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Review Article

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Prevention of Cervical Cancer in Women: Human Papillomavirus DNA Testing in Atypical Pap Smears

Massimo Origoni[1*], Guia Carminati[1], Chiara Gelardi[1], Francesca Occhi[1] and Chiara Stefani[1]

Summary

Human papillomavirus (HPV) is definitely recognized as the necessary cause for the development of cancer of the uterine cervix and the detection of HPV-DNA in cervical samples is demonstrated to own a significantly higher sensitivity towards preneoplastic lesions than conventional cytology (Pap test). Screening, management of atypical Pap tests and follow up of treated patients, represent the optimal settings where HPV-DNA testing has been demonstrated of clinical value. Atypical Pap tests account for cases in which the cytological alterations cannot clearly be attributed neither to negative nor to positive cytology; in these cases HPV-DNA testing has been demonstrated to have a sensitivity very close to 100% in identifying patients with an histologically proven intraepithelial preneoplastic lesion of high grade (CIN2-CIN3). Despite this, specificity of HPV-DNA positive testing lacks of significance and the referral rate to second-level colposcopy is too high. Different options have been tested to improve the specificity and the overall performance of HPV-DNA testing in cases of equivocal cervical cytology; the present paper aims to collect and present data from the recent literature, in order to better clarify the present state of the art in this particular aspect of cervical cancer prevention.

Keywords

Human papillomavirus; HPV; HPV-DNA testing; Cervical cancer prevention; Pap test; Cervical cytology; ASC-US; Atypical cytology

Introduction

Cervical cancer is the third malignancy for frequency in women worldwide. It also represents the second most common cancer and the second most common cause of cancer-related deaths in women in developing countries [1]. Among causal agents, different sexually transmitted pathogens, such as bacteria (*Chlamydia trachomatis*), protozoa (*Trichomonas vaginalis*) and viruses (*herpes simplex virus type 2*) have been implicated and studied, until in the early 1980s, Zur Hausen identified human papillomavirus (HPV) in biopsies of cervical cancer. However, only in the late 1990s HPV was convincingly established as a common sexually transmitted infection with potential risk of carcinogenicity towards the female cervical cells [2]. It is now widely accepted, and strong evidence exists, that nearly 100% of cervical cancer cases test positive for HPV [3], and that the persistence of cervical infection with high-risk HPV genotypes represents the most important risk factor for the development of cervical cancer and its immediate precursor lesion, cervical intraepithelial neoplasia (CIN). Cervical cancer prevention strategies mainly rely upon cytological screening (Pap test) and, according to this, the incidence of invasive cancers of the uterine cervix has dramatically decreased in countries were screening programs have been realized on a large scale. In recent years, prevention of cervical cancer has changed its target, focusing the efforts towards the identification of women at high risk for developing cervical cancer rather than women with preinvasive lesions; in this view, HPV testing has strongly emerged as a powerful tool to detect oncogenic viral strains long before the neoplastic transformation would occur.

HPV and cervical cancer pathogenesis

Human papillomavirus (HPV) is a non-enveloped double-stranded DNA-virus that belongs to the Papillomaviridae family [4]. More than 150 different HPV genotypes have been isolated and more than 40 of these subtypes can infect the female genital epithelial and mucosal surfaces, such as the uterine cervix [5]. Among these, high-risk (e.g. HPV16 and 18) and low-risk genotypes (e.g. HPV6 and 11) can be distinguished, the first involved in the transformation to cervical cancer and the latter mainly associated to the onset of benign genital warts. The HPV virion is composed by 8-kb circular genome that is enclosed in a capsid shell which comprises a major (L1) and minor capsid protein (L2). The genome also encodes for several early genes (E1, E2, E4, E5, E6 and E7) that enable viral transcription and replication and interact with the host genome [6,7]. In the majority of immunocompetent individuals, the viral genome does not interact with the host cells nuclear DNA, resulting in a transient and subclinical infection (resolving within 1 year in 70% of cases and within 2 years in about 90% of cases) [8]. However, it is not clearly understood why HPV infection is spontaneously cleared in certain individuals while progresses towards more severe lesions in others. The host immune system has been demonstrated to play a key role in this issue. The recognition of a causal link between HPV and cervical cancer, along with the understanding of the epidemiology and natural history of HPV infection, has led to a new model for cervical carcinogenesis: 1) HPV acquisition, 2) HPV persistence (vs clearance), 3) progression to precancerous lesions, and malignant invasion [6-9]. The natural history of HPV-induced cervical carcinogenesis begins with the primary infection of the proliferating/differentiating basal cells of the squamous cervical epithelium. If the infection is determined by a high-risk HPV type and if the host local and systemic immune response fails to control and clear the infection, HPV infection persists accumulating genomic instability and leading to neoplastic transformation of the epithelium. These phenomena are influenced by the viral genome integration within the genome of the epithelial cells, resulting in an interruption of the genes encoding for E1 and E2. These genes encode proteins which have a crucial role in regulating the expression of E6 and E7. The increasing expression of the viral oncogenes E6 and E7 abrogate cell cycle control and apoptosis mechanisms, signaling the transition from a viral infection to a

