Value of TCT combined with HPV and CA125 in early cervical cancer screening in a medical examination population

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ABSTRACT

This research aimed to explore the clinical value of thin prep cytologic test (TCT) combined with human papillomavirus (HPV) and carbohydrate antigen 125 (CA125) in early cervical cancer screening in the physical examination population. For this purpose, a total of 3587 females who received gynecological physical examination in the outpatient department of Ganzhou people's Hospital from January 2018 to March 2022 were included and underwent TCT, HPV and carbohydrate antigen 125 upon admission. Colposcopy biopsy was performed on patients who tested positive for any of the three indicators. Then using pathological diagnosis as the gold standard, the three methods applied alone or in combination were evaluated in terms of sensitivity, specificity, diagnostic yield and Youden index. Results showed that Among the 3587 females, 476 (13.27%) were HPV positive, 364 (10.14%) CA125 positive, and 314 (8.75%) TCT positive. Furthermore, 738 tested positive for any of the three indicators and underwent cervical biopsy. Among the 738 cases, 280 (39.94%) developed chronic cervicitis, 268 (36.31%) low-level cervical intraepithelial neoplasia (CIN), 173 (23.44%) high-level CIN, and 17 (2.30%) cervical cancer. HPV+TCT+CA125 combined screening showed higher sensitivity (94.54%), specificity (83.92%), diagnostic coincidence rate (87.46%) and Youden index (0.760) than single-indicator examinations. Also, it had the largest area under the receiver operating characteristic (ROC) curve, 0.673 (0.647, 0.699), compared to any other screening method. In conclusion, The combined detection of CA125, HPV and TCT is of clinical significance due to its higher sensitivity and accuracy in early screening of cervical cancer in the physical examination population.

Introduction

Cervical cancer is the fourth most common malignant tumor in women worldwide, after breast cancer, colorectal cancer and lung cancer. It is estimated that in 2018, an estimated 570,000 women were diagnosed with cervical cancer globally and about 311,000 died from the disease., accounting for 3.15% of all malignant tumor cases and 3.26% of all malignant tumor deaths (1). In 1974, Zur was the first to propose that human papillomavirus (HPV) infection is closely related to cervical tumors. A large number of epidemiological and molecular biological studies have also suggested HPV infection as an indispensable factor leading to cervical cancer and precancerous lesions (2). By PCR method, Li et al. combined primers GP5+/GP6+ with type-specific primers to perform HPV genotyping on surgical biopsy specimens collected from 198 women. In the case of HPV-16 infection, the most common type of cervical cancer, the severity of cervical lesions was strongly correlated with the frequency of HPV-16 integration. Following HPV DNA detection by HC II, HPV genotyping and physical condition detection were beneficial to predict the prognosis of cervical precancerous lesions and improve the accuracy of cervical cancer screening (3).

Wang et al. leveraged a nested polymerase chain reaction to detect HPV-16 and HPV-18 DNA in 48 cases of cervical cancer, 55 of high-grade CIN, 6 of low-grade CIN and 29 of infectious CIN. The results revealed a high rate of plasma HPV-DNA detection in patients with cervical cancer, particularly in those with lymph node metastases. And dual infection with HPV-16 and HPV-18 were more common in patients with cervical cancer (4). In their study on the relationship between HPV and cervical cancer, Munoz et al. attributed more than 90% of cervical cancer to certain HPV types, with HPV 16 accounting for the largest proportion (about 50%), followed by HPV 18 (12%), HPV 45 (8%) and HPV 31 (5%) (5). Additionally, several studies have shown long-term precancerous lesions before cervical lesions are developed into malignant tumors, hence a time series relationship between HPV infection and cervical cancer (6). Therefore, the detection of HPV infection can indicate the risk of cervical cancer, which is of great significance for the early diagnosis and prevention of the disease.

