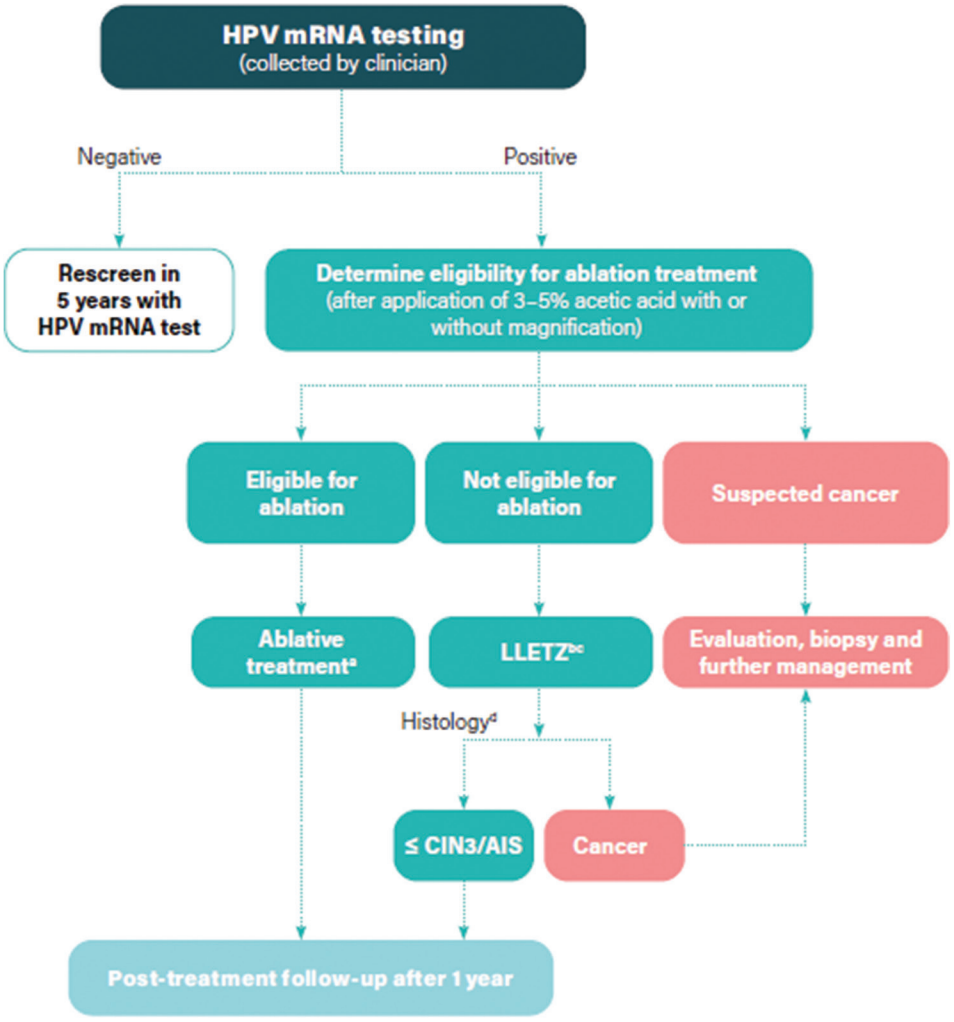
Cervical Screening Using HPV mRNA: A New Modality

Cervical cancer is one of the leading causes of mortality among women. In India, the incidence of cervical cancer is 18.5/100,000.[1] According to the WHO, it is estimated that 45,300 women died of cervical cancer in India in 2019.[1] According to the GLOBOCON 2020, Asia has 60% of cervical cancer cases with a mortality of 57.3%.[2] Reduction in mortality and morbidity caused by cervical cancer can be reduced to a great extent by screening, early detection, and management.

Screening of precancerous lesions of the cervix plays a major role in reducing the incidence of cervical cancer. Screening tests should be able to detect early cancers and preinvasive lesions, and early treatment should be more advantageous than treatment given during clinical presentation.

