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Primary HPV screening for cervical cancer

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A B S T R A C T

Cytology-based cervical screening had unequivocal success in reducing the incidence and mortality of cervical cancer in the last century. The recognition of the role of human papillomavirus (HPV) as a necessary cause of cervical cancer led to the development of HPV testing. Gradually, there has been a shift from reflex HPV testing for mild cytological abnormalities, to co-testing with cytology and HPV, and lately to primary HPV screening, based on evidence from well-designed large randomized controlled trials and meta-analyses. Advantages of primary HPV screening include higher sensitivity to detect pre-neoplastic lesions, better reassurance with a negative test, and safe prolongation of screening intervals. However, clinicians and policy makers must ensure the availability of clinically validated HPV assays and triage protocols of screen positive cases prior to implementation of primary HPV screening. This is likely to reduce potential harm from over-treatment as well as extra burden on the health care system.

Introduction

Cervical cancer continues to be an important public health problem with an estimated 569,847 new cases and 311,365 deaths globally in the year 2018 [1]. Nearly 85% of the disease burden lies in low/middle-income countries (LMICs), which lack universal screening programs. Developed countries implemented cytology-based screening programs since the middle of the last century but the main decline in cancer incidence was witnessed only when national call-and-recall programs enabled

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coverage over 70% [2]. Repeated screening is necessary because cytology has poor sensitivity, reported to be ~53% according to a meta-analysis from Europe and North America [3]. The discovery that persistent infection with oncogenic human papillomavirus (HPV) is the necessary cause of cervical cancer led to the development of HPV tests and vaccines. HPV tests were found to have higher sensitivity than cytology (96.1% vs. 53.0%) but somewhat lower specificity (90.7% versus 96.3%) [3]. Thus, HPV testing was better suited as a screening test [4]. Nevertheless, there was an initial reluctance to accept it as a primary screening modality. Initially, HPV testing was incorporated as a method for triage of atypical squamous cells of undetermined significance (ASC-US) cytology results by the American Society for Colposcopy and Cervical Pathology (ASCCP) [4]. Later the concept of co-testing with cytology emerged, and finally, it found acceptance as primary screening test for cervical cancer [5,6]. This article traces the history of these developments and discusses the advantages and disadvantages of the new paradigm.

HPV and cervical cancer

HPV is a non-enveloped double-stranded deoxyribonucleic acid (DNA) virus with more than 100 genotypes. The viral genome codes for early regulatory proteins (E1, E2, E5, E6, and E7) and late structural proteins (L1 and L2) [7]. Transmission occurs predominantly by the sexual route, and it is estimated that approximately 80% of women will acquire the infection during their lifetime [7]. The majority (~90%) clear the infection by their innate immunity, usually within 9–12 months. Those with persistent infection with high-risk genotypes are at risk of developing neoplasia. Of the 30–40 HPV types that infect the anogenital tract, there are 13 high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and 5 likely high-risk (53, 66, 70, 73 MM9, and 82 MM4) HPV types [8]. HPV16/18 are the most aggressive and likely to persist, and they integrate with the host genome and cause cervical neoplasia. They have been associated with ~70% cases of cervical cancer globally, though there is some geographical variation in this proportion [9]. The other HPV strains are low-risk variants (e.g., 6/11/40/42/43/44/54/61/72) associated with anogenital warts and laryngeal papilloma [6,7,10,11].

HPV testing

Understanding the technical aspects of HPV assay is an integral part of the successful implementation of HPV-based screening because it is prudent to choose a clinically validated test [5]. HPV DNA tests are multiplex assays that detect DNA of targeted high-risk HPV types, using a cocktail of probes, either by direct genomic detection or by amplification of a viral DNA fragment using polymerase chain reaction (PCR) [11,12]. HPV genotyping identifies specific viral types (usually HPV 16 and 18), thereby identifying those at greatest risk of persistence and progression. HPV mRNA tests detect the expression of E6 and E7 onco-proteins, a marker of viral integration [11].

