New strategies for human papillomavirus-based cervical screening.

Lorincz, A; Castanon, A; Wey Lim, AW; Sasieni, P

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Prof Attila Lorincz, Dr Alejandra Castanon, Dr Anita Wey Wey Lim, Prof Peter Sasieni

Centre for cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University of London, Charterhouse Square, London, EC1M 6BQ

Professor Attila Lorincz, Professor of Molecular Epidemiology

Dr Alejandra Castanon, MD MsC, Epidemiologist

Dr Anita Wey Wey Lim, PhD, Senior Epidemiologist

Professor Peter Sasieni , Professor of Biostatistics and Cancer Epidemiology

Corresponding Author

Peter Sasieni

Centre for cancer Prevention, Wolfson Institute of Preventive Medicine,

Queen Mary University of London,

Charterhouse Square,

London, EC1M 6BQ

Tel +44 (0) 20 7882 3544

Fax +44 (0) 20 7882 3890

Email: p.sasieni@qmul.ac.uk

Summary

Human papillomavirus (HPV) testing has been shown to be far more sensitive and robust in detecting CIN2+ (and CIN3+) for cervical screening than approaches based on either cytology or visual inspection, but there are a number of issues that need to be overcome if it is to substantially reduce the morbidity and mortality from cervical cancer at a population level. The two main issues are coverage (increasing the numbers of women who participate in screening) and management of women who test high-risk HPV (hrHPV) positive. This article will review the potential for vaginal self-collection to improve coverage and the options for triage of hrHPV-positive women in high-resource and low-resource settings.

Key words

Cancer Prevention, screening for cervical cancer, human papillomavirus testing, triage of hrHPV-positive women, vaginal self sampling

Executive Summary

HPV Testing

- High risk HPV(hrHPV) infection is needed for cervical cancer to develop and since hrHPV DNA is still present during the development of cancer, looking for hrHPV DNA should be a good approach to identifying women with high-grade CIN.
- The advantages of hrHPV testing over cytology (currently the most widely used screening method) are that HPV negative women are extremely unlikely to develop cervical cancer over the next 5-10 years and infrequent screening would be safe. Additionally HPV testing does not depend on collection of samples with good morphology.
- Screening for cervical cancer using HPV testing is more sensitive than screening using cytology but considerably less specific. A greater proportion of women will test HPV positive on a single screen compared to the proportion found to have abnormal cytology. Therefore a good way of managing HPV positive women is essential.

Management of women positive for HPV

- A positive HPV test in primary screening should only trigger a triage test not automatic referral to colposcopy. The most widely studied triage test in this situation is cytology. Triage with cytology can be done on the same sample as the HPV test without the need for women to attend for another clinic visit.
- HPV positive women with normal cytology need not be retested at a short interval, but neither should they be re-screened only every 3 or 5 years. A repeat test after an interval of 12-24 months seems reasonable.
- Genotyping for HPV 16 and 18 has been proposed as a good alternative for women who test HPV positive and cytology negative because the risk of CIN3+ is genotype dependent and HPV types 16 or 18 are found in over 70% of cervical cancer. However being HPV positive, but negative to HPV16 and 18 confers an increased risk of cervical disease and these women cannot be returned to routine screening.
- One particularly promising triage test is the immunocytochemical detection of over-expression of the $p16^{INK4a}$ tumour suppressor gene as a surrogate marker for the transforming activity of hrHPV onco-proteins that are essential for the initiation and maintenance of the neoplastic process. Currently the potential of this test is limited by the lack of standardised reporting and a need for substantial expert interpretation.
- DNA methylation of a panel of both human and HPV gene markers to be used as triage tests is an exciting area of current research.

Vaginal self-collection for HPV-testing

- Testing for HPV in vaginal self-collected samples offers a relatively cheap and effective way of improving cervical screening coverage in many settings.
- The performance of the HPV test on self collected samples, in terms of clinical sensitivity and specificity for CIN2+ in the screening setting, is not as good as HPV testing on clinician-taken cervical specimens; in most studies the sensitivity or specificity are quite variable, showing values between 60 to 90%. However it is fairly clear that HPV testing on self collected samples is as sensitive and sometimes more sensitive than developed-country cytology-based screening of cervical specimens; however, cytology is the more specific test.

Conclusions

- HPV testing is the preferred approach to primary cervical screening in both high and low-resource settings.
- The best well-validated option for triage of HPV positive women is cytology. Genotyping for HPV16 and some other HPV types or alternatively p16 alone or p16 in combination with Ki-67 are also currently under evaluation as possible triage options for high risk HPV positive women.
- Testing for HPV in vaginal self-collected samples offers a relatively cheap and effective way of improving cervical screening coverage in many settings.
- In the future DNA methylation may provide a suitable triage assay for vaginal self-collected samples.

Background

Cervical screening works by detecting and treating high grade cervical intraepithelial neoplasia (CIN), typically CIN2 and above (CIN2+) or in some settings CIN3 and above (CIN3+). In the absence of treatment some, but not all, CIN3 would progress to cervical cancer.[1, 2] Cervical cancer is rare in women previously adequately treated for CIN.[3, 4]

For many years cervical screening was done by (conventional) cytology: a sample was collected using a spatula and smeared onto a glass slide before being analysed under a microscope in a laboratory. Today many screening programmes use liquid based cytology (LBC) instead: samples are now collected using a small plastic broom and stored in a liquid transport medium. Glass slides are prepared from the cells in this transport medium in the laboratory. LBC is considered to be better than conventional cytology in that it is easier and quicker to see what is on the slide, but research studies have not shown that it is better than conventional cytology at detecting high-grade CIN.[5]

The success of effective screening programmes stem from their good coverage (eg in the UK around 80% of the target population has been screened in the last five years) and the high-level of quality assurance. Cervical cytology depends on the collection of good samples and the skilled interpretation of cells under the microscope. Both activities require extensive training and continuous quality assurance. Examining cells under a microscope is a skilled job that relies on concentration (not to miss something) and human judgement to interpret what is seen. Cervical screening has been ineffective in most low-resource countries because of an inability to obtain high coverage with a reliable screening test.