***Corresponding author:** Dr. Massimo Origoni, MD, Department of Gynecology & Obstetrics, Vita Salute San Raffaele University School of Medicine, Ospedale San Raffaele Scientific Institute, Via Olgettina 60, 20132 Milan, Italy, Tel: +39.02.2643.2579; Fax: +39.02.2643.2759; E-mail: massimo.origoni@hsr.it

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malignant process. Further genetic alterations involving the loss of tumor suppressor genes and changes in cellular growth modulation result in the progression from CIN lesions to overt malignancy [9]. As previously detailed, two different subgroups of HPVs have been identified: high-risk HPVs and low-risk HPVs; high-risk types only have biological relevance in the onset of cervical cancer, as only these types present integration of their DNA in the host cells promoting neoplastic transformation. HPV16 is the most carcinogenic HPV genotype and accounts for approximately 55% to 60% of all cervical cancers. HPV18 is the second most carcinogenic HPV genotype, accounting for approximately 10% to 15% of cervical cancers [10]. Approximately 10 other HPV genotypes [11-19] are correlated with the remaining 25% to 35% of all cervical cancers. HPV18 causes a greater proportion of glandular cancers, adenocarcinoma and adenosquamous carcinoma, than squamous cell carcinoma (approximately 32% vs 8%, respectively) [20]. The persistent infection of high-risk genotypes determines significant risk of developing precancerous lesions, compared to others HPV types. One-year [21] and 2-year HPV persistence [22] especially by HPV16, strongly predict CIN3 or more severe diagnoses (CIN3+) in the subsequent years (e.g. a 20%-30% risk of CIN3+ over 5 years). Untreated CIN3 has a 30% probability of becoming invasive cancer over a 30-year period, while only about 1% of treated CIN3 will progress to invasive cancer [23].

HPV testing in cervical cancer prevention

Cervical cancer screening began in the 1950s with the introduction of the Papanicolaou smear, which has to be considered a milestone in cancer prevention efforts. Cytologic screening has been very successful in significantly lowering cervical cancer incidence and mortality in countries where good-quality screening is available; however, the overall performance of Pap test is characterized by a very high specificity (>90%) but a significantly lower sensitivity (50-60%). Thus, the rate of false negative tests is actually considered unsatisfactory in terms of screening success. The issue of improving the sensitivity of cervical screening is one of the most debated among clinicians and researchers of the last ten years. An increased understanding of the association between HPV and cervical cancer risk has led to the development of several molecular tests for HPV that offer increased sensitivity albeit lower specificity compared with cytology [24]. Moreover, HPV tests may better forecast which women will develop CIN3+ over the following 5-15 years than cytology [25-28]. Twenty years have now passed since the first studies using human papillomavirus (HPV) testing began in clinical settings. As a result, the American Cancer Society (ACS) guidelines for the early detection of cervical cancer included HPV-DNA testing in its review and update in 2002 [29,30]. Since that time, several studies have been published supporting changes to recommended age-appropriate screening as well as the management of abnormal screening results; all the results are consistent in demonstrating the superior sensitivity of HPV testing compared to cervical cytology [31]. Thus, it became clearly evidenced that HPV-DNA detection results in predicting the risk of cervical cancer and its precursors, better and in advance than cytological or colposcopic abnormalities, which are "signs" of HPV-correlated transformation of cervical tissues. Today, the validated use of HPV-DNA testing essentially covers three different situations: 1) cervical screening 2) management of atypical (ASC-US) cytology and 3) follow up after treatment of preneoplastic lesions (CIN) [31]. According to published data, screening and follow up after treatment seem to have reached some kind of definite management with the use of HPV-DNA testing, while the "borderline" category of atypical pap smear still deserves attention and is object of increasing interest and speculations.

The atypical Pap smear

Communication to women of uncertain or equivocal findings at the Pap test, such as minimal cytologic squamous atypia, and their clinical significance have been among the greatest challenges related to the Pap test throughout its history. These cells share some but not all of the cytologic features of cancer precursor and are not easily distinguishable from inflammatory or reactive changes. In 1998, Kinney demonstrated that these cytological abnormalities harboured a high percentage of high-grade dysplasia [32]. In the original classification system, developed by Papanicolaou, these "abnormal" cells have been included in the class II category, within five degrees of classes including progressively more high-grade alterations, from normal cytology to the detection of cancer. The class II comprised reactive and reparative changes, koilocytic atypia, inflammation, non-specific atypia and other specific infections besides HPV [33]. A revised classification of cytologic abnormalities was introduced in 1988 with the Bethesda system. This led to the dismantling of Papanicolaou classes: the inflammatory and reactive findings were included in the category of "within normal limits" cells, while the koilocytic atypia and mild dysplasia were combined in the termed "low-grade squamous intraepithelial lesions" (LSIL). The other class II benign atypia were included in the new category "atypical squamous cells of undetermined significance" (ASCUS) which represented a diagnosis of exclusion when cytopathologic findings were not definitely clear-cut to allow a more specific diagnosis. Smear characteristics may be "favour reactive" or "favour dysplasia" [34]. In 2001 the terminology was revised and "undetermined significance" (ASC-US) and "high-grade dysplasia cannot be excluded" (ASC-H) were introduced. This was done with the aim of better describing cellular changes that do not fulfill criteria for HSIL cytology, but for which a high-grade lesion cannot be excluded [35]. The Bethesda system also introduced a new category for atypical glandular cells termed "atypical glandular cells of undetermined significance" (AGC-US), which was previously included in Papanicolaou class II as a vaguely defined category of endocervical glandular atypia. The risk of detection of a histological high-grade squamous intraepithelial lesions (HSIL) is 2-3 times greater in the follow up of AGC-US than in that of an ASC-US or LSIL Pap smear [36]. Nowadays, the diagnosis of ASC-US should be expected in no more than 5% of all performed cervical smears or should not exceed 2-3 times the rate of SIL [37,38]. A rate exceeding these parameters is likely due to an overreading benign reactive and inflammatory change in atypia. Approximately 5% of all ASC diagnosis are ASC-H lesions [39], while an AGC-US diagnosis is reported in the 0.13-0.8% of all Pap smears [11,40].

