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A novel highly sensitive and specific flow cytometry system for cervical cancer screening

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HIGHLIGHTS

- A new automated FCM cervical screening system showed a high sensitivity and specificity.
- The CPIx increased statistically with the lesion degrees.
- This FCM testing has the potential to be a cervical cancer and precancerous screening method.

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ABSTRACT

Purpose. This study assessed the performance of a novel flow cytometry (FCM) cervical cancer screening system compared with human papillomavirus (HPV) Hybrid Capture 2 (HC2).

Methods. Chinese women aged 20 years or older were enrolled in this study at Fudan University Shanghai Cancer Center. All participants underwent cytology/pathology testing (gold standard), HPV HC2 testing and FCM testing involving analysis of cell proliferation index (CPIx).

Results. Among 437 women enrolled in this study, 185 women (42.3%) were diagnosed as "gold standard positive" by pathology with diseases including cervical intraepithelial neoplasia (CIN) grade 2 (n = 11), CIN3 (n = 41), squamous cell carcinoma (SCC; n = 115), adenocarcinoma in situ (n = 2) and adenocarcinoma (n = 16). The remaining 252 cases were deemed "gold standard negative". The sensitivity was 87.6% (95% CI, 82.8–92.3) for FCM testing and 89.7% (95% CI, 85.4–94.1; $p = 0.5121$) for HPV HC2 testing. The specificity of FCM testing was 90.5% (95% CI, 86.2–94.7), which was superior to the specificity of HPV HC2 testing (84.5%, 95% CI, 79.3–89.7; $p = 0.04$). In the 20–29 years old group, the sensitivity and the specificity of FCM testing were 90.0% (95% CI, 71.4–100.0) and 92.9% (95% CI, 76.9–100.0), respectively. The FCM testing CPIx statistically increased with the transition from normal cervical specimens to SCC specimens.

Conclusions. Our results showed that the FCM screening system had high sensitivity and specificity for women of various ages. The FCM CPIx was able to evaluate the severity of disease quantitatively.

1. Introduction

Cervical cancer is a worldwide health problem for women. In 2012, the World Health Organization announced that the global incidence of cervical cancer was 528,000 with an annual mortality of 266,000. Fortunately, the incidence and mortality of cervical cancer have distinctly decreased in countries which carried out Pap testing for large-scale screening over the last century [1,2]. Since the popularization of cervical cytology screening in the United States in the 1950s, cervical cancer has dropped from the first to the fourteenth most common cause of cancer death [3]. In recent years, liquid-based cytology developed from conventional cytology has optimized the accuracy and reliability of testing. However, cervical cancer is still a leading cause of death in many developing countries due to the lack of high-quality cytology screening and experienced cytologists.

Thus, there is great demand for an automated-screening system that exhibits high sensitivity, high specificity and high-throughput. Since the

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revelation of pathogenic processes involving the cervical epithelium and cervical carcinoma, several studies have proven that human papillomavirus (HPV) testing is an efficient screening method for cervical cancer [4,5]. Such testing is now a basic part of all recommended guidelines for cervical precancerous lesion and cancer screening. However, HPV testing has a much lower specificity than cytology for detecting squamous intraepithelial neoplasia. Therefore, most guidelines discourage using HPV testing of women less than 29 years old due to an unacceptable high false positive rate.

To decrease the burden on cytologists and meet the ever-increasing screening load, we have been using a new flow cytometry screening system for cervical cancer that addresses the problems mentioned above and contributes to the early detection of cervical cancer [6–9]. This system implements efficient screening by both sorting out the majority of normal specimens and detecting abnormal specimens with high sensitivity. Subsequently, the abnormal specimens can be subjected to more detailed testing. This automated flow cytometry screening system includes specimen pre-treatment technology and cell analysis technology. The specimen pre-treatment technology is a combination of methods to disperse cells (chemical, mechanical, and physical), adjust cell density, and collect cells. The cell analysis technology is based on fluorescent signals and scattering from DNA staining and flow cytometry measurements, respectively.

Our goal was to identify a more accurate, less expensive, and more automated screening method for the detection of cervical cancer and high-grade cervical neoplastic lesions. In this paper, we report the findings of our clinical study that assessed and compared the performance of HPV Hybrid Capture II (HC2) and flow cytometry testing in the same subject group.