Cervical cancer is caused by around 15 high risk types of the human papilloma virus (hrHPV), notably types 16 and 18 which together give rise to about 70% of all cases. Although infection of the cervix with HPV is very common, there would be no (or virtually no) cervical cancer without HPV infection.[6] In most cases, the body's immune system clears a cervical HPV infection within a year

or two.[7] hrHPV infection may lead to high grade CIN and high grade CIN sometimes progresses to cervical cancer. The time from hrHPV infection to cancer (in women developing cancer) varies hugely, but it is very rare for cancer to develop in less than 7 years and it is not uncommon for cancer to develop more than 30 years after infection.

HPV testing

Since hrHPV infection is needed for cancer to develop and since hrHPV DNA is still present during the development of cancer, looking for hrHPV DNA should be a good approach to identifying women with high-grade CIN. Additionally, since the time from hrHPV infection to cancer is long, a woman who is HPV negative is extremely unlikely to develop cervical cancer over the next 5-10 years and infrequent screening would be safe. Thus, there are two potential uses for HPV testing: (i) to identify those likely to have disease now (and would benefit from treatment); and (ii) to identify those who might develop disease in the next few years (or by elimination those who are extremely unlikely to develop disease over the next several years and therefore do not need to be screened again for several years). However, any HPV assay used for screening purposes will need to be clinically validated for the detection of CIN2+. Most commercial HPV tests are based on DNA but two are based on RNA. One of these tests is restricted to five HPV types and has lower sensitivity and the other has similar sensitivity to the DNA based test.[8]

There have been many studies comparing HPV testing to cytology for the detection of high grade CIN. All large studies have found that HPV testing is at least as good as cytology at detecting high-grade CIN and most studies find HPV testing to be better (ie, it is more sensitive).[9-12] The disadvantage of HPV testing is that it is less specific than cytology. That is, among women without high grade CIN more will test positive for HPV than will have abnormal cytology.

Another advantage of HPV testing is that it does not depend on the preservation of good quality morphology. In stark contrast to cytology, reasonable results can be obtained by using HPV testing

on samples that women collect themselves. [13] Further, HPV testing does not rely on human judgement and the results are reproducible in different laboratories. In studies comparing cytology to HPV testing in Europe and North America[11], the sensitivity of cytology varied from less than 40% to about 80% (average 53%) whereas the sensitivity of clinician-based HPV testing was consistently above 85% (average 96%). The specificity of both tests increased with age, however cytology was on average more specific (96%) than HPV testing (91%).

Longitudinal studies show that the rate of high-grade CIN after a negative HPV test is considerably less than the rate after negative cytology.[14-17] In a joint European study the cumulative risk of CIN3+ six years after a negative HPV test was significantly less (0.27%) than the cumulative risk three years after a negative cytology (0.51%).[14] One study of cervical cancer incidence in the five years following screening with both cytology and HPV testing reported half the rate of cervical cancer following a negative HPV test compared to following a negative cytology test.[18]

Randomised controlled trials (RCTs) have shown that the additional cases of high-grade CIN picked up by HPV testing but missed by cytology are not simply indolent disease that would not have progressed to cancer in the short term. In all four RCTs of cytology versus HPV testing within established screening programmes with published results for the second round of screening, the rate of high-grade CIN detected at round two (i.e. between one and five years after enrolment) was substantially lower (approximately half) in those individuals originally screened by HPV testing compared to those originally tested by cytology alone.[19-22] Thus by treating more disease in the first round, the trials found that there was less detected in the second round. Further, the limited data on the incidence of cervical cancer in RCTs suggest that HPV testing does indeed result in fewer cases of cervical cancer on follow-up. In the Italian trial [22] there were 9 cancers in the cytology arm compared to 0 in the HPV arm after the initial screen and in the Dutch [21] trial there were 14 versus 4. Results in the Swedish trial[20] including those detected at first screen, found five squamous cell carcinomas in the cytology arm and one in the HPV arm.

Initially, many people believed that HPV testing was too expensive to be used in middle and low-income countries and that cytology or simply looking at the cervix would be adequate to greatly reduce the burden of cervical cancer. There was much interest in visual inspection after application of dilute acetic acid (VIA). Indeed for many years VIA was the favoured approach of the Alliance for Cervical Cancer Prevention; one of the main organizations working to prevent cervical cancer in developing countries. However, a trial in the San Martin region of Peru that compared conventional cytology, VIA and HPV testing to screen over 5000 women found that the sensitivity of HPV testing was far superior to that of cytology and VIA, and that the specificity of VIA was considerably lower.[23] Further a cluster randomised trial in India, reported –"34 deaths from cervical cancer in the HPV-testing group, compared with 64 in the control group (hazard ratio, 0.52; 95% CI, 0.33 to 0.83). No significant reductions in the numbers of advanced cancers or deaths were observed in either the cytology group or the VIA group, as compared with the control group".[24]

Management of women positive for HPV

The great advantage of HPV testing for most women is that HPV negative women can have their screening interval extended to over 3 years.[14] By extending the screening interval to six or even 10 years, the proportion of women who will test positive on at least one HPV screen by age 65 will be similar to the proportion that test positive on at least one cytology screen in current programmes. Since the rate of disease following an abnormal HPV test is half the rate of disease following an abnormal cytology, where screening interval is 5 years, extending the screening interval to 10 years should roughly result in the same number of women testing positive as they currently do.