HPV-DNA testing in ASC-US management

The triage of ASC-US cytology has been the first validated clinical application of HPV-DNA testing approved by the FDA [12]. As already mentioned above, cytologic interpretation of ASC-US represents a category of morphologic uncertainty. It means "some, but not all" of the features of an LSIL and as such, includes both poorly sampled and poorly represented LSIL and the many morphologic mimics of LSIL. Because of its equivocal nature, it is a much lesser reproducible diagnosis than other cytological abnormalities and is frequently associated with spontaneously resolving, self-limited disease or no disease at all. The current American Society of

Colposcopy and Cervical Pathology (ASCCP) recommendations [41] for the management of ASC-US cytology indicate the use of HPV-DNA testing in these cases to better stratify the risk of development of cervical cancer precursor lesions. In fact, atypical squamous cells of undetermined significance and LSIL cytology represent an important clinical issue, as 15–20% of these lesions may be histologically positive for a high-grade preneoplatic lesion of the cervix (CIN2+) [13]. According to a recent meta-analysis, the absolute risk of underlying high-grade cervical intraepithelial neoplasia (CIN2 and CIN3+) among women with ASC-US cytology is, on average, 9–10% for CIN2 and 4–5% for CIN3+. For women with LSIL, these risks are about 1.5 to 2 times as high [42]. Therefore, it is essential to triage and select women who need more intensive follow up among those with ASCUS and LSIL cytology. Several studies have evaluated the efficacy of HPV testing as a triage tool for atypical pap smear. Manos evaluated one of the most widely performed test for HPV-DNA detection in cervical samples, the HC2® test, (Hybrid Capture 2 – QIAGEN Inc., USA) after an ASC-US cytology and demonstrated that the HC2® sensitivity for the detection of CIN2+ lesions in comparison to repeated Pap smear was 89.2% versus 75.8% [43]. Schiffman and Adrianza from the National Cancer Institute (NCI), coordinated the largest government-funded randomized trial on abnormal Pap test management, the ASCUS/LSIL Triage Study (ALTS) [44]. The purpose of this large multicenter randomized trial was to evaluate whether ASC-US would have been most efficiently and safely managed by referral to immediate colposcopy, by repeated Pap test or by HPV-DNA testing. In this setting of almost 3,500 women, sensitivity rates for CIN3 and colposcopy referrals rates were 53.6% and 100% for immediate colposcopy, 54.6% and 12.3% for repeated cytology, and 72.3% and 55.6% for HPV-DNA triage, respectively; these results were demonstrated to have statistical significance [26]. Of note, it was found that a single enrollment HPV-DNA test identified 92.4% of the women affected by a histologically proven CIN3; repeated cytology would have required two rounds of follow up to achieve a similar sensitivity (95.4%), referring 67.1% of women to colposcopy [26]. This trial, as confirmed also by other similar studies, demonstrated that HPV-DNA testing for ASC-US triage had a significantly higher sensitivity for CIN 3+ than repeated cytology. Based upon these results, both the American Society for Colposcopy and Cervical Pathology (ASCCP) consensus guidelines and European Guidelines, actually recommend HPV-DNA testing as a viable options for the management of ASCUS [14,45,46]. ASC-H cases management, because of their higher risk of hiding high-grade lesions or cancer, are best referred to immediate colposcopy irrespective of the HPV testing result [36].

HPV-DNA testing in LSIL management

In the case of baseline LSIL cytology, however, as many as 80% of women in the ALTS trial were found to be HPV positive; in this group, HPV-DNA testing fails to discriminate between clinically insignificant cytological abnormalities and those representing true cervical cancer precursors [44]. The ALTS trial, as well as a meta-analysis of published studies [47] and data from a very large randomized italian study, the New Technologies for Cervical Cancer screening group (NTCC) [48], concluded that HPV testing was of no value in managing women with LSIL cytology. As a matter of fact, the American Society for Colposcopy and Cervical Pathology recommends that all women with LSIL cytology should undergo immediate colposcopy instead of HPV testing [45].

