



Compare two different usages of FRD for detecting high-grade cervical lesions and invasive cancer

Yuping Dai¹ · Lirong Wang¹ · Donghong Li²

Received: 25 May 2017 / Accepted: 2 February 2019 / Published online: 5 March 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

Abstract

Objective To evaluate two usages of the folate receptor-mediated staining solution (FRD) for detecting high-grade cervical lesions and invasive cancer, and compared with cytology test (TCT) and human papillomavirus (HPV) testing.

Methods FRD sampling and FRD direct staining methods were used for detecting high-grade cervical lesions and invasive cancer. As a comparison, TCT and HPV testing were also applied for screening high-grade cervical lesions and invasive cancer. The sensitivity and specificity of TCT, HPV testing, and staining results of FRD were analyzed by the SPSS software.

Results In this study, 317 patients with biopsy were collected. The positive rate of FRD sampling method was 35.33% (112/317), and positive rate of FRD direct staining was 48.90% (155/317). Area under the curve (AUC) of TCT, HPV testing, FRD sampling and FRD direct staining were 0.53, 0.55, 0.58, and 0.75, respectively. The sensitivity of TCT, HPV, FRD sampling and FRD direct staining was 69.72%, 97.25%, 64.22% and 81.65%, respectively, and the specificity was 37.98%, 12.98%, 79.81% and 68.27%, respectively.

Conclusion Compared with TCT and HPV testing, two usages of FRD methods have compatible sensitivity and high specificity to detect high-grade cervical lesions and invasive cancer. FRD direct staining may be comfortable for routine cervical cancer screening

Keywords Folate receptor-mediated staining solution (FRD) · High-grade cervical lesions · Invasive cancer · Cytology test · Human papillomavirus testing

Introduction

Cervical cancer is the third most common cancer among women worldwide. There were 275,000 deaths due to cervical cancer with an estimate of approximately 530,000 new cases [1]. It is the most common cause of cancer death and years of life lost owing to cancer in many less developed countries [2]. The key genetic event in development of cervical cancer is human papillomavirus (HPV) integration, and one of the most important risk factors for cervical carcinoma

development is that HPV DNA is integrated into the host genome [3].

Various approaches have been considered for screening cervical carcinoma. International Agency for Research on Cancer has made recommendations that cytology test (TCT) or HPV testing is supported as primary screening [4]. However, many studies showed that HPV testing is considerably more sensitive than TCT to screen cervical intraepithelial neoplasia grade 2 (CIN2) and grade 3 (CIN3) and cancer [5, 6]. However, TCT has some limitations, including that low sensitivity of single smear fails to detect high-grade precursor lesions [7]. HPV testing has low reproducibility which results in increased false positives [8].

Folate receptor-mediated staining solution (FRD) is the epithelia staining agent target to cervical cancer cell which is found to be a rapid, simple, and accurate diagnosis method to identify patients with cervical cancer [9, 10]. In this study, compared with TCT and HPV testing, detected abilities of FRD sampling and direct staining methods for detecting CIN2+ are studied in 317 subjects.

✉ Donghong Li
donghong2017@163.com

¹ Department of Obstetrics and Gynecology, Affiliated Guangren Hospital, School of Medicine, Xi'an Jiaotong University, Xi'an, China

² Department of Gynecology Oncology, The Affiliated Shaanxi Provincial Cancer Hospital, School of Medicine, Xi'an Jiaotong University, No. 309, Yantaxi Road, Yanta District, Xi'an 710061, Shaanxi, China

Material and methods

Subjects

Within the scope of this study, a total of 317 women who had been diagnosed by histopathological diagnosis as gold standard were enrolled from May 2016 to March 2017, at the Affiliated Guangren Hospital, School of Medicine, Xi'an Jiaotong University. All these women with the positive HPV test result and/or abnormal TCT test will undergo colposcopy. Inclusion criteria: women between 25 and 65 years, non-pregnant, not in menstrual period. Exclusion criteria: undergone hysterectomy, cervical contact with bleeding and large bleeding, received cervical surgery (including conization, LEEP, infrared, microwave, etc.), pregnant, acute inflammation more than 7 days, diagnosed with CIN2+. Subjects were detected by FRD sampling and direct staining, and then, colposcopy was performed; finally, they all were detected by histopathological diagnosis. The detail flow chart is shown in Fig. 1. This study was proved by the institutional review ethics boards of Guangren Hospital. All women provided written informed consent before undergoing any study procedures.

