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Simon Leeson[a,*], Remi Alalade[a], Neelam Singh[a], Pekka Nieminen[b], Margaret Cruickshank[c], Xavier Carcopino[d], Christine Bergeron[e]

[a] *Department of Obstetrics and Gynaecology, Betsi Cadwaladr University Health Board, Wales, LL57 2PW, UK*
[b] *Department of Obstetrics and Gynecology, Helsinki University Hospital, Finland and University of Helsinki, 00029, HUS, Finland*
[c] *Aberdeen Centre for Women's Health Research, Aberdeen Maternity Hospital, Aberdeen, AB25 2ZD, UK*
[d] *Department of Obstetrics and Gynaecology, Hôpital Nord, APHM, Aix-Marseille University (AMU), Univ Avignon, CNRS, IRD, IMBE UMR 7263, 13397, Marseille, France*
[e] *Département Anatomocytopathologie, 95066, Paris, France*

ABSTRACT

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The development of human papillomavirus (HPV)-based screening should detect more pre-cancerous changes and so reduce the incidence and mortality from cervical squamous carcinoma and cervical adenocarcinoma. However, many more women are high risk HPV (hrHPV) screen positive compared to cytology-based screening, especially in younger age-women. A variety of tests have become available which may triage into those hrHPV test-positive women who need immediate referral to colposcopy from those who need early repeat HPV tests or recall on the basis of their disease status.

We performed a literature review of publications and a manual search from 2010, reporting cytology, HPV partial genotyping, dual-staining and DNA methylation for triage of hrHPV positive tests, including their comparative performance between these methods as well as the effectiveness of some triage combinations with reference to HPV-based screening services in Europe. Cost effectiveness and the structure of triage algorithms for colposcopists also have been considered. From one report evaluating four options for triage as single options or as combined algorithms, partial genotyping for HPV 16 and 18 with dual-staining yielded the highest risk of cervical intraepithelial neoplasia grade three or worse within an HPV positive population and with an acceptable colposcopy rate. From a separate paper, this option appeared cost effective. However, publications were difficult to compare objectively.

All options have their merits but a combination triage involving any two of cytology, HPV partial genotyping or dual-staining seems most efficient at present. HPV vaccination may impact upon the performance of future partial genotyping. DNA Methylation may become an acceptable future option.

Contents

Introduction

Several national programmes have converted from a cytology-based cervical cancer screening programme to one based upon human papillomavirus (HPV) testing. Other European states are considering this change. Four European prospective randomised screening studies have shown a 70% reduction in cervical cancer rates for those with negative tests including adenocarcinoma [1]. Only a minority of all positive high-risk HPV (hrHPV) cases have pre-cancerous lesions requiring treatment. There are various triage tests available to prioritise those women requiring colposcopic assessment as opposed to surveillance in the community based on the risk of underlying cervical intraepithelial neoplasia grade two or worse (CIN2+) or high grade squamous intraepithelial lesions or worse (HSIL+). A plethora of published data has compared the performance of triage options in a range of scenarios. As screening programmes vary in a number of ways such as target populations, screening intervals and the level of risk to recommend referral, it is difficult to determine which may be the ideal triage for each programme. Time and resources may be wasted as a consequence in attempting to improve colposcopic diagnostic performance. Furthermore, the colposcopic identification of HSIL from within the group of women referred with low grade squamous intra-epithelial lesion (LSIL) cytology is often difficult with a poor sensitivity in part due to low prevalence of pre-cancerous disease in this group [2]. A Eurogin Roadmap examining triage strategies has stressed the importance of a clear triage strategy of each national screening programme either as a single or as a combination option. Choices are dependent upon the complexity of the consequent management algorithms and cost [3].

Turkey was the first European state to introduce an HPV-based cervical screening programme in 2014 [4]. The Netherlands followed in 2017 with various regions in Finland, Italy and Sweden converting from cytology [5,6] with France converting in 2020. In the UK, Wales converted in 2018 with the remaining UK nations converting in 2020. It is important to recognize that there are service issues impacting upon newly developed HPV-based screening programmes. The HPV positive test rate varies in differing screening programmes with a range of five to 16% [7–10] with 13% in the English Sentinel Sites pilot [11]. However, pre-cancerous lesions defined as CIN2+ or HSIL+ are present consistently in less than 2% of each HPV screening round [12]. Triage of those women within the hrHPV positive group is required to avoid overwhelming colposcopy services with referrals who will mostly have a low risk of pre-invasive or invasive disease and avoid unnecessary colposcopy with associated physical and psychological impacts on women themselves. Suitable triage also reduces lead time delay to diagnosis of significant lesions [13]. A switch of cytology from the primary screen to become the triage test of hrHPV positive cases is a logical choice but there are other options.

