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, CI = 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
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.[12] 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.[13] It is also affected by other factors, such as the number,[14] 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.[15-16] 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[17,18] 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-Xiangyang, 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 1.0pg 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 unsatisfactory 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, VA, 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 WA, positive self- or physician-HC2, or an abnormal AFB, or a positive Pap test (≥ASC-US): 4-quadrant biopsies and ECC if necessary | CICAMS |
| 3   | SPOCCS 3 - Shanxi | 2006; Xiangyuan County, Shanxi Province | 884          | 16–54   | HC2 (self, physician), LBC, VA | (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 | Same as SPOCCS 3-Shanxi |
| 4   | SPOCCS 3 - Beijing | 2006; Beijing | 795          | 16–54   | HC2 (physician), LBC, VA | (1) Positive VIA: colposcopy and directed biopsy, ECC if necessary; (2) positive physician-HC2 and ≥LSIL 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, VA | 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, VA | Same as SPOCCS 3-Shanxi | CICAMS; blinded international review (only histology) |
| 7   | SPOCCS 3 - Shanghai | 2007; Shanghai | 774          | 16–54   | HC2 (physician), LBC, VA | (1) Positive WA: 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, VA | Positive WA, positive HC2 or ≥LSIL on LBC: colposcopy and 4-quadrant biopsies and ECC if necessary | CICAMS; blinded international review |
| 9   | START 2004 | 2004; Xuishu County, Jiangxi Province | 2499         | 30–49   | HC2 (physician), LBC, VA, MIU, colposcopy | (1) Positive WA, MIU or colposcopy; 4-quadrant biopsies and ECC. (2) Negative WA/MIU but HC2 positive or ≥LSIL on LBC: Repeat colposcopy and 4-quadrant biopsies and ECC if necessary | Jiangxi; Blended CICAMS and international review |
| 10  | START 2005 | 2005; Wudu County, Gansu Province | 2053         | 30–49   | HC2 (physician), LBC, VA, MIU | (1) Positive WA or MIU: colposcopy and directed biopsy, and ECC if necessary. (2) Negative WA/MIU 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, VA, MIU, colposcopy | Same as START 2005 | CICAMS; blinded international review |
| 12  | START 2007 | 2007; Xiangyuan and Wuxiang County, Shanxi Province | 2530         | 30–54   | HC2 (physician), caripPV™, LBC, VA, colposcopy | (1) Positive WA or colposcopy: directed biopsy, and ECC if necessary. (2) Negative WA but HC2 or caripPV™ positive or ≥LSIL on LBC | CICAMS; Blinded international review |

(continued)
| No. | Study name       | Study year and location                          | No. screened | Age (y) | Screening tests | Follow-up procedure                                                                 | Cytology/histology location and review |
|-----|------------------|--------------------------------------------------|--------------|---------|-----------------|------------------------------------------------------------------------------------|----------------------------------------|
| 13  | IARC-Shenzhen    | 2005; Shenzhen, Guangdong Province               | 1137         | 15–59   | HC2 (physician), LBC, VIA, colposcopy | repeat colposcopy and 4-quadrant biopsies and ECC if necessary                    | Shenzhen; Blinded CICAMS review        |
|     |                  |                                                  |              |         |                 | (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 |                                      |
| 14  | IARC - Yangcheng | 2004; Yangcheng County, Shanxi Province          | 745          | 15–59   | HC2 (physician), fluorescence test, LBC, VIA, VIU, 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  | FastHPV trial    | 2007; Qinxian County, Shanxi Province            | 818          | 30–50   | HC2 (physician), careHPV™, LBC, VIA, VIU | (1) Either VIA or VIU or careHPV™ was positive: colposcopy and directed biopsy, and ECC if necessary; (2) VIA and VIU and careHPV™ 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 WA: colposcopy and directed biopsy, and ECC if necessary; (2) negative WA but ≥LSIL on LBC: colposcopy and 4-quadrant biopsies, and ECC if necessary; (3) negative WA 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 | (1) Positive WA or VIU: directed biopsy, and ECC if necessary; (2) negative WA or VIU 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 = Amplens’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; VIU = 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 $\chi^2$ 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 positive HR-HPV, 53.7%...
Cytology diagnoses and disease outcomes stratified by high-risk HPV status in women with abnormal screening results and negative colposcopy.

### Table 3

| Cytology diagnoses | Normal | CIN1 | CIN2 | CIN3 | Cancer | Total | CIN2+ | CIN3+ |
|--------------------|--------|------|------|------|--------|-------|-------|-------|
| Negative           | 200 (98.0) | 4 (2.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 204 (100.0) | 0 (0.0) | —     | 0 (0.0) | —      |
| ASC-US/LSIL        | 455 (92.1) | 35 (7.1) | 3 (0.6) | 1 (0.2) | 0 (0.0) | 494 (100.0) | 0 (0.0) | —     | 0 (0.0) | —      |
| POS                | 633 (88.7) | 89 (11.3) | 5 (0.7) | 1 (0.1) | 1 (0.2) | 701 (100.0) | 1 (0.1) | 1 (0.1) | 1 (0.1) | 1 (0.1) |
| ASC/AGC-H/HSIL+    | 26 (63.9) | 12 (28.6) | 2 (4.9) | 0 (0.0) | 1 (2.3) | 31 (100.0) | 1 (3.2) | 1 (3.2) | 1 (3.2) | 1 (3.2) |
| POS                | 107 (38.2) | 52 (18.6) | 61 (21.8) | 56 (20.0) | 4 (1.4) | 280 (100.0) | 118 (42.6) | 35.4 (66.2) | 59 (21.3) | 69.7 (27.7-175.5) |
| Total              | 681 (93.4) | 84 (11.6) | 5 (0.7) | 1 (0.1) | 1 (0.1) | 729 (100.0) | 7 (10.0) | 1 (1.2) | 1 (1.2) | 1 (1.2) |
| POS                | 1886 (76.9) | 376 (15.1) | 134 (5.4) | 83 (3.3) | 5 (2.2) | 2484 (100.0) | 222 (8.9) | 10.1 (6.1-21.6) | 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, HSIL = high-grade squamous intraepithelial lesion, LSIL = low-grade squamous intraepithelial lesion, OR = odds ratio.

— HSL+ included HSIL and cancer.

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% [77/729]; CIN3+: 0.3% [27/729], 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+, 0.3 [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 CIN 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 CIN3+.
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%. 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%.[25] 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.[26] 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]

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).[21] 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 < 0.001).[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+.[11,26,33,34] 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.[33] 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.[16,37] 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

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### 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.

| CIN2+, n (%) | CIN3+, n (%) |
|-------------|-------------|
| 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 | 90.7 |

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### 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) |

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, LSL = 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, 2 ASC-H, 2 LSIL, 1 HSIL, and 1 Cancer), 14 CIN2 (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.

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The sensitivity of colposcopy for CIN detection is in

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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) was higher than the average level reported in a European and North American pooled study [19] (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.

Acknowledgments

Thanks for the generous support from the Cleveland Clinic, Program for Appropriate Technology in Health, the International Agency for Research on Cancer, and the Bill & Melinda Gates Foundation to Chinese cervical cancer prevention research for the 17 original studies. The authors also thank the women who participated in our studies from Beijing, Gansu, Guangdong, Jiangsu, Jiangxi, Henan, Shanghai, Shanxi, and Xinjiang, as well as the local health workers and the staffs in the research team for cervical cancer prevention in CICAMS. They also thank Dr. Jerome L. Belinson for his contributions in SPOCCS projects and the valuable advices on this paper. And, also Ayling Dominguez from University of Chicago for language editing.

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