The cytological screening was the first cervical cancer screening technique, which is effective in detecting early cervical cancer. Pap smears are a clinical tool and a routine screening test for cervical cancer. The traditional Pap

smear is performed by a gynaecologist using a cervical spatula or cervical brush to collect cervical shed cells, the accuracy of which is subject to sampling, smearing, staining and film reading. Thinprep cytologic test (TCT) improves the Pap smear technique by storing exfoliated cells in a cell preservation solution and raising the efficiency of smear taking and production through centrifuging. With higher diagnostic accuracy, it can be effectively used for early cervical cancer screening (7). Although TCT is effective in reducing the incidence of cervical cancer, it is less sensitive to low-grade CIN and therefore will result in a proportion of missed diagnoses. Currently, the combination of HPV and TCT is mostly used clinically to improve the detection rate of cervical lesions. Liu et al. performed TCT combined with HPV-DNA testing and found that the combination had higher sensitivity and accuracy in cervical cancer screening than single testing (8). A similar study by Sun also gave the same conclusion (9).

CA 125, a member of the mucin family glycoproteins encoded by the MUC16 gene, has been recognized as an important marker for the diagnosis and monitoring of other gynecological tumors such as ovarian and cervical cancer (10). Zhang et al. studied the expression of squamous cell carcinoma (SCC) antigen, CA 125 and Ki 67 in cervical cancer and precancerous lesions. The results showed that the expression rates of SCC, CA 125 and Ki 67 in cervical cancer tissue were significantly higher than those in the cervical epithelium benign group and control group (11). By analyzing the preoperative CA 125 level in cervical adenocarcinoma patients with unfavorable pathological features, Kim et al. noted a link between the serum CA 125 level at diagnosis and recurrence to the illness state and prognosis (12). Takeda et al. showed that preoperative serum SCC, CA125 and CA19-9 levels were significantly correlated with the FIGO stage of cervical squamous cell carcinoma, while serum SCC and CA 125 levels were closely associated with tumor diameter, cervical interstitial invasion depth, lymphatic space invasion and lymph node metastasis (13). As a serum tumor marker, CA125 consequently plays an important role in the screening, diagnosis and monitoring of certain gynecological tumors.

HPV-DNA testing, TCT and CA 125 level testing are all important tools for screening cervical cancer, but a single method has low diagnostic value [14]. This paper focused on the value of TCT combined with HPV-DNA and CA125 tests in early cervical cancer screening.

Materials and Methods

General data

A total of 3587 women who received gynecological physical examinations in the outpatient department of Ganzhou people's Hospital from January 2018 to March 2022 were included in the study. Inclusion criteria: cervical ulcer, contact bleeding, erosion; sexual life; conforming to the diagnostic indications of TCT, HPV-DNA and CA125. Exclusion criteria: patients with recurrence of cervical cancer; patients with other malignant lesions; patients during pregnancy or lactation. The eligible subjects then underwent TCT, HR-HPV, and CA125 examinations before a colposcopy biopsy were performed on those testing positive for any of the three indicators. Pathological diagnosis served as the gold standard. Enrolled patients were required to fill in a case report form and provide

written informed consent. This study was approved by the hospital ethics committee. The patients were aged 20-65 years, with an average age of 35.5 ± 4.6 years. There was no statistical difference in general data.

TCT examination

Cervical cells were collected from the subjects as follows: rotate the collection brush clockwise evenly for 5 circles at the junction of the cervix and the cervical column, keep it for 3-5 seconds, and then transfer the cervical cells collected using the brush into the cell preservation solution, and stain it with a fully automatic production stainer. Finally, a test report was issued by a cytologist in the pathology department. According to the TBS criteria recommended by the International Cancer Society (2001), the patients were categorized by the normal or inflammatory, low-level squamous intraepithelial lesion (LSIL), high-level squamous intraepithelial lesion (HSIL), and atypical squamous cells of undetermined significance (ASCUS), squamous cell carcinoma and adenocarcinoma (CA).

HPV test

HPV test shared the sample collection method with TCT. The cervical exfoliated cell samples were detected by PCR reverse dot hybridization assay to identify a total of 13 high-risk virus subtypes -- HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. The operation steps and result interpretation were carried out according to the requirements of the kit.

CA125 test

At admission, 10 ml of peripheral fasting venous blood was drawn from all patients, and 5 ml of the samples were centrifuged at 3 000 r/min for 20 min to obtain the upper serum to detect the level of CA125 by enzyme-linked immunosorbent assay.