We aim to describe the updated performance of triage tests which are currently validated and commercially available since the publication of the 2017 Eurogin Roadmap to develop recommendations for practice. To achieve this, a literature search was performed, using MEDLINE requesting articles published since 2010 written in English from Europe, North America and Australia. We used a combination of Mesh Subject Headings and free text search terms Papillomaviridae, HPV, human papilloma virus, triage, triage test, DNA methylation, dual stain, cytology triage, partial genotyping, human papillomavirus 16/ or type 16 and human papillomavirus 18/ or type 18 for female human studies. Seventy-four papers were obtained on 21.05.2020, of which 13 were relevant and in addition to a manual search.

The performance of cytology, HPV partial genotyping, p16 and Ki-67 dual-staining, DNA methylation and combinations have been reviewed to see what effects they have on detecting CIN2+ or HSIL+ as well as keeping women at low risk in the community so reducing the need for unnecessary colposcopy and associated interventions. These triage options were rated according to the reproducibility of published findings as detailed in the latest and earlier editions of the American Society for Colposcopy and Cervical Pathology (ASCCP) consensus guidelines (see Table 1).

Options for triage of HPV positive cases

Cytology

Compared to cytology-based screening, in an unblinded cross-sectional and longitudinal randomized controlled trial of 58,076 women in a population screening programme in Finland, HPV testing with cytology as triage was more sensitive for the detection of CIN3+ for all age groups (relative rate in HPV arm for CIN3+ 1.44 (95% confidence interval, CI 1.01–2.05) for all women as the intention to treat group and 1.77 (1.16–2.74) for those that attended (per protocol). For those with negative hrHPV testing the relative rate was 0.28 (0.04–1.17) [14]. These findings have been reported in other cross-sectional and longitudinal studies [15–19].

Cytology as triage leads to an acceptable colposcopy referral rate (36.4% of hrHPV positive tests) in the Kaiser Permanente cytology and HPV co-testing screening programme [20], 38.6% in the Finnish population-based screening programme [21] and 33.1% in the English Sentinel Sites Pilot [22]. The importance of negative cytology as triage is that approximately eight percent of each screening round is returned to early recall. Women with hrHPV positive and cytology negative testing overall accounts of 7.1–8.6% of each round of cervical screening in the Sentinel Sites Pilot and in Wales. The abnormal cytology rate has decreased in Wales following conversion to HPV-based screening in 2018 as hrHPV negative cases do not have cytology triage. Moreover, delayed referral of those with negative cytology potentially prevents overtreatment of regressive SIL as approximately 40% of these women clear HPV in 12 months [23]. In the UK, women would be referred to colposcopy with HPV persistency at 12 or 24 months in the absence of any cytological abnormality. This group added 54% extra referrals to the ongoing colposcopy workload of the in the Sentinel Sites Pilot [22] and 36% of all colposcopies in Italy [24]. The same effect has been reported in France (Carcopino; personal communication). Despite this significant increase in colposcopy workload, the risk of CIN2+ in this group is only three to seven percent after a single test and is below the agreed threshold for colposcopy referral of 10–20% [24–26].

The value of cytology as triage was reported in a Dutch sub-study comparing 14 triage strategies for hrHPV positive women from the VUSA-screen study. Cytological triage and follow-up with cytology for initial cytology negative women at 12 months was recommended. This provided a high negative predictive value

Table 1
American Society for Colposcopy and Cervical Pathology rating of published evidence.

Strength of recommendation	Descriptor
A.	Good evidence for efficacy and substantial clinical benefit support recommendation for use.
B.	Moderate evidence for efficacy or only limited clinical benefit supports recommendation for use.
C.	Evidence for efficacy is insufficient to support a recommendation for or against use, but recommendations may be made on other grounds.
D.	Moderate evidence for lack of efficacy or for adverse outcome supports a recommendation against use.
E.	Good evidence for lack of efficacy or for adverse outcome supports a recommendation against use.

Quality of evidence	Descriptor
I.	Evidence from at least one randomized, controlled trial.
II.	Evidence from at least one clinical trial without randomization, from cohort or case-controlled analytic studies (preferably from more than one center), or from multiple time-series studies, or dramatic results from uncontrolled experiments.
III.	Evidence from opinions of respected authorities based on clinical experience, descriptive studies, or reports of expert committees.