The greater proportion of women testing HPV positive on a single screen (compared to the proportion with abnormal cytology) need not be a problem as demonstrated by the very large trials of primary HPV testing that have been run successfully in several countries (including England). The first solution is good management of HPV positive women. A positive HPV test in primary screening

should only trigger a triage test not automatic referral to colposcopy. The most widely studied triage test in this situation is cytology. Triage with cytology can be done on the same sample as the HPV test without the need for women to attend for another clinic visit (when the sample is taken using liquid based cytology transport medium). Effectively the same women (those positive on both cytology and HPV testing) are immediately referred to colposcopy regardless of whether one screens using cytology triaged by HPV testing or HPV testing triaged by cytology.[25] In practice, because cytology is subjective, more women may be considered morphologically abnormal when they are known to be HPV positive. Evidence of this was observed in the Finish trial[12], where women were referred to colposcopy based in their cytology result, in particular women under age 35. Introduction of primary HPV testing should be closely monitored to assess whether the increase in morphologically abnormal cytology is associated with the learning curve or whether it will affect referral rates long term Conversely, a very small number of women who would be referred to colposcopy with high-grade cytology, without HPV triage, will test negative for HPV and will not have cytology triage. HPV positive women with normal cytology will be re-screened at a shorter interval (e.g. every 12 months). In this way the sensitivity of HPV testing will be maintained but the numbers referred to colposcopy need not be excessively increased.

Current proposed strategies to deal with HPV positive/cytology negative women suggest they should be retested 12-24 months later (with HPV and cytology). Women who are still HPV positive or those that develop cytological abnormalities upon retesting are referred to colposcopy .[26-28] Support for this strategy comes from cohort study evidence that suggest 90% of HPV infections will be self clearing in a period of 1 to 2 years.[15, 16] Nevertheless referring all women who test HPV positive/cytology negative twice 12 months apart leads to higher colposcopy referral rates than those observed in cytology screening programmes.

Genotyping for HPV 16 and 18

Genotyping for HPV 16 and 18 has been proposed as a good alternative for women who test HPV positive/cytology negative because the risk of CIN3+ is genotype dependent[25, 29, 30] and HPV types 16 or 18 are found in over 70% of cervical cancer.[31] HPV 16 (but not HPV18) is associated with a high cross-sectional rate of CIN2+ and CIN3+.[8] Women with HPV 18 and HPV 45 are more likely to develop disease (particularly adenocarcinomas in the endocervical canal) than are women with other high risk types.[31, 32] The risk of CIN3 reaches 10% over one to four years for HPV16 positivity and over 2 to 5 years for HPV18 positivity.[30, 33] By contrast the 12 months risk of CIN3+ after a HPV positive/cytology negative test ranges from 0.8% to 4.1%.[26]

Studies have shown that HPV DNA testing followed by triaging with cytology and screening for persistent HPV type-specific infection (genotyping) has a considerably higher sensitivity for detecting CIN3+ than screening by cytology alone with a modest increase in screening tests and referrals.[25, 28] These results have been validated as part of the ATHENA trial[9] in a scenario where HPV is the primary test. Being hrHPV positive but negative to HPV16/18 carries an increased risk of CIN3 in the next two years of 2.9%.[21] For this reason the lack of HPV16/18 cannot be used to return women to routine screening.

Genotyping for triage has been primarily investigated in women over the age of 30. In younger women, the proportion testing HPV16/18 positive may be too high to justify referring them all; however, the proportion of CIN3+ harbouring HPV16 also decreases in older women, indicating the need for careful follow-up of hrHPV women regardless of HPV type.[9]

Immunocytohemical detection of p16INK4a

Triage using other biomarkers has been less comprehensively evaluated. One particularly promising test is the immunocytochemical detection of over-expression of the $p16^{INK4a}$ tumour suppressor gene as a surrogate marker for the transforming activity of hrHPV onco-proteins that are essential for the

initiation and maintenance of the neoplastic process. A number of studies looking at $p16^{INK4a}$ staining of cervical cytology showed that women with CIN1 positive for p16 were more likely to be subsequently diagnosed with CIN2+ .[34-36] Unfortunately the sensitivity of p16 varies hugely in the literature due to difficulties in reading the slides, lack of standardised reporting and a need for substantial expert interpretation. In general p16 is less sensitive for CIN2+ or CIN3+ than is HPV testing, but most studies have found it to be more specific at identifying women with high-grade disease.[8, 37-39]

A more recent development has been double staining for $p16^{INK4a}$ and ki-67. Several studies investigating the performance of the paired markers $p16^{INK4a}$/ki-67 found that the results were quite similar to $p16^{INK4a}$ alone. The main difference between the single and the paired markers is that $p16^{INK4a}$ should be read by an expert and requires considerable interpretative skills to rule out false positives due to staining of endocervical and other cells. In contrast the $p16^{INK4a}$/ki-67 marker pair is read differently and can be more easily interpreted correctly by a less experienced cytopathologist.[38, 40-42] A study by Carozzi F et al [38] is of particular interest since good results on $p16^{INK4a}$ were demonstrated in women aged 25-34, showing that $p16^{INK4a}$ may be considered as a triage marker for these hrHPV -positive women. A recent paper from the same study with additional follow-up showed that $p16^{INK4a}$ positivity in hrHPV-positive women had a cumulative 3 year absolute risk for CIN2+ of 19.5% with a longitudinal sensitivity of 75.6%.[43] The data suggest that $p16^{INK4a}$ may be considered as an option for triage of hrHPV- positive women

DNA Methylation

DNA methylation (DNAme) of host cell DNA has been proposed as an alternative triage for hrHPV-positive women because DNAme plays an essential role in gene transcription and in genomic stability. Aberrant methylation leads to cell immortality and malignant transformation.[44] The use of DNAme for triage would have the advantage of being an automated, objective test that could be run on the same sample as the HPV assay.

Of the more than 50 human genes tested so far in cervical tissue, [45] 15 have been reported in five or more studies and three genes (CADM1, DAPK1 and RARB) have been repeatedly shown to have elevated methylation in cervical cancers. . Hesselink et al[46] evaluated CAD1/MAL methylation to triage HPV positive women in a large population-based screening study. They found it to be as effective at detecting CIN3+ in HPV positive women as cytology with HPV16/18 genotyping. Studies of host cell methylation use different clinical specimens, methylation assays, assay thresholds and/or selected promoter regions making consistency among results difficult .[44]

The methylation of HPV genes, especially HPV16, in disease progression has been a major focus of research. Elevated methylation of the HPV 16 L1 and L2 open reading frames (ORF) in particular is associated with high grade CIN and invasive cancer.[47-49] Recently the association between specific patterns of DNAme in HPV16 L1 and L2 and high-grade CIN has been validated in a small prospective study.[50] Further validation in large studies is required. The potential utility of methylation of other regions of the HPV16 genome as a biomarker for CIN2+ is less clear because studies have been small and the results were highly variable. To expand the potential utility of HPV16 methylation assays for predicting risk of cancer additional research needs to be directed to other HPV types such as 18, 31, 33 and 45.

