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Diagnostic accuracy of novel folate receptor-mediated staining solution detection (FRD) for CIN2+

A systematic review and meta analysis

Yuan-xing Li, MD, Hai-xia Luo, MD, Wei Wang, MD, Zhe Wang, MD, PhD, Wei-hong Zhao, MD, PhD, Min Hao, MD, PhD*

Abstract

Background: Early detection and diagnosis of high-grade cervical intraepithelial neoplasia grade 2 or higher (CIN2+) is critical for a good prognosis and appropriate treatment. The chief aim of our study was to evaluate the diagnostic performance of folate receptor-mediated staining solution detection (FRD) for CIN2+.

Methods: We conducted a systematic review and meta-analysis by searching the PubMed and EMBASE databases for studies published until May 2020, which assessed the diagnostic accuracy of FRD, human papilloma virus (HPV) testing, and ThinPrep cytology test (TCT) for the detection of CIN2+. Bivariate models were used to compare the diagnostic performance of FRD, HPV, and TCT.

Results: Six studies involving 2817 patients were included in this meta-analysis. The pooled specificity of FRD was higher than that of HPV and TCT for detecting CIN2+ (0.65, 0.12, and 0.39, respectively). The summary area under the receiver operating characteristic curve values using FRD, HPV, and TCT for detecting CIN2+ were 0.79, 0.95, and 0.77, respectively, indicating that FRD was superior to TCT. The diagnostic odds ratios of FRD, HPV, and TCT were 6 (95% CI: 5–7), 3 (95% CI: 2–5), and 3 (95% CI: 2–4), respectively, demonstrating that FRD had good diagnostic accuracy.

Conclusion: FRD showed good diagnostic accuracy and higher specificity than HPV and TCT for detecting CIN2+. Based on our results, we propose that FRD could be a candidate for cervical screening, especially in underdeveloped countries.

Abbreviations: AUC = area under the receiver operating characteristic curve, CIN = cervical intraepithelial neoplasia, CIN2+ = cervical intraepithelial neoplasia grade 2 or higher, DOR = diagnostic odds ratio, FN = false negatives, FP = false positives, FR = folate receptor, FRα = folate receptor subtype alpha, FRD = folate receptor-mediated staining solution detection, HIC = high-income countries, HPV = human papillomavirus, HR-HPV = high-risk human papillomavirus, LMICs = low- and middle-income countries, MOOSE = Meta-analysis of Observational Studies in Epidemiology, NLR = negative likelihood ratio, PLR = positive likelihood ratio, PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-analyses, PROSPERO = Prospective Register of Systematic Reviews, QUADAS-2 = Quality Assessment of Diagnostic Accuracy Studies, SROC = summary receiver operating characteristic curve, TBS = the Bethesda System, TCT = ThinPrep cytology test, TN = true negatives, TP = true positives, VIA = visual inspection with acetic acid, VILI = visual inspection with Lugol's iodine.

Keywords: cervical neoplasia, developing world, diagnosis, meta-analysis

1. Introduction

Cervical cancer remains a common public health problem worldwide.[1] It has been established that high-risk human papillomavirus (HR-HPV) is the main cause of cervical lesions. Although high-income countries (HIC) have reduced their morbidity and mortality rates through screening and vaccination, HR-HPV is still a serious threat to the health of women in low- and middle-income countries (LMICs),[2] where current screening systems are limited and less successful due to the scarcity of infrastructure, skilled laboratory professionals, and financial resources.[3,4]

Cervical cancer predominantly affects underscreened women in LMICs; thus, a substantial effect on cervical cancer incidence and mortality requires the identification of effective outreach strategies. Current cervical screening tests are usually conducted with HPV testing, ThinPrep cytology testing (TCT), or co-testing.[5,6] However, their efficacies are still questionable. Although HPV testing is accurate and has higher sensitivity, recent doubts about its efficacy in an era of vaccination[7] have called for the need to improve this method. It also had higher false positives (FP) and colposcopy rates compared with TCT, which may lead to unnecessary treatments and potential psychological harm.[8] TCT shows low sensitivity for detecting high-grade lesions and requires skilled laboratory professionals, making it less accessible to women in LMICs.[9] LMICs still face barriers to satisfactory screening coverage, such as high operating costs and logistic challenges.[10,11] Thus, a new assay with high sensitivity and specificity, simplicity, and low workload and costs is needed for screening cervical cancer in LMICs.