Cervical biopsy

Vaginal and cervical surface secretions were wiped off with a cotton ball. For those with normal colposcopy images, cervical canal scraping was performed by taking biopsies at 3, 6, 9, and 12 points of the neck. In addition, 2-5 biopsies were selected from those with abnormal colposcopy images. The specimens were subsequently sent for histopathological examination. CIN is divided by the degree of allotyping and the extent of epithelial involvement into CIN I with mild dysplasia and cellular atypia; CIN II, moderate dysplasia and marked cellular atypia; CIN III, severe dysplasia and marked cellular atypia. Histology above low levels of CIN was defined as positive.

Observation indicators

The three methods employed alone or in combination were compared in terms of sensitivity, specificity, diagnostic rate and Jorden index.

Statistical analysis

Using SPSS.19 software, the specificity and sensitivity (%) of screening for cervical cancer were compared between groups by $\chi2$ test. $P<0.05$ was considered statistically significant.

Results

Colposcopic biopsy results

Of the 738 patients with cervical lesions, 458 (62.05%) were found positive by colposcopy, of which 268 (36.31%) had low-level CIN, 173 (23.44%) high-level CIN, 17 (2.30%) cervical cancer and the remaining 280 (39.94%) chronic cervicitis.

TCT results

There were 314 abnormal TCTs in 3587 patients, of which 212 developed ASCUS, 30 LSIL, 60 HSIL and 12 SCC (Table 1).

HPV results

Among the 3587 patients, 476 tested positive for HPV infection, accounting for 13.27% of the total. HPV-positive patients included 139 with chronic cervicitis and squamous metaplasia, 185 with CIN I, 73 with CIN II, 62 with CIN III, and 17 with invasive cervical cancer. The higher the pathology grade, the higher the rate of high-risk HPV infection (Table 2).

CA125 results

Of the 3587 patients, 364 were positive for CA125. The diagnostic accuracy of CA125 alone was 10.14% and the sensitivity of CA125 alone was 73.56% with a specificity of 81.42%.

Comparison of different screening strategies

Pathological diagnosis was used as the gold standard to jointly assess the sensitivity, specificity, diagnostic yield, and Jorden index of HPV, TCT, and CA125 for lesions above CIN I (Table 3). TCT+HR-HPV+CA125 sensitivity, specificity, diagnostic compliance, and Jorden index were higher than any other strategy. In addition, the area under the curve (AUC) for TCT+HR-HPV+CA125 was greatest
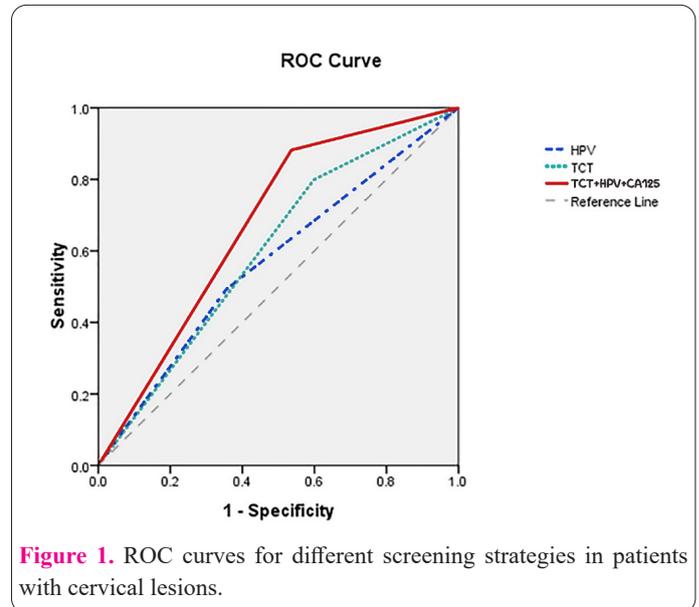

Figure 1. ROC curves for different screening strategies in patients with cervical lesions.

(0.675 (0.646, 0.697)) according to the ROC curve (Figure 1).

Discussion

Cervical cancer can be defined as a complex pathogenetic mechanism with an increasingly growing incidence rate. It tends to develop among younger females and thus seriously threatens their reproductive health. Effective diagnosis and treatment not only avoid unnecessary suffering caused by missed diagnosis and misdiagnosis but also reduce mortality (15-18). Its change stages usually include CIN I, CIN II, CIN III, cervical cancer in situ, early invasive cervical cancer, and invasive cervical cancer (19-21). CIN is closely related to invasive cervical cancer, which may reflect the continuous process of cervical cancer occurrence and development. Therefore, it is of great signi-

Table 1. Comparison of TCT and histopathological examination.

