The current status of HPV DNA testing

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Summary

Infection with high-risk types of HPV underlies most cases of high-grade cervical intraepithelial neoplasia (CIN) and practically all cases of invasive cervical cancer. Currently, cervical HPV DNA is detected by means of PCR and sandwich capture molecular hybridization methods. Research has focused on the potential role of HPV testing in three conditions: screening for cervical neoplasia, triage of women with low-grade lesions and follow-up after conservative surgical treatment for CIN. Concerning the first condition, HPV testing does not seem to offer an obvious advantage over traditional cytology screening, mainly due to false positive results in younger women with transient HPV infection. A possible exemption to this is the case of middle-aged women and low-resource settings, where the excellent sensitivity of a HPV test is desirable. Although data are controversial regarding low grade lesions, results from randomized studies indicate that HPV testing could be useful in a triage of women with an initial cytological diagnosis of ASCUS, where detection of DNA of a high-risk type should lead to colposcopy. Although there is a lack of randomized controlled trials in this field, data from observational studies indicate that HPV DNA testing after conservative surgical treatment for CIN may be very sensitive and detect early residual and recurrent disease.

Key words: HPV; PCR; Hybrid Capture; Screening; Triage; Follow-up.

Introduction

Introduction of the Pap test has largely decreased the incidence of cervical cancer in the last decades. However, the Pap test has an innate false negative rate of 20-30% [1] and cervical cancer still remains a problem, with a global annual incidence of approximately 370,000 cases and annual death rate of 190,000 cases [2]. A meta-analysis addressing the diagnostic performance of the Pap test showed that it may be unable to achieve concurrently high sensitivity and specificity, as their estimates are highly negatively correlated [3]. Moreover, the diagnostic accuracy of the Pap test seems to be poorest among early precancerous lesions, especially low grade squamous intraepithelial lesions (lgSIL) and atypical squamous cells of undetermined significance (ASCUS).

It is widely recognized that certain types of human papillomavirus (HPV) are found in the majority of high grade SIL and in practically all cases of cervical cancer [4-6], and it is virtually impossible for a woman to develop cervical cancer without having a pre-existing HPV infection [6-8]. Thus, it is reasonable that HPV DNA detection in cervical samples would increase the diagnostic performance of existing screening methodology and help in the management of most equivocal tests (i.e., lgSIL and ASCUS). Moreover, Elfgren et al. showed that HPV DNA is regularly eliminated after successful treatment of cervical intraepithelial neoplasia (CIN), whereas it persists in cases of recurrent disease [9]. Several studies have confirmed these findings, indicating that HPV DNA testing could be considered in follow-up after treatment for CIN as well.

However, there still important issues concerning utilization of HPV DNA testing. For example, HPV DNA detection is more frequent in younger women, indicating most often a transient infection and thus limiting the specificity of the test [10]. Moreover, it is possible that high risk HPV types are often present in healthy women as well [11, 12], furthermore limiting the specificity of the test.

The current fields of research on HPV DNA testing concern its use 1) in screening for cervical cancer, either alone or as an adjunct to traditional screening methods, 2) its use for a triage of cases of equivocal Pap tests (mainly lgSIL and ASCUS), and, 3) its use for follow-up after conservative surgical treatment for CIN.

Methods of HPV DNA testing

Detection of HPV is not feasible with conventional tests used for infectious factors, e.g., serology and cultures [13]. Indeed, local detection of HPV DNA in cervical samples has demonstrated superior diagnostic accuracy to serology [14-16]. Initial experience with filter in situ hybridization (FISH), prototypic DNA to DNA

in situ hybridization and early polymerase chain reaction (PCR) methodology yielded disappointing results [13]; however, current hybridization-based techniques, essentially PCR and Hybrid Capture II (HC II) array (Digene Corp., Beltsville, MD, USA), have overcome most drawbacks and are considered reliable and accurate.