DNAme of a panel of both human and HPV gene markers is an exciting area of current research.

Vaginal self-collection for HPV testing

For years conventional cervical screening has relied on clinician-collected cervical specimens, however, vaginal self-collection for HPV testing is likely to become an integral part of screening in both the developed and developing world. This is because self collection has the potential to overcome two major problems inherent in current screening strategies: cost and coverage. It is difficult to maintain the large numbers of clinics required for mass population screening, especially in resource-constrained regions. Even in countries that manage to do this well, 80% compliance with

the recommended screening intervals is considered extremely good. Women who are not screened or attend infrequently are at highest risk of developing cervical cancer.[51] Studies have shown that self collection for HPV testing is a practical and effective way to collect exfoliated cell specimens from the vaginal tract and cervix that is broadly acceptable to women [52-55] and eliminates the cost of visiting a clinician.

Acceptability of vaginal self collection

Numerous studies in a wide variety of countries and ethnic groups over the past decade have reported that self collection is well-accepted by women and in some more educated groups may be preferred to speculum-based cervical sampling. [56-61] Virtually all measures of acceptability, including pain, embarrassment and anxiety favour the self collection approach. However, a consistent negative theme across the studies is that 50 to 70% of women worry they will not take the sample properly.[56-58, 62] This is actually a misconception, it is easy for a woman to take a self collected sample correctly and the samples are almost always good. There do not appear to be any specific cultural or religious barriers to self-sampling,[62, 63] although one study in the UK found that Muslim women were reluctant to use the self collection approach.[52]

The main problem in high-income countries with well-established screening programmes is low coverage and falling participation rates. A well-recognised barrier to cervical screening attendance is the need to undergo a pelvic examination, which some women find to be uncomfortable, invasive or unacceptable (for emotional or cultural reasons).[54] However, practical barriers may be more predictive of non-attendance behaviour.[64] The self collection approach allows the women to obtain samples in privacy and comfort with the additional benefit of being able to choose the time and place of sampling.

The devices most commonly used for self collection are swabs, which may be made of spun polyester fibre or treated cotton on a plastic shaft or special flocked swabs with a somewhat rough surface; untreated cotton swabs should not be used. Another device is a small soft nylon brush

similar in shape to an artist's brush but with much wider diameter bristles (Rovers® Viba-Brush) or a small soft nylon brush similar to a Christmas tree-shaped mascara brush. Yet other approaches use plunger-like devices (Delphi device) to do a vaginal lavage. Women may be reluctant to use devices that look uncomfortable or appear like medical implements; to some extent even simple swabs may be resisted by some women. There are now several companies focused on delivering elegant self collection devices, with a look and feel that is more attractive to feminine sensibilities (for example, the Evalyn sampler, Rovers Medical). While such devices may cost more they may be more acceptable, more readily used and returned at higher rates to the clinicians, thus promoting greater compliance with screening and saving costs in the long run.

Sensitivity and specificity of vaginal self sampling

Overall, the reviews which compared self collection versus clinician-collected samples found comparable values for HPV prevalence , [65-67] although self collection may detect more low risk HPV types.[65, 68] The performance of the HPV test on self collected sample, in terms of clinical sensitivity and specificity for CIN2+ in the screening setting, is not as good as HPV testing on clinician-taken cervical specimens. Of the 10 studies comparing self collected samples vs. clinician collected samples reported by Snijders et al[13] nine found the clinician collected sample to be more sensitive and one found it to be as sensitive. In most studies the sensitivity or specificity of self collected samples are quite variable, showing values between 60 to 90%.[13] Best results, in terms of clinical sensitivity and specificity, depend on the combination of the sampler, transport conditions (medium and conduits) and the assay. Clinical validation of a combination, rather than just its individual components, should be required before it is used in routine practice[13]

It is fairly clear that HPV testing on self collected samples is as sensitive and sometimes more sensitive than developed-country cytology-based screening of cervical specimens; however, cytology is the more specific test.[13] In contrast, in resource constrained regions where routine cytology is often quite poor, HPV testing on self collected samples is usually more sensitive than cytology-based screening. A large randomized clinical trial in Mexico[69] and several smaller observational studies in

China[70] have shown that HPV testing of self collected specimens is a much more sensitive approach than local routine cytology screening and may address issues related to the lack of access of poor women to screening. The study by Lazcano et al[69] in Morelos State Mexico randomized 25,061 women living in 540 poor communities to either routine clinic-based cytology or self collection in their homes followed by HPV testing, women positive on either test were referred to routine colposcopy and biopsy of any abnormal areas of the cervix. Among the 11,054 participants of the cytology arm there were 38 CIN 2+ discovered of which 8 were invasive cancers, while among the 9,292 participants in the HPV arm there were 108 CIN2+ detected of which 28 were cancers; overall 3.4 (95% CI=2.4-4.9) times more high grade CIN was detected by HPV testing on self collected samples than by routine cytology. A report from China[70] presents a pooled analysis of the diagnostic accuracy of self collection in five small to medium population-based studies of mostly poor rural women. The studies had a colposcopy reference standard with biopsy as appropriate on women positive by any screening test, including liquid based cytology, visual inspection by acetic acid, self collection and physician-based cervical HPV testing, using the same HPV assay as in Mexico. Of 13,004 women in the pooled analysis 507 were diagnosed with CIN2+. Self collection with HPV testing had a sensitivity of 86.2% and a specificity of 80.7% as compared to a sensitivity of 97% and a specificity of 82.7% for physician-based HPV testing. Liquid based cytology was significantly less sensitive than either HPV test.