TCT

ThinPrep system (Cytoc Corporation) was used for liquid-based TCT. According to the manufacturer's instructions, one slide was prepared for one subject. Cytological diagnoses were performed according to the previous study [11]. These classifications included NILM, ASC-US, LSIL, ASC-H, and HSIL.

HPV testing

HPV testing was performed by Digene Hybrid Capture 2 (HC2, Qiagen; Crawley, UK) test, which was blind to TCT

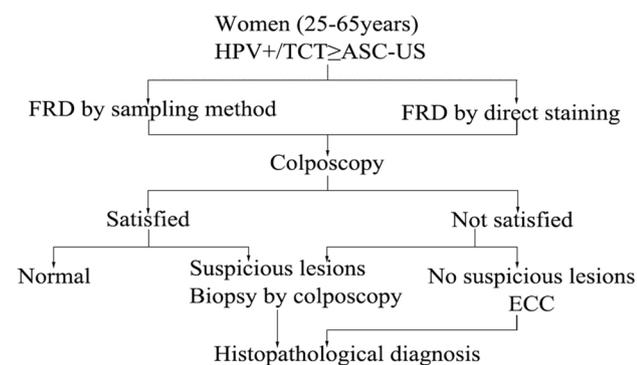


Fig. 1 Diagram of this study. ECC endocervical curettage

results. Digene Microplate Luminometer 2000 (DML 2000; Qiagen, Crawley, UK) was used for reading and calculating results of HPV testing.

FRD testing

Major components of FRD (Shaanxi Gaoyuan Medical Equipment Service Co., Ltd.) are folic acid, methylene blue, and acetic acid.

FRD sampling method: sampling is carried out by the Epithelium Staining Applicator in patient's cervix, and then dip the applicator into the FRD staining solution for 30 s. After staining, place the applicator into the FRD Colorimeter and it will scan color changes of the applicator. Based on scanning results, staining results are judged.

FRD direct staining: sampling is carried out by the Epithelium Staining Applicator which is dipped with the FRD staining solution in patient's cervix. Sampling is completed, and staining is completed at the same time. After completion, applicator is placed in the FRD Colorimeter and scanned. Based on scanning results, staining results are judged.

Statistical analysis

SPSS (version 16.0, SPSS Inc, Chicago, IL, USA) was used for statistical analysis. Chi-square test was for categorical variables. ROC analysis was used to assess sensitivity and specificity of TCT, HPV testing and staining results of FRD.

Results

Results of histopathological diagnosis

Results of histopathological diagnosis are shown in Table 1. TCT included NILM in 103 women (32.49%), ASC-US in 130(41.01%), LSIL in 51 (16.09%), ASC-H in 12 (3.79%), and HSIL in 21 (6.62%). As shown in Table 2, 317 women with histological findings were included in the study, including inflammation (178), CIN1 (30), CIN2 (59), CIN3 (34), and cancer (16).

Screening results of HPV testing, TCT, and FRD methods

As for inflammation, positive rates of HPV testing, TCT, FRD sampling, and FRD direct staining were 87%, 60%, 17%, and 30%. As for CIN1, positive rates of HPV testing, TCT, FRD sampling, and FRD direct staining were 87%, 73% (22/30), 40%, and 43%. As for CIN2, positive rates of HPV testing, TCT, FRD sampling, and FRD direct staining were 97%, 73%, 58%, and 76%. As for CIN3, positive rates of HPV testing,

Table 1 Histopathological diagnosis of TCT test results in this study

Pathology	NILM	ASCUS	ASC-H	LSIL	HSIL	Total
Inflammation	71	74	5	26	2	178
CIN1	8	16	0	5	1	30
CIN2	16	22	3	10	8	59
CIN3	5	12	3	9	5	34
Cancer	3	6	1	1	5	16
Total	103	130	12	51	21	317