Hybrid Capture test (the digene HPV test, hc2, Qiagen Inc., USA) was the first HPV DNA test to be approved by FDA in 1997 for reflex testing of women with ASC-US cytology. In 2003, it was approved for co-testing along with cytology in women aged >30 years. In 2009, Cervista (Hologic, Inc., USA) was approved for co-testing, and APTIMA (Gen-Probe, USA) and Cobas (Roche Molecular Systems Inc., USA) were later approved for the same indications as hc2 [13]. The hc2 test is commercially available in a 96-well microplate format and uses specific probe cocktails for 13 high-risk types. HPV DNA is first hybridized with RNA probes, which are then captured by specific antibodies. The immobilized hybrids are detected by a series of reactions that give rise to a luminescent product. The intensity of emitted light, expressed as relative light units (RLUs), is proportional to the amount of target DNA in the specimen, making it a semi-quantitative test. The clinically significant cut-off value for a positive test is 1.0 (equivalent to HPV DNA 1 pg/mL sampling buffer, corresponding to 5000 genomes per test well) [11,12].

Currently, Cobas is the only FDA-approved test for primary HPV screening in women aged >25 years. It has robust, automatic end-to-end encrypted procedural steps, with excellent reproducibility [14]. Additionally, it has a primer pair and probe, which targets the human β-globin gene and serves as an internal control to provide a measure of specimen adequacy and monitor the quality of extraction and amplification. For interpretation of results, cycle threshold cut-offs are set at 40 for HPV16, and

40.5 for HPV18 and other 12 genotypes. It also includes genotyping for HPV16/18 in two separate channels, thereby providing an immediate triage option for HPV-positive women [5,12,14].

Limitations of HPV-based screening include cost, need for laboratory infrastructure, and time taken to process, which impacts the feasibility of a single-visit approach (SVA). Widespread implementation of primary HPV screening in LMICs needs affordable and point-of-care (POC) HPV test to permit a SVA. careHPV (Qiagen) and Gene Xpert (Sunnyvale, CA, USA) have been evaluated as POC tests. Test performance was comparable with gold standard tests. However, the cost needs to be reduced to make them affordable in LMICs [15]. AVantage E6 strip (Arbor Vita Corporation) test is a rapid POC diagnostic test with target assay time <90 min that detects E6 oncoproteins from cervical swabs. It enables detection of the E6 gene of seven HPV types most prevalent in cervical cancer [15,16]. Salient features of the commercially available HPV tests are summarized in Table 1.

Table 1
Overview of HPV tests [11,12,15,16].