Folate is a key nutrient for maintaining normal biological functions. Recent studies have indicated that folate receptor subtype alpha (FRα) is overexpressed in the membranes of gynecological tumor tissues and is correlated with tumor development and prognosis.[12,13] To function in the body, folate must enter cells through folate receptors (FR). Folate is compatible with both organic and inorganic matter, without modification. Based on these characteristics, folate receptor-mediated staining solution detection (FRD) has been developed and is gradually being applied clinically to detect cervical intraepithelial neoplasia (CIN) or cervical cancer. The FRD reagent consists of methylene blue, folate, vitamin C, neutral red, and other components. It can target cervical lesion cells via endocytosis of the FR,[14,15] which changes the color of the cotton swab from the original brown. The test results can be determined immediately (within 60 s) after staining the cervix, and a blue, dark blue, or black swab indicates CIN grade 2 or higher (CIN2+).

Recent studies have estimated the diagnostic performance of FRD for predicting CIN2+. However, due to the limited sample size in these studies, the data may be insufficient for verifying the ability of the FRD assay, and the comparisons between FRD, HPV, and TCT were inconsistent. To resolve this disparity, we performed a systematic review and meta-analysis to generate a more comprehensive understanding of the diagnostic performance of FRD in cervical cancer screening, in comparison with HPV testing and TCT.

2. Materials and methods

This meta-analysis was designed, implemented, analyzed, and reported following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA)[16] and the Meta-analysis of Observational Studies in Epidemiology (MOOSE) protocol.[17] The protocol of this study was registered in the International Prospective Register of Systematic Reviews (PROSPERO, registration number: CRD42020185357). This study is a systematic review and meta-analysis based on published data, therefore, ethics approval and written informed consent were not needed.

2.1. Search strategy

A protocol was developed prior to conducting this systematic review and meta-analysis. We conducted a comprehensive systematic search in PubMed and EMBASE for studies that evaluated the diagnostic accuracy of FRD for cervical lesions until May 2020 using the following search terms: ('Folate Receptor' OR 'FR') and ('Diagnosis' OR 'Sensitivity' OR 'Specificity') and ('Uterine Cervical Neoplasms' OR 'Cervical Neoplasm' OR 'Cervical Cancer' OR 'Cervical Intraepithelial Neoplasm' OR 'CIN'). We searched these databases for original, English language research articles that studied the diagnostic accuracy of FRD in cervical screening of women.

2.2. Selection criteria

Only articles that met following criteria were included in this meta-analysis:

1. cervical lesion-related FRD studies;
2. related data can be obtained or calculated to construct a 2 × 2 table, including true positives (TP), FP, true negatives (TN), and false negatives (FN);
3. the diagnosis of CIN was confirmed based on histology or the appropriate dyeing characteristics as defined by accepted guidelines; and
4. article is in English.

Studies were independently excluded based on the following exclusion criteria:

1. non-related studies;
2. non-diagnostic studies;
3. literature reviews, editorial pieces, conference abstracts, letters, comments, or case reports; and
4. animal or cellular experiments.

2.3. Data extraction

Two reviewers independently extracted the following relevant information via electrical form (Microsoft Access) from the included studies: first author, publication year, age range (years), number of participants, proportion of patients with CIN2+, sensitivity, specificity, TP, FP, FN, TN, and the results. Any discrepancies were discussed and resolved by consensus.

2.4. Assessment of study quality

We used the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) checklist to assess the methodological quality of the included studies. Two authors independently assessed the risk of bias and applicability, and discrepant results were resolved in a consensus meeting.

