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Full lenght article

Comparison of the cervista HPV HR test and luminex XMAP technology for the diagnosis of cervical intraepithelial neoplasia

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ABSTRACT

Objectives: This study was designed to compare the Cervista high risk (HR) human papillomavirus (HPV) test with Luminex XMAP technology for the detection of the relationship between HPV infection and cervical intraepithelial neoplasia.
Methods: In total, 3280 patients in a cervical specialty clinic were divided into two groups for either Cervista (1855 patients) or Luminex (1425 patients). Subsequent colposcopy examinations were performed in 1270 women with cytologic results showing ASCUS or higher level lesions. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were evaluated.
Results: The positive rates of the Cervista and Luminex groups were 61.48% and 67.43%, respectively, and occurrence in those 30–40 years old was most common. The typing of HPV showed that A9 positive cases were the most prevalent genotype (33.53%), followed by A5/A6 (16.44%) and A7 (11.37%) in the Cervista group, and HPV-16 was the most prevalent genotype (25.81%), followed by HPV-18 (18.6%) and HPV-31 (8.6%) in the Luminex group. Moreover, the overall concordance rate was 96.26% (95% confidence interval, 93.51–99.00%) in the 187 women with cytologic results of ASCUS or higher. There were no significant differences in the positive rates of HPV between the Cervista and Luminex groups, and both had a high sensitivity and NPV.
Conclusions: The results showed a high good concordance between the two methods in diagnostic accuracy. Among the patients in the cervical specialty clinic, both the A9 group of HPV and HPV-16 showed the highest positive rate. Cervista and Luminex shared similar a clinical value in the detection of CIN2 or higher.

Introduction

Cervical cancer is one of the most common malignancies among women in China and also remains a leading cause of mortality [1]. It is believed that the persistently high risk for human papillomavirus (HR-HPV) infection worldwide contributes to the development of cervical precancerous lesions and cancer [2]. To date, more than 200 HPV genotypes have been identified [3], And the 14 variants referred to as high risk HPVs include HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68, which are responsible for most cervical cancers [4]. In particular, the main causes of cervical cancer are HPV-16 and HPV-18, which account for 60–70% of the cases [5,6].

The guidelines of the American Cancer Society, the American Society for Colposcopy and Cervical Pathology, and the American Society for Clinical Pathology recommend joint detection (cytologic and HPV testing) for cervical cancer screening in women aged between 30 and 65 years every 5 years [7]. The US Food and Drug Administration (FDA) approved HPV testing, and cervical cytologic testing has been used for cervical cancer screening, leading to the development and investigation of innovative HPV detection technology [8]. Currently, several HPV detection methods have been applied in cervical cancer screening, significantly decreasing mortality from cervical cancer. The global cervical cancer incidence has increased from 378,000 cases in 1980–454,000 cases in 2010, and mortality has decreased with 200,000 fatalities among women in 2010 [9]. Thus, early cervical screening shows positive significance because the progression from precursor lesion to invasive cancer occurs over 5 years [10].

It has been documented that there is high clinical sensitivity with approximately 95% of HR-HPV testing for CIN2 or higher in

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screening populations [11]. However, few studies have been carried on the Cervista HPV HR test and Luminex XMAP technology for the detection of HPV infection. Recently, Cervista has been used to detect 14 HR HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and has been approved by the Food and Drug Administration [12]. Additionally, it shows great specificity and sensitivity in clinical trials [13,14]. Luminex is a method based on polymerase chain reaction (PCR) using primers GP5+/5′- biotinylated GP6+ to detect 27 genotypes. As a part of the validation process, we compared the two technologies for the diagnosis of cervical intraepithelial neoplasia.

Materials and methods

Patients

A total of 3280 cervical samples were collected from women at Shanghai Tenth People's Hospital between March 2014 and March 2016, including 1855 samples tested by Cervista and 1425 samples tested by Luminex. To compare the sensitivity and specificity of Cervista and Luminex, we screened 745 samples tested by Cervista and 525 samples tested by Luminex. The cytology results of these 1270 women were ASCUS and higher, in which there were 187 samples tested by both technologies.

TCT (Thinprep cytologic test) and cervical biopsy

All cervical samples were collected in a SurePath (Yaneng Bioscience (Shenzhen) Co., Ltd, China) liquid-based cytology solution or a ThinPrep PreservCyt (Hologic, Inc.) medium using the Cervix broom and were tested by the ThinPrep 2000 Processor (Hologic, Inc.) following the manufacturer's instructions in the pathology department. For all cervical specimens, HPV testing and cytologic testing were performed from the same liquid-based cytology solution. The cytological results were diagnosed according to the 2001 Bethesda classification system [15] as follows: a) negative, b) atypical squamous cells of undetermined significance (ASCUS), c) low-grade squamous intraepithelial lesion (LSIL), d) atypical squamous cells that cannot exclude HSIL (ASC-H), and e) high-grade squamous intraepithelial lesion (HSIL). Colposcopies were performed combined with a multiple punch biopsy with indications including either cytology results shown as ASCUS and higher or a positive HPV test. According to Richard's classification system [16], the pathology grades were classified as inflammation, CIN1, CIN2, CIN3, and squamous-cell carcinoma or adenocarcinoma, in which CIN2 and CIN3 were defined as HSIL.

