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Pooled analysis on the necessity of random 4-quadrant cervical biopsies and endocervical curettage in women with positive screening but negative colposcopy

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Abstract

Controversy remains over whether random cervical biopsies and endocervical curettage (ECC) should be used in women with positive screening but negative colposcopy. Our paper aims to determine the indications for random biopsies and ECC among these screened positive women.

Three thousand two hundred thirteen women with any positive screening test result but negative colposcopy, who received random 4-quadrant biopsies, were pooled from 17 population-based cervical cancer screening studies done in China from 1999 to 2008. The detection rates of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) and CIN grade 3 or worse (CIN3+) stratified by cytology and high-risk human papillomavirus (HR-HPV) status were assessed, as well as the false negative rates for CIN2+ and CIN3+ by random biopsies without ECC.

Compared with women with negative cytology and positive HR-HPV, those with atypical squamous cells of undetermined significance/low-grade squamous intraepithelial lesion (ASC-US/LSIL) and negative HR-HPV had the equivalent lower risks of CIN2+ and CIN3+, but ascending risks were observed in the groups of ASC-US/LSIL and positive HR-HPV, and atypical glandular cells/atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion/high-grade squamous intraepithelial lesion or worse (AGC/ASC-H/HSIL+). If random biopsies were only taken without ECC, 9.3% of CIN2+ and 18.5% of CIN3+ would have been missed.

For women with any positive screening but negative colposcopy, in areas with good cytological infrastructure, it was necessary to perform random biopsies plus ECC on those with cytological ASC-US/LSIL and positive HR-HPV, AGC, ASC-H, or HSIL+. In contrast, those with other results should be followed up.

Abbreviations: ADC = adenocarcinoma, AGC = atypical glandular cells, AIS = adenocarcinoma in situ, ASC-H = atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion, ASC-US = atypical squamous cells of undetermined significance, CICAMS = Cancer Institute/Hospital, Chinese Academy of Medical Sciences, CIN2+ = cervical intraepithelial neoplasia grade 2 or worse, CIN3+ = CIN grade 3 or worse, CIs = confidence intervals, ECC = endocervical curettage, HC2 = hybrid capture 2, HR-HPV = high-risk human papillomavirus, HSIL = high-grade squamous intraepithelial lesion, IARC = International Agency for Research on Cancer, LBC = liquid-based cytology, LSIL = low-grade squamous intraepithelial lesion, OR = odds ratios, PATH = Program for Appropriate Technology in Health, SCC = squamous cell carcinoma, SPOCCS = Shanxi Province Cervical Cancer Screening Study, START = Screening Technologies to Advance Rapid Testing, VIA = visual inspection with acetic acid.

Keywords: cervical intraepithelial neoplasia, colposcopy, endocervical curettage, random biopsy

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1. Introduction

Cervical cancer is the fourth most common cancer in women worldwide, with an estimated 528,000 new cases and 266,000 deaths in 2012.[1] Due to the longer duration of precancerous lesions and several available screening methods, cervical cancer is the kind of malignant tumor fit for screening. Cervical cancer has a standardized screening and diagnosis procedure—that is screening, colposcopy, and biopsy in that order. During this procedure, the colposcopy examination is an indispensable technique. However, a colposcopy examination is subjective. Its accuracy to a great extent relies on the physician's experience, and there are large variations between physicians' performances.[2] It is also affected by other factors, such as the number,[2] the size and scope of the lesions, cytological result before colposcopy, high-risk human papillomavirus (HR-HPV) testing result, the number of biopsies under colposcopy, the type of transformation zone, and so on.[3–6] Moreover, colposcopy has several limitations, such as the limited perspective for the lesions in the endocervical canal, the difficulty to identify infiltration under the presence of cervical epithelium, and the uncertainty of colposcopy image. Hence, these lead to a quantity of false negatives[7,8] and poor reproducibility of a colposcopy examination.[9,10] Because of the significant values of colposcopy in the management of cervical abnormalities, improving the sensitivity of colposcopy and detection rates for cervical cancer and precancerous lesions is one of key points when promoting screening quality.

Although some studies demonstrated that performing up to 4 random biopsies and/or endocervical curettage (ECC) increased the yield of cervical intraepithelial neoplasia grade 3 or worse (CIN3+) regardless of skill,[2,11] we cannot take multiple biopsies and ECC on every woman screened, so the indication of random biopsies and ECC need to be explored. Moreover, the most amount of controversy surrounds whether women with positive screening result but negative colposcopy (normal-appearing cervix) should take random 4-quadrant biopsies or not, or whether we should combine and use random 4-quadrant biopsies and ECC together or not. Currently, there is little direct research on the diagnostic value of random 4-quadrant biopsies and ECC in these screened positive women. Thus, we pooled the individual data from 17 population-based studies in China to explore the clinical indications related with the detection of CIN grade 2 or worse (CIN2+) or CIN3+ in women with positive screening result but negative colposcopy and to determine the necessity of random 4-quadrant biopsies and ECC in these screened positive women.