2.5. Data synthesis and statistical analysis

To evaluate the diagnostic accuracy of FRD for cervical lesions, we calculated the pooled sensitivity and specificity, diagnostic odds ratio (DOR), and area under the receiver operating characteristic curve (AUC) based on the bivariate mixed effects models. We constructed a summary receiver operating characteristic (SROC) curve, and a diagnostic tool was defined as perfect (if AUC = 1.00), excellent (AUC > 0.90), very good (AUC > 0.80), or good (AUC < 0.80). The DOR combined the strengths of sensitivity and specificity, and a higher estimate indicates a stronger discriminatory ability between patients and healthy individuals.[18]

2.6. Assessment of heterogeneity and publication bias

The heterogeneity between studies was assessed using the inconsistency index (I^2) and the Cochran Q test. $I^2 > 50\%$ or $I^2 > 25\%$ with a P-value < .10 indicated that the heterogeneity was substantial. As heterogeneity can be caused by two effects (threshold or non-threshold), the Spearman correlation coefficient was calculated to determine whether there was a threshold effect. When there was a non-threshold effect between the included studies, the $\chi2$ test was used to further analyze the statistical heterogeneity among the included studies, and the amount of heterogeneity was quantitatively judged in conjunction with I^2. The fixed-effect model was used for combined analysis if $I^2 < 50\%$, and the random-effect model was used otherwise.

To test for possible publication bias, we constructed Deeks' effective sample size funnel plots versus the DOR and performed a regression test of asymmetry. All statistical tests were two-sided, and statistical significance was defined as P-value < .05. All statistical analyses were performed using STATA version 15.1 (StataCorp, College Station, TX, USA).

3. Results

3.1. Search results and study characteristics

Of the 1335 articles identified (1177 in PubMed and 158 in EMBASE), we removed 1329 studies that did not fulfill the inclusion criteria, leaving six studies for inclusion in the quantitative synthesis.

Table 1 shows the characteristics of the included studies. The six studies in this meta-analysis consisted of 2817 individuals and were published between 2015 and 2020. All studies were conducted prospectively and were based on a cervical screening system in a hospital. Regarding the reference tests, five studies[19–23] defined the 'gold standard' as colposcopy biopsy pathological results, while one study[24] used cytologic diagnoses according to the Bethesda System (TBS 2001). The proportion of patients with CIN2+ among the studies ranged from 16.09% to 37.30%, and the number of participants ranged from 169 to 1504. Only one study[21] was a multi-center study.

3.2. Quality assessment

Methodological quality was assessed using the QUADAS-2 tool (see Table S1, Supplemental Digital Content, http://links.lww.com/MD/G149 and Figure S1, Supplemental Digital Content http://links.lww.com/MD/G147, which illustrates the quality assessment scores of the six studies). For the risk of bias in the reference standard, all studies were defined as "high" risk because no cases were difficult to diagnose, and inappropriate exclusions could not be avoided. Regarding the domain of the index test, five studies were scored "low" risk because the results were always conducted and interpreted prior to the reference standard. One study defined cytologic results as the "gold standard" but had lower accuracy than pathological results; therefore, this study was labeled as "high" risk. For flow and timing domains, five studies scored "low" since they clearly defined the appropriate interval between the index test and reference standard. As for applicability, all studies had patient selection criteria that were in accordance with our analysis inclusion criteria and scored "low" risk. The reference standard and index test domains scored well for five of the six included studies.

3.3. Quantitative data synthesis

Six studies were included to compare the diagnostic accuracy of FRD, HPV, and TCT for CIN2+ in the same enrolled patients. Among them, five studies compared the diagnostic efficiency of FRD against HPV for CIN2+, and all six compared the diagnostic efficiency of FRD against both HPV and TCT. Table 2 shows the


Table 1

Characteristics of the included studies.

Author, year	Age (years)	N	CIN2+ (%)	FRD Se, Sp (%)	TP	FP	FN	TN	HPV Se, Sp (%)	TP	FP	FN	TN	TCT Se, Sp (%)	TP	FP	FN	TN
Lu 2015[24]	19–68	169	33.73	71.93, 66.07	41	38	16	74	–	–	–	–	–	73.68, 61.61	42	43	15	69
Dai 2018[19]	25–65	216	37.16	80.41, 68.29	78	52	19	112	93.81, 16.46	91	137	6	27	76.29, 35.98	74	105	23	59
Dai 2019[20]	25–65	317	34.38	81.65, 68.27	89	66	20	142	97.25, 12.98	106	181	3	27	69.72, 37.98	85	129	24	79
Xiao 2019[22]	20–69	404	16.09	80.00, 51.92	52	163	13	176	96.90, 7.08	63	315	2	24	90.77, 39.53	59	205	6	134
Zhao 2019[21]	20–76	1504	37.30	77.72, 60.02	436	377	125	566	95.54, 14.95	536	772	25	171	80.39, 30.12	451	659	110	284
Qi 2020[23]	20–73	207	35.75	75.68, 63.91	56	48	18	85	93.22, 7.56	69	123	5	10	82.09, 35.34	61	86	13	47