Commercial Name (Company Details)	Comments
DNA based HPV assays	
Direct genome detection	
Hybrid Capture 2 (hc2) (Qiagen Gaithersberg Inc., MD, USA)	Reference standard for HPV screening, US FDA approved, detects 13 hr-HPV types 16/18/31/33/35/39/45/51/52/56/58/59/68 (probe B); 5 low-risk types (6/11/42/43/44) (probe A); does not determine specific type
**care*HPV (Qiagen Gaithersberg Inc., MD, USA)	US FDA-approved affordable rapid test, detects 14 hr-HPV types (above mentioned and type 66), takes 2.5 h to process 90 samples.
Amplification of HPV L1 fragment	
Cervista HPV-HR (Hologic, Inc., Bedford, MA, USA)	US FDA approved, tests hr-HPV DNA of 14 types (16/18/31/33/35/39/45/51/52/56/58/59/66/68), cannot determine specific subtype.
BD Onclarity HPV assay (Becton Dickinson, Sparks, MD, USA)	US FDA approved, detects 14 hr-HPV types, detects types 16/18/45 specifically as well as types 31/33/35/39/51/52/56/59/66/68.
Amplification and genotyping of HPV 16 and 18	
Cervista HPV 16/18 (Hologic Inc., Bedford, MA, USA)	Identifies 16/18 subtypes, US FDA approval for co-testing in 2009.
Cobas HPV test (Roche Molecular Systems Inc., Alameda, CA, USA)	Reports pooled results from 12 hr-HPV types (31/33/35/39/45/51/52/56/58/59/66/68) and specifies also HPV 16/18. US FDA approved, rapid test, can process 96 samples in 5 h. Only test approved presently for primary HPV screening from age 25 years in USA.
*Xpert HPV Cepheid (Sunnyvale, CA, U.S.A.)	Detects DNA encoding for E6/E7 of 14 h types. Sample processing time is usually 1 h. It identifies HPV16 and 18/45 in two distinct channels and other 11 types in a pooled result. Each kit can process 10 samples.
Real-time high-risk HPV assay (Abbott Molecular, Des Plaines, IL, USA)	Concurrent genotyping for HPV 16/18 and pooled detection of 12 other HPV types (31/33/35/39/45/51/52/56/58/59/66/68). The turn-around time is 6–8 h, and can process 96 samples in one cycle.
PapilloCheck (Greiner Bio-One, Frickenhausen)	For qualitative detection and genotyping of 24 HPV (13 high-risk, 5 intermediate-risk and 6 low-risk) types
RNA based HPV assays	
Amplification of E6/E7 proteins	
Aptima HPV assay (Gen-Probe, San Diego, CA, USA)	Allows detection of E6/E7 mRNA transcripts of 14 HPV types.
PreTect HPV-Proofer assay (Proofer NorChip)	Detects HPV, E6/E7 oncogene mRNA from hr-HPV 16/18/31/33/45; high specificity to triage ASCUS cytology.
Monoclonal antibodies	
AVantage HPV E6 Test (Arbor Vita Corporation)	Point-of-care test detects E6 oncoprotein of HPV16/18/45/31/33/52/58, useful for low resource settings, can process 45 samples within 2–2.5 h [16].

**care*HPV and *Xpert HPV Cepheid are also included in the WHO list of prequalified in vitro diagnostic products [17].

Currently, approximately 250 distinct commercial HPV tests are available in the global market, but only some are clinically validated [7,18,19]. Performance of an HPV test depends on a large number of factors, including sample collection procedure, nucleic acid extraction methodology, primers, and use of internal controls. The chosen assay should be type-specific and HPV genome region-specific (L1, E1/E2, and E6/E7). During the process of genome integration, E6/E7 always maintain their expression, but L1 expression may sometimes be lost; therefore, tests designed only for detecting L1 expression may erroneously miss 10% invasive cancer cases [7]. Any new HPV assay (candidate test) chosen for primary screening should be comparable with a gold standard HPV test that has been validated in an randomized controlled trial (RCT) [11,12]. For clinical validation of HPV DNA tests international guidelines have been developed based on Meijer's criteria [12]. Similarly, VALGENT (Validation of HPV Genotyping Tests) provides an international standard and resource for facilitating validation of the HPV genotyping assays [21]. Currently, hc2, Cobas 4800, Aptima, and BD Onclarity are the clinically validated tests available for primary cervical cancer screening [12]. The assays that are validated by Meijer's protocol include APTIMA HPV assay (Gen-Probe, San Diego, CA, USA), Abbott Real-Time High-Risk HPV test, BD Onclarity HPV assay (Becton Dickinson, Sparks, MD), Cervista HPV HR Test (Hologic Inc., Bedford, MA, USA), cobas 4800 HPV test (Roche Molecular Systems Inc., Alameda, CA, USA), E6/E7 mRNA by RT-PCR, and PapilloCheck (Greiner Bio-One, Frickenhausen) [20,21]. Similarly, the genotyping assays that are validated using Valgent criteria include GP5+/6+ LMNX, *care*HPV test (Qiagen Gaithersburg Inc., MD, USA), MALDI-TOF (Sequenom, Inc., San Diego, CA), Pretect ™ HPV-Proofer (Nor Chip Norway), Anyplex II HPV HR (Seegene Inc, Seoul, Korea), and Xpert HPV (Sunnyvale, CA, USA) [20,21].

