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Comparison of human papillomavirus testing and cytology for cervical cancer screening in a primary health care setting in the Democratic Republic of the Congo

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ABSTRACT

Objectives. We compared the screening performance of conventional Pap cytology and two human papillomavirus (HPV) DNA assays, the original Hybrid Capture 2 (HC2) and an expanded version that tests for 4 additional HPV types (HC2 + 4; Qiagen Corporation), in the detection of cervical neoplasia among unscreened women in a primary care setting in a suburb of Kinshasa, Democratic Republic of the Congo.

Methods. All women 30 years or older residing in the area were invited to participate, and 1528 were evaluated by Pap cytology and the two HPV assays, conducted at a European and US reference laboratory, respectively, followed by colposcopy. Cervical biopsies were obtained from all women with abnormal colposcopy and from 290 randomly chosen women with normal colposcopy (to correct for verification bias).

Results. Using a relative light unit of 1 as the cutoff for positivity, 169 and 168 (11%) women tested positive using HC2 and HC2 + 4, respectively. HC2 and HC2 + 4 were in agreement in 98.6% of cases (Kappa = 0.94; 95% confidence interval: 0.91–0.96). Both assays were sensitive (~83%) and specific (~91%) for the detection of cervical intraepithelial neoplasia-2 or worse disease. Irrespective of the cutoff point used to define positivity, Pap cytology was both less sensitive and more specific than HC2 or HC2 + 4. For instance, cytology was 63% sensitive and 97% specific when a cutoff point of low-grade squamous intraepithelial lesions or worse was used.

Conclusions. Among unscreened women, HC2 and HC2 + 4 had similar screening accuracy for cervical neoplasia, and both were more sensitive but less specific than Pap cytology.

Introduction

As the second most frequent cancer among women worldwide, cervical cancer is an important global public health problem [1]. Women in developing countries are affected disproportionately by this disease. Nearly 80% of all invasive cervical cancer cases occur in developing countries [1]. In most developed countries, Pap cytology screening for the early detection of cervical neoplasia has been successful in reducing cervical cancer incidence and mortality, especially in jurisdictions with organized screening programs [2]. However, Pap cytology as a primary screening tool has important limitations. In addition to low sensitivity and poor reliability [3,4], cytology-based screening programs require technical expertise and financial resources that are not readily available in most developing countries [5]. In the Democratic Republic of the Congo (DRC), the scarcity of pathologists and cytotechnologists has impeded the implementation of cytology-based screening programs, which may have contributed to that country's high incidence of invasive and advanced stage cervical cancer [6].

Abbreviations: HC2, Hybrid Capture 2; HPV, human papillomavirus; PPV, positive predictive value; NPV, negative predictive value; CIN, Cervical intraepithelial neoplasia; RLU, relative light units; ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade SIL; ROC, Receiver operating characteristic.

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The identification of HPV as a necessary cause of cervical cancer has raised the prospect of HPV DNA testing being used to screen for cervical cancer. The FDA-approved Hybrid Capture 2 (HC2) assay (Qiagen, MD) is one of the most widely used HPV DNA assays [7]. The diagnostic performance of HC2 has been evaluated in clinical trials and observational studies [8–11]. HC2 tests for the presence of 13 high-risk (HR) oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68, a core set of HR-HPVs that have been targeted in all subsequent commercial HPV assays used in clinical applications. The International Agency for Research on Cancer lists a total of 25 HPV types as possible, probable, or confirmed carcinogens [12]. Yet, little is known about the performance of HPV assays that expanded on the above core set of HR types in detecting cervical lesions.

We conducted a population-based study of an unscreened population in the DRC to evaluate the diagnostic performance of several screening tests as compared to cervical cytology for the detection of cervical neoplasia. Detailed findings on the performance of visual inspection methods as screening tools were presented elsewhere [6]. In this paper, we present results of analyses comparing the diagnostic performance of Pap cytology, HC2, and an expanded version of HC2 that probes for 17 HR-HPV types (henceforth designated as HC2 + 4).

Methods

The methods of this population-based cross-sectional study were described in detail elsewhere [6,13]. Briefly, between November 2003 and April 2004, 1699 women aged 30 and older residing in a suburb of Kinshasa, DRC (population ~25,000 with approximately 2000 women in the chosen age range) were invited to participate in health education sessions on cervical cancer prevention at the local health care center, and 1571 (92.5%) participated. After obtaining informed consent, eligible women were enrolled in the study. Women were eligible if they were 30 years or older and had an intact uterus but were not pregnant. Nineteen women were ineligible, in most cases due to pregnancy or hysterectomy. Twenty four other women, who did not return for screening after menstruation, were excluded. The remaining 1528 women were included in this analysis. Ethics approval was obtained from the PATH Human Subjects Protection Committee and the Institutional Review Board of the Kinshasa School of Public Health.