2. Materials and methods

2.1. Participants

From 1999 to 2008, Cancer Institute/Hospital, Chinese Academy of Medical Sciences (CICAMS) (Beijing, China), in collaboration with the Cleveland Clinic (Cleveland, OH), Program for Appropriate Technology in Health (PATH) (Seattle, WA), and International Agency for Research on Cancer (IARC) (Lyon, France), screened 30,371 women from 9 provinces (4 urban and 10 rural areas) of China in 17 population-based, cross-sectional, cervical cancer screening studies. The 17 studies were: 7 projects from the Shanxi Province Cervical Cancer Screening Study (SPOCCS 1; SPOCCS 2; SPOCCS 3-Xiangyuan, -Beijing, -Henan, -Xinjiang, and -Shanghai) performed with the Cleveland from 1999 to 2007,[2,12,13] 5 projects from the Screening Technologies to Advance Rapid Testing (START 2003, 2004, 2005, 2006, and 2007) performed with PATH from 2003 to 2007,[14–16] 2 projects performed with IARC in Yangcheng in 2004 (IARC-Yangcheng) and Shenzhen in 2005 (IARC-Shenzhen)[17,18], the fast HPV trial performed in 2007,[19] the Prevalence Survey performed in Jiangsu in 2008[20] and the Hybrid Capture 2 clinical trial (HC2 clinical trial) performed in 2008.[21] Eligible women were 15 to 59 years old, had sexual history, were not pregnant, had an intact uterus, and had no history of CINs, cervical cancer, or pelvic irradiation. Most women included had not been screened in the past 5 years. Written informed consent was obtained from all women. Seventeen population-based studies were approved by the institutional review boards of CICAMS and other cooperative institutions before implementation. This present paper only involved the women with positive screening result but negative colposcopy who had 4-quadrant random biopsies for analysis, some of whom received ECC concurrently.

2.2. Procedures

The main information of every individual study is listed in Table 1 and has been published in great detail.[22] All participants concurrently underwent liquid-based cytology (LBC; Sure-PathTM, BD Diagnostics, Franklin Lakes, NJ or ThinPrep, Hologic, Bedford, MA), HR-HPV DNA testing (HC2, Qiagen, Gaithersburg, MD), and visual inspection with acetic acid (VIA). Cytology results were graded according to the Bethesda system. The cytological classifications were: within normal limits (negative), atypical squamous cells of undetermined significance (ASC-US), atypical glandular cells (AGC), atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), squamous cell carcinoma (SCC); adenocarcinoma in situ (AIS), or adenocarcinoma (ADC). HPV DNA testing was performed using the high-risk probe of the HC2 test. HPV DNA positive was defined according to the manufacturer's recommended positive cut point of 1.0 relative light units per cutoff (approximately equal to 1.0 pg DNA per mL). Positivity for VIA was defined as distinct, dense, non-moveable acetowhite areas in the transformation zone near the squamocolumnar junction, visible 1 minutes after application of 3% to 5% acetic acid. All visual inspection was performed by trained Chinese gynecologists.

Women positive for any of the 3 screening tests received colposcopy and biopsy if necessary, which were performed by trained Chinese gynecologists. Electronic colposcopy (SLC-2000, Goldway, China) was used. The diagnosis of colposcopy was assessed based on color, turbidity, boundary, and outline of the white vinegar epithelium and vascular characteristics comprehensively. Each quadrant of the cervix was graded separately as negative (no lesion seen), low-grade (HPV or CIN1), high-grade (CIN2 or 3), cancer, or unsatisfied colposcopy. Colposcopy-directed biopsy was used in cases of visible lesions. When study protocol included random 4-quadrant punch biopsies (Table 1), biopsies were taken at positions of 2, 4, 8, and 10 o'clock at the squamocolumnar junction if the colposcopic examination showed no lesion in a quadrant. Cervical biopsy was performed using a 2-mm bronchoscopy biopsy instrument. In SPOCCS 1, SPOCCS 2, and START 2003 and 2004, women with 4-quadrant biopsies concurrently underwent ECC. In the other studies, ECC was performed on women if they had an unsatisfactory colposcopy (squamocolumnar junction could not be completely visualized), if the lesion extended into the endocervical canal in its entirety or was inaccessible to biopsy, or if the lesion was glandular. Histological diagnoses were graded by CIN terminology as normal (no CIN lesions), CIN1, CIN2, CIN3, SCC, AIS, or ADC. The worst histopathological result of biopsies and ECC was taken as the final diagnosis.

Table 1

Characteristics of pooled studies.