PCR is an extremely sensitive method, based on the selective amplification of certain, known DNA sequences (targets). The procedure starts with denaturation of a DNA target. A specific primer set (i.e., oligonucleotides that bind to their complementary sequence of DNA-target) binds to the opposite ends of target sequence and it initiates synthesis of the complementary strand of DNA-target, which is promoted by a DNA polymerase and it is directed towards the opposite primer. Cycles of denaturation, hybridization and replication repeat, and initial DNA sample is amplified in an exponential way. Amplified DNA is then detected by various methods. Primers could be type-specific (i.e., detect a specific HPV type) or consensus primers (i.e., primers which can detect multiple types of HPV, like the MY09-MY11-HMB01 L1 primer system). When excluding sources of extrinsic error (e.g., contamination), the sensitivity of PRC can be extremely high, depending on the number of amplification cycles. Given that a large proportion of women, especially in the younger ages, may have transient or latent HPV infection without eventually developing dysplasia or cancer, it is necessary to establish a trade-off between the sensitivity and specificity of the test.

Hybrid Capture is a non-radioactive immunoassay that employs RNA probes to detect single-stranded target RNA [13]. A new version of this test, HC II, has become available in recent years, showing improved sensitivity when compared to HC [17]. HC II is a sandwich capture molecular hybridization assay that utilizes chemiluminescent detection [18]. RNA:DNA hybrids are captured on the surface of a well coated with a specific antiRNA: DNA antibody. The complex then reacts with an alkaline phosphatase antibody and it is incubated in a chemiluminescent substrate. Alkaline phosphatase cleaves the substrate and light is emitted, which is measured by a luminometer and expressed as relative light units. HC II allows for DNA detection of 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) or five low-risk types (6, 11, 42, 43 and 44) in a single assay. In order to optimize the trade-off between sensitivity and specificity, the clinical test cut-off is set to 5,000 HPV genomes.

The two tests have similar sensitivity and specificity and show a satisfactory level of agreement (kappa 0.65-0.89) [19, 20].

HPV DNA testing and screening for cervical cancer

Screening refers to utilization of diagnostic means for early detection of disease, before symptoms occur. Desirable attributes of a screening test include a reasonable diagnostic accuracy (trade-off between sensitivity and specificity), ability to detect common disease, ability to detect curable disease, wide availability, low morbidity/high safety and a favorable cost-to-effectiveness relationship [21].

The Pap test has performed well as a screening test, since morbidity and mortality from cervical cancer have substantially declined during the last decades; however, its performance is far from being ideal.

A single Pap test may miss nearly half of all cases of CIN [22]. In a meta-analysis of 62 published studies, the estimates of sensitivity and specificity of the Pap test ranged from 11 to 99% and 14 to 97%, respectively, and the summary receiver operating characteristic curve suggested that the Pap test may be unable to achieve concurrently high sensitivity and specificity [3]. In an analysis of 22,439 paired cytology-biopsy specimens, the sensitivity of the Pap test was 89.4%, while the specificity was only 64.8%, thus the diagnostic accuracy of the Pap test becomes particularly low concerning low-grade lesions [23]. Among patients with a cytological diagnosis of ASCUS, 37% had a normal cervix and 13% had high-grade CIN, whereas among patients with a diagnosis of low-grade SIL, a normal cervix was found in 13% and high grade CIN in 18% of cases. Similarly, in a review of 137 smears initially classified as atypical glandular cells of undetermined significance (AGUS), only 32% were re-classified as AGUS, and review histology showed that 77% of cases were normal [24]. Finally, in a prospective study addressing the correlation of cytology, colposcopy and histology, only 17% of cases of hgSIL and 38% of cases of invasive cancer followed Papanicolaou smears suggesting hgSIL or cancer [25].

Liquid-based cytology has been developed as an advance to conventional cytology and it utilizes proprietary fixative solutions into which the collection devices are placed. Although confidence intervals overlap, this technique appears at least as sensitive as conventional cytology [21]. Moreover, residual samples from liquid-

base cytology can be efficiently used for detection of HPV DNA [26]. For samples diagnosed as ASCUS or above, reflex HPV testing can be performed on the remaining stored cytology specimen, thus eliminating the cost of a second visit [12].