Cervical screening using vaginal self-sampling for HPV testing

Conventional cervical screening has never been successfully transferred to developing or low-income countries because of the need for infrastructure and the associated costs. Unsurprisingly, 85% of the global burden of cervical cancer arises from these areas.[71] However, self collection is comparatively resource-efficient and has the potential to provide effective widely available screening to these largely unscreened populations. [69, 72, 73]

Studies mainly in the Netherlands and Scandinavia have shown a way to implement self collection in developed countries that is complementary to current screening systems, regardless of whether the primary screening is based on cytology or HPV testing. The main problem in these countries is a lack of adherence to screening guidelines. Several studies have shown that offering self collection to non-compliant women was superior to a recall invitation for cytology in attracting them to participate again in the screening program.[13, 58] Nine studies reporting response rates to self-sampling amongst non-attendees showed that between 8 and 39% of non-attendees of the cervical screening program provided self collection samples to the laboratory.[74-82] Giorgi et al[74] found a substantial decrease in participation among Italian women when self-sampling was available as an opt-in versus providing self collection devices directly to the non-attendees (8.7% versus 19.6%). An interesting study of self collection in the Netherlands[78] used the national postal service to deliver and return the devices. The research team studied 26,409 non-compliant women of whom 26,145 were offered self collection while a control group of 264 women were offered a re-invitation to cytology. They found a 30.8% return of the self collection devices versus 6.5% of the control group who attended cytology screening. Subsequently 89% of the HPV positive women took a triage cytology test, with 95.8% of those with abnormal cytology going to colposcopy. The rate of CIN2+ and invasive cancer discovered on colposcopy in the self collection group was 1.5% and 0.1% respectively, rates which are similar to those seen in the developing world. The next challenge for self collection in countries with an established screening programme will be to see if the increased coverage observed in research settings will translate into population screening. Achieving high rates of women attending triage following an HPV positive self-sampling test (reflex cytology is not possible from the self-sample) presents a particular challenge. This is why a molecular test (such as DNA methylation or genotyping) from the self-collected sample is attractive because it can be done without requesting another sample or an examination from the woman. Currently the only commercially available molecular triage is genotyping for HPV16 and perhaps HPV18, although the

latter has questionable specificity, however in the future methylation may provide a suitable triage assay for vaginal self-collected samples.

Overall the studies support the use of self collection to improve cervical cancer prevention in non-compliant and poor women worldwide. It appears that the next logical step is large-scale pilot implementation of HPV testing on self collected samples in developed countries, as well as in resource-constrained regions and poor countries that have sufficient economic capacity and provider skills with appropriate infrastructure. Prior to implementation there should be a thoughtful analysis of the program including: 1) the screening groups and means of contact, 2) the primary testing and triage approach, 3) rendering of accurate final diagnoses, 4) treatment, follow-up and documentation.

Conclusions

HPV testing is seen to be the preferred approach to primary cervical screening in both high and low-resource settings.[83] Testing for HPV in vaginal self-collected samples offers a relatively cheap and effective way of improving cervical screening coverage in many settings. Currently the best well-validated option for triage of HPV positive women is cytology, but that requires a clinician-collected sample from the cervix. Genotyping for HPV16 and some other HPV types or alternatively p16 alone or p16 in combination with Ki-67 are also currently under evaluation as possible triage options for hrHPV positive women. In the future DNA methylation may provide a suitable triage assay for vaginal self-collected samples. Even with affordable HPV testing, middle and low income countries will have to overcome the infrastructure challenges needed to ensure good follow-up, triage and treatment of HPV positive women.

Future Perspectives

HPV co-testing has become the predominant method of screening in the United States. Eventually HPV testing is likely to become the primary screen in the USA with cytology used as the triage test on hrHPV positive women. In the next 5 to 10 years HPV testing will be introduced as the primary screening test in organised screening programmes in many countries in Europe. HPV screening in Asia and Latin America is also taking hold, there will be a diversity of tests and approaches and in general adoption will be quite slow and be region specific. Triage of HPV positive women, using various combinations of genotyping, immunochemistry and molecular markers, will begin to be introduced as part of some population based screening programmes. Vaginal self-collection will be used to boost coverage wherever HPV testing is used.

The impact of HPV vaccination on women of HPV screening age (those over age 30) is still 5 to 10 years away and more data on the population-based efficacy of the vaccine for preventing cervical cancer will be needed before recommendations on altering current screening algorithms can be made. Rational algorithms will distinguish between women known to have been vaccinated (3 doses) whilst under age 15 years and those not known to have been vaccinated or known not to have been vaccinated against HPV 16 and 18. Nevertheless, a combination of HPV vaccination and HPV testing will be the cornerstone of cervical cancer control globally.

Reference

1. Mccredie MR, Sharples KJ, Paul C *et al.*: Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. *Lancet Oncol* 9(5), 425-434 (2008).
2. Sasieni P, Castanon A, Parkin DM: How many cervical cancers are prevented by treatment of screen-detected disease in young women? *Int J Cancer* 124(2), 461-464 (2009).
3. Soutter Wp, Sasieni P, Panoskaltsis T: Long-term risk of invasive cervical cancer after treatment of squamous cervical intraepithelial neoplasia. *Int J Cancer* 118(8), 2048-2055 (2006).
4. Evans HS, Newnham A, Hodgson SV, Moller H: Second primary cancers after cervical intraepithelial neoplasia III and invasive cervical cancer in Southeast England. *Gynecol Oncol* 90(1), 131-136 (2003).