Table 2

Summary of diagnostic accuracy for cervical intraepithelial neoplasia grade 2 or higher.

	Se (95% CI)	Sp (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)	AUC (95% CI)
FRD	0.75 (0.70, 0.80)	0.65 (0.58, 0.72)	2.2 (1.8, 2.6)	0.38 (0.33, 0.43)	6 (5, 7)	0.79 (0.75, 0.82)
HPV	0.95 (0.93, 0.97)	0.12 (0.08, 0.17)	1.1 (1.0, 1.1)	0.38 (0.22, 0.64)	3 (2, 5)	0.95 (0.92, 0.96)
TCT	0.80 (0.76, 0.83)	0.39 (0.32, 0.47)	1.3 (1.2, 1.5)	0.52 (0.42, 0.64)	3 (2, 4)	0.77 (0.73, 0.80)

AUC = the area under the receiver operating characteristic curve, CI = confidence interval, DOR = Diagnostic odds ratio, FRD = Folate receptor-mediated staining solution detection, HPV = human papilloma virus, NLR = negative likelihood ratio, PLR = positive likelihood ratio, Se = sensitivity, Sp = specificity, TCT = thinPrep cytology test.
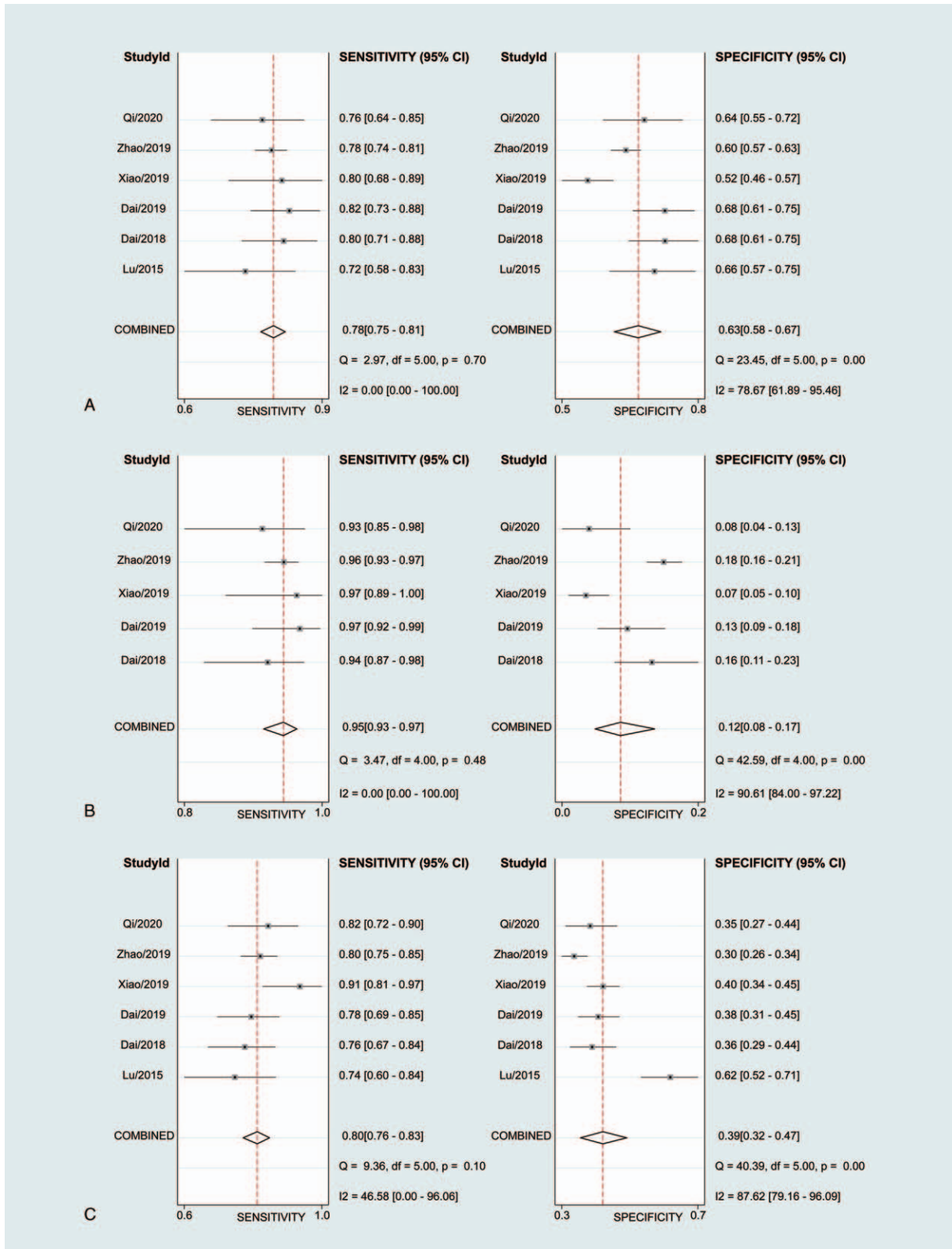
Figure 1. Meta-analysis of the FRD (A) HPV (B) and TCT (C) performance (sensitivity and specificity) for CIN2+ detection. CIN2+ = cervical intraepithelial neoplasia grade 2 or higher, FRD = folate receptor-mediated staining solution detection, HPV = human papillomavirus, TCT = ThinPrep cytology test.

