THE NEW ERA OF PRIMARY HPV SCREENING FOR PREVENTION OF INVASIVE CERVICAL CANCER

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Abstract

We now know that persistent cervical infections by certain types of human papillomavirus (HPV) designated as high-risk, carcinogenic or cancer-associated, cause virtually all invasive cervical cancer. The discovery of oncogenic HPV as the necessary cause of invasive cervical cancer has led to revolutionary advances in prevention, including the development of sensitive molecular HPV testing for cervical cancer screening. Using high-risk HPV testing for the primary screen shifts the use of the Pap test from the general population to those women at risk of cervical cancer, high-risk HPV positives. In high-resource settings, using high risk HPV testing as the primary cervical cancer screening test could increase the efficiency of current screening programs, more effectively identify women at risk for adenocarcinoma, and combined with self-collection, reach medically unserved populations that experience a disproportionate burden of invasive cervical cancer.

HPV natural history: rational basis for intervention

Since the discovery of human papillomavirus (HPV) in the tissue from invasive cervical cancer (ICC) by Harold Zur Hausen (2008 Nobel Laureate in Medicine) and colleagues 30 years ago,[1] there have been rapid advances in our understanding of ICC and its cause. We now know that persistent cervical infections by certain types of HPV, designated as high-risk, carcinogenic or cancer-associated, cause virtually all ICC everywhere in the world.[2] HPV also causes a significant number of vulvar, vaginal, anal, penile and oropharyngeal cancers.[3] Approximately 5% of the human burden of cancer is caused by HPV.[3] HPV16 is the most important HPV genotype, responsible for 55-60% of ICC.[4] HPV18 is the next most important HPV genotype, responsible for 10-15% of ICC, including 30% of adenocarcinoma of the cervix,[4] which is on the rise in western countries.[5,6] Together, HPV16 and HPV 18 account for approximately 70% of ICC and the same 12-15 HPV types cause 95-99% of ICC on all continents.[4] Thus, an important corollary of these findings is that HPV does not discriminate by race or ethnicity, and there is no evidence of significant genetic predisposition. Thus, the only two important causes of ICC are persistent cervical infections by high-risk HPV genotypes and a lack of access to preventive services.

The natural history of HPV and cervical carcinogenesis can be represented by a simple, causal schema, which is composed of four, reliably-measured stages: 1) HPV acquisition; 2) HPV persistence (versus clearance); 3) progression to precancer (CIN3, AIS); and 4) invasion.[2] HPV infection is a very common, perhaps universal exposure, among sexually active populations, but on a per infection event basis, is an uncommon cause of cancer. Most (~90%) HPV infections are benign and are cleared or controlled within two years. Although there is now evidence that some infections may become quiescent (latent) or undetectable,[7] the clinical importance of their re-emergence in peri- and post-menopausal women is uncertain and possibly lower because of the absence of hormones thought to contribute to the carcinogenic process.[8]

The key step in cervical carcinogenesis is overt, measurable high-risk HPV (hrHPV) persistence, which even after a year or two strongly predicts the development of cervical precancer, cervical intraepithelial neoplasia grade 3 (CIN3) or adenocarcinoma in situ (AIS).[9,10] Importantly, the longer an infection persists, the greater the risk for development of precancerous changes in the epithelium and for the development of frank malignancy. At some unknown average duration, HPV persistence becomes synonymous with cervical precancer and cancer, but the transition between the two states is imperfectly understood because of the less than perfect sensitivity of colposcopy and biopsy to detect errors in the pathologic diagnosis of cervical precancer, especially the earliest and smallest precancerous lesions with low malignant potential that must arise from the persisting infection.[11]

Finally, untreated precancerous lesions in older women (median age = 38 years), about 10 years after the earliest, smallest precancerous lesions can be found in the population by screening, have about a 30% risk of becoming invasive over the next 30 years.[12,13] The carcinogenic process for cancer to develop from incident HPV infection on average takes quite a long time, approximately 10 years at a minimum and 20-25 years on average, which makes it possible to successfully screen, diagnose, and treat most women with precancerous changes prior to invasion, even if with only moderately sensitive screening and diagnostic tests and procedures.