Traditional methods of screening for cervical cancer have been cytology (Pap smear and liquid-based cytology). In positive results, a colposcopy is done and treatment is given after biopsy of suspicious lesions and



[a] Ablative treatment includes cryotherapy and thermal ablation.
[b] Cold knife conization (CKC) if LLETZ not available.
[c] LLETZ and LEEP (loop electrosurgical excision procedure) indicate the same procedure.
[d] Histology may not be available in certain settings; women should be advised to attend follow-up after 1 year or to report earlier, if they have any of the symptoms of cervical cancer.
AIS: adenocarcinoma in situ; CIN: cervical intraepithelial neoplasia; HPV: human papillomavirus; LLETZ: large-loop excision of the transformation zone.

Algorithm 1: Primary human papillomavirus messenger RNA screening (screen-and-treat approach): For the general population of women. HPV: Human papillomavirus, mRNA: Messenger RNA, AIS: Adenocarcinoma *in situ*, CIN: Cervical intraepithelial neoplasia, LLETZ: Large loop excision of the transformation zone

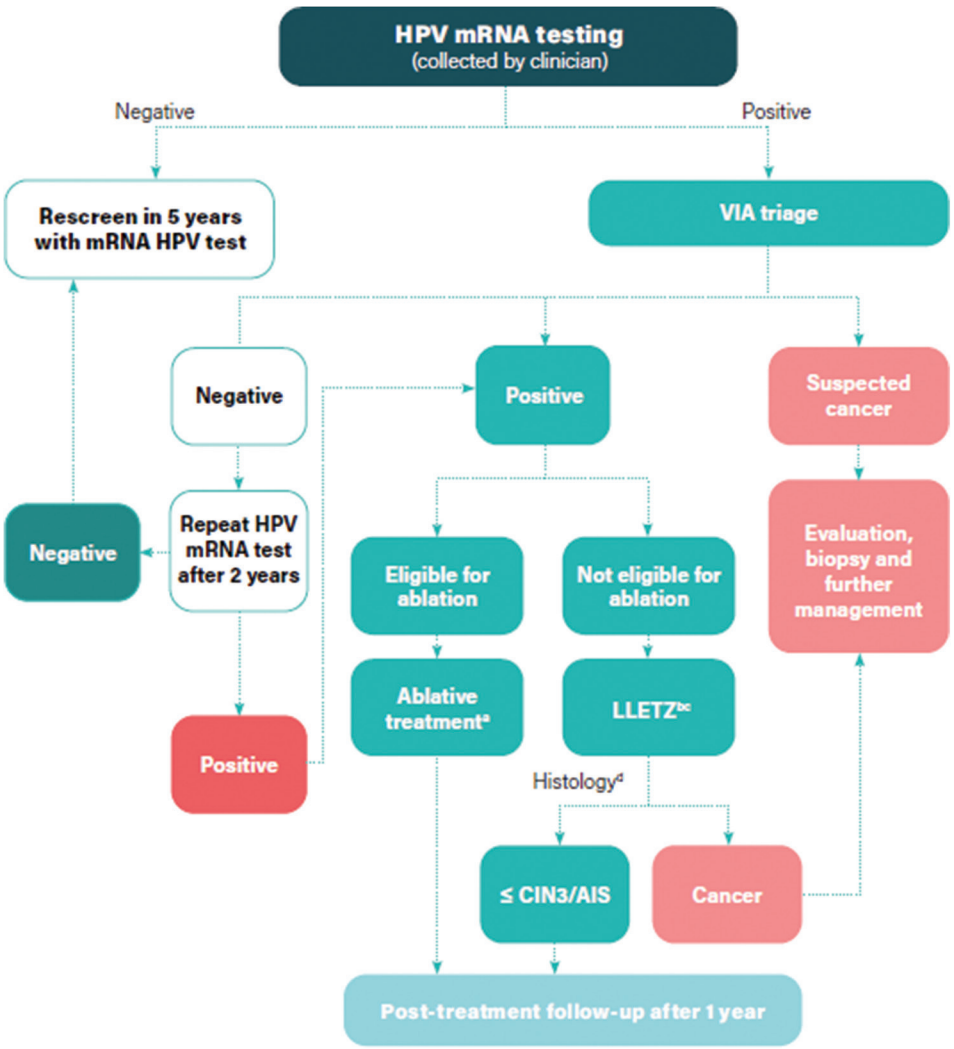
histopathological confirmation. Visual inspection with acetic acid (VIA) and Lugol's iodine is used in low-setting areas. Newer screening tests for the detection of cervical cancer include molecular tests, mainly high-risk human papillomavirus (HPV) DNA-based tests.[3]

More recently, even newer tests and techniques have been developed:

(i) Other molecular tests which include detecting HPV messenger RNA (mRNA), oncoprotein, or DNA methylation
(ii) Test done on cytological samples such as p16/Ki-67 dual staining[4]
(iii) Advanced visual inspection tests which are based on artificial intelligence/machine learning platforms.[3]

This article focuses on the use of newer methods of HPV detection, i.e. HPV mRNA tests (HPV E6/E7 mRNA detection).