pooled sensitivity (Se), specificity (Sp), positive likelihood ratio (PLR), negative likelihood ratio (NLR), DOR, and AUC. We also constructed forest plots of the sensitivities and specificities (Fig. 1) and compared the SROC plots of FRD, HPV, and TCT (Fig. 2). The pooled specificity using FRD (65%) was higher than that using HPV (12%) and TCT (39%) for detecting CIN2+.
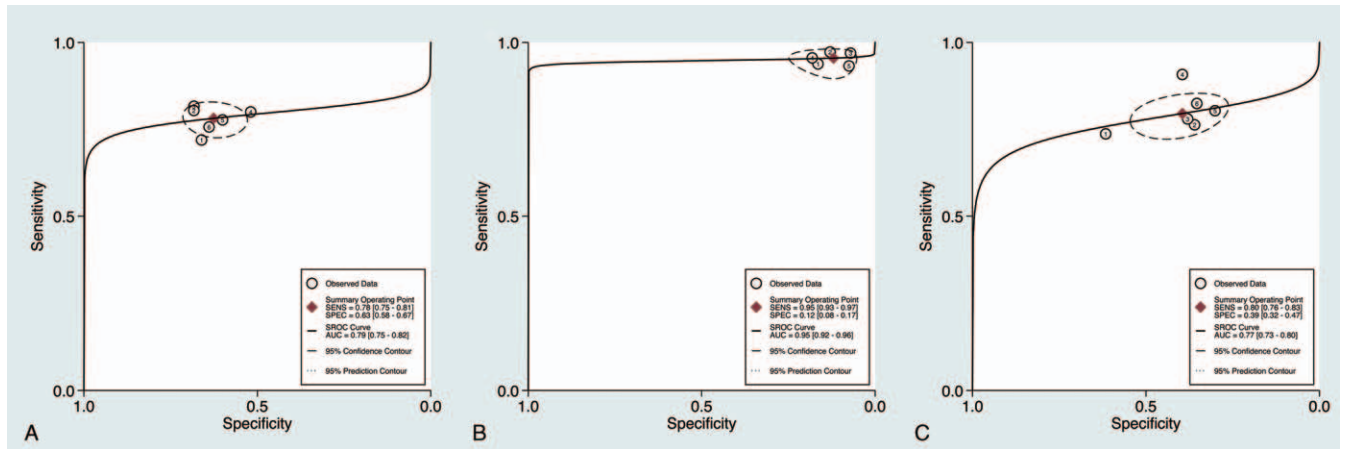
Figure 2. SROC curve for the diagnostic accuracy of FRD (A) HPV (B) and TCT (C) for CIN2+. CIN2+ = cervical intraepithelial neoplasia grade 2 or higher, FRD = folate receptor-mediated staining solution detection, HPV = human papillomavirus, SROC = summary receiver operating characteristic curve, TCT, ThinPrep cytology test.