HPV-DNA testing in AGC-US management

Despite in case of an AGC-US cytology the risk of a high-grade lesion is higher than in case of an ASC-US smear, the majority of women are not found to have a neoplastic disease. However, considering that squamous dysplasia, AIS (*adenocarcinoma in situ*) and cervical cancer are found in 20-50% of AGCUS, all women are usually referred to immediate colposcopy, and if over 35 years they are also referred to hysteroscopy with endometrial biopsy [49]. In these situations, the use of HPV-DNA testing is more controversial and unclear, because patients are immediately referred to colposcopic evaluation (due to the higher risk for cancer), and because this cytological diagnosis is very rare. Ronnett reported a positive predictive value and sensitivity of HPV-DNA testing for all high-grade glandular and squamous lesions of 41% and 94% respectively, compared to 22.7% and 62.5% for repeated cytology [15]. In this context, the utility of HPV-DNA testing may be linked to an increased reassurance that no significant disease have been missed when the colposcopic and hysteroscopic evaluation are negative, whereas a positive HPV-DNA testing may require due diligence.

Limits of HPV-DNA testing

As described before, HPV-DNA testing provides the major advantage of significantly improving the sensitivity for the detection of histologically confirmed CIN2/CIN3 in the triage of ASCUS cytology; this is reported to be as high as 85-100% according to published series. However, this high sensitivity almost always corresponds to a low specificity (~60%) and low positive predictive value (PPV) [42], determining high referral rates to second-level colposcopy and biopsy. Meta-analyses including trials that followed the ALTS study confirmed the same findings in terms of higher sensitivity for detection of CIN2/3 of HPV-DNA testing compared to repeated cervical cytology (95% vs 82%), but found a moderate-poor specificity for both approaches (67% and 58%, respectively). The Trial Of Management of Borderline and Other Low grade Abnormal smears trial (TOMBOLA) [50] started in 1999 and was a 7-year, multicentre trial run from Dundee, Aberdeen and Nottingham. It evaluated the contribution of HPV-DNA testing to the effectiveness and efficacy of the existing procedures for the management of women with borderline cytological results. The cross-sectional and longitudinal results of the TOMBOLA trial partially contrast with those from the ALTS study. The sensitivity for prevalent CIN2+ and CIN3+ was substantially lower in the TOMBOLA trial compared with the ALTS study results and the following meta-analysis; this may be explained by differences in specific HPV-DNA testing assay sensitivity or differences in outcome assessment. In the TOMBOLA trial no significant interaction was found by study arm between CIN2+ detection in HPV-positive compared with HPV-negative women (similar relative risks). The authors interpreted their data as evidence for not recommending more aggressive management for HR-HPV-positive women. The TOMBOLA trial results do not support guidelines recommending HPV-based triage of women with equivocal cervical cytology because of the high number of false positives cases. For triage of LSILs, the situation is less clear. In conclusion, these results may be reassumed stating that although HPV-DNA testing-based triage increases sensitivity compared with repeated cytology, its specificity remains low for the triage of borderline (ASCUS) cases.