No.	Study name	Study year and location	No. screened	Age (y)	Screening tests	Follow-up procedure	Cytology/histology location and review
1	SPOCCS 1	1999; Xiangyuan County, Shanxi Province	1997	35–45	HC2 (self, physician), fluorescence test, LBC, VIA, colposcopy	All women received 4-quadrant biopsies and ECC under colposcopy	CICAMS; Blinded international review (only histology)
2	SPOCCS 2	2001–2002; Xiangyuan and Yangcheng County, Shanxi Province	8497	35–50	HC2 (self, physician), LBC, VIA, AFB	Positive VIA, positive self- or physician-HC2, or an abnormal AFB, or a positive Pap test (≥ASC-US): 4-quadrant biopsies and ECC	CICAMS
3	SPOCCS 3 - Shanxi	2006; Xiangyuan County, Shanxi Province	884	16–54	HC2 (self, physician), LBC, VIA	(1) Positive VIA or positive self- HC2: colposcopy and directed biopsy, ECC if necessary; (2) positive physician- HC2 or ≥LSIL on LBC: colposcopy and 4-quadrant biopsies, ECC if necessary	CICAMS; blinded international review (only histology)
4	SPOCCS 3 - Beijing	2006; Beijing	795	16–54	HC2 (physician), LBC, VIA	(1) Positive VIA: colposcopy and directed biopsy, ECC if necessary; (2) positive physician-HC2 and ASC-US on LBC or ≥LSIL: colposcopy and 4-quadrant biopsies, ECC if necessary	Peking University People's Hospital; blinded CICAMS and international review (for cytology, only blinded CICAMS review)
5	SPOCCS 3 - Xinjiang	2006; Yutian County, Xinjiang Uygur Autonomous region	883	16–54	HC2 (self, physician), LBC, VIA	Same as SPOCCS 3-Shanxi	Cytology: People's Hospital of Xinjiang Uygur Autonomous Region, blinded CICAMS review; histology: CICAMS; blinded international review
6	SPOCCS 3 - Henan	2006; Ximi, Henan Province	879	16–54	HC2 (self, physician), LBC, VIA	Same as SPOCCS 3-Shanxi	CICAMS; blinded international review (only histology)
7	SPOCCS 3 - Shanghai	2007; Shanghai	774	16–54	HC2 (physician), LBC, VIA	(1) Positive VIA: colposcopy and directed biopsy, ECC if necessary; (2) positive HC2 or ≥LSIL: colposcopy and 4-quadrant biopsies, ECC if necessary	Shanghai; blinded CICAMS and international review (for cytology, blinded by CICAMS only)
8	START 2003	2003; Xiangyuan County, Shanxi Province	2005	30–49	HC2 (physician), LBC, VIA	Positive VIA, positive HC2 or ≥LSIL on LBC: colposcopy and 4-quadrant biopsies and ECC	CICAMS; blinded international review
9	START 2004	2004; Xiushui County, Jiangxi Province	2499	30–49	HC2 (physician), LBC, VIA, VILI, colposcopy	(1) Positive VIA, VILI or colposcopy: 4-quadrant biopsies and ECC. (2) Negative VIA/VILI but HC2 positive or ≥LSIL on LBC: Repeat colposcopy and 4-quadrant biopsies and ECC	Jiangxi; Blinded CICAMS and international review
10	START 2005	2005; Wudu County, Gansu Province	2053	30–49	HC2 (physician), LBC, VIA, VILI	(1) Positive VIA or VILI: colposcopy and directed biopsy, and ECC if necessary. (2) Negative VIA/VILI but HC2 positive or ≥LSIL on LBC: colposcopy and 4-quadrant biopsies and ECC if necessary	Cytology: CICAMS, blinded international review. Histology: Gansu Cancer Hospital, blinded CICAMS and international review
11	START 2006	2006; Qinxian County, Shanxi Province	2500	30–49	HC2 (physician), LBC, VIA, VILI, colposcopy	Same as START 2005	CICAMS; blinded international review
12	START 2007	2007; Xiangyuan and Wuxiang County, Shanxi Province	2530	30–54	HC2 (physician), *care*HPV™, LBC, VIA, colposcopy	(1) Positive VIA or colposcopy: directed biopsy, and ECC if necessary. (2) Negative VIA but HC2 or *care*HPV™ positive or ≥LSIL on LBC:	CICAMS; Blinded international review

(*continued*)

Table 1

(continued).

No.	Study name	Study year and location	No. screened	Age (y)	Screening tests	Follow-up procedure	Cytology/histology location and review
						repeat colposcopy and 4-quadrant biopsies and ECC if necessary	
13	IARC-Shenzhen	2005; Shenzhen, Guangdong Province	1137	15–59	HC2 (physician), LBC, VIA, colposcopy	(1) Positive colposcopy: directed biopsy, and ECC if necessary; (2) negative colposcopy, but HC2 positive and ASC-US, or ≥LSIL on LBC: repeat colposcopy with directed biopsies, and ECC if necessary	Shenzhen; Blinded CICAMS review
14	IARC - Yangcheng	2004; Yangcheng County, Shanxi Province	745	15–59	HC2 (physician), fluorescence test, LBC, VIA, VILI, colposcopy	(1) Positive colposcopy or fluorescence test: directed biopsy, and ECC if necessary; (2) negative colposcopy, but HC2 positive and ASC-US, or ≥LSIL on LBC: repeated colposcopy with 4-quadrant biopsies and ECC if necessary	Yangcheng County Cancer Hospital. Blinded CICAMS and international review (for histology, only blinded CICAMS review)
15	*Fast*HPV trial	2007; Qinxian County, Shanxi Province	818	30–50	HC2 (physician), *care*HPV™, LBC, VIA, VILI	(1) Either VIA or VILI or *care*HPV™ was positive: colposcopy and directed biopsy, and ECC if necessary; (2) VIA and VILI and *care*HPV™ were negative, or colposcopy was negative, but HC2 positive or ≥LSIL on cytology: 4-quadrant biopsies, and ECC if necessary	CICAMS
16	Prevalence survey	2008; Binhai and Jintan County, Xuzhou city, Jiangsu Province	316	18–25	HC2 (physician), LBC, VIA	(1) Positive VIA: colposcopy and directed biopsy, and ECC if necessary; (2) negative VIA but ≥LSIL on LBC: colposcopy and 4-quadrant biopsies, and ECC if necessary; (3) negative VIA but HC2 positive and ≤ASC-US on LBC: colposcopy and directed biopsy, and ECC if necessary.	CICAMS
17	HC2 clinical trial	2008; Xiangyuan County, Shanxi Province	1059	30–59	HC2 (physician), LBC, VIA, VILI	(1) Positive VIA or VILI: directed biopsy, and ECC if necessary; (2) negative VIA or VILI but HC2 positive and ASC-US, or ≥LSIL on LBC: colposcopy and 4-quadrant biopsies, and ECC if necessary; (3) either HC2 positive or ASC-US on LBC: directed biopsy, and ECC if necessary	CICAMS