The laboratory should comply with quality assurance, quality control, and external quality assessment standards. The candidate test should have at least 90% sensitivity and 98% specificity for CIN2+ abnormality as compared to the reference standard in women aged $\geq$30 years. For achieving 90% sensitivity (in comparison with hc2), the test in question should perform well in $\geq$50 samples and for achieving 98% specificity, the candidate test should be tested in 800 cases. There should be good intra- and inter-laboratory agreement (>87%) [11,12].

HPV genotyping

HPV genotyping is helpful to further triage primary HPV and co-test positive women. HPV 16 and 18 have the highest risk of persistence and progression and merit immediate referral to colposcopy. ASC-US positive cases with other high-risk types represent lower risk and can be recommended 12-month follow-up and repeat testing. Thus, 40% of ASC-US cases can be deferred and approximately half of them are likely to clear the infection in this period. This strategy will reduce the colposcopy burden substantially [21].

Various HPV genotyping assays include INNO-LiPA HPV Genotyping extra (Innogenetics), Linear array HPV genotyping test (Roche), PapilloCheck HPV-Screening Test (Greiner Bio-One), Clart HPV 2 (Genomica), and Infiniti HPV genotyping assay (Auto Genomics) [20,21].

Primary HPV screening: best practice for the future

Several well-designed clinical trials have investigated the performance of primary HPV screening [14,19,22,23]. Data from over 1.2 million women have been pooled, and evidence has emerged to confirm the safety and efficacy of primary HPV screening.

HPV test versus cytology

The Canadian cervical cancer screening randomized trial consisted of 10,154 participants and provided most of the initial evidence to demonstrate the higher sensitivity (95% vs 55%), but slightly lower specificity (94% vs 97%) of HPV (hc2) test over conventional cytology for the detection of CIN2+ lesions [19].

Dilner et al. pooled data from seven studies from six countries (Germany, Sweden, Denmark, UK, France and Spain). The cumulative incidence rates (CIR) of CIN3+ after 6 years of follow up increased consistently in HPV-positive women. The CIR of CIN3+ in various cohorts was as follows: both HPV-

and cytology-positive cohort, 34%; HPV-positive, cytology-negative cohort, 10%; HPV-negative, cytology-positive cohort, 2.7%; both HPV- and cytology-negative cohort, 0.28%. CIR of CIN3+ were compared among those with co-test-negative, HPV-negative, and cytology-negative results. At six years follow-up, rates were lowest for HPV-negative cohort (0.27%;0.12%–0.45%), followed by cytology-negative cohort (0.97%; 0.53%–1.34%). At three, four, five and six years respectively, CIN3+ rates in women with negative cytology were 0.51%, 0.69%, 0.83% and 0.97% and with negative HPV were 0.12%, 0.19%, 0.25% and 0.27%. A consistently low six-year CIR in the HPV-negative cohort supported six-yearly screening [24].

Ronco et al. pooled data from four European RCTs (Swedescreen, POBASCAM, NTCC, ARTISTIC) with 176,464 participants, randomly assigned to HPV or cytology screening. Swedescreen and POBASCAM used GP5+/GP6+ PCR, while ARTISTIC and NTCC used hc2 for primary HPV screening. Screening interval was 3 years in all except 5 years in POBASCAM. During the first 2.5 years, the pooled detection rate of invasive disease was similar in the two arms with pooled rate ratio for cancer detection being 0.79 (0.46–1.36). After 2.5 years, it was 0.45 (0.25–0.81), favoring HPV arm. HPV testing prevented more cases of adenocarcinoma, as the pooled rate ratios were lower for adeno-than for squamous cell carcinoma {0·31 (0·14–0·69) vs 0·78 (0·49–1·25)}. HPV-based screening from age 30 years provided 60–70% better protection than cytology. The observed lower incidence at 5.5 years indicated that 5-yearly primary HPV screening might provide adequate reassurance against pre-cancer and cancer, compared to 3-yearly cytology or 5-yearly co-testing [25].