Improving HPV-DNA testing specificity

Several studies have considered different options in the attempt

of increasing the tests' specificity; positive effects upon tests' performance by increasing the age at which testing is performed and HPV genotyping are among the most widely studied [51]. In a meta-analysis that considered a total of 20,810 women, Khan et al. [52] showed that the 10-year cumulative incidence of CIN3 or worse was 17.2% (95% CI - 11.5% to 22.9%) among HPV16+ women and 13.6% (95% CI - 3.6% to 23.7%) among HPV18+16– women, but only 3.0% (95% CI - 1.9% to 4.2%) among women HC2® positive but negative for both HPV16 and HPV18; the 10-year cumulative incidence among HC2® negative cases was 0.8% (95% CI - 0.6% to 1.1%). A sub-analysis among women aged ≥ 30-years with normal cytology at enrollment strengthened the observed risk differences. The authors concluded that HPV-negative women at baseline were substantially less likely to develop high-grade CIN over 10 years of follow up than women who were HPV-positive. The risk was higher with HPV16 and 18 subtypes and lower with other HPV types. In terms of prognostic significance, HPV genotyping identifies about one-tenth of women at risk of developing CIN2+ disease, and a closer follow up has been recommended for this subset. The 2012 guidelines of the American Society of Clinical Pathology (ASCP) stated that among cytology-negative women aged ≥ 30-years who test HPV-DNA positive (for any of the HR-HPV types detected by the HR-HPV assays), molecular genotyping assays detecting HPV16 and 18 would be clinically useful for identifying cases to be referred for immediate colposcopy, and cases to be followed up by co-testing with repeated cytology and HPV-DNA testing in 12 months [53]. On the other end, genotyping is inappropriate for women <30-years or who have already been diagnosed a positive Pap test (ASC-US+); in these situations HPV genotyping would not add clinical usefulness or improve management, and thus the ASCP guidelines do not recommend the use of HPV genotyping in women with ASC-US who are HR-HPV positive. In this context, Ronco et al. reported a study on 22,708 women, computing the sensitivity and the specificity of HC2® testing for histology-confirmed CIN2 or more severe lesions (CIN2+) separately among women with ASC-US or AGC-US and with LSIL cytology, and among two age groups (25-34 and 35-60). HC2® results were expressed as the ratio of each specimen's light emission compared to the average of three concurrently tested controls, each containing 1 pg/ml of HPV-DNA. This ratio is quantified as relative light units/cut off (RLU/CO). For each group of patients the receiver operating characteristic (ROC) curves were also computed, using different log-RLU values as cut-off. The area under the curves was taken as an overall measure of test accuracy. It was observed that the area under the ROC curve was significantly lower among women aged 25-34 years, than in those older, either considering ASC-US/AGC-US ($p = 0.0355$) and LSIL ($p = 0.0009$) cytology; additionally, the area under the curve for LSIL was significantly lower compared to the area for ASC-US in this age groups ($p = 0.0084$). Authors concluded that a higher specificity of HPV-DNA testing for ASC-US triage was observed with increasing age and that, considering that the ROC curve is very low in women under 35 years, triaging for LSIL cytology should not be performed in women ≤ 35 years. For older women, the utility of HPV-DNA triaging should also be considered in LSIL cases [48]. The issue of increasing the cut-off value of HPV-DNA testing in order to improve its specificity seemed to be a simple and reasonable option to reach the goal; in this view, several authors have investigated the performance of the tests according to different levels of increased cut-off values of positivity. Together with expected significant improvements in terms of specificity, these attempts have however almost always been correlated with lowering sensitivity and Negative Predictive Value (NPV) [16,17,54,55]. The determination of the viral load in HPV-DNA positive samples, as a marker of subjects at higher risk for intraepithelial cervical lesions, has been proposed as a viable tool to discriminate between simple HPV infections and HPV-induced cervical pre-neoplastic lesions. In fact, a close correlation had already been reported between high HPV viral load levels and high-grade CIN or cervical cancer [24,27,28]. Moberg et al. [56] examined 2747 archived Pap smears specimens and found that the risk of CIN3 directly and significantly correlated with HPV16 viral load. In a recently published experience, we tested and confirmed the hypothesis that the determination of HPV viral load could be of use in the management of atypical pap smear to improve the specificity of HPV-DNA testing without lowering its sensitivity and NPV. We designed a study to investigate the correlation between the semi-quantitative measurement of HPV viral load, expressed as relative light units (RLU) obtained using the HC2® assay, and the presence and grade of cervical preneoplastic lesions in ASC-US cases. Our goal was to identify the prognostic significance of HPV viral load figures in 614 consecutive ASC-US cases. HC2® RLU/CO values, categorized into five classes, were correlated to clinical outcomes and statistically analysed. A significant correlation ($p < 0.0001$) was observed between increasing RLU values and the prevalence of high-grade CIN (CIN2/CIN3) [57]. In a subsequent work we also demonstrated that ASC-US cases with RLU/CO ratios below 10.0 were associated with a significantly lower rate of histologically proven CIN2/3 compared to RLU/CO ratios > 10.0 (4.6% vs 24.2% - $p = 0.0002$) [18]. Our results are consistent with those reported by Jarboe et al. [58] who reported 3.2% and 17.3% ($p = 0.047$) in the same groups of patients respectively, with no differences in CIN1 prevalence. Moreover, our results came from a high-risk subgroup of patients from the age-related standpoint (mean age 38.3 yrs), and thus seemed to be bias-free. We concluded that a "low-grade positivity" of HPV viral load in ASC-US cytology identifies women at low-risk for CIN 2/3. These results could represent an interesting matter of debate to suggest modifications of the standard algorithm of management of ASC-US cases. Another interesting option, tested in recent years, aimed to the improvement of HPV-DNA testing specificity in atypical Pap smears, has been the use of new biomarkers ($p16^{ink4}$-Ki67) directly implicated in HPV effects of host cells; these new markers, although without any direct interaction with HPV-DNA tests, may represent a promising tool for the overall improvement of the efficacy of conventional cervical cytology. In fact, recent papers have strongly supported the significant correlation with high-grade cervical lesions (CIN2-CIN3) in case of $p16^{ink4}$-Ki67 positive cervical smears [19,59-61].

Cost-effectiveness of HPV-DNA testing in clinical practice

The issue of the cost-effectiveness analysis for the use of HPV-DNA testing in clinical practice has been one of the most debated topics of the last decades; in particular, the hypothesis that the introduction of this new technologies could have been associated with a significative increase in costs, even if correlated with relevant clinical advantages, has contributed to the delay of the tests' acceptance by clinicians and health organizations. Another related major concern has been identified with regard to the inherent low specificity of the tests, which may result in over referral to second-level diagnostic procedures (i.e. colposcopy/biopsy/histology). In this view, several studies have been performed, either in developed and in low-resources settings, according to mathematical models and simulations aimed to the cost-benefit evaluation of different HPV-DNA testing utilizations. All the available data in the recent literature are consistent in identifying a

cost-effective result when cytology-based screening programmes are switched to HPV-DNA testing alone [62-66]. The obvious primary endpoint is the reduction of cervical cancer incidence and the cancer-related costs for clinical management. Moreover, the major advantages are direct consequences of the lengthening of the screening intervals (up to 6 years), the lowered referral to colposcopy only limited to cases of HPV persistence for at least two years, and the exclusion from long and expensive follow-ups of HPV-negative patients after treatment. Another positive effect, not of secondary importance mainly from the patients' point of view, is the reduced anxiety determined by the reduced need for histological confirmation of an abnormal pap test.