NILM non-invasive load monitoring, *ASCUS* atypical squamous cells of undetermined significance, *ASC-H* atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesion; *LSIL* Low-grade squamous intraepithelial lesion, *HSIL* high-grade squamous intraepithelial lesions

Table 2 Screening results of HPV testing, TCT, and FRD methods for inflammation, CIN1, CIN2, CIN3, and cancer

Pathology	HPV testing		TCT		FRD sampling		FRD direct staining		Total
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	
Inflammation	155	23	107	71	30	148	53	125	178
CIN1	26	4	22	8	12	18	13	17	30
CIN2	57	2	43	16	34	25	45	14	59
CIN3	33	1	29	5	20	14	28	6	34
Cancer	16	0	13	3	16	0	16	0	16
Total	287	30	214	103	112	205	155	162	317

TCT, FRD sampling, and FRD direct staining were 97%, 85%, 59%, and 82%. As for cancer, positive rates of HPV testing, TCT, FRD sampling, and FRD direct staining were 100%, 81%, 100%, and 100%.

Diagnostic analysis of TCT, HPV testing, and FRD methods

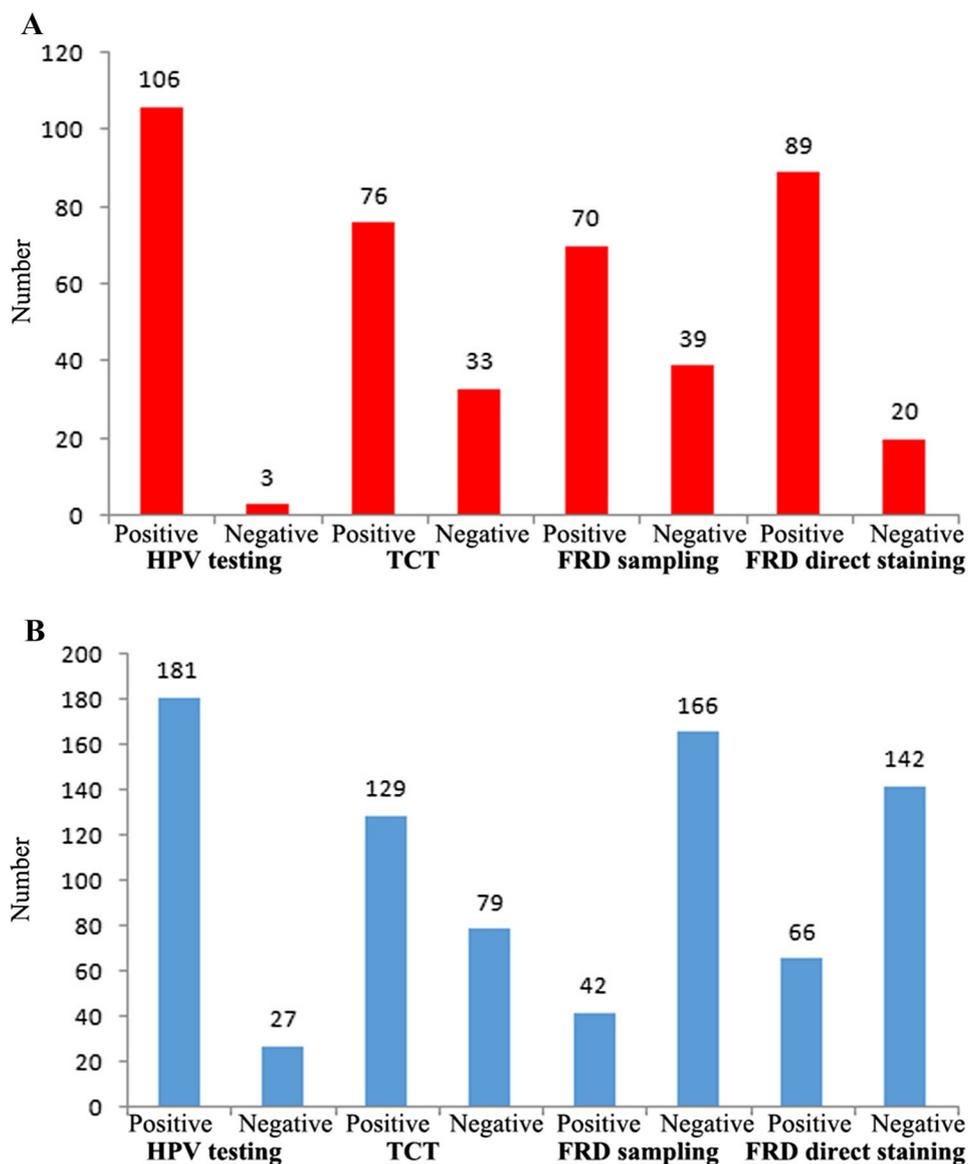
As for positive biopsy results (Fig. 2a), positive rates of HPV testing, TCT, FRD sampling, and FRD direct staining were 97%, 70%, 64%, and 82%, which were statistically significant ($P < 0.0001$). As for negative biopsy results (Fig. 2b), negative rates of HPV testing, TCT, FRD sampling, and FRD direct staining were 13%, 37%, 80%, and 68%, which were statistically significant ($P < 0.0001$). As for ROC analysis, area under the curve (AUC) of TCT, HPV testing, FRD sampling, and FRD direct staining was 0.53, 0.55, 0.58, and 0.75, respectively, indicating that FRD direct staining showed the best diagnostic abilities among four methods. The sensitivity and specificity of TCT, HPV testing, FRD sampling and FRD direct staining were 69.72% and 37.98%, 97.25% and 12.98%, 64.22% and 79.81%, and 81.65% and 68.27%, respectively (Fig. 3 and Table 3).