AFB = Ampersand's fluorescent biomolecular markers; ASC-US = atypical squamous cells of undetermined significance; CICAMS = Cancer Institute/Hospital, Chinese Academy of Medical Science; ECC = endocervical curettage; HC2 = hybrid capture 2; IARC = International Agency for Research on Cancer; LBC = liquid-based cytology; LSIL = low-grade intraepithelial neoplasia; SPOCCS = Shanxi Province of Cervical Cancer Screening; START = Screening Technologies to Advance Rapid Testing; VIA = visual inspection with acetic acid; VILI = visual inspection with Lugol's iodine.

In all studies, laboratory personnel performing HC2 were blinded to other test results, and cytopathologists and histopathologists made diagnoses without knowledge of other test results. Colposcopists were blinded to the results of all screening tests but were aware 1 test was positive, except in SPOCCS 1, in which all women received colposcopy regardless of test positivity. In all of the studies, cytology and biopsy results were read or reviewed at CICAMS, although some cytologies or biopsies were read first by local pathologists. Cytology results in 6 studies and biopsy results in 11 studies were reviewed for quality control by international experts (Table 1).

2.3. Statistical methods

Firstly, the detection rates of CIN2+ and CIN3+ were calculated from different cytological groups. The odds ratios (OR) for CIN2+ and CIN3+ detection and their 95% confidence intervals (95% CIs) were also assessed by comparing with cytological negative. Secondly, the detection rates of CIN2+ and CIN3+ in different cytological groups were calculated, stratified by HR-HPV status. In each stratified group, the OR value and 95% CI compared with those with negative cytology and positive HR-HPV was evaluated. Thirdly, we used McNemar χ^2 test to compare the detection rates of CIN2+ or CIN3+ by random 4-quadrant biopsies with or without ECC in women undergoing random 4-quadrant biopsies and ECC concurrently, and calculated the false negative rate for CIN2+ or CIN3+ by random biopsies alone without ECC. A P value less than .05 (2-sided) was considered to be statistically significant. SAS 9.2 (SAS Institute, Cary, NC) was used for all analyses.

3. Results

A total of 30,371 women were screened in 17 population-based studies. Of them, 27,158 women were excluded due to negative screening results (21,081, 77.6%), abnormal colposcopy or lack of colposcopy examination (4647, 17.1%), lack of random 4-quadrant biopsies (1379, 5.1%), and unsatisfactory cytology, or missing HR-HPV results (51, 18.8%). Thereby, 3213 women with any positive screening result (VIA, LBC, or HR-HPV) but negative colposcopy were included for final analysis, including 503 with 4-quadrant random biopsies but no ECC and 2710 with both random 4-quadrant biopsies and ECC (Fig. 1). Among these 3213 women, 77.3% (2484/3213) had postive HR-HPV, 53.7%