The HPV FOCAL (HPV for cervical cancer screening) randomized clinical trial investigated the efficacy of HPV test (hc2) in comparison with liquid-based cytology (LBC). After the initial round of screening, the detection rates for CIN3+ lesions supported primary HPV screening, risk ratio being 1.61 (95% CI: 1.09–2.37). The absolute difference in incidence rate was 2.67/1000 (95% CI: 0.53–4.88) at the start and −3.22/1000 (95%CI: −5.12 to −1.48) at 48-months exit round, which favored primary HPV screening. Colposcopy referral rates, were higher in the primary HPV group (HPV: 57.0 [95% CI: 52.5–61.9] versus LBC: 30.8 [95% CI: 27.5–34.5]) after initial screening, but after 48 months were lower in the HPV group (HPV: 49.2 [95% CI, 45.0–53.7]; LBC: 70.5 [95% CI, 65.5–75.8]; the absolute difference being −21.3 [95% CI, −28.3 to −14.8]). The initial high rates reflect the impact of initial implementation of HPV-based screening when both prevalent and incident HPV infections are detected, which decline with ongoing HPV-based screening [26].

HPV test versus co-testing (combined HPV and cytology test)

Arbyn et al. reviewed 48 studies, including 8 RCTs in a systematic meta-analysis. Majority of the studies used hc2 or GP5+/6+ PCR. The detection rates for CIN2+ were 42% higher and for CIN3+ were 33% higher using co-testing than cytology alone at ASC-US threshold. The corresponding specificity of co-testing was 6% and 8% lower than cytology. Addition of cytology to HPV raised the sensitivity by only 5% for CIN2+ and 2% for CIN3+ compared to HPV testing alone. This improvement in sensitivity was at the expense of considerable loss of specificity with a ratio of 0.95 (95% CI: 0.94–0.96) for CIN2+ and 0.93 (95% CI: 0.92–0.95) for CIN3+ disease [27].

Similar results were obtained from a study of 331,818 women enrolled for co-testing at Kaiser Permanente (Berkeley, CA, USA). The risk predicted by HPV test alone when compared with cytology was significantly higher at both 3 years (5.0% vs. 3.8%; $p = 0.046$) and 5 years (7.6% vs. 4.7%; $p = 0.001$). A negative cytology result did not decrease the risk of CIN3+ further for HPV-negative women at both 3 years (0.047% vs 0.063%, $p = 0.6$) and 5 years (0.16% vs 0.17%, $p = 0.8$). Positive cytology could modify the risk for positive HPV test after 3 years (10% vs. 3.1%, $p < 0.0001$) and 5 years (12.1% vs 5.9%, $p < 0.0001$) but not for HPV-negative test at neither 3 years (0.53% vs 0.047%, $p < 0.0001$) nor 5 years (0.86% vs 0.16%, $p = 0.004$). They concluded that a negative HPV test was enough reassurance for low risk of CIN3 or invasive disease. An additional Pap-negative result does not provide extra reassurance. Hence, there was limited benefit of adding cytology to HPV testing [28].

Schiffman et al. assessed the relative contribution of HPV test and cytology in detection of CIN and cancer. The HPV component alone identified a significantly higher proportion of preinvasive and invasive disease than cytology. Only 3.5% of pre-cancers and 5.9% of cancers were preceded by HPV-

negative, cytology-positive results. Thus, the cytology component contributed only five cases per million women per year to the sensitivity of the combined test [29].

HPV test versus VIA (visual inspection with acetic acid)

Although VIA has been widely considered a valuable method for screening in LMICs, HPV testing can provide more effective coverage, even in under-resourced areas as there is an option for self-sampling. HPV test when compared with VIA has better pooled sensitivity {95% (95% CI: 84–98) vs. 69% (95% CI: 54–81), respectively} and similar pooled specificity {84% (95% CI: 72–91) vs. 87% (95% CI: 79–92), respectively} [30]. In a landmark cluster-randomized trial of 131,746 women aged >30 years from rural India, Sankaranarayanan et al. compared a single round of screening with HPV, cytology, or VIA testing with a control group and found the positive predictive value (PPV) for the detection of CIN2+ disease in HPV and VIA testing group was 11.3% and 7.4% respectively. The death rate due to cervical cancer per 100,000 person-year was 12.7, 20.9, 21.5 and 25.8 in HPV, VIA, cytology and control groups respectively. The hazard ratio (HR) for the detection of invasive disease was 0.47 (95% CI, 0.32 to 0.69) and for death was 0.52 (95% CI, 0.33 to 0.83) in the HPV screened cohort but there was no reduction in the detection rates {1.04 (0.72–1.49)} and death rates {0.86 (0.60–1.25)} of cervical cancer in the VIA screened cohort, and the gap widened during the follow up. The number of subsequent new cancer cases after negative results or after 3 months of positive test results were least in the HPV test cohort (22/34,126) as compared to cytology (27/32,058) and VIA group (34/34,074) [31].