Terminology used for recommendations	Descriptor
Recommended.	Good data to support use when only one option is available.
Preferred.	Option is the best (or one of the best) when there are multiple options.
Acceptable.	One of multiple options when there is either data indicating that another approach is superior or when there are no data to favor any single option.
Not recommended.	Weak evidence against use and marginal risk for adverse consequences.
Unacceptable.	Good evidence against use.

Reproduced with permission from Perkins RB, Guido RS, Castle PE, Chelmow D, Einstein MH, Garcia F et al. 2019 ASCCP risk-based management consensus guidelines for abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis 2020; 24: 102-131.

(NPV 99.3%) but the lowest colposcopy referral rate of 33.4% compared to other strategies including HPV partial genotyping in more than 25,600 women aged 30–60 years [27]. Co-testing as recommended in the US with HPV and cytology both as a primary screen does not improve performance [10], albeit with slight (4.7%) increase in sensitivity but a 35.2% increase of screen positives compared to hrHPV testing alone [28].

Cytology provides grading of risk that current colposcopists are familiar with from previous cytology-based screening. HPV-based screening using cytology as triage is effective in reducing the incidence of cervical adenocarcinoma [1] where women who were hrHPV positive and had abnormal cervical cytology, as well as those who showed hrHPV persistency were referred for colposcopy [29]. However, cytology is subject to inter-observer variation particularly with atypical squamous cells of undetermined significance (ASCUS) [30,31] and prior HPV status may influence cytology interpretation by cytologists [27,32]. This has not been the universal view [33]. Variations in referral practice to colposcopy amongst women who were HPV positive ranging from 20-57% have also been reported in Italy, although histological endpoints were not assessed [34]. Laboratories which had a high proportion of low grade samples with cytology-based screening appeared to continue with a high rate of low grade samples after converting to HPV-based screening in England. This behaviour can be closely scrutinized with robust quality assurance (Tidy, personal communication on behalf of the Sentinel Sites Pilot). Cytology as a triage with referral to colposcopy with any cytological abnormality has a large volume of evidence validating its role. Despite cytological examination being a subjective assessment producing variations in referral rates to colposcopy and detection rates of CIN2+, it is the favoured single triage option (preferred: ASCCP strength A; quality II).

HPV 16/18 typing

HPV types 16 or 18 has been detected in 63% of CIN3 and 91% of cervical glandular intraepithelial neoplasia [9]. Genotyping for HPV 16 and 18 may allow for modified management depending upon genotype status [35] and is not dependent on cytotechnician interpretation of findings unlike cytology [13]. The ATHENA study of 47,208 women aged 21 years or older having routine screening with cytology and HPV testing reported on 4,219 who were hrHPV positive and cytology negative in women older than 30 years of age (including a subset of 886 hrHPV negative women). The prevalent CIN2+ rate of HPV 16/18 positive cases was 11.4%; the rate for those hrHPV positive for all types was 6.1 % and 0.8% for those who were hrHPV negative. The prevalent rate for CIN2+ for the 12 non-16/18 oncogenic genotypes was 4.6% [36]. From the same study of 41,955 women older than 25 years of age, HPV 16 or 18 positive or LSIL cytology positive women had an increased sensitivity for detection of CIN3+ ($p < 0.0001$) compared to ASCUS + cytology only [28]. Typing for HPV 16/18 in addition to cytology led to the detection of around 25% of hrHPV positive CIN3+ one year earlier than with repeat testing at expense of 1.5% drop in positive predictive value (PPV) [13]. Other hrHPV positive types could be managed with early recall rather than with colposcopy.

There is an increased risk of CIN2+ if same virus type persists for two years (odds ratio or OR of 813.0, CI 168.2−3,229.2) in prospective study of 10,758 women 20–29 years [37]. Immediate referral to colposcopy of HPV types 16, 18, 31, 33 and 45 with no further triage will possibly lead to a high colposcopy referral rate [27].

There appears to be age trend in the prevalence of HPV 16 and 18. HPV 16/18 associated CIN3+ appears to be more common in women under 30 years of age compared to women older than 45

years of age as reported in a Finnish population-based cohort study of 1279 women referred to colposcopy for abnormal cytology. Other hrHPV types than HPV16/18 (HPVO) accounted for 19.3% (11/57) of CIN3+ in women under 30 years and 44.7% (17/38) in women over 45 years of age. For women over 45 years the risk ratio of having HPV16/18+ associated CIN3+ was 0.61 (95% CI 0.43–0.88) compared to women under 30 [38].

