (11.2–23.0)	0.80 (0.69–0.92)	100.0 (100.0–100.0)	1.03 (1.00–1.06)	69.0 (62.7–74.6)
8	HPV results across rounds-a[a] and genotyping (16/18)	92.6 (82.7–100.0)	1.04 (0.87–1.25)	51.0 (44.1–57.9)	0.90 (0.76–1.08)	20.2 (13.1–27.2)	0.94 (0.76–1.17)	98.1 (95.5–100.0)	1.01 (0.97–1.05)	54.1 (47.7–60.5)
9	HPV results across rounds-a[a] and genotyping (16/18/31/33/45/52/58)	92.6 (82.7–100.0)	1.04 (0.87–1.25)	30.7 (24.3–37.1)	0.54 (0.43–0.69)	15.2 (9.7–20.6)	0.71 (0.57–0.87)	96.9 (92.6–100.0)	0.99 (0.94–1.05)	72.1 (65.9–77.5)
10	HPV results across rounds-b[b] and cytology	100.0 (100.0–100.0)	1.13 (0.98–1.29)	48.0 (41.1–54.9)	0.85 (0.79–0.92)	20.5 (13.6–27.3)	0.95 (0.84–1.08)	100.0 (100.0–100.0)	1.03 (1.00–1.06)	57.6 (51.2–63.9)
11	HPV results across rounds-b[b] and genotyping (16/18)	81.5 (66.8–96.1)	0.92 (0.72–1.17)	73.3 (67.2–79.4)	1.30 (1.12–1.50)	28.9 (18.8–39.1)	1.35 (1.03–1.77)	96.7 (93.9–99.5)	0.99 (0.95–1.03)	33.2 (27.4–39.5)
12	HPV results across rounds-b[b] and genotyping (16/18/31/33/45/52/58)	92.6 (82.7–100.0)	1.04 (0.87–1.25)	43.6 (36.7–50.4)	0.77 (0.63–0.95)	18.0 (11.6–24.4)	0.84 (0.67–1.05)	97.8 (94.7–100.0)	1.00 (0.96–1.05)	60.7 (54.2–66.8)

[a]Women with persistent HPV within 4–15 years were referred to colposcopy and women with incident HPV within 4–15 years were triaged by other tests or without any interventions.
[b]Women with persistent HPV within 9–15 years were referred to colposcopy and the remaining were triaged by other tests.

compared with simulated baseline screening round. We also observed the HPV prevalence and PPV to detect prevalent CIN2+ varied dramatically depending on the duration of HPV persistence. All triage algorithms presented acceptable test performance in triaging HPV-positive women at fourth screening round in a real-life context, with varied balance on screening accuracy and efficiency.

The significant reduction of CIN2+ in subsequent screening rounds, especially when HPV-based screening was adopted, has been reported in several clinical trials and organized cervical cancer screening programs (1, 6, 7). Results from ARTISTIC trial showed CIN2+ incidence rate reduced from 2.39% in round one to 0.74% in round three (1). The reduction observed in our study, using the simulated cotesting screening strategy with HPV and cytology (ASC-US) was less evident. Lack of early recalls to positive screening results after 6–12 months in our cohort limits a dramatic reduction as observed in ARTISTIC trial. Besides, we used prevalent CIN2+, rather than incident CIN2+, as the endpoint during data analysis. Although 30%–40% CIN2+ lesions diagnosed, but not treated, could regress, some CIN2+ cases might persist or progress to more severe forms and would be detected again subsequently (26). These cases were included in this analysis to reflect a real-life effectiveness after sequential screening, considering that treatment compliance is an important factor negatively impacting the effectiveness in roll-out of an organized cervical screening program. Under the global call of cervical cancer elimination, treatment of preinvasive lesions and invasive cancer of 90% has been proposed as one of the 2030 triple-intervention coverage targets (4). Recent studies on "see-and-treat" approach using thermal ablation also showed promising effect in alleviating the situation (27, 28). The trend of detecting lesions at a lower grade was observed in this cohort and supports that, with implementation of screening rounds, many more women will be diagnosed at earlier grade when treatment is less invasive and more effective, which will reduce the likelihood of death, disability, and infertility and reinforce the effectiveness of organized cervical cancer screening (29).