Shi et al. conducted a modeling analysis of studies performed in rural China comparing *care*HPV and VIA. *care*HPV was more effective than VIA in preventing invasive disease and death. Once in a lifetime screening done at the age of 35 years with *care*HPV was more effective in preventing mortality due to cervical cancer (12% versus 8%). Regular screening at 10-yearly intervals was likely to reduce cervical cancer-related deaths by 19–28% [32]. The American Society of Clinical Oncology (ASCO) resource-stratified clinical practice guidelines have recommended HPV tests as being suitable for all settings, including limited and basic resource settings [32]. Women in limited resource settings should undergo HPV testing every 10 years from 30 to 49 years of age which corresponds to two to three times per lifetime. Women aged 30–49 years in basic resource settings should be screened with HPV test at least once, but no more than thrice, in a lifetime [33].

Management of HPV-positive women

Primary HPV screening is now incorporated in several international guidelines for cervical cancer and 5-yearly HPV screening is recommended from age 25 years onwards in enhanced and maximal settings [33–36]. HPV testing is highly sensitive but cannot discriminate between transient and persistent infections. A negative test result indicates low probability for developing CIN3+ disease in the next 5–10 years with accuracy, but a positive test result only indicates the presence of an essential risk factor [18]. Therefore, if all HPV-positive cases are referred for colposcopy, the burden of colposcopy referrals and associated procedures will be very high (57.2 colposcopies per 1000 tests), which is of particular concern in younger women [14,26]. Hence, a triage strategy is essential to segregate clinically unimportant lesions that do not require colposcopy [37].

The ATHENA trial investigated various triage strategies for HPV-positive cases. Women in the co-testing arm with HPV-positive and cytology-negative results underwent repeat co-testing after one year; if either test was positive, colposcopy was advised. Women in the primary HPV testing arm were triaged with HPV genotyping and reflex cytology. If HPV16/18 was positive, colposcopy was advised, but if any of the other 12 HPV types were positive, reflex cytology was done: if reported as ASC-US or worse, colposcopy was performed; conversely, if it was normal, women were rescreened with co-testing after one year. Although, this strategy led to a reduction in the number of colposcopies, referrals were still higher in the primary HPV arm (3769 colposcopies per 294 cases) compared to cytology (1934 colposcopies per 179 cases) or co-testing (3097 colposcopies per 240 cases) in women $\geq$ 25 years. A similar trend was seen in women $\geq$30 years with the maximum number of colposcopies in the HPV-alone arm (2522 colposcopies per 192 cases), followed by co-test (2457 colposcopies per 189 cases) and cytology (1294 colposcopies per 128 cases) [14].

A. Cytology Triage:

The high specificity of cytology makes it suitable for triaging HPV-positive cases, but the subjective nature of cytology affects the interpretation of abnormalities. Awareness of positive HPV status may lead to more accurate detection or sometimes even over-detection of abnormalities [12,38]. According to the VASCAR trial (The Viral Study Alone with Papanicolaou triage for the Screening of Cervical Cancer in Routine Practice), despite higher colposcopy referrals in the HPV screening and cytology triage group (19.36 versus 14.54 per 1000 women), the median time from positive cytology report to colposcopy was significantly reduced (3.14 months versus 10.98 months) in comparison with cytology screening group [39]. In another trial, the HPV test was repeated after one year in HPV-positive and cytology triage-negative women. Only persistent high-risk HPV-positive cases were subjected to colposcopy. Cytology triage led to 30% improvement in detection rates with marginal rise (12%) in colposcopy burden [40].

