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DNA- versus RNA-based methods for human papillomavirus detection in cervical neoplasia

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Abstract

Objective. To compare DNA-based and mRNA-based methods for detection of high-grade cervical neoplasia in Norway.

Methods. HPV prevalence was analyzed in 383 women with positive index cytology, selected from gynecology clinics. All patients were investigated by a new PAP smear, histology, and two commercially available HPV tests: Hybrid Capture II (Digene, Gaithersburg, MD) and the Pre Tect HPV-Proofer (NorChip AS). Cases with positive DNA test and negative mRNA test and cases with high-grade histology and negative HPV tests were retested with PCR and sequencing. We regarded the infection as latent or transient if sequencing revealed an HPV type included in both assays.

Results. High-risk HPV was detected in 99.7% of the histological confirmed high-grade lesions (CIN2+) (290/291). The DNA test was positive in 95% (275/291), and the mRNA test was positive in 77% (225/291) of the histological confirmed high-grade lesions. All invasive carcinomas were mRNA positive. The DNA test was significantly more often positive in benign and low-grade lesions, some of which were found to be false positive due to cross-contamination with unrelated types. High-grade histology was detected in 83% of women with normal cytology and positive mRNA test. Latent or transient infections were detected in 11 low-grade and 12 high-grade preinvasive lesions. Sequencing revealed high-risk HPV types included only in the DNA test in 35 high-grade preinvasive lesions, HPV 52 and 58 were the most prevalent HPV types.

Conclusions. These HPV tests have the potential to improve the detection rate of high-grade cervical neoplasia, with some limitations. The mRNA test seems to be more appropriate for risk-evaluation. Larger scale, population based studies are necessary to evaluate the predictive values of HPV testing in Norway.

Keywords: HPV; DNA; mRNA; CIN; Carcinoma

Introduction

It has now been firmly established that infection with high-risk human papillomavirus (HPV) types is the primary cause of almost all cervical cancers [1–3]. Persistent infection with high-risk HPV types is the first step in the carcinogenesis [4]. However, the vast majority of women infected by high-risk HPV will not develop cervical cancer, as most HPV infections are transient, especially in the young, sexually active population. Women with active HPV infection will express E6/E7 oncogenes, which are required for malignant transformation by inhibiting the tumor suppressors p53 and RB [4]. E6/E7 mRNA transcripts are detected by mRNA-based molecular techniques [5,6].

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Standardized HPV DNA-based testing has shown that high-risk HPV DNA detection has higher sensitivity for histological verified high-grade cervical neoplasia compared to classical cytology [7–13]. However, the specificity as well as the positive predictive values (PPV) in these studies is low, especially when young women are tested. Persistent expression of E6/E7 oncogenes could serve as an indicator of progression to cervical intraepithelial neoplasia (CIN) and invasive cancer [14,15]. RNA-based testing to detect E6/E7 mRNA transcripts may therefore be of higher prognostic value and improve the specificity and PPV compared to HPV DNA testing in screening. While DNA-based methods have been extensively evaluated in screening programs, the predictive values of mRNA based methods are unknown. Few studies concerning the extent of oncogenic expression in cervical neoplasia have so far been published [14–19].

Recent meta-analyses have shown that the accuracy of PAP test varied greatly with sensitivity ranging from 30–87% [20,21]. The high rate of false negative cytology has important public health and legal implications. Consequently, there has been substantial interest in the use of HPV testing in order to improve the efficiency of population-based screening programs for cervical cancer. It has been discussed if HPV testing may be used as primary screening for cervical cancer, as triage for equivocal or low-grade lesions and in the follow-up after treatment for CIN [22]. Secondary HPV testing will be implemented in the Norwegian cervical cancer screening program in 2005. The aims of the present study were to evaluate two commercially available assays for HPV testing to detect high-grade cervical neoplasia. The outcome of DNA-based and mRNA-based testing was compared with cytology and histology, using histology as the "gold-standard".

Patients and methods

Study population and collection of specimens

Women were recruited from 5 hospital based outpatients clinics and 10 gynecologists in private practice in Norway. Enrolment took place from October 2002 through October 2003. Included in this study were 383 women with median age 35 years (range, 19–85). The study population comprised women with positive cytology referred for colposcopy or conization. Both squamous and glandular lesions were included in the study. The Regional Committee for Medical Research Ethics, South Region, approved the study protocol and written informed consent was obtained from the study subjects.

Cervical specimens were collected with Cervex-Brush (ROVERS Medical Devices B.V.Oss-The Netherlands). A conventional Pap smear was taken first, and the remaining material on the brush was transferred to a PreservCyt™ vial (Cytec Corporation, Boxborough, MA) for HPV analyses.

HPV testing

Testing for high-risk HPV DNA (type 16, 18, 31, 33,35, 39, 45, 51, 52, 56, 58, 59, and 68) was performed with the Hybrid Capture II assay (Digene, Gaithersburg, MD) as described by the manufacturer. The results were given as the ratio of relative lights units (RLU), and the chosen positive threshold of this test was 1.0 pg of HPV DNA/ml. The performance of HC II has been shown to be broadly equivalent to that of well-established PCR protocols for the detection of HPV in clinical specimens, and the analytical sensitivity is 0.2 pg HPV DNA which is equivalent to approximately 20,000 HPV genomes equivalents [23].

Total mRNA was extracted using the RNeasy Mini-protocol (Qiagen, cat. no. 74104). Individual identification of E6/E7 mRNA full-length transcripts from HPV 16, 18, 31, 33, and 45 was performed with the Pre Tect HPV-Proofer assay (NorChip AS, Klokkarstua, Norway) as described by the manufacturer. Artificial and standardized oligos, corresponding to the viral sequence, are included as positive control for each HPV type. To avoid false negative results due to degradation of RNA, a primer-set and probe directed against the human U1 small nuclear ribonucleoprotein (snRNP) specific A protein (U1A mRNA) is used as the performance control. The analytical sensitivity of the Pre Tect HPV-Proofer is less than 10 SiHA cells, equivalent to 20 copies of HPV 16 and less than 1 HeLa cell equivalent to 25 copies of HPV 18. The analytical sensitivity is based on random testing of HPV types, and no cross reactivity has been discovered between HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, and 58 (Molden et al., unpublished results).

Sequencing

Additional testing with PCR and sequencing was performed in 40 cases with benign/low-grade histology, positive DNA test, and negative mRNA test, in 59 cases with high-grade histology (CIN2+), positive DNA test, and negative mRNA test, and in 7 cases with high-grade histology and both HPV tests negative. Sequencing was done on the PCR product obtained by using the consensus primers Gp5+/6+ and CpI/CpIIG [24,25]. An aliquot of the PCR product was sequenced using Sanger's chain-termination method. This was accomplished by the cycle sequencing reaction using the DNA sequencing kit (PE Applied Biosystems, Foster City, CA) [26] and the PCR primers as sequencing primers. The products of the sequencing reaction were analyzed using the Applied Biosystems Prism 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA). The identification of the sequences was performed by sequence comparison to the NCBI Sequence Database. Ominga sequence analysis software (Accelrys, San Diego, CA) was used for the sequence analysis. Ninety-one out of these 106 cases were successfully sequenced. We regarded the infection as latent