5. Karnon J, Peters J, Platt J, Chilcott J, Mcgoogan E, Brewer N: Liquid-based cytology in cervical screening: an updated rapid and systematic review and economic analysis. *Health Technol Assess* 8(20), iii, 1-78 (2004).
**6. Walboomers JM, Jacobs MV, Manos MM *et al.*: Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 189(1), 12-19 (1999). *First paper to show that HPV was necessary for developing cervical cancer*
7. Moscicki AB, Shiboski S, Broering J *et al.*: The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. *The Journal of pediatrics* 132(2), 277-284 (1998).
8. Szarewski A, Mesher D, Cadman L *et al.*: A comparison of seven tests for high grade cervical intraepithelial neoplasia in women with abnormal smears: the Predictors 2 study. *J Clin Microbiol*, (2012).
9. Castle PE, Stoler MH, Wright TC, Jr., Sharma A, Wright TL, Behrens CM: Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. *Lancet Oncol* 12(9), 880-890 (2011).
10. Cuzick J, Arbyn M, Sankaranarayanan R *et al.*: Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. *Vaccine* 26 Suppl 10, K29-41 (2008).
**11. Cuzick J, Clavel C, Petry KU *et al.*: Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int J Cancer* 119(5), 1095-1101 (2006). Overview of the sensitivity and specificity of cytology and HPV testing. First to show that HPV testing is uniformly more sensitive for detection of high grade CIN.
12. Leinonen M, Nieminen P, Kotaniemi-Talonen L *et al.*: Age-specific evaluation of primary human papillomavirus screening vs conventional cytology in a randomized setting. *J Natl Cancer Inst* 101(23), 1612-1623 (2009).
**13. Snijders PJ, Verhoef VM, Arbyn M *et al.*: High-risk HPV testing on self-sampled versus clinician-collected specimens: A review on the clinical accuracy and impact on population attendance in cervical cancer screening. *Int J Cancer* 132(10), 2223-2236 (2013). Good reference for those wanting to know more about HPV self-sampling.
*14. Dillner J, Rebolj M, Birembaut P *et al.*: Long term predictive values of cytology and human papillomavirus testing in cervical cancer screening: joint European cohort study. *BMJ* 337, a1754 (2008). Good paper on the longer term predictive value of cytology and HPV testing.
15. Mesher D, Szarewski A, Cadman L *et al.*: Long-term follow-up of cervical disease in women screened by cytology and HPV testing: results from the HART study. *Br J Cancer* 102(9), 1405-1410 (2010).
16. Sherman ME, Lorincz AT, Scott DR *et al.*: Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. *J Natl Cancer Inst* 95(1), 46-52 (2003).
17. Kjaer S, Hogdall E, Frederiksen K *et al.*: The absolute risk of cervical abnormalities in high-risk human papillomavirus-positive, cytologically normal women over a 10-year period. *Cancer research* 66(21), 10630-10636 (2006).
18. Katki HA, Kinney WK, Fetterman B *et al.*: Cervical cancer risk for women undergoing concurrent testing for human papillomavirus and cervical cytology: a population-based study in routine clinical practice. *Lancet Oncol* 12(7), 663-672 (2011).
19. Kitchener HC, Almonte M, Thomson C *et al.*: HPV testing in combination with liquid-based cytology in primary cervical screening (ARTISTIC): a randomised controlled trial. *Lancet Oncol* 10(7), 672-682 (2009).
20. Naucler P, Ryd W, Tornberg S *et al.*: Human papillomavirus and Papanicolaou tests to screen for cervical cancer. *N Engl J Med* 357(16), 1589-1597 (2007).