Discussion

In this study, FRD sampling and FRD direct staining methods were used for detecting high-grade cervical lesions and invasive cancer. To our knowledge, this is first study to compare HPV testing, TCT, FRD sampling and FRD direct staining. As for inflammation, CIN1, CIN2, and CIN3, positive rates of HPV testing were higher than the other methods. However, for cancer, positive rates of HPV testing, FRD sampling and FRD direct staining were the same (100%). In negative biopsy results of subjects, negative rates of FRD sampling and FRD direct staining were higher than HPV testing and TCT. Compared with TCT and HPV testing, FRD sampling and FRD direct staining methods had compatible sensitivity and high specificity to detect high-grade cervical lesions and invasive cancer, but FRD direct staining had the best diagnostic abilities with $AUC = 0.75$.

HPV testing is an increasingly important part of cervical screening [12, 13]. TCT includes conventional TCT and liquid-based TCT. The liquid-based TCT could detect more cases of histological high-grade squamous disease

Fig. 2 Screening results of HPV testing, TCT and FRD methods for positive (a) and negative (b) biopsy results



than conventional TCT [14, 15]. Therefore, in this study, liquid-based TCT was used as compared method for detect high-grade cervical lesions and invasive cancer. As for inflammation and CIN1, HPV testing and TCT showed similar positive rates (87% and 61%, 87% and 73%, respectively). Our results showed that HPV testing for high-risk types such as CIN2, CIN3, and cancer (97–100%) was more sensitive than TCT (58–85%) (Table 2). There are similar results found in many studies which focused on comparison HPV testing and TCT, and found that sensitivity of HPV testing was higher than TCT, and specificity of HPV testing was lower than TCT [16–18].

Folic acid receptor can serve as imaging and therapy of target for cancer and inflammatory diseases [19]. FRD (sampling method) was used as comparable diagnostic method for screening cervical cancer among 169 patients (10). As

for inflammation, CIN1, CIN2, and CIN3, the positive rates of FRD sampling and FRD direct staining were lower than TCT and HPV testing, but in turn, the negative rates of FRD sampling and FRD direct staining were higher than TCT and HPV testing (Table 2). Compared with TCT, HPV testing, and FRD sampling, FRD direct staining showed the best diagnostic abilities. Compared FRD sampling and FRD direct staining, sensitivity of the former was lower than that of the latter, but specificity of the former was higher than the latter. As for HPV testing and TCT, the specificity, PPV, NPV, CR, and PLR of two FRD methods were higher (Table 3). All the above results indicated that two FRD methods were comparable methods to screening high-grade cervical lesions and invasive cancer, and FRD direct staining had the most diagnostic abilities for distinguishing positive and negative subjects among four methods.