A trained research nurse interviewed all women using a standardized questionnaire, and obtained cervical specimens for conventional cervical cytology and HPV testing using a cytobrush and an Ayre's spatula. Pap smears, prepared with both devices, were fixed and shipped to the “Groupement de Recherche Cytologique” in Lyon, France, where they were stained and read by a cytology technician and reviewed by a cytopathologist. Cytological diagnoses were based on the Bethesda system [14], and were made without knowledge of disease status or the results of the other screening tests.

The same cytobrush that was used to make the Pap smear was then placed in a specimen transport medium (Qiagen, Germantown, MD) kept at 4 °C and shipped to the laboratory of one of the authors (AL at Digene, Inc., Gaithersburg, MD, later acquired by Qiagen) in the United States for HPV testing. The standard commercially available HC2 was performed according to the manufacturer's instructions. Specimens were considered positive if the ratio of Relative Light Units (RLUs) of the specimen to the mean RLUs of triplicates of a positive control was equal to or greater than 1 (equivalent to 1 pg/ml of HPV DNA/ml) [8]. An expanded probe set version of HC2 that included types 26, 66 73, and 82, in addition to the 13 HR-HPVs targeted by HC2, was used experimentally (designated HC2 + 4). This new HC test was conducted with the same standard protocol and cutoff analyses as routinely used for the HC2 test.

Following the collection of cervical specimens, all participants underwent direct visual inspection of the cervix after the application of 5% acetic acid solution (VIA). This was followed by reapplication of 5% acetic acid and colposcopic examination by an experienced gynecologist (GSL) who had previously undertaken colposcopy training at the Gynecologic-Oncology Department of the Centre Hospitalier de l'Universite de Montreal, Canada. Colposcopic impressions were classified, according to widely accepted colposcopic patterns, as normal (including inflammation), low-grade cervical intraepithelial neoplasia (CIN), high-grade CIN, or probable invasive cancer [15]. When lesional epithelium was visible, a biopsy was taken. Finally, visual inspection with Lugol's iodine (VILI) of the cervix was performed. Because the findings from the visual inspection may increase the propensity for the colposcopist to identify lesional tissue resulting in verification bias [16], cervical biopsies were performed in a random 20% sample of women who had normal colposcopic findings. This allowed us to correct for verification bias by examining the rate of disease in those with normal colposcopy and using it to adjust the overall distribution of lesions conditional on test results [17].

Cervical biopsy specimens were fixed in Bouin solution and embedded in paraffin. Paraffin blocks were shipped to the Department of Pathology, Jewish General Hospital, Montreal, Canada. Histological sections were stained with hematoxylin and eosin, and slides were read independently by two pathologists (AF and NHS). The cytopathologists and pathologists were blinded to the results of the screening tests and to the colposcopic impressions. The reports were based on the WHO criteria for classification of histological diagnosis of CIN.

Statistical analysis

Conventional screening indexes, such as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), and their asymptotic 95% confidence intervals (95%CI) were calculated using standard formulae for individual tests and combinations of tests. Two sets of reference diagnoses were used: the first was based on the histological examination alone whereas the second was based on histological examination supplemented by colposcopic diagnosis if histology was not available. Screening indexes were calculated for two thresholds of disease severity as defined by the reference diagnostic method: CIN 2 or worse, and CIN 3 or worse (including invasive cancer). For Pap cytology, calculations were performed for three thresholds of test positivity: “atypical squamous cells of undetermined significance” (ASCUS) or worse, low-grade squamous intraepithelial lesion (LSIL) or worse, and high-grade SIL (HSIL) or worse.

Because we tested for the presence of CIN or cancer in a random sample of women who tested negative by colposcopy, we were able to derive verification bias-corrected estimates of sensitivity and specificity for the various tests [16]. Correction for verification bias was done using validated formulae [8,17] calculated, along with bootstrapped 95% confidence intervals (95%CI), using the VALIDES Stata package (Stata Corporation, TX, USA) [18].

Results

Table 1 shows the distribution of socio-demographic and reproductive characteristics for the 1528 women who had colposcopy according to their biopsy verification status. About 29% of women were 50 or older and 33% were post-menopausal. Most women (75%) were married and most had 6 or more children. Most women had one lifetime sexual partner, and 71% first had sexual intercourse before the age of 18. Generally, there were no significant differences in the distribution of the listed variables between women who had a biopsy due to abnormal colposcopy and women who were selected randomly for biopsy, except that the former group was, on average, slightly younger (Table 1).

Table 2 shows the joint distribution of screening test results and cervical biopsy results. Colposcopic examinations were completed