Importantly, reported data has largely been from unvaccinated populations. The performance of hrHPV typing can be only speculative when the vaccinated population against hrHPV types enters the screening age range. Typing performance may be different for the recently introduced nonavalent vaccine as opposed to the older bi- and quadrivalent vaccines. However, an effect may be seen shortly in the younger colposcopy referrals. The prevalence of hrHPV types 16/18 in all assays in SHEVa study of women up to 21 years of age in the Scottish Cervical Screening Programme (where screening of women from 20 years continued to 2016) dropped by more than 75% in vaccinated cohorts with no change in non-16/18 types compared to the non-vaccinated cohort [39]. There is a more than 90% uptake of the three dose vaccine schedule in Scotland. The current two dose vaccination rate from 2015 to 2017 was up to 86% in England and Wales for 12 and 13-year old girls but uptake in other European states is inconsistent, for instance with less than 50% in France and Germany [40]. Whilst Scotland has reported reduction in HPV16/18 infections, CIN2+ detection and in colposcopy referrals and treatments, this must be seen in light of high vaccine uptake. Vaccination may not have an impact upon colposcopy referral rates for 10 years or more until a vaccinated cohort predominates in the under 40-year old referrals. Furthermore, the Sentinel Sites Pilot reported a 1.2% (CI 0.6–2.0) improved detection rate of CIN2+ at the expense of 5.9% extra colposcopy referrals with the use of HPV 16/18 partial typing. The conclusion was that within a quality assured screening programme and with women reliably returning for early recall with low grade cytology as triage, partial genotyping with HPV 16/18 did not significantly improve detection of pre-cancerous lesions [11]. Nonetheless, partial genotyping would be a suitable triage option for populations where vaccination coverage is below an acceptable threshold (such as less than 80%) for an effect in preventing CIN2+ [41]. In the unvaccinated population partial genotyping appears more sensitive than cytology and testing is automated (acceptable: ASCCP strength A; quality II).

p16/Ki-67

Differential expression host cell cycle regulatory proteins may have a role as biomarkers for dysplasia. A cyclin-dependent kinase inhibitor, p16 is upregulated with expression of HPV E7 protein. Levels of p16 correlate with the grade of abnormal cytology and the degree of CIN [42]. Over-expression is seen in 12% of normal cytology and two per cent of normal biopsies from a systematic review of 61 studies [42].

Triage of hrHPV tests with p16 had a significantly higher relative sensitivity for detection CIN2+ compared to cytology as triage but especially for the 25 to 34-year age group with over three years of follow-up (3.01; CI 1.82−5.17) without a significant change of colposcopy referral rate as part of the Italian NTCC HPV-based screening trial [43]. This was also reported in a prospective US study of triage of 3,225 hrHPV positive women having co-testing as screening [44] meaning that short term retesting could be avoided [13,45]. For triage of ASCUS and LSIL smears in cytology-based screening programmes, the sensitivity of p16 was equivalent to hrHPV triage but with a higher specificity [46].

Dual-staining with p16 and Ki-67, a proliferation marker, may also have a higher specificity compared to p16 alone [47]. Dual-staining had a sensitivity for CIN2+ 91.9% and a specificity of 82.1%

in the Wolfsburg HPV-based screening trial, a study of 425 women older than 30 years of age who were hrHPV positive and cytology negative [48]. The performance of dual-staining as triage was also investigated for hrHPV positive women in a longitudinal study of 1549 women with mean age of 42.2 years at enrolment. This study reported a significantly higher cumulative risk of CIN2+ (31.0 v 25.0%) if dual-stain positive and a significantly lower risk of CIN2+ (8.5 v 12.3%) if negative compared to cytology with a median follow-up of 3.7 years [49]. Alternatively, a longer retesting interval at three years is an option for negative dual-staining which was the same as the risk for negative cytology at one year, so potentially reducing referral to colposcopy. This provides reassurance following earlier concern [13] that negative dual-staining in the context of HPV infection can predict those at low risk of developing significant disease. Dual-staining may be a triage option in the HPV vaccinated population [44,45].