Fig. 3 ROC analysis of HPV testing, TCT and FRD methods for positive and negative biopsy results

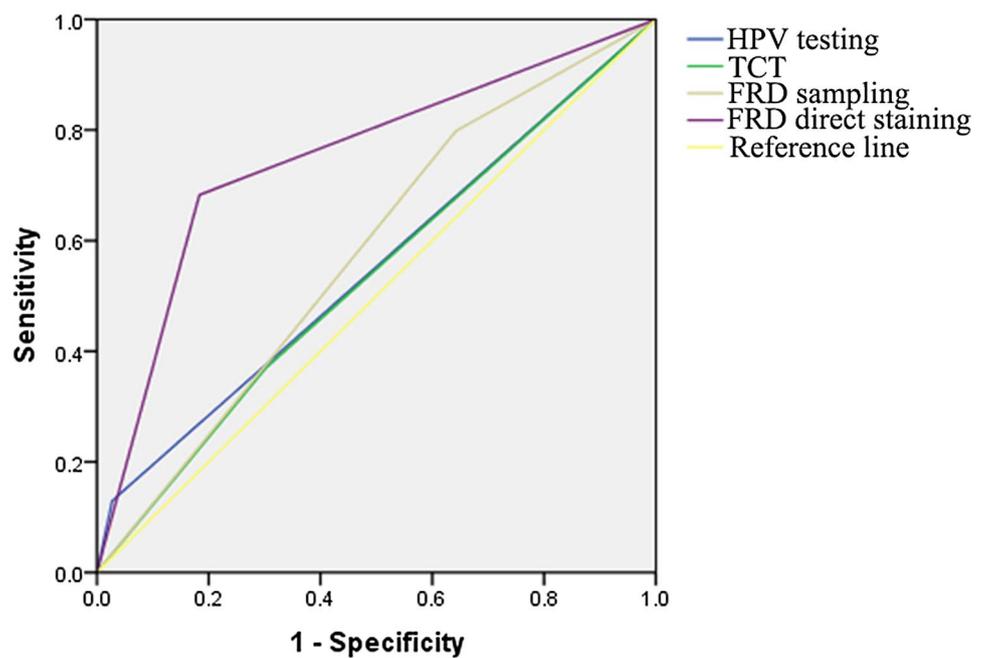


Table 3 Diagnostic significance of HPV testing, TCT and FRD methods

Indicators	HPV testing (%, 95% CI)	TCT (%, 95% CI)	FRD sampling (%, 95% CI)	FRD direct staining (%, 95% CI)
AUC	0.55 (0.51–0.59)	0.53 (0.50–0.57)	0.58 (0.54–0.62)	0.75 (0.72–0.78)
Sensitivity	97.25 (92.22–99.06)	69.72 (60.55–77.56)	64.22 (54.88–72.59)	81.65 (73.35–87.8)
Specificity	12.98 (9.08–18.23)	37.02 (30.75–43.76)	79.81 (73.83–84.7)	68.27 (61.66–74.21)
PPV	36.93 (31.56–42.66)	36.71 (30.45–43.47)	62.5 (53.26–70.91)	57.42 (49.55–64.93)
NPV	90 (74.38–96.54)	70 (60.88–77.77)	80.98 (75.05–85.76)	87.65 (81.7–91.86)
CR	41.96 (36.65–47.45)	48.26 (42.82–53.75)	74.45 (69.37–78.94)	72.87 (67.72–77.47)
PLR	1.12 (1.11–1.13)	1.11 (1.08–1.14)	3.18 (2.99–3.39)	2.54 (2.49–2.66)
NLR	0.21 (0.07–0.67)	0.82 (0.74–0.91)	0.45 (0.4251–0.4728)	0.27 (0.24–0.30)
kappa	0.07 (0.02–0.12)	0.06 (– 0.04–0.15)	0.44 (0.33–0.55)	0.45 (0.35–0.59)

AUC area under the curve, PPV positive predictive value, NPV negative predictive value, PLR positive likelihood ratio, NLR negative likelihood ratio

In general, compared with TCT and HPV test, two FRD methods have compatible sensitivity and high specificity to detect high-grade cervical lesions. Furthermore, FRD direct staining method had the best diagnostic abilities, and the sensitivity is more important in cervical cancer screening, so the direct staining method is more comfortable

in clinical. In addition, FRD is a very inexpensive and easy method, which can be used in less-developed countries or areas that lack the resources and trained personnel required for routine cervical screening.

