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Letters to the Editor / Cartas al Editor

HPV DIAGNOSIS IN THE CLINICAL SETTING. CORRELATION AND DISCREPANCIES BETWEEN MOLECULAR TECHNIQUES

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To the Editor:

Cervical cancer is the second most common cancer in women worldwide, and it is the principal cancer of women in most developing countries, where 80 percent of cases occur. Molecular epidemiologic evidence clearly indicates that certain types of human papillomavirus (HPV) are the principal cause of invasive cervical cancer and cervical intraepithelial neoplasia. More than 80 HPV types have been identified, and about 40 can infect the genital tract. About 100 HPV genotypes are known to date and different types are classified on the basis of the amount of DNA homology. Genital HPV types have been subdivided into low-risk types, which are found mainly in genital warts, and high-risk types, which are frequently associated with invasive cervical cancer¹. In order to meet clinical requirements, molecular assays to detect and type HPV genomes in biopsy and cytological specimens have been developed and standardised. HPV diagnostic tests are based mainly on direct hybridisation assays with signal amplification and on nucleic acid amplification methods².

Several HPV DNA detection methods have been described during the last decade, each of which allows the detection of a wide spectrum of HPV types, but none has fulfilled all expectations. Amplification-based methods, mainly PCR, are currently the most sensitive methods for detection of HPV DNA³ but they are time consuming and less applicable to large number of samples. The HPV tests that are most widely applied are based on two principles. The first, as used in the HCII assay, involves hybridization of HPV target DNA with a cocktail of full-length HPV type-specific RNAs, followed by capture of DNA/RNA hybrids on a solid phase. Subsequently, signal amplification is achieved by the binding of hybrids to multiple conjugated antibodies that specifically recognize DNA/RNA hybrids. The principle of the consensus PCR methods is based on PCR amplification of HPV target DNA directed by so-called consensus or general primers that bind to highly conserved regions within the L1 open reading frame of all genital HPV genotypes. Several read-out systems have been described for the latter assays, but enzyme immuno-assays (eg EIA, DEIA) using type specific oligoprobes either individually or in a cocktail or reverse line blot assays (eg LIPA, RLB) are now most commonly used⁴.

In the present study we comparatively described the diagnostic correlation observed between the technique currently applied for screening of HPV DNA and those for typing positive cervical samples from woman suspected to have gynecological lesion selected to HPV.

METHODS:

The HPV DNA tests considered in this study were the Hybrid Capture 2 (HC2) test (Hybrid Capture 2, Digene, Gaithersburg, USA), the PCR (polymerase chain reaction) and Inno-LiPa HPV Genotyping (Innogenetics, USA). The HC2 test is a standardized test approved by the Food and Drug Administration (FDA) that has been used extensively in research studies and has been in routine clinical use for more than 4 years. A total of 990 genital smears were obtained in a solution hybridisation assay. Hybrid Capture 2 (HC2) is a sensitive test for the detection of cervical intraepithelial neoplasia grade 3 (CIN3) and cervical cancer (CIN3+)⁵. The applied hybridisation technique classifies the virus on those associated to a low or high-moderate risk of cell transformation. We analysed the agreement between these results and those obtained by PCR and subsequent typing by means of digestion with restriction enzymes (HPVfast, Genomica S.A.U., Madrid, Spain). Polymerase chain reaction relies on the enzymatic amplification of HPV DNA to allow the detection of very low levels of HPV infection. We used a LIPA technique in 100 positive smears analysed by hybridisation in order to evaluate this assay with a previous one.

RESULTS:

The results obtained by hybridisation were distributed as follows: 31,2% of the smears revealed the presence of HPV DNA of high-moderate risk of cell transformation, 3,1% was associated to a HPV DNA of low risk of transformation and the remaining 6,8 % were positive for both HPV DNA. PCR was able to detect 49,3% (N=205) of positive samples by hybridisation techniques. 49,5% (N=153) of HPV of high-moderated risk (H-M risk) were not detected by the PCR used for identification. The figures for the rest of positive samples were as follows: 77,4%(N=24) among low-risk HPV, being 49,3%(N=33) of those positive for both HPV groups (p<0,05) not detected by means of PCR. In samples analysed by LIPA, high-moderate risk HPV was found in 35%, low-risk HPV in 4% and 7,2% of both type groups. 91% of positive smears by means of hybridization were confirmed by LIPA and the agreement between both techniques on the classification of HPV was of 89%.

CONCLUSIONS:

The PCR identification technique lack to detect a significant number of HPV positive samples, mainly on the HPV H-M risk and less for the others. The use of a LIPA test leads to a more accurate identification. Then there is a better correlation between LIPA and DNA hybridisation and more discrepancies among PCR and hybridization.

Competing interests: The authors declare that they have no competing interests.

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HPV MOLECULAR DIAGNOSIS

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To the Editor:

We read with interest the letter by [Dominguez-Gil et al.](#) regarding the molecular diagnosis of human papillomavirus (HPV). In Costa Rica, the Guanacaste study Project¹ was designed to investigate the role of HPV infection in the development of cervical neoplasia and evaluate new cervical cancer screening technologies. This project identified that HPV-16 alone accounts for approximately half of all cervical cancers in Guanacaste likewise².

The diagnosis of HPV infections in patients at risk of disease in a clinical setting requires a different approach from that used for epidemiological studies, vaccination trials, and natural history studies³. The main interest in HPV relates to its causative role in cervical cancer, one of the most common cancers in women, with an annual incidence of almost half a million and a mortality rate of approximately 50%. The development of highly sensitive DNA detection assays over the past years has revolutionized the diagnosis of HPV and allowed various crucial aspects of HPV infections to be studied. However, diagnostic test results should be interpreted with caution and require careful laboratory validation⁴. There is a clear need for well characterized international quality control panels to compare the various diagnostic methods. The implications of HPV-DNA detection for patient management purposes needs to be addressed properly. Recent studies have shown that the prevalence of HPV-DNA and of multiple HPV genotypes in the same patient is higher than expected. Also, the efficacy of large community-based HPV screening studies depends on the accuracy and predictive values of the diagnostic assays used. To identify women with an increased risk for cervical neoplasia, it is clear that detection of HPV-DNA alone is insufficient and novel algorithms are being developed which combine cytological screening and HPV-DNA analysis, to optimize the positive and negative predictive values for development of disease⁵.

Two different pathways can be used of molecular HPV diagnostics⁶. The first application is aimed at identifying women at risk of developing cervical cancer, either in community-based screening programs or in the clinical setting. Here, a highly sensitive HPV detection assay will greatly overestimate the proportion of women who have low-grade cytological abnormalities. A less sensitive or a quantitative assay might be more effective in identifying women at risk of progression, thus improving both negative and positive predictive values⁷. It is also important that the assay detects only high-risk genotypes, which are associated with a significantly increased risk for cervical carcinoma. The interesting study by Dominguez-Gil and co-workers is of great importance because represents an effort to assess better tests to identify HPV and its clinical correlation.

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