Like cytology, dual-staining requires visual interpretation of the degree of staining which is dependent upon subjective assessment and may be merely the presence of a single dual-stained cell. This technique may vary depending on the precise method used and reproducibility is a key issue. Quality assurance of laboratory testing is needed as standardization of the reliability of testing is variable especially for p16 staining for low grade cytology [42]. As opposed to immunohistochemistry which is traditionally used, in-situ hybridisation for p16 measures gene copy numbers and may have greater accuracy and sensitivity with equivalent specificity compared to cytology for predicting CIN2+ for hrHPV positive women [50]. Other techniques for standardisation of dual-staining have been described including cytotechnician training and automated slide reading [44,51,52].

HPV testing provides a long term risk of CIN2+ but dual-staining within the same cell provides identification of E6 and E7 mediated inactivation of tumour suppressor proteins and cell cycle deregulation relevant for immediate outcomes [46,53]. Dual-staining, like cytology is a subjective assessment but appears a reasonable option for triage of hrHPV positive women. Several small studies report improved sensitivity and specificity compared to triage with cytology (acceptable: ASCCP strength C; quality I).

Methylation testing

DNA methylation is an epigenetic mechanism for the control of gene expression for many cellular functions. Identification of specific sites may identify those that have an altered risk of HSIL+ as opposed to those that possess oncogenic virus. High methylation can equate with reduced risk of CIN3 and invasion at some sites [54] but other sites confer a high risk via the nuclear gene promoters in the host or L1 and L2 genes in hrHPV [55]. At present it is unclear if methylation of the viral genome or that of the host is more important but a combination of test sites may improve test accuracy [55]. Methylation at certain sites usually of the L1 open reading frame generally identifies HPV infection with progression to CIN3+ [56,57] as well for apparent longer lasting CIN3.

Host microRNAs (miRs) with methylation are responsible for silencing target genes such as tumour suppressor activity. Kocsis et al. [58] reported improved sensitivity at a similar specificity using POU4F3 as a host methylation marker to detect CIN3+ compared to cytology for triage of HPV positive samples. Aberrant methylation of the host gene site miR-124 provides a biomarker for cervical cancer [59]. MAL/ miR-124-2 genes on hrHPV positive self-samples had a sensitivity non-inferior to cytology triage for detection of CIN2+ [60]. A test kit identifying hyper-methylation at FAM19A4/ miR-124-2 is now commercially available. The high sensitivity recorded for pre-cancer detection is independent of genotype and appears to show a sensitivity and specificity for the detection of CIN2+ similar to that of HPV testing [61]. This study

Table 2
Triage studies reporting single triage methods in this review.