However, the pooled sensitivity of FRD was inferior to that of HPV (95%) and TCT (80%). The summary AUC values using FRD, HPV, and TCT for detecting CIN2+ were 0.79, 0.95, and 0.77, respectively, indicating that FRD is slightly superior to TCT but inferior to HPV. FRD had moderate diagnostic performance for CIN2+. The DORs of FRD, HPV, and TCT were 6 (95% CI: 5–7), 3 (95% CI: 2–5), and 3 (95% CI: 2–4), respectively.

3.4. Investigation of heterogeneity

No heterogeneity existed in the sensitivities of FRD and HPV; however, there was significant heterogeneity in the specificities of FRD, HPV, and TCT (Fig. 1). Visual inspection of the SROC curves (Fig. 2) and calculation of Spearman's correlation coefficients ($\rho = -0.37$, 0.1, 0.31; $P = .47$, .87, and .54, respectively) indicated that there was no threshold effect contributing to the heterogeneity of FRD, HPV, and TCT.

Regarding the non-threshold effect, we performed a subgroup analysis by patient source (whether multi-center study), sample size (≥ 400), and the proportion of CIN2+ ($> 30\%$) to confirm the possible heterogeneity source of specificities. The results of the subgroup analysis (Table 3) revealed that the proportion of CIN2+ and the number of participants accounted for the heterogeneity

of FRD specificity, and the number of participants may have contributed to the heterogeneity of HPV specificity. The heterogeneity of TCT specificity may have had no relationship with the proportion of CIN2+, the number of participants, and the patient source.

3.5. Publication bias

We performed Deeks' funnel plots of FRD, HPV, and TCT, and explored the regression tests of asymmetry of the included studies (see Figure S2, Supplemental Digital Content http://links.lww.com/MD/G148, which illustrates the funnel plots of FRD, HPV, and TCT). There was no publication bias for FRD, HPV, and TCT for detecting CIN2+ ($P = .54$, .16, and .14, respectively).

4. Discussions

A total of six studies and 2817 patients were included in this meta-analysis. Our results suggest a good overall diagnostic performance of FRD for CIN2+ based on the following: 1) the pooled specificity of FRD was higher than those of HPV and TCT for detecting CIN2+; 2) the summary AUC values using FRD, HPV, and TCT for detecting CIN2+ were 0.79, 0.95, and 0.77,

Table 3

Subgroup analysis of folate receptor-mediated staining solution detection, human papilloma virus testing and ThinPrep cytology test specificities.

	No of studies	FRD		HPV		TCT	
		Sp (95% CI)	Heterogeneity, *P*-value	Sp (95% CI)	Heterogeneity, *P*-value	Sp (95% CI)	Heterogeneity, *P*-value
No of participants							
≥400	2	0.58 (0.55, 0.61)	$I^2 = 85\%$, $P < .01$	0.17 (0.15, 0.19)	$I^2 = 91\%$, $P < .01$	0.33 (0.30, 0.35)	$I^2 = 89.90\%$, $P < .01$
<400	4	0.67 (0.63, 0.71)	$I^2 = 0\%$, $P = .83$	0.13 (0.10, 0.16)	$I^2 = 64.6\%$, $P = .06$	0.41 (0.37, 0.45)	$I^2 = 87.30\%$, $P < .01$
CIN2+							
≥30%	5	0.63 (0.60, 0.65)	$I^2 = 53.3\%$, $P = .07$	0.16 (0.14, 0.18)	$I^2 = 77.1\%$, $P < .01$	0.34 (0.32, 0.37)	$I^2 = 90.8\%$, $P < .01$
<30%	1	0.52 (0.46, 0.57)	–	0.07 (0.05, 0.10)	–	0.40 (0.34, 0.45)	–
Patient source							
Single centre	5	0.62 (0.58, 0.65)	$I^2 = 81.5\%$, $P < .01$	0.10 (0.08, 0.13)	$I^2 = 76.50\%$, $P < .01$	0.41 (0.37, 0.44)	$I^2 = 83.2\%$, $P < .01$
Multi-centre	1	0.60 (0.57, 0.63)	–	0.18 (0.16, 0.21)	–	0.30 (0.26, 0.34)	–