2. Materials and methods

2.1. Patient groups and screening procedure

This study was conducted at Fudan University Shanghai Cancer Center (Shanghai, China) between 2012 and 2013 after the protocol had been approved by the Ethical Committee of this center. We selected non-pregnant Chinese women 20 years of age and older who intended to undergo cervical cytology testing for health examination or cervical disease confirmation. Female subjects who had received cervical conization, hysterectomy, radiotherapy or chemotherapy were excluded. Eligible women were enrolled in this study after they submitted written informed consent.

All subjects first underwent cervical exfoliated cell collection. These samples were tested separately by cytology, HPV, and flow cytometry. If there were cytology findings of atypical squamous cells of undetermined significance (ASC-US) or ASC-US with HPV positivity, the patient required colposcopy and biopsy (see Fig. 1). For the management of cervical intraepithelial neoplasia (CIN), cancer, and HPV HC2 (+), the principal investigators or doctors in attendance complied with standard treatment procedures.

2.2. Clinical specimens

Two vials of cervical liquid-based cytology samples were obtained with a ThinPrep Pap Test Physician's Kit (70136–001) and Hologic brush devices simultaneously. One vial was gathered for cytology and flow cytometry testing, and the other was used for HPV testing. ThinPrep vials were aliquoted into a 5 ml volume for flow cytometry

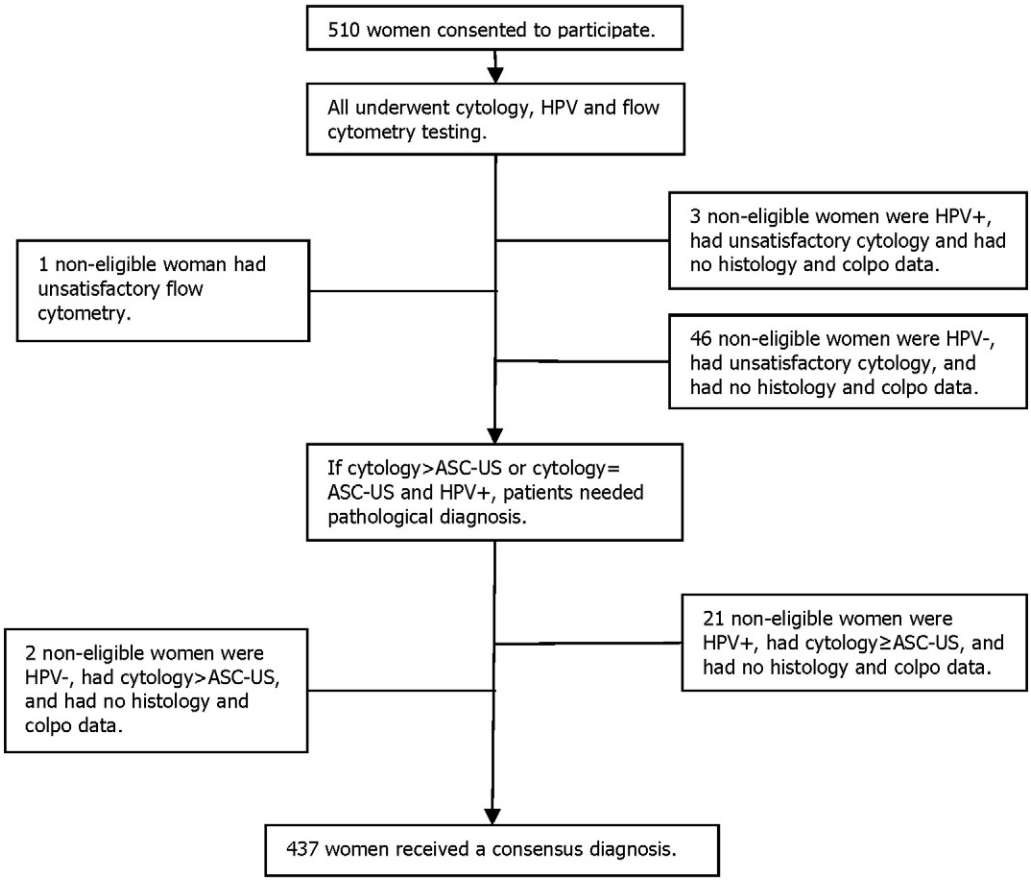

Fig. 1. Study profiles of enrollment. HPV = human papillomavirus, ASC-US = atypical squamous cells of undetermined significance, and Colpo = colposcopy.