The HPV DNA test detects either the viral DNA using the hybridization technique or detects L1 capsid protein or the E genes using polymerase chain reaction. In contrast, HPV mRNA tests detect transcripts of the viral E6 and E7 oncoproteins. These oncoproteins



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AIS: adenocarcinoma in situ; CIN: cervical intraepithelial neoplasia; HPV: human papillomavirus; LLETZ: large-loop excision of the transformation zone; VIA: visual inspection with acetic acid.

Algorithm 2: Primary human papillomavirus messenger RNA screening and visual inspection with acetic acid triage (screen, triage, and treat approach): For the general population of women. HPV: Human papillomavirus, mRNA: Messenger RNA, AIS: Adenocarcinoma *in situ*, CIN: Cervical intraepithelial neoplasia, LLETZ: Large loop excision of the transformation zone, VIA: Visual inspection with acetic acid

are responsible for the HPV-mediated oncogenic transformation of epithelial cells.[3]

The process of carcinogenesis starts with HPV virus entering the basal layer of the cervical epithelium and integrating HPV DNA with host DNA in cells. This integration leads to expression of E6 and E7 oncoproteins.[5] E7 oncoprotein, in turn, degrades retinoblastoma (pRb) tumor suppressor protein, which leads to uncontrolled activation of the cell cycle.[3,5] E6 oncoprotein degrades p53 tumor suppressor protein, which leads to inhibition of apoptosis and upregulating telomerase activity.[5,6] This leads to the neoplastic transformation of cervical intraepithelial cells.

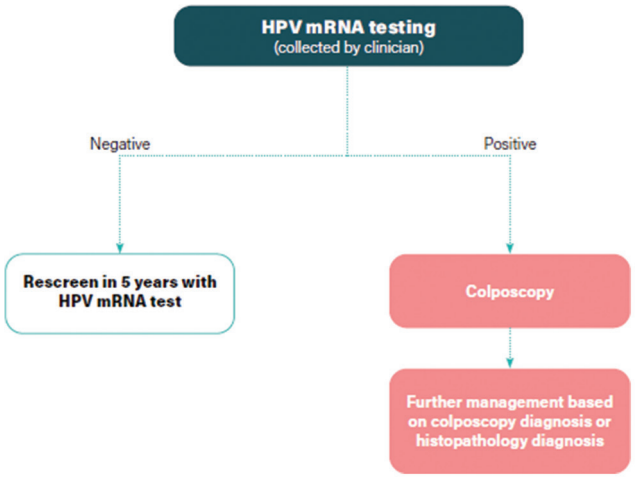
Since this expression of HPV mRNA leading to the production of E6 and E7 oncoproteins is directly involved in neoplastic transformation, detection of these oncoproteins could be more specific than detection of HPV viral DNA. Hence, HPV mRNA tests directly correlate with actual viral replication, which occurs years after HPV infection, which in turn leads to the precancerous stage; detection of these proteins appears to be more specific.[6]

The WHO suggests that HPV mRNA tests can be used as a primary screening test with or without triage in place of HPV DNA, provided that the samples are taken by a health-care worker to prevent cervical cancer in the general population of women with regular screening every 5 years.[3]