Author contributions DHL: guarantor of integrity of the entire study, study concepts and design, definition of intellectual content, literature research, statistical data analysis, manuscript preparation, editing, and review. YPD: clinical studies and studies. LRW: clinical studies and data acquisition.

Funding This study was not funded.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics, 2012. *CA Cancer J Clin* 61:69–90
- Waggoner SE (2003) Cervical cancer. *Lancet* 361:2217–2225
- Hu Z, Zhu D, Wang W et al (2015) Genome-wide profiling of HPV integration in cervical cancer identifies clustered genomic hot spots and a potential microhomology-mediated integration mechanism. *Nat Genet* 47:158
- Organization WH (2005) IARC handbooks of cancer prevention, vol 10. Cervix cancer screening, pp 117–120.
- Berkhof J, Meijer CJ (2007) Human papillomavirus DNA versus papanicolaou screening tests for cervical cancer. *N Engl J Med* 358:642 (**author reply 3**)
- Naucler P, Ryd W, Törnberg S et al (2008) Human papillomavirus and Papanicolaou tests to screen for cervical cancer. *N Engl J Med* 357:1589
- Nanda K, Mccrory DC, Myers ER et al (2000) Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. *Ann Intern Med* 132:810
- Staples M (2014) HPV-based screening for prevention of invasive cervical cancer. *Lancet* 383:1294
- Zhang CH, Xue XL, Dang RF, Gynecology DO (2014) Application value of folic acid receptor mediated FRD in diagnosis of cervical intraepithelial neoplasia and cancer. *Chin J Lab Diagn* 8:7830–7836
- Lu MH, Hu LY, Du XX et al (2014) An special epithelial staining agents: folic acid receptor-mediated diagnosis (FRD) effectively and conveniently screen patients with cervical cancer. *Int J Clin Exp Med* 8:7830
- Anonymous (2010) The 1991 Bethesda system for reporting cervical/vaginal cytological diagnoses. *Diagn Cytopathol* 9:235–246
- Bosch FX, Broker TR, Forman D et al (2013) Comprehensive control of human papillomavirus infections and related diseases. *Vaccine* 31(Suppl 5):F1
- Goodman A (2015) HPV testing as a screen for cervical cancer. *BMJ* 350:h2372
- Davey E, Denton (2007) Accuracy of reading liquid based cytology slides using the ThinPrep Imager compared with conventional cytology: prospective study. *BMJ* 335:31–35
- Akamatsu S, Kodama S, Himeji Y, Ikuta N, Shimagaki N (2012) A comparison of liquid-based cytology with conventional cytology in cervical cancer screening. *Acta Cytol* 56:370–374
- Girianelli VR, Thuler LC, Szklo M et al (2006) Comparison of human papillomavirus DNA tests, liquid-based cytology and conventional cytology for the early detection of cervix uteri cancer. *Eur J Cancer Prev* 15:504–510
- Kitchener HC, Gilham C, Sargent A et al (2011) A comparison of HPV DNA testing and liquid based cytology over three rounds of primary cervical screening: extended follow up in the ARTISTIC trial. *Eur J Cancer* 47:864
- Ronco G, Cuzick J, Pierotti P et al (2007) Accuracy of liquid based versus conventional cytology: overall results of new technologies for cervical cancer screening: randomised controlled trial. *BMJ* 335:28
- Low PS, Henne WA, Doorneweerd DD (2008) Discovery and development of folic-acid-based receptor targeting for imaging and therapy of cancer and inflammatory diseases. *Acc Chem Res* 41:120–129

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.