A 4-6 year follow-up of 1,643 women with normal cytology showed that women with a positive PCR HPV DNA test at baseline were 116 times more likely to develop CIN3 than women with a negative DNA test [27]. However, in about 40% of women, HPV infection will spontaneously regress and only a minority will demonstrate abnormal cytology or colposcopy [28]. Testing for high-risk HPV may allow predicting 80% of CIN2/3 three years before the cytological diagnosis and two-thirds six years before [29]. Thus, if HPV DNA testing identifies women at risk for cervical cancer, it could be a part of the screening policy, either alone or in combination with conventional methods, i.e., cytology. Multiple studies have addressed this challenging hypothesis in recent years, focusing mainly on potentially low specificity, availability of resources and cost-effectiveness.

A drawback in this field is that none of these studies was a randomized controlled one. As a rule, multiple tests (cytology, HPV test, cervicography, visual inspection, and colposcopy) were performed in each patient, and the diagnostic performance of any individual test, or of their combination, was assessed. HPV DNA testing had constantly higher sensitivity than cytology in predicting CIN [30-34]. Sensitivity ranged between 73 and 100% across the aforementioned studies, depending mainly on the grade of CIN. Another constant finding was that HPV DNA testing demonstrated lower specificity compared to cytology. The diagnostic performance of the two tests may become similar when the cytological threshold for referral to colposcopy is expanded towards benign lesions e.g., mild koilocytosis or mild dyskeratocytosis [35]; however, this would substantially increase the demand for colposcopy. It seems that the negative prognostic value of HPV test may be of more value than its positive one, so that a negative result, in conjunction with a negative cytological result may allow for a longer screening interval, especially in middle-aged women [29-31, 33, 34]. Alternatively, the high sensitivity of the HPV DNA test may be very useful in settings in which sensitive detection of high-grade lesions and cancer is of paramount importance, e.g., in high-risk populations [36], or in low-resource settings [32]. In a cost effectiveness analysis in low-resource settings it was concluded that HPV testing followed by treatment of screen positive women in a second visit was a more effective and cost-saving policy than cytology and treatment [37]. Elimination of the need for colposcopy is the main reason for cost reduction when utilizing the HPV DNA test in screening policies.

HPV DNA testing and triage for low-grade lesions

Although ASCUS is a rather frequent cytological diagnosis, its management is very often controversial. As already mentioned, either a completely normal cervix or a high grade lesion may underlie cytological diagnosis of ASCUS [23]. Given the high rate of false positive results, a repeat smear would be a reasonable option. However, this could lead to a delay of treatment in case of underlying high grade lesion. So, many authorities would suggest immediate colposcopy; however, this policy would entail a substantial economic burden.

A high-grade lesion scarcely, if ever, develops without underlying HPV infection. Thus, HPV DNA testing could be a triage for women with equivocal cytological diagnoses.

In their study of 4,075 women attending Parenthood Clinics for regular cervical screening, Kulasingam *et al.* found that, compared with referral for colposcopy of all women with ASCUS or higher, HPV DNA testing with signal amplification of women with ASCUS and referral of those with a positive result was significantly more specific (82.5% vs 88.9%, respectively) [34]. In a recent randomized trial, the efficacy of HPV DNA testing in women with low-grade cytological abnormalities was compared to that of a repeat Pap-test at six months [38]. HPV DNA testing was associated with higher sensitivity (87.5% vs 55.6%) and better compliance (loss to follow-up 17.1% vs 32.7%); however it was more costly (incremental cost of $3,003 per additional case identified). The most powerful study on this matter up to date is the ALTS Study, an American multicenter randomized trial. The first part of this study concerned 642 women with a cytological diagnosis of lgSIL at enrollment. The results showed that there is limited potential of HPV DNA testing to direct decisions about the clinical management of these women because DNA of a high-risk HPV type was detected in 83% of cases, so that the cost of HPV testing of all women with a cytological diagnosis of LSIL would outweigh savings gained from avoiding colposcopy for only 20-27% of women [19]. On the contrary, the second arm

of the ALTS study, including 3,488 women, showed that HPV DNA testing seems to be a useful tool for a triage of women with a cytological diagnosis of ASCUS [39]; having as an outcome measure the diagnosis of CIN3 or above, HPV DNA testing had a sensitivity of 96.3%, with 56% of women referred for colposcopy, while the corresponding rate for repeat cytology was 44%, with 7% referred. With a triage threshold of ASCUS or above, cytology had a sensitivity of 85.3%, with a referral rate of 58.6%.