B. HPV genotyping triage:

HPV16/18 are the most common types associated with cervical squamous cell carcinoma, while HPV18/45 are more commonly associated with cervical adenocarcinoma. Other HPV types including 35/39/51/56/59/66 and 68 have a low risk of progression to pre-invasive or invasive disease and thus can be recommended follow-up at 12 months. Thus, triaging HPV-positive women with genotyping to detect oncogenic types is useful in risk stratification [37]. In the ATHENA trial, CIN3+ was identified in 17.8% (95% CI, 14.8–20.7%) of HPV16-positive women at baseline, and the CIR increased to 25.2% (95% CI, 21.7–28.7%) after three years. The 3-year CIR of CIN3+ was only 5.4% (95% CI, 4.5–6.3%) in women with HPV genotypes other than 16/18. HPV18-positive women had a 3-year CIR that was intermediate between women with HPV16 and women with 12 other genotypes [14]. Hence, HPV16/18 positive cases should be referred for immediate colposcopy, and negative cases should be followed up with cytology [37].

C. Other markers:

Newer molecular tests for identifying proliferating cells (Ki-67), methylated target host genes or methylated viral genome can be used to triage HPV-positive cases. P16/Ki 67 dual staining is effective, but it cannot be used to triage self-collected samples as reflex cytology is not possible [41]. Several other host methylation markers that identify the specific host genes, including CADM1, MAL, and miR-124-2, have been investigated. These are more specific, reproducible, and can triage self-collected samples too as they are based on molecular methylation analysis [42]. HPV genome methylation, especially in the E2, L2, and L1 regions, can be used to differentiate the transient and pre-cancerous infections. E6/E7oncoproteins can be used to triage *care*HPV-positive cases in low resource settings [43].

D. VIA Triage:

VIA has been used to triage HPV-positive cases in low-resource settings with an additional benefit of providing treatment at the same visit [44]. VIA and cytology triage of *care*HPV-positive cases were compared. VIA detected 58% of CIN2+, 73% of CIN3+, and all invasive lesions. Cytology detected 80% CIN2+ and 88% of CIN3+ lesions but missed 7.7% of cases of invasive cancer. VIA triage alone missed 74% CIN2 lesions, but 40% of CIN2+ lesions were missed by cytology and colposcopy triage as well. VIA triage was effective in reducing colposcopy referrals from 7.6% to 1.5% [44]. Hence, VIA is an effective and viable option to triage women screened with POC HPV tests. who may be offered treatment at the same visit according to the criteria of SVA [44].

Roll-out of primary HPV-based screening programs: real world implications

Primary HPV screening is currently recommended by many organizations including the European Union (EU), ASCO, World Health Organization (WHO), American College of Obstetrician &

Gynaecologist (ACOG), etc. [33–36]. The Federation of Obstetrics & Gynaecologic Societies of India (FOGSI), in its resource-based guidance, endorses primary HPV screening using a validated HPV test [45]. Several countries including Australia, Norway, Italy, The Netherlands, Sweden, Finland, and Germany have already implemented primary HPV screening programs, and many others like the United Kingdom are in the process of transition [7,36,46].

Australia was the first country to introduce universal HPV vaccination into the national immunization program. In 2017, Australia switched to primary HPV screening starting at age 25 years, every 5 years up to 69 years of age. It has been estimated, that if the current vaccination and screening coverage is maintained, cervical cancer rates are likely to be as low as 4/100,000 in Australia by 2035 [47].

The WHO call to action for global elimination of cervical cancer recommends that each country should screen at least 70% of women with a high-performance HPV test at ages 35 and 45 years by 2030 [48]. HPV-based self-sampling has been beneficial in improving participation in cervical cancer screening (RR: 2.13, 95% CI 1.89 to 2.40) [49]. Self-sampling has shown good concordance with physician-sampling and is acceptable and feasible in low-resource settings as well [49,50]. However, self-sampling has not led to any additional benefit to improve linkage with treatment (RR: 1.12, 95% CI 0.80 to 1.57) [49]. This can only be possible with POC tests.