Conclusions

The identification and detection of HPV-DNA has represented a significative improvement in the effort of optimizing the results and performance of cervical cancer prevention strategies in different settings: screening, management of atypical cervical cytology and follow up of conservatively treated patients. It is now widely demonstrated and accepted, with the inclusion in many international guidelines, that the use of HPV-DNA testing is significantly superior in terms of sensitivity towards high-grade cervical cancers precursors, compared to conventional cytology. High sensitivity, very close to 100%, is actually recognized as the most valuable positive feature of HPV-DNA detection in cervical samples. Many studies have consistently demonstrated that, being human papillomaviruses the necessary cause for the onset of cervical cancer, the virus identification represents the optimal target for the identification of at-risk women, longer before the onset of preneoplastic disease. As a matter of fact, HPV-DNA testing is still characterized by intrinsic limitations, the most important of which is the relatively low specificity and positive Predictive Value (PPV). In this view, different options aimed to increasing the tests specificity have been evaluated, and satisfactory results obtained. It is reasonable to believe that, together with the substantial change in the scenario of cervical cancer prevention of the last years, the near future will be marked by the definite availability of the best-performing preventive strategies.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, et al. (2011) Global cancer statistics. CA Cancer J Clin 61: 69-90.
2. Parkin DM, Bray F, Ferlay J, Pisani P (2005) Global cancer statistics. CA Cancer J Clin 55: 74–108.
3. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, et al. (1999) Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 189: 12-29.
4. Markowitz LE, Dunne EF, Saraiya M, LawsonHW, Chesson H, et al. (2007) Quadrivalent human papillomavirus vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 56: 1–24.
5. Schiffman M, Castle PE (2003) Human papillomavirus: epidemiology and public health. Arch Pathol Lab Med 127: 930–934.
6. ZurHausen J (2000) Papillomavirus causing cancer: evasion from host-cell control in early events in carcinogenesis. J Natl Cancer Inst 92: 690–698.
7. Stanley M, Lowy DR, Frazer I (2006) Chapter 12: prophylactic HPV vaccines: underlying mechanisms. Vaccine 24: 106–113.
8. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD (1998) Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 338: 423–428.
9. Snijders PJ, Steenbergen RD, Heideman DA, Meijer CJ (2006) HPV-mediated cervical carcinogenesis: concepts and clinical implications. J Pathol 208: 152–164.
10. Munoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, et al. (2003) Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 348: 518-527.
11. Kennedy AW, Salmieri SS, Wirth SL, Biscotti CV, Tuason LJ, et al. (1996) Results of the clinical evaluation of atypical glandular cells of undetermined significance (AGCUS) detected on cervical cytology screening. Gynecol Oncol 63: 14-18.
12. Stoler MH, Schiffman M (2001) Atypical Squamous Cells of Undetermined Significance– Low-grade Squamous Intraepithelial Lesion Triage Study (ALTS) Group. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. JAMA 285: 1500-1505.
13. Bhatla N, Singla S, Awasthi D (2012) Human papillomavirus deoxyribonucleic acid testing in developed countries. Best Pract Res Clin Obstet Gynaecol 26: 209–220.
14. Wright TC Jr, Schiffman M, Solomon D, Cox JT, Garcia F, et al. (2004) Interim guidance for the use of human papillomavirus DNA testing as an adjunct to cervical cytology for screening. Obstet Gynecol 103: 304–309.
15. Ronnett BM, Manos MM, Ramesley JE, Fetterman BJ, Kinney WK, et al. (1999) Atypical glandular cells of undetermined significance (AGCUS) : cytologic features, histopathologic results and human papillomavirus DNA detection. Hum Pathol 30: 816-825.
16. Sargent A, Bailey A, Turner A, Almonte M, Gilham C, et al. (2010) Optimal threshold for a positive hybrid capture 2 test for detection of human papillomavirus: data from the ARTISTIC trial. J Clin Microbiol 48: 554-558.
17. Castle PE, Lorincz AT, Mielzynska-Lohnas J, Scott DR, Glass AG, et al. (2002) Results of human papillomavirus DNA testing with the Hybrid Capture 2 assay are reproducible. J Clin Microbiol 40: 1088-1090.
18. Origoni M, Carminati G, Sideri M, Clementi M, Rolla S, et al. (2012) "Low-grade positivity" of HPV viral load after atypical squamous cells of undetermined significance (ASC-US) cytology identifies women at low-risk for cervical intraepithelial neoplasia grade 2 and 3. Eur J Gynaecol Oncol 33: 261-264.
19. Yoshida T, Sano T, Kanuma T, Inoue H, Itoh T, et al. (2011) Usefulness of CINtec® PLUS p16/Ki-67 Double-Staining in Cytological Screening of Cervical Cancer. Acta Cytol 55: 413-420.
20. De Sanjosé S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, et al. (2010) Retrospective International Survey and HPV Time Trends Study Group. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 11: 1048-1056.
21. Castle PE, Rodriguez AC, Burk RD, Herrero R, Wacholder S, et al. (2009) Short term persistence of human papillomavirus and risk of cervical precancer and cancer: population based cohort study. BMJ 339: b2569.
22. Kjaer SK, Frederiksen K, Munk C, Iftner T (2010) Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. J Natl Cancer Inst 102: 1478-1488.
23. McCredie MR, Sharples KJ, Paul C, Baranyai J, Medley G, et al. (2008) Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. Lancet Oncol 9: 425-434.
24. Nishino HT, Tambouret RH, Wilbur DC (2011) Testing for human papillomavirus in cervical cancer screening: a review of indications and methodology. Cancer Cythopathol 119: 219-227.
25. Castle PE, Fetterman B, Thomas Cox J, Shaber R, Poitras N, et al. (2010) The age-specific relationships of abnormal cytology and human papillomavirus DNA results to the risk of cervical precancer and cancer. Obstet Gynecol 116: 76-84.
26. ASCUS-LSIL Triage Study (ALTS) Group (2003) Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. Am J Obstet Gynecol 188: 1383-1392.
27. Sherman ME, Lorincz AT, Scott DR, Wacholder S, Castle PE, et al. (2003) Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. J Natl Cancer Inst 95: 46-52.