Using data from published studies, Kim *et al.* [40] compared four strategies for the management of women with ASCUS: immediate colposcopy; HPV triage (colposcopy if high-risk HPV DNA is detected); repeat cytology (colposcopy with abnormal results); reclassification of ASCUS as normal. With the exception of the reclassification strategy, the least costly strategy was HPV DNA testing, which resulted in a reduction in total cancer incidence of 86% for conventional cytology and 90% for liquid-based cytology.

The cytological diagnosis of ASCUS can be both poorly reproducible and deceiving, covering a wide spectrum of cervical changes. The evidence indicates that HPV DNA testing can be used for a triage of these women, allowing for satisfactory discrimination between those who carry a high-risk HPV type, thus having a potential for progressing disease and meriting colposcopy, and those who do not carry such a viral type and do not need immediate further workup.

In 2001, a panel of 121 experts concluded that two repeat cytology tests, immediate colposcopy and DNA testing are all acceptable in women with ASCUS; however, HPV testing is the preferred method when liquid-based cytology is used for screening [41].

HPV DNA in follow-up after treatment for CIN

The overall success rate of conservative methods of treatment for CIN is 90-95% [42]. However, these women are still at a four to five-fold risk for developing invasive cancer than the general population, and this risk is stable throughout eight postoperative years [43]. This, together with the risk of primary treatment failure or recurrence of the disease, makes the need for an efficient postoperative surveillance imperative.

The status of excisional margins cannot be a good predictor for primary treatment failure since residual or recurrent disease can develop with both involved and clear margins, especially in older women [44-46]. HPV testing may have a role in the follow-up of these women. Elfgren et al. showed in 1996 that HPV DNA is regularly eliminated after successful treatment of CIN [9], and more recently found that this clearance most often occurs within three postoperative months [47]. A review of MEDLINE and EMBASE yielded 11 studies evaluating the use of HPV DNA testing for postoperative surveillance of women with CIN [9, 48-57]. None of these studies was a randomized controlled one, and there was marked heterogeneity in their methodology, inclusion criteria and follow-up protocols. In a total of 900 patients who underwent conservative surgical treatment for different grades of CIN, 696 were considered as having successful treatment, whereas 204 had residual or recurrent disease. Positive results in postoperative HPV DNA testing occurred in 15% and 83%, respectively. The sensitivity of HPV DNA testing in detecting treatment failures was quite good in most studies, reaching 100% in four of them [9, 51, 52, 54], whereas a modest performance, in the range of 50%, was demonstrated in two studies [50, 57]. The specificity of the test differed across the studies, ranging from 44% to 95%. A positive HPV DNA test often preceded an abnormal smear. In studies that provided relevant data, this happened in 25-50% of cases.

Although the HPV DNA test seems to detect treatment failures efficiently and early, the present data come from observational studies with markedly heterogeneous results. As a result, a comparison of different follow-up strategies cannot be made at the moment – not even a valid evaluation of the diagnostic performance of HPV DNA testing. Cytology and colposcopy may still be needed in order to rule out false-positive and false-negative results. An ongoing Multicenter European Study aims at reaching a conclusion about an optimal follow-up algorithm and at assessing whether such a strategy would ultimately reduce the incidence of post-treatment invasive cancer.

Conclusion

HPV DNA testing does not seem to be obviously superior to cytology screening, with the possible exemption of low-resource settings or middle-aged women. Although data are also controversial regarding low-grade intraepithelial lesions, HPV DNA testing may be useful for a triage of women with an initial cytolo-

gical diagnosis of ASCUS. Finally, preliminary data indicate that HPV DNA testing after conservative treatment of CIN may detect residual or recurrent disease earlier and more sensitively than cytology; however more data are needed.

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