21. Rijkaart DC, Berkhof J, Rozendaal L *et al.*: Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial. *Lancet Oncol* 13(1), 78-88 (2012).
22. Ronco G, Giorgi-Rossi P, Carozzi F *et al.*: Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. *Lancet Oncol* 11(3), 249-257 (2010).
23. Almonte M, Ferreccio C, Winkler JL *et al.*: Cervical screening by visual inspection, HPV testing, liquid-based and conventional cytology in Amazonian Peru. *Int J Cancer* 121(4), 796-802 (2007).
*24. Sankaranarayanan R, Nene BM, Shastri SS *et al.*: HPV screening for cervical cancer in rural India. *N Engl J Med* 360(14), 1385-1394 (2009). Although there are many good trials of HPV screening this is the only one assessing its impact on mortality from cervical cancer.
25. Naucler P, Ryd W, Tornberg S *et al.*: Efficacy of HPV DNA testing with cytology triage and/or repeat HPV DNA testing in primary cervical cancer screening. *J Natl Cancer Inst* 101(2), 88-99 (2009).
26. Saslow D, Solomon D, Lawson HW *et al.*: 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. *CA Cancer J Clin*, (2012).
27. Sasieni P, Cuzick J: Could HPV testing become the sole primary cervical screening test? *J Med Screen* 9(2), 49-51 (2002).
28. Rijkaart DC, Berkhof J, Van Kemenade FJ *et al.*: Evaluation of 14 triage strategies for HPV DNA-positive women in population-based cervical screening. *Int J Cancer* 130(3), 602-610 (2012).
29. Schiffman M, Herrero R, Desalle R *et al.*: The carcinogenicity of human papillomavirus types reflects viral evolution. *Virology* 337(1), 76-84 (2005).
30. Kjaer SK, Frederiksen K, Munk C, Iftner T: Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. *J Natl Cancer Inst* 102(19), 1478-1488 (2010).
31. De Sanjose S, Quint WG, Alemany L *et al.*: Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol* 11(11), 1048-1056 (2010).
32. Bosch FX, Manos MM, Munoz N *et al.*: Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. *J Natl Cancer Inst* 87(11), 796-802 (1995).
*33. Khan MJ, Castle PE, Lorincz AT *et al.*: 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(14), 1072-1079 (2005). This paper addresses the increased risk of cervical cancer associated with certain HPV sub-types.
34. Negri G, Bellisano G, Zannoni GF *et al.*: p16 ink4a and HPV L1 immunohistochemistry is helpful for estimating the behavior of low-grade dysplastic lesions of the cervix uteri. *Am J Surg Pathol* 32(11), 1715-1720 (2008).
35. Ozaki S, Zen Y, Inoue M: Biomarker expression in cervical intraepithelial neoplasia: potential progression predictive factors for low-grade lesions. *Hum Pathol* 42(7), 1007-1012 (2011).
36. Wang JL, Zheng BY, Li XD, Angstrom T, Lindstrom MS, Wallin KL: Predictive significance of the alterations of p16INK4A, p14ARF, p53, and proliferating cell nuclear antigen expression in the progression of cervical cancer. *Clin Cancer Res* 10(7), 2407-2414 (2004).
37. Denton KJ, Bergeron C, Klement P, Trunk MJ, Keller T, Ridder R: The sensitivity and specificity of p16(INK4a) cytology vs HPV testing for detecting high-grade cervical disease in the triage of ASC-US and LSIL pap cytology results. *Am J Clin Pathol* 134(1), 12-21 (2010).
*38. Carozzi F, Confortini M, Dalla Palma P *et al.*: 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(10), 937-945 (2008). For those wanting to find out more about p16, also see the follow-up from this study (reference 43).
39. Bergeron C, Ordi J, Schmidt D, Trunk MJ, Keller T, Ridder R: Conjunctive p16INK4a testing significantly increases accuracy in diagnosing high-grade cervical intraepithelial neoplasia. *Am J Clin Pathol* 133(3), 395-406 (2010).
40. Petry KU, Schmidt D, Scherbring S *et al.*: Triaging Pap cytology negative, HPV positive cervical cancer screening results with p16/Ki-67 Dual-stained cytology. *Gynecol Oncol* 121(3), 505-509 (2011).
41. Schmidt D, Bergeron C, Denton KJ, Ridder R, European CCSG: p16/ki-67 dual-stain cytology in the triage of ASCUS and LSIL papanicolaou cytology: results from the European equivocal or mildly abnormal Papanicolaou cytology study. *Cancer cytopathology* 119(3), 158-166 (2011).
42. Tsoumpou I, Arbyn M, Kyrgiou M *et al.*: p16(INK4a) immunostaining in cytological and histological specimens from the uterine cervix: a systematic review and meta-analysis. *Cancer treatment reviews* 35(3), 210-220 (2009).
43. Carozzi F, Gillio-Tos A, Confortini M *et al.*: Risk of high-grade cervical intraepithelial neoplasia during follow-up in HPV-positive women according to baseline p16-INK4A results: a prospective analysis of a nested substudy of the NTCC randomised controlled trial. *Lancet Oncol* 14(2), 168-176 (2013).
*44. Lorincz AT: The Promise and the Problems of Epigenetics Biomarkers in Cancer. *Expert Opin Med Diagn* 5(5), 375-379 (2011). A simple overview of Epigenetics including DNAme
45. Wentzensen N, Sherman ME, Schiffman M, Wang SS: Utility of methylation markers in cervical cancer early detection: appraisal of the state-of-the-science. *Gynecol Oncol* 112(2), 293-299 (2009).
46. Hesselink AT, Heideman DA, Steenbergen RD *et al.*: Combined promoter methylation analysis of CADM1 and MAL: an objective triage tool for high-risk human papillomavirus DNA-positive women. *Clin Cancer Res* 17(8), 2459-2465 (2011).
47. Kalantari M, Calleja-Macias IE, Tewari D *et al.*: Conserved methylation patterns of human papillomavirus type 16 DNA in asymptomatic infection and cervical neoplasia. *J Virol* 78(23), 12762-12772 (2004).
48. Brandsma JL, Sun Y, Lizardi PM *et al.*: Distinct human papillomavirus type 16 methylomes in cervical cells at different stages of premalignancy. *Virology* 389(1-2), 100-107 (2009).
49. Mirabello L, Sun C, Ghosh A *et al.*: Methylation of Human Papillomavirus Type 16 Genome and Risk of Cervical Precancer in a Costa Rican Population. *J Natl Cancer Inst*, (2012).
50. Lorincz AT, Brentnall AR, Vasiljevic N *et al.*: HPV16 L1 and L2 DNA methylation predicts high-grade cervical intraepithelial neoplasia in women with mildly abnormal cervical cytology. *Int J Cancer*, (2013).
51. Sasieni PD, Cuzick J, Lynch-Farmery E: Estimating the efficacy of screening by auditing smear histories of women with and without cervical cancer. The National Co-ordinating Network for Cervical Screening Working Group. *Br J Cancer* 73(8), 1001-1005 (1996).
52. Szarewski A, Cadman L, Ashdown-Barr L, Waller J: Exploring the acceptability of two self-sampling devices for human papillomavirus testing in the cervical screening context: a qualitative study of Muslim women in London. *J Med Screen* 16(4), 193-198 (2009).
53. Igidbashian S, Boveri S, Spolti N, Radice D, Sandri MT, Sideri M: Self-collected human papillomavirus testing acceptability: comparison of two self-sampling modalities. *J Womens Health (Larchmt)* 20(3), 397-402 (2011).
54. Waller J, Mccaffery K, Forrest S *et al.*: Acceptability of unsupervised HPV self-sampling using written instructions. *Journal of Medical Screening* 13(4), 208-213 (2006).
55. Gravitt PE, Belinson JL, Salmeron J, Shah KV: Looking ahead: a case for human papillomavirus testing of self-sampled vaginal specimens as a cervical cancer screening strategy. *Int J Cancer* 129(3), 517-527 (2011).