28. Schiffman M, Glass AG, Wentzensen N, Rush BB, Castle PE, et al. (2011) A long-term prospective study of type-specific human papillomavirus infection and risk of cervical neoplasia among 20,000 women in the Portland Kaiser Cohort Study. Cancer Epidemiol Biomarkers Prev 20: 1398-1409.

29. Debbie S, Diane S, Herschel WL, Maureen K, Shalini LK, et al. **(**2012) American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology Screening Guidelines for the Prevention and Early Detection of Cervical Cancer. Am J Clin Pathol 137: 516-542.

30. Saslow D, Runowicz CD, Solomon D, Moscicki AB, Smith RA, et al. (2002) American Cancer Society. American Cancer Society guideline for the early detection of cervical neoplasia and cancer. CA Cancer J Clin 52: 342-362.

31. Schiffman M, Wentzensen N, Wacholder S, Kinney W, Gage JC, et al. (2011) Human Papillomavirus Testing in the Prevention of Cervical Cancer. J Natl Cancer Inst 103: 1–16.

32. Kinney WK, Manos MM, Hurley LB, Ransley JE. (1998) Where's the high grade cervical neoplasia? The importance of the minimally abnormal Papanicolaou smear. Obstet Gynecol 91: 973-976.

33. Papanicolaou GN, Traut HF (1943) Diagnosis of uterine cancer by the vaginal smear. Yale J Biol Med 15: 924.

34. National Cancer Institute Workshop (1989) The 1988 Bethesda System for reporting cervical/vaginal cytologic diagnosis. JAMA 262: 931-934.

35. Solomon D, Davey DD, Kurman R, Moriarty A, O'Connor D, et al. (2002) The 2001 Bethesda System: terminology for the reporting results of cervical cytology. JAMA 287: 2114-2119.

36. Cox T (2001) Management of atypical squamous cells of undetermined significance and low-grade squamous intraepithelial lesion by human papillomavirus testing. Best Pract Res Clin Obstet Gynecol 15: 715-741.

37. Kurman RJ, Solomon D (1993) The Bethesda System for reporting cervical/vaginal cytologic diagnosis. Definitions, criteria and explanatory notes for terminology and specimen adequacy. New York, Springer-Verlag Editors.

38. Kurman RJ, Henson D, Herbst A, Noller KF, Schiffman MF (2004) Interim guidelines for management of abnormal cervical cytology. JAMA 271: 1866-1869.

39. Schifmann M, Solomon D, Liaw KL, Sherman M (1998) Why, how and when the cytologic diagnosis of ASCUS should be eliminated. J Low Gen Tract Dis 2: 165-169.

40. Raab SS, Snider TE, Potts SA, Mc Daniel HL, Robinson RA, et al. (1997) Atypical glandular cells of undetermined significance: diagnostic accuracy and interobserver variability using select cytologic criteria. Am J Clin Pathol 107: 299-307.

41. Wright TC Jr, Schiffman M (2003) Adding a test for human papillomavirus DNA to cervical cancer screening. N Engl J Med 348: 489-490.

42. Arbyn M, Sasieni P, Meijer CJ, Claviel C, Koliopoulos G, et al. (2006) Chapter 9: Clinical applications of HPV testing: a summary of meta-analyses. Vaccine 24: S78–S89.

43. Manos MM, Kinney WK, Hurley LB, Sherman ME, Shieh-Ngai J, et al. (1999) Identifying women with cervical neoplasia: using human papillomavirus testing for equivocal Papanicolaou results. JAMA 281: 1605-1610.

44. Schiffman M, Adrianza ME (2000) ASCUS-LSIL Triage Study. Design, methods and characteristics of trial participants. Acta Cytol 44: 726-742.

45. Wright TC Jr, Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, et al. (2007) 2006 consensus guidelines for the management of women with abnormal cervical screening tests. Am J Obstet Gynecol 197: 346-355.

46. Barzon L, Giorgi C, Buonaguro FM, Palu G (2008) Guidelines of the Italian Society for Virology on HPV testing and vaccination for cervical cancer prevention. Infect Agent Cancer 3: 14.