Luminex XMAP technology

Luminex is a method using polymerase chain reaction (PCR) in a commercial HPV genotyping kit (Yaneng Bioscience (Shenzhen) Co., Ltd, China) according to the manufacturer's recommendations. A total of 27 HPV genotypes were identified based on PCR using the primers GP5+/5′- biotinylated GP6+ as described elsewhere. Probes with an amino group at the 5′ end of each of the 27 HPV types were coupled to carboxylated beads. The beads were labeled with fluorescence and analyzed in a Lumix 200 reader. The results were demonstrated using median fluorescence intensities (MIF) of $\geq$100 beads for each sample. For each probe, reactions with net MIF over 5 were considered positive, whereas MIF values in reactions with no PCR products attached to the hybridization mixture were defined as background values.

Cervista HPV HR test

Cervista is an automated method with a sequence-specific probe and Invader (Third Wave Technologies, Inc., Madison, WI) oligonucleotides that cycle rapidly on and off with the 14 HR-HPV target DNA sequences, creating a substrate for the proprietary Cleavase enzyme (Hologic, Inc.), which is a 50-flap endonuclease enzyme. In this procedure, the Gendfind kit (Hologic, Inc) was first used to extract DNA, and then Invader (Hologic, Inc) chemistry was applied, which comprised two simultaneous isothermal and signal-amplification reactions to detect specific nucleic acid sequences [17].

Cervista could detect 14 HR HPV subtypes simultaneously in 3 different groups (A5/A6: 51, 56, and 66; A7: 18, 39, 45, 59, and 68; and A9: 16, 31, 33, 35, 52, and 58).

Statistical analysis

Statistical analysis was performed using the SPSS 21.0 statistical package (SPSS Inc., Chicago, Illinois) and Prism 6.0 (GraphPad Software Inc., La Jolla, CA, USA), 95% confidence intervals were calculated. Continuous variables were reported as means $\pm$ standard deviation (SD), when categorical data were reported as numbers (percentages). Positive rates of HR-HPV were calculated and were stratified according to patient age. To determine the level of concordance between the two tests, the concordance rates were calculated as well. The x^2 test or Fisher exact test was used to analyze the relationship between Cervista and Luminex. A $P < 0.05$ indicated a statistically significant difference. In addition, the sensitivity, specificity, PPV and NPV values were calculated.

Results

In total, 1855 samples (mean age, 40.25 years; age range, 18–78 years) were tested by Cervista and 1425 samples (mean age, 40.43 years; age range, 18–79 years) were tested by Luminex. The percentage of the women aged between 30 and 40 was 33.69% (625/1855, Cervista) and 33.82% (482/1425, Luminex), in which the positive rate was 20.11% for the Cervista group and 21.05% for the Luminex group (Fig. 1). Within both groups, a similar discrepancy was observed in that the HPV positive rate was significantly different at different ages ($x^2 = 25.58$, $P < 0.01$ in the Cervista-tested group and $x^2 = 23.59$, $P < 0.01$ in the Luminex-tested group). However, between the two tested groups, there were no significant differences in the total positive rates, which were 57.26% for Cervista and 60.77% for Luminex.

Subsequent colposcopy examinations were performed in the 1280 women (745 women tested by Cervista and 525 women tested by Luminex). Among ASCUS or higher level lesions, the results showed 81.74% with cervicitis, 1.56% with CIN1, 3.62% with CIN2, 9.40% with CIN3, and 3.76% with SCC or AC in the Cervista group and 70.26% with cervicitis, 8.76% with CIN1, 9.14% with CIN2, 7.43% with CIN3, and 4.38% with SCC or AC in the Luminex group (Fig. 2). We calculated the positive rates among all the pathology categories between the Cervista and Luminex groups and found that the HPV positive rates gradually increased with increasing pathology levels (Cervicitis $<$CIN1 $<$CIN2 $<$CIN3 $<$SCC or AC), and there were large significant differences in each pathology category (CIN1, CIN2, CIN3) compared to cervicitis (Fig. 2). The HPV positive rates in CIN1, CIN2, CIN3 and SCC or AC patients were significantly higher than those in cervicitis patients, and the positive rate in SCC or AC reached 100% in both test groups. The overall positive rates of the two HPV detection methods were 61.48% (Cervista, 95% confidence intervals [CI], 54.64–100.00%) and 67.43% (Luminex, 95% confidence intervals [CI], 34.60–78.44%).