Targeting HPV

The discovery of hrHPV as the necessary cause of ICC has led to revolutionary advances in ICC prevention, including the development of prophylactic HPV vaccines and sensitive molecular hrHPV testing for cervical cancer screening. hrHPV testing is more sensitive and reliable for detection of CIN3, AIS or invasive ICC (≥CIN3) of the cervix than Pap testing.[14-22] The increased sensitivity of hrHPV testing over Pap testing for (≥CIN3) translates into two important benefits: 1) earlier detection of CIN3/AIS lesions that if treated, results in a reduced incidence of ICC within 4-5 years and related death within eight years;[23,24] and 2) greater reassurance against cancer (lower cancer risk) following a negative result for many years,[23-27] which permits screening at an extended interval of 5-10 years, depending on the acceptable minimum cancer risk. Thus, using hrHPV testing for the primary ICC screen, women would only need one to a few screens in their lifetimes to significantly reduce the burden of ICC.[28] hrHPV testing offers other important advantages, including easier implementation because these molecular tests do not require specialised medical training i.e. molecular tests are processed by machine and therefore do not require a large network staffed by cytopathologists. These advantages make the introduction of hrHPV testing for cervical cancer screening into low and middle income countries more feasible than cytology.

One of the important limitations of hrHPV testing is that it is less specific and therefore has a lower positive predictive value for cervical precancer and cancer than high-quality Pap testing. hrHPV testing detects 'clinically relevant hrHPV', equal or above a threshold that was established for one test and has become the benchmark for all tests.[29-31] However, clinical hrHPV testing does not distinguish between benign hrHPV infections that are destined to clear or be controlled versus those that have or will cause ≥CIN3. So although hrHPV testing detects 25-40% more ≥CIN3 than Pap in high-resource settings, in unvaccinated populations typically approximately twice the number of women will test hrHPV positive compared to Pap positive (atypical squamous cells of undetermined significance [ASC-US] or more severe cytologic abnormalities [≥ASC-US]).[14,32] While some of these additional pick-ups of CIN3/AIS by hrHPV testing represent true precursors to cancer, as evident by the reductions in ICC incidence and mortality when acting clinically to all hrHPV positives as discussed above, it may be impractical and/or unacceptable to send all these hrHPV-positive women to colposcopy or treat all of them using a screen-and-treat strategy.[33,34] In some settings, it may be desirable to use a secondary, triage test to 'rule in' hrHPV-positive women who need immediate follow-up and care. That is, hrHPV testing is used to 'rule out' ≥CIN3 in the generally health population, and a secondary, more specific test is used to 'rule in' those hrHPV-positive women who need colposcopy and biopsy or immediate treatment (figure 1A)