Index	Case	Chronic inflammation	CIN I	CIN II	CIN III	SCC
≤ASCUS	212	150	45	17	0	0
LSIL	30	12	11	6	1	
HSIL	60	0	2	19	38	1
SCC	12	0	0	0	0	12
In total	314	162	58	42	39	13

Table 2. Comparison of HPV-DNA screening and histopathological tissue screening (%).

Index	Case	HPV-DNA positive	Ratio
Chronic inflammation	280	139	48.7%
CIN I	268	185	69.0 %
CIN II	102	73	71.5%
CIN III	71	62	87.3%
SCC	17	17	100%

Table 3. Comparison of the efficacy of TCT, HPV-DNA and CA125 protein alone and in combination for the diagnosis of early cervical cancer [%,(n1/n2).

Index	Sensitivity	Specificity	Diagnostic coincidence rate	Youden index
CA125	73.56% (337/458)	81.42% (228/280)	75.46% (572/758)	0.574
HPV	90.61% (415/458)	72.85% (204/280)	81.66% (619/758)	0.635
TCT	53.27% (244/458)	78.57% (220/280)	61.21% (464/758)	0.321
TCT+ HPV+CA125	94.54% (435/458)	83.92% (235/280)	87.46% (663/758)	0.760

ficance to identify the nature of cervical lesions in patients as soon as possible to improve their prognosis outcomes.

Currently used cervical cancer screening methods comprise exfoliative cytology (Pap smear, TCT), HPV testing (HCII, HR-HPV typing), cytology and HPV testing (22). While playing an important role in cervical cancer screening due to simple operation, high smear collection rate and convenient operation with a high detection rate of abnormal cervical cells (23,24), TCT testing is highly influenced by subjective factors. For the same smear, different cytologists may have different readings. The diagnosis is based on cytomorphological features instead of histological features, thus increasing the rate of misdiagnosis.

HPV is a small double-stranded DNA virus. Currently, more than 200 HPV subtypes have been discovered and identified (25). HPV infection has been widely recognized as the main cause of cervical cancer and its precancerous lesions. HPV infections in the genital tract and anus are classified into low-risk and high-risk types by carcinogenic risk. Its infection rate is also associated with the incidence of cervical cancer (26). In recent years, it has been well documented that persistent HPV infection is a high-risk factor for cervical cancer, with high-risk HPV infection among the causes of HSIL and CIN (27,28). HPV testing is extremely sensitive, however, the false-positive rate is also very high. This may just be a viral infection, not necessarily the result of cervical cytology and pathology. Therefore, a safe and effective combined detection should be adopted for early diagnosis of cervical cancer.

CA 125 is a highly glycosylated mucin encoded by the MUC 16 gene. In healthy women, it is mainly synthesized by mesothelial cells and the lining of the female reproductive tract (29,30). Irritations of the mesothelial layer, such as ascites and pelvic inflammation, are common causes of elevated serum CA 125 levels (31). In contrast, cervical cancer cells synthesize and secrete CA 125 in large quantities. Serum CA 125 level in cervical cancer patients was positively correlated with cancer tissue staining (32), suggesting that cancer tissue is the main source of serum CA 125. It is well known that the glycosylation machinery changes markedly in malignant tumor tissues, resulting in abnormal glycosylation of glycoproteins synthesized in these cancer tissues (32). Thus, CA 125 level is valued as an auxiliary screening index for cervical cancer diagnosis.

The results showed no significant difference in the specificity and accuracy of TCT examination and HPV-DNA in the diagnosis of early cervical cancer. Further, HPV-DNA examination was more sensitive and accurate in diagnosing early cervical cancer than TCT, with a lower rate of missed diagnosis. The study also revealed that the sensitivity, accuracy, specificity and Youden index of TCT in combination with HPV-DNA and CA125 were higher than those of single examinations. It was because the combined examination integrated the advantages of the three methods, thereby reducing missed diagnoses and misdiagnoses and improving diagnostic efficiency.

In conclusion, TCT combined with HPV-DNA and CA125 was more effective than single examinations in diagnosing early cervical cancer.

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