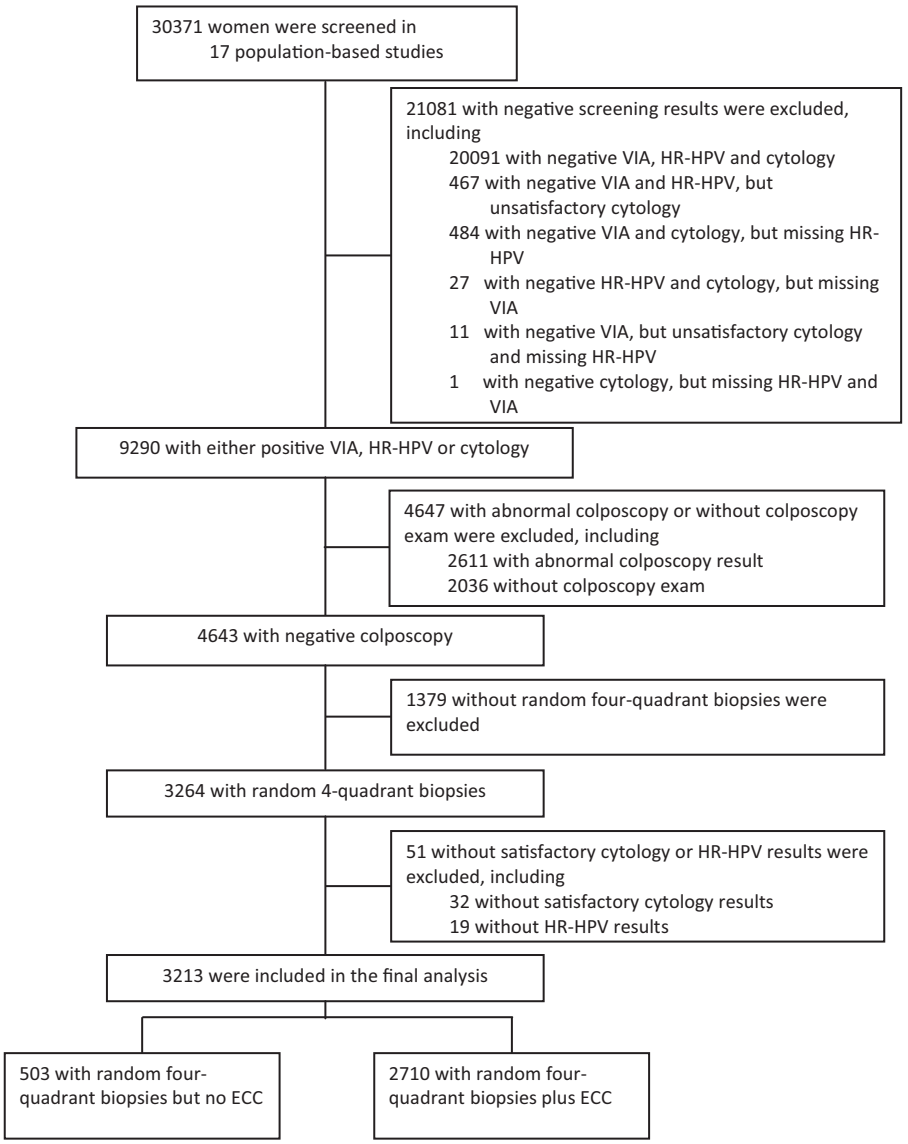

Figure 1. Flowchart of study participants. ECC = endocervical curettage, HR-HPV = high-risk human papillomavirus, VIA = visual inspection with acetic acid.

Table 2

Concordance between cytology diagnoses and disease outcomes in women with abnormal screening results and negative colposcopy.

Cytology diagnoses	Normal	CIN1	CIN2	CIN3	Cancer	Total	CIN2+		CIN3+	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	OR (95%CI)	n (%)	OR (95%CI)
Negative	1346 (90.5)	114 (7.7)	22 (1.5)	5 (0.3)	0 (0.0)	1487 (100.0)	27 (1.8)	1	5 (0.3)	1
ASC-US/LSIL	1088 (76.9)	249 (17.6)	54 (3.8)	23 (1.6)	1 (0.1)	1415 (100.0)	78 (5.5)	3.2 (2.0–4.9)	24 (1.7)	5.1 (1.9–13.4)
AGC/ASC-H/HSIL+*	133 (42.8)	54 (17.4)	63 (20.3)	56 (18.0)	5 (1.6)	311 (100.0)	124 (39.9)	35.9 (23.0–55.9)	61 (19.6)	72.3 (28.8–181.8)
Total	2567 (79.9)	417 (13.0)	139 (4.3)	84 (2.6)	6 (0.2)	3213 (100.0)	229 (7.1)		90 (2.8)	

AGC = atypical glandular cells, ASC-H = atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion, ASC-US = atypical squamous cells of undetermined significance, CI = confidence interval, CIN = cervical intraepithelial neoplasia, CIN2+ = cervical intraepithelial neoplasia grade 2 or worse, CIN3+ = cervical intraepithelial neoplasia grade 3 or worse, HSIL = high-grade squamous intraepithelial lesion, LSIL = low-grade squamous intraepithelial lesion, OR = odds ratio.

* HSIL+ included HSIL and cancer.

(1726/3213) had abnormal cytology, and 12.9% (416/3213) had abnormal VIA. The average age of the women included was 40.7 ± 5.3 years (range: 20–57 years), and 0.7% (24/3213), 94.9% (3048/3213), and 4.4% (141/3213) were in 20 to 24, 25 to 49, ≥50 age groups, respectively. Most women were married (97.9%, 3144/3212) and had never smoked (97.0%, 3117/3211). 9.4% (293/3125) were menopause and only 1.1% (35/3211, 2 with missing data) had ever used oral contraceptive.