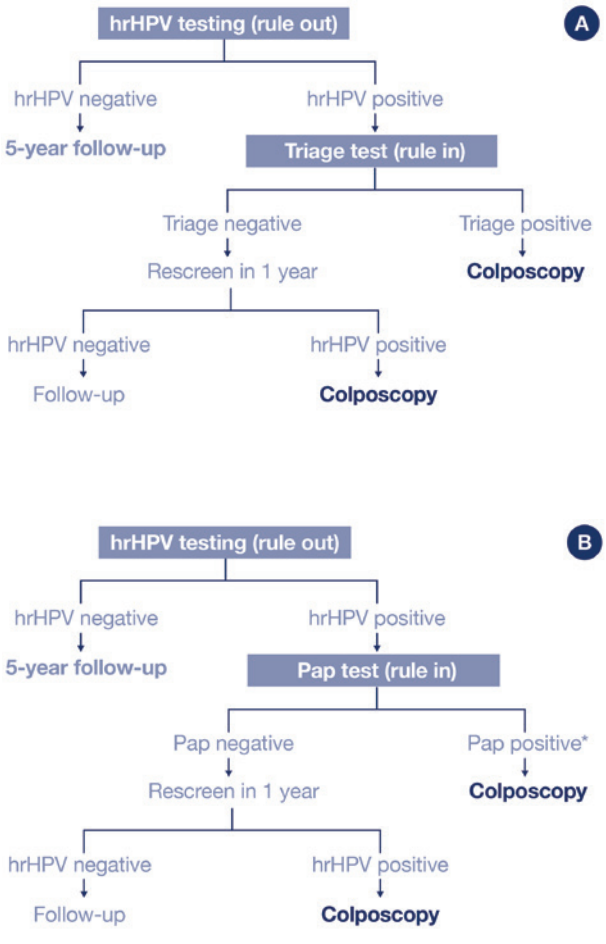
Paradigm shift from 'Pap on everyone' to 'Pap on hrHPV positives'

Pap is the obvious first choice as a triage test for hrHPV positives where good cytology is already available (figure 1B). Essentially, this shifts the use of Pap from the general population at short intervals to only the small fraction of those who have the necessary cause of ICC i.e. hrHPV, and women who are hrHPV negative are screened at longer intervals. Thus, there is a shift in focus and resources to those women who are truly at risk of ICC. However, Pap as a triage test still has limited sensitivity, unless the slides are more heavily scrutinised because they are hrHPV positive.

To glean more of the benefits of hrHPV testing, women who test hrHPV positive but Pap negative (hrHPV+/Pap-) should be followed more intensively than routine screening until there is evidence of persistent hrHPV infection, which even after a year or two strongly predicts the development of ≥CIN3,[9,10] or overt cytologic abnormalities. In the US, it is recommended that hrHPV+/Pap- return for re-screening

***Figure 1**: Algorithms for primary high-risk human papillomavirus (hrHPV) testing to 'rule out' cervical precancer and cancer and a secondary, triage test to 'rule in' cervical precancer and cancer among hrHPV-positive women. Shown are four different scenarios for triage: a generic algorithm with no specific triage test specified (A); Pap testing (B); detection of hrHPV genotypes HPV16, HPV18, and/or HPV45 (C); or combining HPV16, HPV18 and/or HPV45 detection and Pap testing (D).*

**Pap positive is the threshold of abnormality that is currently being used for referral to colposcopy in the Pap-based screening program.*



in a year.[35,36] Following a hrHPV+/Pap- result, the longer the interval, the lower the percentage of women testing hrHPV a second time and the greater the risk of CIN3+ and of frank invasive ICC among the repeat hrHPV positives.[37] Thus, in an organised screening program that can achieve excellent follow-up of patients, it may be desirable to extend the interval of rescreening hrHPV+/Pap-, but recognising that there naturally is a concomitant incremental increase in cancer with the longer surveillance interval.

Although, there has been a general agreement to limit hrHPV testing to women 30 or 35 years and older, there is no theoretical reason not to use hrHPV testing in women at any recommended age of screening, provided that clinical management is based on the triage test results and not on the hrHPV test results alone.

HPV tests

The available tests were previously reviewed, but the market and available products are rapidly evolving.[38] Currently, there are four US Food and Drug Administration (FDA) approved hrHPV tests: Hybrid Capture 2 (Qiagen, Germantown, MD, USA) (approved in 2003); Cervista (Hologic, Bedford, MA, USA) (approved in 2009); cobas4800 (cobas, Roche Molecular Systems, Pleasanton, CA, USA) (approved in 2011); and Aptima (Gen-Probe/Hologic, San Diego, CA, USA) (approved in 2011). The FDA recently approved one hrHPV test, cobas 4800, for primary cervical cancer screening.[39] A laboratory-developed preliminary chain reaction test based on GP5+/6+ primers meets the benchmarks of validity.[30,31]

A number of other tests have received CE marking and/or Chinese FDA-equivalent authority approvals, including a manual, lower-cost test developed for LMICs (careHPV, Qiagen), some of which are undergoing or will undergo pre-marketing approval evaluations for FDA approval. Speculatively, given the comparability of many of these assays, more tests will receive FDA approval for use in cervical cancer screening, and as the primary cervical cancer screening test or at least be accepted as comparable and therefore interchangeable by guidelines developed by professional medical organisations.