As observed in our study, HPV persisted in 35%–56% of women following consecutive positive HPV test for 1–3 rounds, among whom 12%–56% were detected with CIN2+. Swedescreen trial also reported similar results in women with one or three years of HPV persistence, but cytology negative at baseline (30). These results suggest that substantial proportion of infections in women with prior positive HPV results are persistent and immediate colposcopy might be efficient after a current positive HPV test without further reflex tests (31, 32). The significant low PPV in women with incident HPV within 4–15 years might suggest that either prolonged recall interval or further triage tests are both acceptable in current HPV-positive women following a recent past negative HPV test. These findings support the potential of prior HPV result as an effective risk stratifier in HPV-positive women in subsequent screening rounds, which is also considered in the 2019 revision of the American Society for Colposcopy and Cervical Pathology Risk-Based Management Consensus Guidelines (33).

A screening test or strategy is supposed to distinguish between high-grade CIN with higher probability of progressing to invasive cancer and minor lesions that probably regress, to provide informative posttest results for different clinical actions (e.g., immediate colposcopic biopsies or treatment, return for repeated screening in 6–12 months, or return for routine screening schedule; ref. 34). The concept of equal management to equal risk provides a rational benchmarking for decision-making in clinical action thresholds, especially in the context of increasing options of emerging tests or test combinations. While the threshold at which to determine immediate colposcopy and to determine the surveillance period vary between countries. In the Netherland, the decision threshold is 2% for 4-year CIN grade 3 or worse (CIN3+) risk after negative triage (1 − NPV) and 20% for immediate CIN3+ risk after positive triage (PPV; ref. 23). In Norway, the threshold of 1% and 24% for cumulative CIN3+ risk over a period of 21 months was proposed (21, 22). While in the new consensus in the United States, a more complicated scenario is utilized (20). If the immediate risk of CIN3+ is 4% or greater, immediate management via colposcopy or treatment is indicated. If the immediate risk is less than 4%, the 5-year CIN3+ risk is examined to determine whether patients should return in 1 year (≥0.55%), 3 years (0.15–0.54%), or 5 years (<0.15%). An intermediate threshold of 1% and 10% for CIN3+ prevalence and 2% and 20% for CIN2+ prevalence was proposed by Arbyn and colleagues (24, 25). Currently in China, there has not yet been a consensus on the clinical threshold to adopt. Considering the CIN2+ prevalence endpoint used in this study and according to a previous triage analysis in Chinese women, we adopted the intermediate benchmark risk levels proposed by Arbyn and colleagues (NPV at 98% and PPV at 20%) to reevaluate triage strategies in managing HPV-positive women at the fourth screening round (24, 25, 35). It is noteworthy that PPV and NPV depend on underlying prevalence in the population screened. In practice, local experts are encouraged to define the respective threshold according to the local risk levels, tolerance to overdiagnosis or overtreatment, adherence to follow-up recommendations, and also taking the resource availability into consideration when making policy decisions.

Findings of triage performance in the fourth screening round demonstrated that test performance remained acceptable on the basis of routinely adopted clinical actions for the mainstream triage algorithms incorporating cytology and genotyping, which were in line with findings from POBASCAM based on two rounds of cervical screening (11). Cytology triage alone achieved a PPV estimate higher than 20% and an NPV estimate slightly below 98% in our study, which suggest that, after sequential rounds of screening, the clinical actions of return at short intervals is still applicable after a negative cytology. Data from Kaiser Permanente Northern California concluded that routinely adopted clinical actions for abnormal cytology in

HPV-positive women might need to be degraded following one or more sequential negative HPV tests, due to insufficient risks in later rounds (36). Our study is not powerful enough for such analysis as only three CIN2+ cases were detected in fourth round following an incident HPV. However, we shed light on the general impact of sequential rounds of screening on mainstream triaging algorithms when prior HPV results are not available. We also added value on the test performance of overall triage algorithms taking prior HPV results into consideration. Triage with single prior HPV results did not significantly improve the test performance compared with reference cytology or genotyping (16/18/31/33/45/52/58), but showed advantage in utilizing previous information without workload on additional triage tests based on all HPV-positive samples compared with cytology and 10% lower colposcopy referrals compared with genotyping (16/18/31/33/45/52/58). Further triaging women following a persistent HPV for 4 years using genotyping 16/18, actually made it the most effective and efficient option among all triage algorithms with acceptable NPV.