56. Dzuba IG, Diaz EY, Allen B *et al.*: The acceptability of self-collected samples for HPV testing vs. the pap test as alternatives in cervical cancer screening. *J Womens Health Gend Based Med* 11(3), 265-275 (2002).
57. Barata PC, Mai V, Howlett R, Gagliardi AR, Stewart DE: Discussions about self-obtained samples for HPV testing as an alternative for cervical cancer prevention. *J Psychosom Obstet Gynaecol* 29(4), 251-257 (2008).
58. Nobbenhuis MA, Helmerhorst TJ, Van Den Brule AJ *et al.*: Primary screening for high risk HPV by home obtained cervicovaginal lavage is an alternative screening tool for unscreened women. *J Clin Pathol* 55(6), 435-439 (2002).
59. Anhang R, Nelson JA, Telerant R, Chiasson MA, Wright TC, Jr.: Acceptability of self-collection of specimens for HPV DNA testing in an urban population. *J Womens Health (Larchmt)* 14(8), 721-728 (2005).
60. Berner A, Hassel SB, Tebeu PM *et al.*: Human Papillomavirus Self-Sampling in Cameroon: Women's Uncertainties Over the Reliability of the Method Are Barriers to Acceptance. *Journal of lower genital tract disease*, (2013).
61. Mitchell S, Ogilvie G, Steinberg M, Sekikubo M, Biryabarema C, Money D: Assessing women's willingness to collect their own cervical samples for HPV testing as part of the ASPIRE cervical cancer screening project in Uganda. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics* 114(2), 111-115 (2011).
62. Forrest S, Mccaffery K, Waller J *et al.*: Attitudes to self-sampling for HPV among Indian, Pakistani, African-Caribbean and white British women in Manchester, UK. *Journal of Medical Screening* 11(2), 85-88 (2004).
63. Howard M, Lytwyn A, Lohfeld L, Redwood-Campbell L, Fowler N, Karwalajtys T: Barriers to acceptance of self-sampling for human papillomavirus across ethnolinguistic groups of women. *Canadian journal of public health. Revue canadienne de sante publique* 100(5), 365-369 (2009).
64. Waller J, Bartoszek M, Marlow L, Wardle J: Barriers to cervical cancer screening attendance in England: a population-based survey. *Journal of Medical Screening* 16(4), 199-204 (2009).
65. Petignat P, Faltin DL, Bruchim I, Tramer MR, Franco EL, Coutlee F: Are self-collected samples comparable to physician-collected cervical specimens for human papillomavirus DNA testing? A systematic review and meta-analysis. *Gynecol Oncol* 105(2), 530-535 (2007).
66. Ogilvie GS, Patrick DM, Schulzer M *et al.*: Diagnostic accuracy of self collected vaginal specimens for human papillomavirus compared to clinician collected human papillomavirus specimens: a meta-analysis. *Sexually Transmitted Infections* 81(3), 207-212 (2005).
67. Stewart DE, Gagliardi A, Johnston M *et al.*: Self-collected samples for testing of oncogenic human papillomavirus: a systematic review. *J Obstet Gynaecol Can* 29(10), 817-828 (2007).
68. Castle PE, Rodriguez AC, Porras C *et al.*: A comparison of cervical and vaginal human papillomavirus. *Sex Transm Dis* 34(11), 849-855 (2007).
69. Lazcano-Ponce E, Lorincz AT, Cruz-Valdez A *et al.*: Self-collection of vaginal specimens for human papillomavirus testing in cervical cancer prevention (MARCH): a community-based randomised controlled trial. *Lancet* 378(9806), 1868-1873 (2011).
70. Zhao FH, Lewkowitz AK, Chen F *et al.*: Pooled analysis of a self-sampling HPV DNA Test as a cervical cancer primary screening method. *J Natl Cancer Inst* 104(3), 178-188 (2012).
71. Ferlay J SH, Bray F, Forman D, Mathers C and Parkin Dm.: GLOBOCAN 2008 v1.2, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet]. (2010).
72. Holanda F, Jr., Castelo A, Veras TM, De Almeida FM, Lins MZ, Dores GB: Primary screening for cervical cancer through self sampling. *International Journal of Gynaecology & Obstetrics* 95(2), 179-184 (2006).

73. Qiao YL, Sellors JW, Eder PS *et al.*: A new HPV-DNA test for cervical-cancer screening in developing regions: a cross-sectional study of clinical accuracy in rural China. *Lancet Oncol* 9(10), 929-936 (2008).
74. Giorgi Rossi P, Marsili LM, Camilloni L *et al.*: The effect of self-sampled HPV testing on participation to cervical cancer screening in Italy: a randomised controlled trial (ISRCTN96071600). *Br J Cancer* 104(2), 248-254 (2011).
75. Szarewski A, Cadman L, Mesher D *et al.*: HPV self-sampling as an alternative strategy in non-attenders for cervical screening - a randomised controlled trial. *Br J Cancer* 104(6), 915-920 (2011).
76. Bais AG, Van Kemenade FJ, Berkhof J *et al.*: Human papillomavirus testing on self-sampled cervicovaginal brushes: an effective alternative to protect nonresponders in cervical screening programs. *Int J Cancer* 120(7), 1505-1510 (2007).
77. Sanner K, Wikstrom I, Strand A, Lindell M, Wilander E: Self-sampling of the vaginal fluid at home combined with high-risk HPV testing. *Br J Cancer* 101(5), 871-874 (2009).
78. Gok M, Heideman DA, Van Kemenade FJ *et al.*: HPV testing on self collected cervicovaginal lavage specimens as screening method for women who do not attend cervical screening: cohort study. *BMJ* 340, c1040 (2010).
79. Gok M, Heideman DA, Van Kemenade FJ *et al.*: Offering self-sampling for human papillomavirus testing to non-attendees of the cervical screening programme: Characteristics of the responders. *European Journal of Cancer* 48(12), 1799-1808 (2012).
80. Virtanen A, Anttila A, Luostarinen T, Nieminen P: Self-sampling versus reminder letter: effects on cervical cancer screening attendance and coverage in Finland. *Int J Cancer* 128(11), 2681-2687 (2011).
81. Wikstrom I, Lindell M, Sanner K, Wilander E: Self-sampling and HPV testing or ordinary Pap-smear in women not regularly attending screening: a randomised study. *Br J Cancer* 105(3), 337-339 (2011).
82. Virtanen A, Nieminen P, Luostarinen T, Anttila A: Self-sample HPV tests as an intervention for nonattendees of cervical cancer screening in Finland: a randomized trial. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 20(9), 1960-1969 (2011).
83. De Kok IM, Van Rosmalen J, Dillner J *et al.*: Primary screening for human papillomavirus compared with cytology screening for cervical cancer in European settings: cost effectiveness analysis based on a Dutch microsimulation model. *BMJ* 344, e670 (2012).