Screening in vaccinated cohorts

None of the available vaccines provide protection against all the high-risk HPV genotypes, hence screening needs to be continued. The prevalence of HPV 16/18/31/33/45 will fall in vaccinated populations. The near absence of the most carcinogenic HPV 16/18 infections will lead to >50% reduction in the point prevalence of CIN3+ lesions. The sensitivity and PPV of cytology will further reduce due to reduced lesion prevalence [51]. HPV would be the most suitable modality to screen vaccinated cohorts. In order to elicit favorable benefit-to-harm ratio, screening will begin at an older age, and longer intervals will be considered. Finally, more specific biomarkers could be used to triage screen-positive women to discriminate benign infections or clinically important hrHPV infections.

Cost-effectiveness of primary HPV screening

The cost-effectiveness of primary HPV testing has been extensively analyzed in both developed and developing countries [47,52]. In a modeled analysis, 294 cases of CIN3+ were detected with 2422 colposcopies, and the incurred cost was $3.47 M. Cytology was more expensive ($4.79 M) and detected fewer cases (285 per 99,549 women). Co-testing detected a higher number of cases (14 more than HPV; 308 per 99,549 screened women) but required 100,277 more cytology tests and 566 more colposcopies with substantial cost increments ($5.85 M; additional cost of $2.38 M). The cost for detecting one case of CIN3+ was higher with co-test; $170,000 for each additional case, i.e., 60% higher with only marginal benefit in efficacy [53]. Reflex cytology triage of HPV-positive cases was less expensive than the co-test, because it was done only for a limited number of cases and there was no need to repeat sampling [53]. The cost-effectiveness of primary HPV screening in low-resource settings is being debated. In a cost-effectiveness analysis of HPV, VIA, and cytology, HPV testing done 10-yearly between ages 30 and 49 years was found to be the most cost-effective strategy with 350–580 international dollars per life year saved (LYS). The cost of three tests per lifetime was calculated as $180–$1600 per LYS [54]. HPV screening in vaccinated population was found to be cost-effective because of reduced screening frequency over a lifetime. It was found that the most cost-effective screening strategy for 9vHPV-and 2/4vHPV-vaccinated women was HPV testing done once or twice in a lifetime, respectively. HPV 16/18 genotyping as a primary screening method was found to be the most cost-effective strategy for cervical cancer screening in a study conducted in Europe, where 76.2% cases of cervical cancer are attributed to HPV16/18. It was calculated that adopting this strategy might save 1.050 million euros annually [55].

Summary

Primary HPV cervical cancer screening is gradually replacing other screening modalities both in developed and developing countries. It detects more CIN lesions at lower cost. The negative results

provide better reassurance against development of CIN and cancer and, therefore, need less frequent screening. For successful implementation of population-based screening, a clinically validated test performed in accredited laboratories should be used and simplified, standardized triage algorithms should be in place. Combined with HPV vaccination, it holds promise for the elimination of cervical cancer.

Declaration of Competing Interest

None with respect to this manuscript.

Practice points

- The high sensitivity of HPV test makes it ideal for population-based cervical cancer screening.
- Primary HPV screening is recommended 5-yearly from age 25 years.
- In order to eliminate cervical cancer, it is recommended that each country should achieve at least 70% screening coverage by an effective HPV screening test by 2030, and 90% of thus detected lesions should be treated.
- HPV test positivity does not imply disease. These women should be triaged according to a pre-defined triage protocol.
- Only clinically validated HPV tests should be used for primary HPV screening.

Research agenda

- Studying the impact of HPV vaccination on cervical cancer screening.
- Developing and validating low-cost, POC HPV tests; linking primary HPV screening with treatment in a SVA.
- Evaluating the place of self-sampling especially in under-served populations.
- Development of new biomarkers that can be efficiently used to triage hr-HPV-positive cases in low resource settings.

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