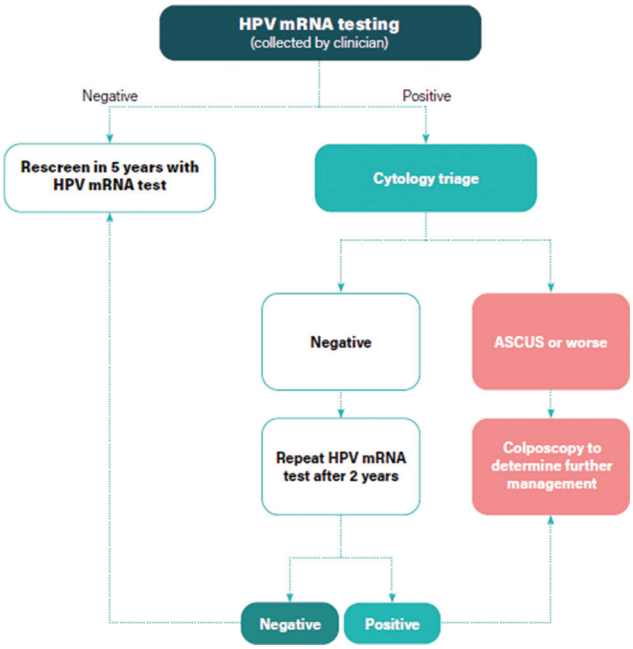
For screening and treatment, different primary screening and triage tests can be combined for better results. The WHO has recommended the following combinations with HPV mRNA testing.[3]

- Screen-and-treat approaches
  - HPV mRNA as the primary screening test [Algorithm 1], followed by treatment.
- Screen, triage, and treat approaches;
  - HPV mRNA as the primary screening test, followed by VIA triage [Algorithm 2], followed by treatment
  - HPV mRNA as the primary screening test, followed by colposcopy triage [Algorithm 3], followed by treatment
  - 4 HPV mRNA as the primary screening test, followed by cytology triage, followed by colposcopy and treatment [Algorithm 4].

According to the WHO, if we compare long-term effect of HPV mRNA testing with HPV DNA testing, in 5-year interval screening, HPV mRNA has 8%–12% higher relative cervical cancer incidence and 6%–8% higher cervical cancer mortality.[3] However, the number of treatments for precancer lesions may be lower as compared to HPV DNA (27%–33% fewer precancer treatments), which leads to lowering of cost (6%–10% lower).[3] HPV infection is usually transient, self-limiting, and gets spontaneously cleared; hence, the presence of this DNA does not necessarily mean that a precancerous abnormality will develop. On the other hand, expression of E6 and E7 proteins occurs later in life and is more related to neoplastic transformation. Hence, the presence of HR-HPV E6/E7 mRNA in cervical cells more accurately detects a risk of developing cervical



Algorithm 3: Primary human papillomavirus messenger RNA screening and colposcopy triage (screen, triage, and treat Approach): For the general population of women. HPV: Human papillomavirus, mRNA: Messenger RNA



Algorithm 4: Primary human papillomavirus messenger RNA screening and cytology triage followed by colposcopy (screen, triage, and treat approach): For the general population of women. HPV: Human papillomavirus, mRNA: Messenger RNA

intraepithelial neoplasia and cervical cancer, than the presence of HPV DNA.[3,7]

If compared to cytology screening or VIA, HPV mRNA screening will result in greater reductions in cervical cancer mortality and incidence.[3] The cost of HPV mRNA and HPV DNA testing is almost the same and both have similar equipment and training needs.[3]

At present, Aptima™ mRNA assay is the only technology commercially available for HPV mRNA testing. This test can qualitatively detect the expression of HPV E6 and E7 mRNA in 14 high-risk types of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) using real-time amplification.[3,7,8] Studies have shown that cost of using the Aptima mRNA HR-HPV assay is less as compared to HPV DNA testing . It also avoids unnecessary HPV repeat testing, cytology testing, and colposcopies.[8]

Research gaps have been identified in HPV mRNA screening tests. First, most of the studies have cross-sectional data on HPV mRNA testing. Long-term effects and results are still awaited. Hence, comparative longitudinal studies which compare HPV mRNA and HPV DNA screening tests should be done. Furthermore, studies conducted up till now have been done for the general population. There are no guidelines for the use of HPV mRNA for screening in HIV-positive women. Hence, longitudinal studies are needed for HIV-positive women.

To summarize, HPV mRNA has been found to be more specific and cost-effective in detecting preinvasive lesions of cancer. Further studies and research are needed to form proper guidelines for using HPV mRNA as a primary screening test in cervical cancer screening.

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Conflicts of interest

There are no conflicts of interest.

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