Adoption

The US was the first country to introduce hrHPV testing into routine screening, as hrHPV and Pap co-testing every three years for women aged 30 years and older, following FDA approval of the first clinical hrHPV test in 2003 and interim guidelines.[40,41] Kaiser Permanente Northern California, a managed care organisation that resembles an organised screening program in many aspects, was an early adopter, rolling out three-year co-testing in women aged 30 and older during 2003-4. The organisation has now screened over one million women 30 and older by co-testing. Some of the key observations from that real-world experience include: 1) although women could opt for annual Pap testing, there was a high degree of adoption (>90%) of triennial cotesting; 2) a negative hrHPV test was more reassuring than a negative Pap, as previously reported;[23,25,26] 3) a negative co-test (hrHPV-/Pap-) was not much more reassuring than a negative hrHPV test;[25,26] and 4) a high proportion of AIS and adenocarcinoma diagnosis was proceeded by hrHPV+/Pap-.

Numerous countries are now either implementing or planning to implement hrHPV testing as the primary screen for ICC in some or all of the country (e.g. Australia, the Netherlands, Argentina, Rwanda and Turkey) or undertaking evaluations (e.g. England, Norway, China, Vietnam, El Salvador and Colombia).[42] Importantly, the World Health Organisation has recently recommended the use of hrHPV testing for primary screening, especially for those places that have the resources to afford hrHPV testing and do not have a high-coverage, effective Pap program.[34] The challenge then, will be developing the financing for and tiered pricing to allow universal access to hrHPV testing and eliminate the historically large cancer health inequities in ICC burden between high-resource countries and low- and middle-income countries.

The introduction of hrHPV testing into high-resource settings, where there is an established and effective Pap test-based screening program, may still lead to some reductions in the burden of cervical cancer. More importantly, using hrHPV testing and extending screening intervals can potentially reduce the harms of screening by permitting newly acquired benign hrHPV infections and associated cytologic abnormalities to go away undetected and avoid triggering clinical action.[35] Screening at longer intervals may also be more cost-effective.[43]

hrHPV testing may address an important limitation of Pap testing, which is identifying women who have or are at risk of having AIS/adenocarcinoma, which has either not declined and in some high-resource settings has increased during the same period that squamous cell carcinoma incidence has declined dramatically.[5,44-46] Several studies have shown that hrHPV testing is more effective in identifying women at risk of AIS/adenocarcinoma than Pap testing,[27,47,48] and a case series report observed that most adenocarcinomas were preceded by hrHPV+/Pap-.[49] However, without good follow-up of hrHPV+/Pap- and concomitant improvements in the diagnosis of AIS and precursors of adenocarcinoma in the endocervical canal, the benefit of hrHPV testing for prevention of adenocarcinoma will not be fully realised.

As mentioned, in most high-resource countries, there is a segment (~20%) of the population in whom a significant fraction of its invasive ICCs occurs because women do not or cannot access routine medical care and are unscreened or under-screened. In fact, elevated ICC incidence and mortality is a general marker for health disparities.[50] hrHPV testing can potentially reduce these disparities because fewer screens in a lifetime will be needed to achieve effective prevention. hrHPV testing also allows for the effective use of self-collected cervicovaginal specimens,[51] which can address a number of barriers to participation including inconvenience, cost and geographical barriers of getting clinic-based screening.