AUC = the area under the receiver operating characteristic curve, CI = confidence interval, DOR = Diagnostic odds ratio, FRD = Folate receptor-mediated staining solution detection, HPV = human papilloma virus, NLR = negative likelihood ratio, PLR = positive likelihood ratio, Se = sensitivity, Sp = specificity, TCT = thinPrep cytology test.

respectively, indicating that FRD was superior to TCT; and, 3) the DORs of FRD, HPV, and TCT were 6 (95% CI: 5–7), 3 (95% CI: 2–5), and 3 (95% CI: 2–4), respectively, demonstrating that FRD had good diagnostic accuracy. Based on these analyses, we conclude that FRD could be a candidate for cervical screening.

Self-sampled screening for HPV DNA and visual inspection with acetic acid or Lugol's iodine (VIA or VILI) have also been suggested as creative screening alternatives for women in LMICs. Self-sampled screening for HPV DNA is highly recommended for those who cannot participate in long-term screening and has proved to be highly acceptable,[25] but the difference in accuracy between self-sampled and clinician-sampled tests is still unclear.[26] VIA and VILI are inexpensive and easy to operate, but their diagnostic accuracy is controversial.[27–30] They also lack reproducibility[31] and are highly dependent on the skill of the observer.[32] FRD, as a novel detection assay for CIN2+, has proved to be a valid diagnostic method based on our data analysis. It does not require a long detection time and complicated medical technique, and it may increase patient compliance with follow-up and facilitate early intervention. In addition, it has higher specificity than HPV, thus possibly reducing unnecessary colposcopy and biopsy and decreasing patient anxiety. Therefore, FRD has the potential to become an affordable alternative for screening in China, as well as in other LMICs or areas that lack medical resources.

This study has some limitations.

1. All of the studies were from China, which is not a folic acid fortification area. Folic acid consumed in fortification areas could plausibly bind to FRα-positive tumors[33] and may impact the detection accuracy. Therefore, our results may be geography specific.
2. There was significant heterogeneity among the specificities of FRD, HPV, and TCT for CIN2+. Although we conducted a subgroup analysis to explore the source of heterogeneity, this only partly explains the heterogeneity. Inconsistencies in HPV assays may contribute to heterogeneity, but further analysis could not be performed due to incomplete data. Consequently, the reliability of these pooled results could be questioned.
3. Although we employed a comprehensive literature search strategy, the number of included studies was inadequate. Further large-scale and well-designed clinical trials are needed to reach a more conclusive result.

Despite the above limitations, the strengths of this meta-analysis are worth mentioning.

1. To our knowledge, this is the first systematic review and meta-analysis to comprehensively assess the diagnostic performance of FRD for CIN2+ and compare FRD, HPV, and TCT.
2. A detailed subgroup analysis was utilized to find the possible sources of heterogeneity.
3. Tests for publication bias also proved the robustness of the results.

5. Conclusions

Our systematic review provides synthetic evidence comparing the diagnostic accuracy of FRD, HPV, and TCT for CIN2+. Based on the results of our meta-analysis, FRD had good diagnostic accuracy and higher specificity than HPV and TCT for detecting CIN2+. We suggest that the implementation of FRD may be conducive to eliminating cervical cancer in LMICs that cannot afford HPV and TCT.

Author contributions

Conceptualization: Yuanxing Li.
Data curation: Yuanxing Li, Haixia Luo, Wei Wang.
Formal analysis: Yuanxing Li.
Investigation: Yuanxing Li.
Methodology: Yuanxing Li.
Software: Yuanxing Li.
Supervision: Min Hao.
Writing – original draft: Yuanxing Li.
Writing – review & editing: Haixia Luo, Wei Wang, Zhe Wang, Weihong Zhao, Min Hao.

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