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Editorial

Role of human papillomavirus genotyping in cervical cancer screening

Papel del genotipado de papillomavirus humano en el cribado del cáncer cervical

The main goal of any cancer screening procedure is to eradicate, eliminate or minimize the impact of the disease or, if none of these is possible, to slow down its progression and reduce morbidity. Cervical cancer can be prevented almost completely through vaccination and screening procedures aimed at detecting precursor lesions in the early phases or invasive cancer in early stages.[1] The success of any screening program depends on the rate of participation of the target population, the screening model (population-based models being more efficient than opportunistic ones), the characteristics of the used test and the quality of the diagnosis and provided treatment. The World Health Organization considers cervical cancer a public health concern worldwide and launched a global initiative in 2020,[2] aimed at eliminating the disease by 2030, especially in lower- and middle-income countries. The organization recommends human papillomavirus (HPV)-DNA detection as the primary method (rather than cytology) in screening.[3] One of the main goals of this strategy is to screen for 70% of the 35–45 years old population with high standard tests.

In the last few decades, major advances in cervical cancer screening models have been made, primarily through the inclusion of HPV-DNA detection techniques. As a result, many countries have changed their pap-smear screening approaches to strategies based on HPV-DNA tests. This change was also partially due to the intrinsic characteristics of cervical cytology and the well-known relationship between persistent high-risk HPV (hrHPV) infection and cervical cancer or its precursor lesions.[4] According to several studies, conventional cytology screening has only 51% (30–87%) sensitivity and 98% (86–100%) specificity for detection of high-grade cervical epithelial lesions.[5] It is estimated that about 30% failures in cervical cancer prevention can be attributed to false negative results in cytology studies. High oncogenic risk HPV types include genotypes 16 and 18, found in more than 70% of all cases of cervical cancer[6] and covered by the three prophylactic HPV-vaccines currently available in the market. It has been established that molecular methods for hrHPV-DNA detection have higher relative sensitivity (1.37; 95% CI 1.20–1.55) and specificity (0.95; 95% CI 0.94–0.97) than cytology – conventional or liquid – for the diagnosis of ASC-US or higher grade findings.[7]

HPV detection has been used for primary cervical cancer screening in several clinical trials and population-based cohort studies over the past two decades. Randomized clinical trials conducted in Europe (Swedescreen, POBASCAM, ARTISTIC and NTCC),[8] with follow-up periods of up to 6 years, plus those carried out in Finland (Finish Trial), Hong Kong (Hong Kong SAR), Australia (Compass trial) and Canada (HPV FOCAL) showed that the use of hrHPV tests significantly reduced detection of high-grade lesions or cancer in a second round of trials, in hrHPV-screened women compared with cytology-screened ones.[1] In addition, negative hrHPV test results were associated to much lower risk of suffering high-grade lesions or long-term cancer than negative cytology results. The low specificity and positive predictive value (PPV) of hrHPV tests cause many positive hrHPV patients with a transient infection bound to resolve on its own, to be referred to unnecessary colposcopy or clinical follow-up and to experience unjustified psychological stress. Therefore, using hrHPV tests is not recommended in patients under 25 years of age. Such weaknesses lead us to search for screening strategies that help us differentiate patients at high risk of developing high-grade lesions, who consequently need colposcopy, from those at an intermediate risk, who need clinical follow-up or those at a low risk, who should return to the established routine screening circuit.[9] In our environment, the screening methods most frequently used in hrHPV positive patients are: reflex cytology, HPV genotyping, dual p16/Ki67 staining and recently, study of the methylation of certain genes and viral load. These methods are used alone or in combination.[10]

Three years ago, the Spanish Ministry of Health updated the primary cervical cancer screening model from a cytology-based approach to molecular detection of hrHPV DNA in women of 35–65 years of age. In this regard, studies like the one published by Hernández-Aguado et al. (CRYGEN 16/18 study)[11] provide valuable information to evaluate the different screening modalities that can be applied to the primary molecular model in order to improve its efficiency.

The CRYGEN 16/18[11] study was a blinded, prospective, interventional study, which included nearly two thousand women of 35 years of age, designed to evaluate ten different primary screening protocols based on molecular methods, cytology or a combination of both. The study followed the European 2015-updated guidelines,[12] which recommend the use of hrHPV tests to screen women over 35 years of age in an organized population-based structure. Potential participants were sent a personal invitation to participate in the study. With a primary molecular hrHPV detection protocol similar to that of the ATHENA study,[13] the CRYGEN

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16/18 study used the Cobas HPV test® (Roche Molecular Diagnostics), which can detect hrHPV types 16 and 18 separately from a set of other hrHPV types (31, 33, 35, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). The efficacy and optimization of the CRYGEN 16/18 strategy was compared with those of 9 other theoretical strategies (2 ones based on cytology with or without subsequent hrHPV test, 3 ones based on co-test and 4 ones based on molecular hrHPV detection in primary screening). In the CRYGEN 16/18 protocol, women with either a positive result for hrHPV 16/18 or a positive result for other hrHPV types plus cytological alterations (≥ASCUS – atypical squamous cell of undetermined significance) were referred to colposcopy. The age of selected subjects was established following current national and international guidelines[12,14,15] (≥30 years). However, selecting subjects within a one-year age range (35 years) is a limitation that may hinder the applicability of results; actually, an evaluation of screening strategies in broader age groups, similar to a real-life setting, is desirable. The prevalence of hrHPV infection and the proportion of hrHPV types 16/18 were similar to those of other population-based studies.[13,16]