Management of hrHPV-positive women

Although Pap testing of hrHPV positives is the first and obvious method of triage, as for primary screening, it has limited sensitivity for CIN2+ in routine practice. Pap testing with the knowledge that a woman is HPV positive could lead to more scrutiny of the slide and increase sensitivity,[52]

i.e. 'screening with prejudice', such an improvement has not been documented and almost certainly would be accompanied by a decrease in specificity.

Next generation hrHPV tests offer at least separate detection for HPV16 and HPV18, or HPV16, HPV18 and HPV45 in various formats (concurrent or sequential testing, individual detection or pooled detection), the three HPV genotypes that cause the most ICCs and have the highest ratio in cancers versus the general population.[4,53,54] There is significant evidence that one-time or two-time detection (persistence) of these types identify a subset of hrHPV-positive women at higher risk of CIN2+ and CIN3+ cross-sectionally and prospectively.[9,17,19,55-57]

The evidence for clinical utility for separate detection of HPV16 is the strongest.[35] HPV16 is the most carcinogenic genotype and identifying HPV18- and HPV45 related precancerous lesions appears to be more difficult than HPV16 related ones. So often HPV18 and HPV45 detections do not distinguish themselves as higher risk than other hrHPV genotypes when CIN2+ or CIN3+ is used as an endpoint.[58] Yet, HPV18 and HPV45 are the second and third leading causes of ICC and contribute a much higher proportion of adenocarcinoma and AIS, which are missed by Pap testing. HPV16, or HPV16 and HPV18 detection has been recommended for the management of hrHPV+/Pap- women in the US.[35] Individual detection of other HPV genotypes does not seem to provide important risk stratification, although several reports have suggested that HPV33 detection is comparable to HPV18 detection and might be useful, without accounting for the fact that HPV33-related precancer is common and probably has a lower risk of becoming invasive than HPV18 and HPV[45.54,59-62]

Thus, partial HPV genotyping could be used alone (figure 1C) or in combination with Pap testing (figure 1D) for the triage of hrHPV-positive women. The choice to use one, the other, or both depends on factors of cost, performance and follow-up rates of hrHPV-positive/triage-negative (e.g. hrHPV+/Pap) women.

New biomarkers

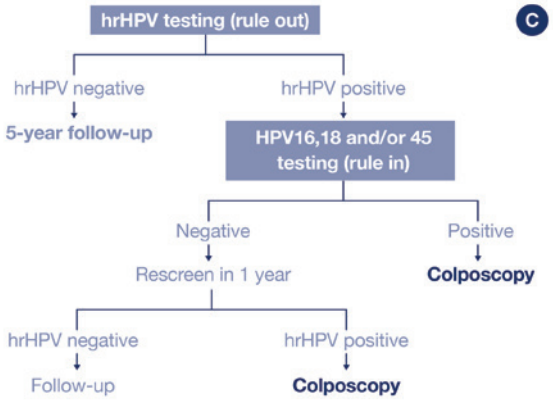
There are a number of promising new biomarkers that might achieve better performance as a triage for hrHPV-positive women than Pap and/or HPV genotyping for the riskiest HPV genotypes. The most advanced of these next-generation biomarkers with respect to validation and readiness for introduction into routine practice is $p16^{INK4a}$ immunocytochemistry. In a number of studies, p16 immunocytochemistry has demonstrated high sensitivity and specificity that is similar or better than Pap testing for CIN2+ and CIN3+ among hrHPV-positive women.[63-65] Ki-67, a cell proliferation marker, has been included with p16 immunocytochemistry as a dual stain to create a morphology-independent test.[65]