Of the 3213 women included in the analysis, 2567 (79.9%) were diagnosed as histological negative, 417 (13.0%) as CIN1, 139 (4.3%) as CIN2, 84 (2.6%) as CIN3, and 6 (0.2%) as cancer. The total detection rates of CIN2+ and CIN3+ were 7.1% (229/3213) and 2.8% (90/3213), respectively, which were increased with the severity of cytological results. Compared with cytological negative, ASC-US/LSIL, AGC/ASC-H/HSIL+, respectively had 3.2 (95%CI: 2.0–4.9) and 35.9 (95%CI: 23.0–55.9) times higher risks of CIN2+, and had 5.1 (95%CI: 1.9–13.4) and 72.3 (95%CI: 28.8–181.8) times higher risks of CIN3+ (Table 2). 12.9% (416/3213) of women had abnormal VIA, in which the detection rates of CIN2+and CIN3+ had no significant differences from those with negative VIA (CIN2+: 7.0% vs. 7.2%, CIN3+: 3.1% vs. 2.8%, all $P > .05$).

In 2484 women with positive HR-HPV but negative colposcopy, the detection rates of CIN2+ and CIN3+ were 8.9% (222/2484) and 3.5% (88/2484), respectively, which were significantly higher than those in 729 women with negative HR-HPV (CIN2+: 1.0% [7/729]; CIN3+: 0.3% [2/729], all $P < .0001$) (OR: 10.1 [95% CI: 4.7–21.6] for CIN2+; 13.4 [95% CI: 3.3–54.4] for CIN3+). Compared with women with negative cytology and positive HR-HPV, those with cytological ASC-US/LSIL and negative HR-HPV had the equivalent lower risk of CIN2+ and CIN3+ (OR:0.4 [95%CI: 0.1–1.1] for CIN2+, 0.5 [95%CI: 0.06–4.4] for CIN3+), but the ascending risks of CIN2+ and CIN3+ were observed in the groups of ASC-US/LSIL and positive HR-HPV, AGC/ASC-H/HSIL+ and negative HR-HPV, and AGC/ASC-H/HSIL+ and positive HR-HPV (OR: 4.1 [95%CI: 2.6–6.4], 5.0 [95%CI: 1.4–17.4], and 35.4 [95%CI: 22.6–55.4] for CIN2+, 6.5 [95%CI: 2.5–17.3], 8.5 [95%CI: 1.0–75.2], and 69.7 [95%CI: 27.7–175.5] for CIN3+) (Table 3).

Of 2710 women with negative colposcopies and 4-quadrant biopsies plus ECC, 540 cases were confirmed as CINs or cancer, and 40 (7.4%, 40/540) cases had worse ECC diagnoses than random biopsies. Of the 40 cases, 14 CIN1, 10 CIN2, 14 CIN3, and 2 micro-invasive cervical cancers were diagnosed by ECC. Nearly, all 40 cases were HR-HPV positive, except for 1 which was cytological ASC-US and diagnosed as CIN1 by ECC. The detection rate of CIN2+ or CIN3+ by random 4-quadrant biopsies plus ECC was higher than that by random 4-quadrant biopsies alone (CIN2+: 7.2% vs. 6.5%; CIN3+: 3.0% vs. 2.4%; all $P < .0001$). If only random 4-quadrant biopsies were taken, the false negative rates for CIN2+ and CIN3+ would have, respectively, been 9.3% (18/194) and 18.5% (15/81) (Tables 4 and 5).

4. Discussion

Our study which included 3213 women with positive screening result but negative colposcopy from 17 population-based studies performed in China, enabled us to evaluate the risks of CIN2+ or

Table 3

Concordance between cytology diagnoses and disease outcomes stratified by high-risk HPV status in women with abnormal screening results and negative colposcopy.

Cytology results	HR-HPV	Normal	CIN1	CIN2	CIN3	Cancer	Total	CIN2+		CIN3+	
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	OR (95%CI)	n (%)	OR (95%CI)
Negative	NEG	200 (98.0)	4 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	204 (100.0)	0 (0.0)	—	0 (0.0)	—
	POS	1146 (89.3)	110 (8.6)	22 (1.7)	5 (0.4)	0 (0.0)	1283 (100.0)	27 (2.1)	1	5 (0.4)	1
ASC-US/LSIL	NEG	455 (92.1)	35 (7.1)	3 (0.6)	1 (0.2)	0 (0.0)	494 (100.0)	4 (0.8)	0.4 (0.1–1.1)	1 (0.2)	0.5 (0.06–4.4)
	POS	633 (68.7)	214 (23.2)	51 (5.5)	22 (2.4)	1 (0.1)	921 (100.0)	74 (8.0)	4.1 (2.6–6.4)	23 (2.5)	6.5 (2.5–17.3)
AGC/ASC-H/HSIL+*	NEG	26 (83.9)	2 (6.5)	2 (6.5)	0 (0.0)	1 (3.2)	31 (100.0)	3 (17.6)	5.0 (1.4–17.4)	1 (5.9)	8.5 (1.0–75.2)
	POS	107 (38.2)	52 (18.6)	61 (21.8)	56 (20.0)	4 (1.4)	280 (100.0)	118 (42.6)	35.4 (22.6–55.4)	59 (21.3)	69.7 (27.7–175.5)
Total	NEG	681 (93.4)	41 (5.6)	5 (0.7)	1 (0.1)	1 (0.1)	729 (100.0)	7 (1.0)	1	2 (0.3)	1
	POS	1886 (75.9)	376 (15.1)	134 (5.4)	83 (3.3)	5 (0.2)	2484 (100.0)	222 (8.9)	10.1 (4.7–21.6)	88 (3.5)	13.4 (3.3–54.4)