HPV16 is the most prevalent genotype in high-grade lesions, followed by HPV33, HPV31 and HPV18, all of which can be used as markers of increased risk in primary screening.[17] The combination of different screening methods has been evaluated in cross-sectional studies, as well as in longitudinal studies, in which the risk of high-grade lesions could be assessed. Currently, the combined genotyping most frequently used for screening hrHPV-positive patients is HPV16/18, which shows lower specificity for ≥ASC-US than cytology, though similar sensitivity.[10] Previous studies showed that partial HPV16/18 genotyping plus reflex cytology is a cost-effective strategy in cervical screening.[18] In a recent study, Stanczuk et al.[10] reported that a two-step screening strategy consisting of initial HPV16/18 genotyping followed by liquid cytology, in patients with positive hrHPV other than 16/18, showed higher sensitivity (90%), specificity (60%) and PPV (22-38%) for ≥CIN2 detection than one-step approaches, in which the same techniques were used alone. With the two-step model, the risk of CIN3 in patients that were negative when they left the screening circuit was 2.3% after 3 years and 1.8% after 5 years. A combined strategy could be proposed,[1] similar to that of the CRYGEN 16/18 study, in which HPV16/18 positive patients (20% risk of CIN3) would be directly referred to colposcopy, while patients who are positive for hrHPV other than 16/18 (8% risk of CIN3) would undergo cytological assessment and would only be referred to colposcopy if ≥ASC-US are detected. With both groups, a sensitivity of 83% for CIN2 and 86% for CIN3 would be reached, with 55% specificity for low-risk lesions.

The results of the study indicate that only three protocols would detect the same number of high-grade lesions ($n = 26$) than CRYGEN 16/18, although with more patients referred to colposcopy. Two of them are co-test strategies (concomitant cytology and HPV test), while with the third protocol, all hrHPV positive patients are referred to colposcopy. None of these three protocols is recommended in current guidelines.[12,14] In the CRYGEN 16/18 study, in which positive 16/18 HPV women were directly referred to colposcopy, 26 high-grade lesions were detected (17 ones positive for hrHPV 16 and 9 positive for hrHPV other than 16/18), with 100% sensitivity, 19.5 PPV, 100% NPV and 133 patients referred to colposcopy. Thus, six high-grade lesions, which had negative cytology but positive hrHPV 16 results, would have passed undetected if only cytology had been used. These results support the authors' suggestion to avoid reflex cytology in HPV 16/18 positive cases.

The official strategy, approved by the Spanish health authorities, for the implementation of a screening protocol based on hrHPV detection is identical to protocol 7 of the CRYGEN 16/18 study, which was considered to detect only 20 cases of high-grade lesions with PPV of 29.1 (compared to 19.5 in the CRYGEN 16/18 study), NPV of 99.5% and 76 patients referred to colposcopy; i.e. nearly 43% less than CRYGEN 16/18. This latter figure should be considered a very important factor in the cost-effectiveness analysis of any cervical screening protocol. In addition, the official protocol includes second follow-up rounds for patients with positive hrHPV results and negative cytology (such as the above mentioned six high-grade cases detected with the CRYGEN 16/18 protocol), who are to be examined 12 months later. Second rounds were proven to detect lesions initially unadvertised due to limitations of cytological examination or to the natural history of the HPV infection with long latency periods.[19] In addition, it should be noted that the prevalence of HPV16 decreases in older women, which compromises its usefulness as a screening tool, and HPV18 does not perform well in triage.[12,20] An important aspect to be considered in designing a screening program is the potential effect of HPV vaccination.[21] According to some studies, the prevalence of viral types (HPV 6, HPV11, HPV16 and HPV18) covered by the earliest approved vaccines (bivalent and quadrivalent) and their associated lesions have declined significantly and some have almost disappeared. As the prevalence of such genotypes and their associated lesions becomes lower, the PPV decreases and the number of false positive results increases, thereby increasing the number of unnecessary diagnostic procedures and treatments. In this scenario, molecular methods for detection of hrHPV other than 16/18 would become a more reliable screening tool.

In conclusion, CRYGEN 16/18 is a valuable study that supports the use of hrHPV molecular detection in cervical screening protocols and shows that hrHPV genotyping improves detection of high-grade cervical lesions. It further suggests that direct referral to colposcopy with positive HPV 16/18 genotyping as well as liquid cytology triage (≥ASCUS) in patients positive for hrHPV other than 16/18 provides substantial advantages that deserve to be verified in our setting. Current cervical cancer screening programs should be based on international[12,14] and national[15] guidelines. Future studies could help us help us modify and adapt the screening protocols dynamically and rigorously, according to the different emerging public health scenarios. Therefore, it is essential to understand and evaluate the epidemiology of the infection, its associated lesions and variants in different populations, the available healthcare personnel and infrastructures, the risks assumed for a chosen screening procedure and the associated costs, in each community.

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