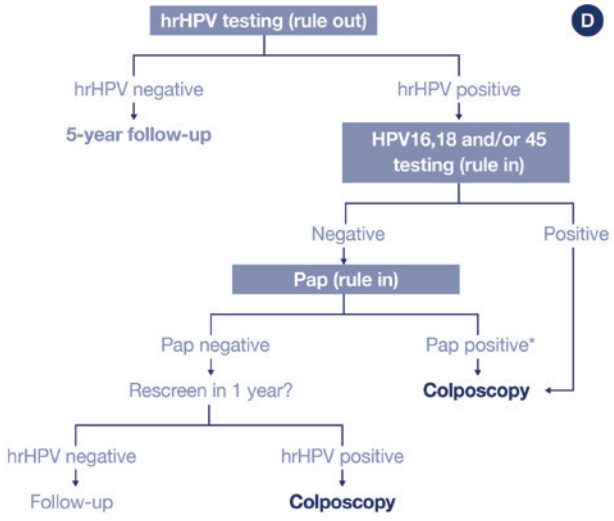
There are a considerable number of additional biomarkers that have not been fully validated. These include but are not limited to viral,[66-69] and host,[66,70-73] methylation, chromosome region 3q amplification,[74-79] and viral integration.[80,81] In addition to needing further validation and demonstration of performance and reliability, these biomarkers must be 'reduced to practice' i.e. translating from a promising biomarker to a test that can be readily used in the clinical laboratory setting.

Integration of HPV vaccination and screening

It is anticipated that in the absence of HPV16 and HPV18 due to HPV vaccination, the predictive values of hrHPV and Pap testing will decline because of a lower prevalence of CIN2+ in the population i.e. a negative test will be more reassuring and a positive test will be less predictive of CIN2+ and CIN3+.[82-84] This is due to approximately 50% of CIN2,[54] 60% of CIN3/AIS,[54] and 70% of ICC,[4,54] caused by HPV16 and HPV18 prevented, while hrHPV positives will only be reduced by 25-30%. In addition, largely due to the absence of HPV16, there will be fewer high-grade Pap results as specific indicators of the presence of cervical precancer or cancer.[85,86]

To adapt screening and maintain the balance of benefits and harms,[35] three strategies might be employed. Using





cancer risk to guide screening and management, as discussed below, HPV16/18-vaccinated populations might start screening later or be screened less frequently.[87] New biomarkers may be useful to increase the accuracy of cervical cancer screening now and in the future, when HPV16/18-vaccinated need to be screened to prevent the residual ~25-30% of ICC not caused by HPV16 and HPV18.

Final comments

In all likelihood, if we cannot prevent and control ICC on a global scale, given the robustness of the tools at our disposal, it seems unlikely that we will have a major impact on reducing the burden of any other cancer, except for those can be largely prevented through behaviour and environmental interventions (e.g. smoking cessation and reducing arsenic exposure, respectively). ICC prevention and control can serve as the flagship for the prevention and control of other cancers and more generally non-communicable diseases. Investment in ICC prevention and control will help build the capacities such as diagnostics, pathology, surgery and oncology necessary to impact these other non-communicable diseases. Specifically, hrHPV testing may be our best chance to reduce the burden of ICC now in both low- and middle-income countries and high-resource settings. In high-resource settings, using hrHPV testing as the primary cervical cancer screening test could increase the efficiency of current screening programs, more effectively identify women at risk for adenocarcinoma, and combined with self-collection, reach medically unserved populations that experience a disproportionate burden of ICC. In low- and middle-income countries, if made affordable and accessible, hrHPV testing could more rapidly reduce the burden of ICC in populations that experience 10-fold greater rates of ICC incidence and mortality compared to high-resource settings. Next generation hrHPV tests often are on testing platforms that include a menu of clinical tests for other medically important analytes (e.g. chlamydia and gonorrhoea, HIV, TB and genetic markers). As a result, on the same platform, hrHPV testing could be introduced where other clinical tests are already being provided or vice versa. Investment in delivery of cervical cancer prevention and control will strengthen the healthcare delivery and systems for other diseases that disproportionately burden these same populations. The challenge going forward is to make the new standard of care for cervical cancer screening, hrHPV testing, accessible to everyone.

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