AGC = atypical glandular cells, ASC-H = atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion, ASC-US = atypical squamous cells of undetermined significance, CI = confidence interval, CIN = cervical intraepithelial neoplasia, CIN2+ = cervical intraepithelial neoplasia grade 2 or worse, CIN3+ = cervical intraepithelial neoplasia grade 3 or worse, HR-HPV = high-risk human papillomavirus, HSIL = high-grade squamous intraepithelial lesion, LSIL = low-grade squamous intraepithelial lesion, OR = odds ratio.

* HSIL+ included HSIL and cancer.

Table 4

Comparison of histopathological diagnoses between random 4-quadrant biopsies and ECC and only random biopsy in women with 4-quadrant biopsies and ECC concurrently*.

	Normal	CIN1	CIN2	CIN3	Cancer	Total	CIN2+	CIN3+
Random 4-quadrant biopsies, n (%)	2199 (81.1)	335 (12.4)	110 (4.1)	62 (2.3)	4 (0.1)	2710 (100.0)	176 (6.5)	66 (2.4)
Random 4-quadrant biopsies + ECC, n (%)	2170 (80.1)	346 (12.8)	113 (4.2)	75 (2.8)	6 (0.2)	2710 (100.0)	194 (7.2)	81 (3.0)
Random 4-quadrant biopsy/random biopsies + ECC, %	101.3	96.8	97.3	82.7	66.7	100.0	90.7	81.5

ASC-H = atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion, ASC-US = atypical squamous cells of undetermined significance, CIN = cervical intraepithelial neoplasia, CIN2+ = cervical intraepithelial neoplasia grade 2 or worse, CIN3+ = cervical intraepithelial neoplasia grade 3 or worse, ECC = endocervical curettage, HR-HPV = high-risk human papillomavirus, HSIL = high-grade squamous intraepithelial lesion, LSIL = low-grade squamous intraepithelial lesion.

* Forty cases had worse ECC diagnoses than 4-quadrant biopsies, including 14 CIN1 (5 cytological negative, 4 ASC-US, 3 LSIL, and 2 HSIL), 10 CIN2 (1 cytological negative, 2 ASC-US, 3 ASC-H, 2 LSIL, 1 HSIL, and 1 Cancer), 14 CIN3 (2 cytological negative, 1 ASC-US, 4 LSIL, and 7 HSIL), and 2 micro-invasive cervical cancers (2 HSIL). Nearly all of them were HR-HPV positive, except 1 which was cytological ASC-US and diagnosed as CIN1 by ECC.

CIN3+ in the groups stratified by different screening results, and to further determine the clinical value of random 4-quadrant biopsies plus ECC in this screened positive population. The study demonstrated that the prevalences of CIN2+ and CIN3+ were 7.1% and 2.8%, respectively, detected by random 4-quadrant biopsies and ECC, and the risks of CIN2+ and CIN3+ among those with both ASC-US/LSIL and positive HR-HPV, or AGC/ASC-H/HSIL+, were significantly higher than the ones among those with cytological negative, or ASC-US/LSIL and negative HR-HPV. It was also confirmed that ECC could contribute to increasing the detection rates of CIN2+ and CIN3+. Therefore, performing random 4-quadrant biopsies plus ECC on high-risk populations among women with positive screening result but negative colposcopy could decrease the false negative rate for CIN lesions.

The sensitivity of colposcopy for CIN detection is influenced by many factors. It was reported that the false negative rate of colposcopy was 14%, and 0% to 8.9% of invasive carcinoma was potentially under-diagnosed, with an average of 2%.[23,24] The misdiagnosis rate for CIN2+ in women diagnosed as CIN1 by directed biopsy under colposcopy was 19% to 55%.[25,26] The accuracy of colposcopy impression was highly related with the number of cervical quadrants with lesions, and the accuracy of 1, 2, and 3/4 quadrant involved was, respectively, 13%, 44%, and 85%.[2] The possible reasons for false negative colposcopy were smaller lesion, thinner lesion epithelium, lower nuclear density, and/or no obvious boundary around the thinner epithelium.[27] Our pooled analysis found that 20.1% of CIN were missed by colposcopy, including 4.3% CIN2, 2.6% CIN3, and 0.2% invasive cervical cancer. These findings indicate that even if colposcopy was negative, the woman still had the risk of CIN or invasive cervical cancer, especially the CIN2+ lesions with smaller size and thinner epithelium, which could not be identified even by the experienced colposcopists and were likely to cause false negatives.[6,27,28]

Table 5

Proportions of random 4-quadrant biopsies and/or ECC showing CIN2+ and CIN3+.