In clinical practice, although cytology is the most routinely adopted triage in national HPV-based screening program in high-income countries, one challenge is the wide variance on diagnostic performance among low-resource settings. There is also concern that when HPV primary screening is implemented in routine practice, unblinded cytologists may have some increased attention in reading HPV-positive slides, resulting in a so-called ascertainment bias (30). Capacity to undertake cytology might also be impaired because of limited resources and manpower allocated in quality control. Besides, reflex cytology requires additional tests based on all HPV-positive samples, which carries a considerable burden and cost, especially in low-resource settings. Genotyping outweighs on these points to enable the objective generalizability as a variety of commercially available HPV assays or kits, as well as the capacity to incorporate primary screening and triage into one test. The high expense of a compromise in sensitivity at fourth screening round for HPV16/18 genotyping alone was observed both in our cohort and in POBASCAM (8, 11), which has already been a concern at baseline screening (35). Nonetheless, such partial genotyping test is useful for risk stratification to refer HPV16/18-positive women to immediate colposcopy. Combination with other triage tests or simply expanding the genotypes to HPV16/18/31/33/45/52/58 would complement the lower NPV because of missed CIN2+ cases associated with other hrHPV types and reach comparable performance with cytology. Of note, expanding genotyping would require consideration according to population burden and attributable fraction in high-grade lesions in specific context, especially when younger birth cohorts enter into the screening program, for settings with HPV national immunization available now (37, 38).

A challenge for incorporating prior HPV results to manage HPV women is the availability of a linked digitalized screening registry and invitation system to enable individual linkage of results from different rounds of screening, which has not yet been established in many settings. Considering surveillance and quality assurance are important indicators in both organized screening program and postvaccination impact evaluation and also require a robust electronic health record system, the prospect of accounting for previous HPV results might be desirable (39). Furthermore, with the rapid development of technology and accumulation of evidence, management would be further improved with additional incorporation of individual demographics, vaccination, medical history, and genetic risk factors (40). This might facilitate to revolutionize prevention by recommending clinical action for either immediate referral or routine screening and minimize amount of recalls in short intervals.

The main strength of our study lies in that it is the longest follow-up cohort in P.R. China with four visits at approximately 5-year interval within 15 years. All women not reaching the CIN2+ endpoint were recalled at next screening round, which allowed us to have more detailed screening and histology data for sequential rounds of evaluation. However, the findings in this report are subject to four limitations. First, the relatively small sample size limits the possibility of using more stringent endpoint on CIN3+. Second, the threshold to determine the early or routine recall is better to be considered with cumulative risks within a given period. This research has only been able to consider the immediate prevalence of CIN2+ due to the absence of retest visits. A more comprehensive evaluation is warranted in longitudinal cohorts with larger sample size and retest visits. Third, the relatively narrow age range restricted more specified stratification by age group. While considering that the starting age of women for national cervical cancer screening program is 35 years old in P.R. China, these findings are supposed to reflect the real-life experience of current screening practice (41). Fourth, colposcopy and four-quadrant biopsies were performed at baseline, which are close to the full baseline disease ascertainment and associated with bias in comparison of CIN2+ prevalence across rounds. Notwithstanding the acknowledged limitations, this work complements the limited evidence regarding triage performance after sequential rounds of screening and clinical value of incorporating prior HPV results in subsequent screening rounds.

In conclusion, triage performance using cytology and genotyping based on routinely adopted clinical action remain suitable to manage HPV women after four rounds of screening. Taking prior HPV results into account helps to distinguish a persistent infection at higher risk of progression from transient infection that may probably clear and optimize the triaging algorithm in a more efficient way.

Authors' Disclosures

No disclosures were reported.

Authors' Contributions

X.-Q. Xu: Conceptualization, formal analysis, visualization, writing–original draft, writing–review and editing. **R. Rezhake:** Writing–review

and editing. **S.-Y. Hu:** Methodology, writing–review and editing. **F. Chen:** Methodology. **X. Zhang:** Methodology, writing–review and editing. **Q.-J. Pan:** Methodology, writing–review and editing. **W.-H. Zhang:** Methodology, writing–review and editing. **J.-F. Ma:** Methodology. **Y.-L. Qiao:** Writing–review and editing. **F.-H. Zhao:** Conceptualization, formal analysis, visualization, writing–original draft, writing–review and editing. **M. Cruickshank:** Writing–review and editing.

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