47. Arbyn M, Paraskevaidis E, Martin-Hirsch P, Prendiville W, Dillner J (2005) Clinical utility of HPV DNA detection: triage of minor cervical lesions, follow-up of women treated for high-grade CIN. An update of pooled evidence. Gynecol Oncol 99: S7-11.

48. Ronco G, Cuzick J, Segnan N, Brezzi S, Carozzi F, et al. (2007) HPV triage for low grade (L-SIL) citology is appropriate for women over 35 in mass cervical cancer screening using liquid base citology. Eur J Cancer 43: 476-480.

49. Cox JT (1997) ASSCP practice guidelines: management of glandular abnormalities in the cervical smear. J Low Gen Tract Dis 1: 41-45.

50. Cotton SC, Sharp L, Little J, Duncan I, Alexander L, et al. (2006) Trial of management of borderline and other low-grade abnormal smears (TOMBOLA): trial design. Contemp Clin Trials 27: 449–471.

51. Sherman ME, Schiffman M, Cox JT (2002) Effects of age and human papilloma viral load on colposcopy triage: data from the randomized Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion Triage Study (ALTS). J Natl Cancer Inst 94: 102–107.

52. Khan MJ, Castle PE, Lorincz AT, Wacholder S, Sherman M, et al. (2005) The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. J Natl Cancer Inst 97: 1072–1079.

53. Wright Jr. TC, Denny L, Kuhn L, Pollack A, Lorincz A (2000) HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer. JAMA 283: 81–86.

54. Ordi J, Alonso I, Torné A, Esteve R, Sierra E, et al. (2005) Human papillomavirus load in Hybrid Capture II assay: does increasing the cutoff improve the test? Gynecol Oncol 99: 313-319.

55. De Cremoux P, Coste J, Sastre-Garau X, Thioux M, Bouillac C, et al. (2003) Efficiency of the Hybrid Capture 2 HPV DNA test in cervical cancer screening. A study by the French Society of Clinical Cytology. Am J Clin Pathol 120: 492-429.

56. Moberg M, Gustavsson I, Gyllensten U (2004) Type-specific associations of human papillomavirus load with risk of developing cervical carcinoma in situ. Int J Cancer 112: 854-859.

57. Origoni M, Carminati G, Rolla S, Clementi M, Sideri M, et al. (2012) Human papillomavirus viral load expressed as relative light units (RLU) correlates with the presence and grade of preneoplastic lesions of the uterine cervix in atypical squamous cells of undetermined significance (ASCUS) cytology. Eur J Clin Microbiol Infect Dis 31: 2401-2406.

58. Jarboe EA, Venkat P, Hirsch MS, Cibas ES, Crum CP, et al. (2010) A weakly positive human papillomavirus Hybrid Capture II result correlates with a significantly lower risk of cervical intraepithelial neoplasia 2,3 after atypical squamous cells of undetermined significance cytology. J Low Genit Tract Dis 14: 174-178.

59. Singh M, Mockler D, Akalin A, Burke S, Shroyer A, et al. (2012) Immunocytochemical colocalization of P16(INK4a) and Ki-67 predicts CIN2/3 and AIS/adenocarcinoma. Cancer Cytopathol 120: 26-34.

60. Donà MG, Vocaturo A, Giuliani M, Ronchetti L, Rollo F, et al. (2012) p16/Ki-67 dual staining in cervico-vaginal cytology: correlation with histology, Human Papillomavirus detection and genotyping in women undergoing colposcopy. Gynecol Oncol. 126: 198-202.

61. Carozzi F, Confortini M, Dalla Palma P, Del Mistro A, Gillio-Tos A, et al. (2008) Use of p16-INK4A overexpression to increase the specificity of human papillomavirus testing: a nested substudy of the NTCC randomised controlled trial. Lancet Oncol 9: 937-945.

62. Goldie SJ, Kim JJ, Myers E (2006) Cost-effectiveness of cervical cancer screening. Vaccine 24: 164-170.

63. Goldie SJ, Kim JJ, Wright TC (2004) Cost-effectiveness of human papillomavirus DNA testing for cervical cancer screening in women aged 30 years or more. Obstet Gynecol 103: 619-631.

64. Kim JJ, Wright TC, Goldie SJ (2005) Cost-effectiveness of human papillomavirus DNA testing in the United Kingdom, The Netherlands, France and Italy. J Natl Cancer Inst 97: 888-895.

65. Kulasingam SL, Myers ER, Lawson HW, McConnel KJ, Kerlikowske K, et al. (2006) Cost-effectiveness of extending cervical cancer screening intervals among women with prior normal pap tests. Obstet Gynecol 107: 321-328.

66. Burger EA, Ortendhal JD, Sy S, Kristiansen IS, Kim JJ (2012) Cost-effectiveness of cervical cancer screening with primary human papillomavirus testing in Norway. Br J Cancer 106: 1571-1578.

Author Affiliations

Top

[1]Department of Gynecology & Obstetrics, Vita Salute San Raffaele University School of Medicine, Ospedale San Raffaele Scientific Institute, Milan, Italy