	CIN2+, n (%)	CIN3+, n (%)
Random 4-quadrant biopsies Yes/ECC Yes*	13 (16.0)	32 (16.5)
Random 4-quadrant biopsies Yes/ECC No	53 (65.4)	144 (74.2)
Random 4-quadrant biopsies No/ECC Yes	15 (18.5)	18 (9.3)
Random 4-quadrant biopsies and/or ECC Yes	81 (100.0)	194 (100.0)

CIN = cervical intraepithelial neoplasia, CIN2+ = cervical intraepithelial neoplasia grade 2 or worse, CIN3+ = cervical intraepithelial neoplasia grade 3 or worse, ECC = endocervical curettage.

* "Yes" means the histological result was CIN2+ or CIN3+, and "No" means less than CIN2 or CIN3, oppositely.

The severity of cytological abnormality and HPV status before colposcopy were the risk factors of false negatives for high-grade lesions by colposcopy.[11,26,29,30] Alvarez RD reported that 84% to 97% of CIN2 were under-diagnosed in CIN1 but cytological HSIL.[29] The multivariate analysis showed that previous cytological HSIL/AGC was an independent risk factor (OR = 4.67).[26] Pretorius RG found that besides colposcopy-directed biopsy, 17.6% CIN2+ could still be detected in cytological HSIL or cancer group by random biopsies, but 1.7% CIN2+ in ASC-US and positive HR-HPV group ($P < .001$).[11] Our pooled study showed that the risk of CIN2+ or CIN3+ also increased with the severity of cytological abnormality in women with negative colposcopy. Meanwhile, our study also indicated women with negative cytological and positive HR-HPV, or ASC-US/LSIL and negative HR-HPV had a relatively lower absolute risk of CIN2+ or CIN3+. This finding implies that these women had a lower clinical value to perform random 4-quadrant biopsies plus ECC, and were fit for follow-up. However, women with cytological ASC-US/LSIL and positive HR-HPV, AGC, ASC-H or HSIL+ had a higher absolute risk of CIN2+ or CIN3+, and should be given more attention and receive 4-quadrant biopsies plus ECC.[31] These actions are in accord with the attention given to those with abnormal cytology in the recent guideline of American Society of Colposcopy & Cervical Pathology, which recommend that women with cytological negative but positive HR-HPV, or ASC-US/LSIL and negative HR-HPV, should be followed up.[32] Our findings also confirmed the necessity of ASC-US/LSIL triage with HR-HPV.

Several studies showed that random 4-quadrant biopsies plus ECC could greatly increase the detection rate of CIN2+ or CIN3+.[14,33,34] Pretorius RG found that random biopsy can detect 22.9% to 37.4% of CIN2+ lesions, and 2.4% to 5.5% of CIN2+ was detected by ECC alone.[35] He also suggests that in women with negative colposcopy, random biopsy was helpful in improving the detection rate of CIN2+, and ECC should be performed at the same time, even if the colposcopy was satisfied.[11] Other studies also recommended performing random biopsies and ECC in non-pregnant women aged >25 years old.[36,37] Our study showed that random 4-quadrant biopsies plus ECC could significantly raise the detection rate of CIN2+ or CIN3+ compared with random biopsies alone in women with negative colposcopy, and 9.3% of CIN2+ and 18.5% of CIN3+ would have been missed without ECC.

The current study has both strengths and limitations. The study pooled more than 3200 individual data with positive screening result and negative colposcopy from 17 population-based studies, which was conducted in 9 provinces and 14 field sites and had a larger sample size compared with other reported

studies. Moreover, our study had the strict quality control for biopsy and cytology reading. The sensitivity of cytology in general women in our study (80.5% for CIN2+, test positivity at LSIL or worse[19]) was higher than the average level reported in a European and North American pooled study[38] (53.0% for CIN2+, test positivity at ASC-US or worse). This ensures the good internal validity of our study. On the other hand, the high accuracy of cytology limits the generalization of our research conclusion to the regions with poor cytological infrastructure; this is the limitation of our study. Therefore, the results of cytology and HPV testing are valuable to determine whether the random 4-quadrant biopsies and ECC are necessary among women with positive screening result but negative colposcopy in settings with good cytological infrastructure, otherwise a HR-HPV testing result, which is objective, should mainly be considered in settings without good cytological diagnosis level.

5. Conclusion

In summary, for women with any positive screening result but negative colposcopy, the risk of CIN2+ or CIN3+ was highly correlated with cytology and HR-HPV results. In the areas with good cytological infrastructure, it was necessary to perform random 4-quadrant biopsies plus ECC on women with cytological ASC-US/LSIL and positive HR-HPV, AGC, ASC-H, or HSIL+. In contrast, immediate random biopsies and ECC could not be performed on women with cytological negative and positive HR-HPV, or ASC-US/LSIL and negative HR-HPV, who should be followed up. For women in areas with poor cytological infrastructure, HR-HPV testing result and other potential biomarkers, for example, HPV genotyping, P16/Ki67 and E6/E7 oncoprotein could be considered to decide whether immediate random biopsies plus ECC or not. This strategy probably helps maximize screening benefits and minimize potential harms. Further studies on cost-effective analysis and prospective trials are required to test the role of random biopsies and ECC.[39]

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