Author	Triage type	Design	Study size	Findings	Conclusion
[14]	Cytology	Prospective population-based screening data.	58,076 women with 95,600yrs follow-up in HPV arm; 95,700yrs follow-up in cytology arm.	76 CIN3+ in HPV arm, 53 cases CIN3+ in cytology arm.	Cytology as triage with HPV screening better than cytology-based screening.
[20]	Cytology	>30 yr HPV, cytology co-testing.	580,289 women.	36.4% of HPV+ve tests had ASCUS+ cytology.	Acceptable colposcopy referral rate for co-testing.
[22]	Cytology	Observational.	578,547 women.	2015 HPV+ve/ cytology+ve @ 12 months & 2186 HPV+ ve/ cytology-ve @ 24 months.	HPV+ve/ cytology-ve represent 54% of extra colposcopy referrals over 2yrs.
[24]	Cytology	Retrospective, longitudinal	46,694 women.	Rate of persistent hrHPV+ve was 58% (830/1,435). A high-grade lesion was detected in 42 (5.1%) at 1-yr repeat.	Only small number of high-grade lesions were detected among the group of hrHPV+ve/ cytology-ve women who repeated testing 1-yr
[27]	Cytology, genotyping	30−60yr retrospective.	25,658 women.	Cytology most efficient triage of 9 strategies with repeat testing with 33.4% colposcopy referral rate & NPV 98.9%.	Lowest colposcopy referral rate with cytology triage and repeat cytology @12 months.
[34]	Cytology	35−64 yr, retrospective.	72,869 women.	Proportion of HPV+ women referred to colposcopy varied across programmes (20−57 %; average 37 %) and so did CIN2+ detection (49−94 %; average 77%).	Repeat HPV testing limits subjectivity of cytology interpretation on overall colposcopy referral and sensitivity.
[36]	Genotyping	HPV+ ve, cytology-ve, >30yr.	4,219 HPV+ve & 886 HPV-ve women.	Prevalence CIN2 + 11.4% HPV 16/18, 6.1% HPV + ve, 0.8% if HPV-ve.	Genotyping useful for cytology -ve women.
[28]	Genotyping vs cytology	>25yr. Prospective sub-study of ATHENA.	41,955 women.	Cobas HPV test more sensitive than LBC cytology for detection of CIN3+ (92.0% vs 53.3%).	Improved sensitivity to detect CIN3 + compared to cytology (p < 0.0001).
[37]	Genotyping	20−29yr prospective.	10,758 women.	Increased risk CIN2+ if persistent same hrHPV type (OR 813; CI 168.2-3,229.2).	Risk of CIN2+ highest with same HPV type on repeat testing.
[43]	p16 vs cytology	Randomised controlled trial.	1,137 HPV+ve women.	Improved relative sensitivity for p16 to detect CIN2+ 3.01 (CI 1.82−5.17).	Improved sensitivity as triage with p16 compared to cytology.
[46]	p16 vs genotyping	Retrospective, triage of ASCUS & LSIL.	810 slides.	Specificity better for p16 compared to HPV for ASCUS or LSIL (both p <.001).	p16 sensitivity = to HPV testing with improved specificity. Efficient for triage of ASCUS and LSIL.
[47]	p16/Ki-67	Retrospective, triage of ASCUS & LSIL.	776 women.	Dual-staining a similar sensitivity but higher specificity compared to hrHPV testing.	Dual-staining improved specificity to p16 alone.
[48]	p16/Ki-67	HPV+ve, cytology-ve, >30yr prospective.	425 women.	Dual-staining sensitivity for CIN2+ 91.9% and specificity of 82.1%.	Dual-staining useful triage for HPV +ve, cytology-ve cases.
[49]	p16/Ki-67 vs cytology	Prospective cohort, HPV/ cytology co-testing >30yr.	1549 HPV+ve women.	Higher 5 yr cumulative risk of CIN2+ (31.0 v 25.0%) if dual-stain +ve and significantly lower risk of CIN2+ (8.5 v 12.3%) if −ve compared to abnormal cytology.	Risk of CIN2+ in dual-stain -ve means that recall can be @ 3yrs.
[57]	Methylation	Prospective, 21−67yr. HPV16+ve.	145 women.	L1 HPV16 mean methylation significantly increased with disease severity (CIN3, 17.9% vs CIN2, 11.6%, p <.001 or vs CIN1, 9.0 %, p <.001).	Elevated methylation level associated with increased disease severity and has good ability to discriminate HPV16+ve women that have CIN3+.
[58]	Methylation	Prospective, 25-65 years.	5,384 samples from HPV+ve women.	For CIN3+ histological endpoint methylation test of POU4F3 achieved relative sensitivities of 1.74 (95% CI: 1.25−2.33) and 1.64 (1.08−2.27), respectively, after verification bias adjustment.	Quantitative triage tool for identification of CIN3+.
[61]	Methylation	Retrospective	447 cytology samples.	hsa-miR-124-2 had best performance of 6 candidate methylation genes for identifying CIN2+ (OR 5.1).	Sensitivity/ specificity for detection of CIN2+ similar to HPV testing. Performance independent of genotype.
[62]	Methylation	Prospective cohort.	149 women with untreated CIN2.	Highest area under the curve (AUC) was 0.735 (95% CI 0.621−0.849) in regression vs. progression outcome with combination of S5 and cytology. HPV16/ 18 or HPV16/18/31/33 genotyping did not provide additional prognostic information.	S5-classifier shows high potential to identify women with progressive CIN2.
[63]	Methylation	Retrospective HPV+ve, HG or persistent LG cytology.	1,493 women.	Methylation of viral and host genes may improve PPV.	Methylation testing can be used as a triage test for HPV+ve to identify CIN2+.
[64]	Methylation	Retrospective, longitudinal.	1,040 women, 29−61yrs.	Kaplan-Meier estimate of 14-year cumulative cervical cancer incidence 1.7% (95 % CI: 0.66−3.0) among baseline methylation -ve and 2.4% (1.4−3.6) among baseline cytology-ve women (risk difference: 0.71% [0.16−1.4]).	Negative FAM19A4/mir-124-2 methylation test provides low cervical cancer risk in HPV+ve women of ≥30 yrs.

Abbreviations: yrs - years; NPV - negative predictive value.

compared six gene loci with miR-124 having the highest OR associated with a diagnosis of CIN2+ (hsa-miR-124-2 (OR = 5.1) including SOX1, TERT and LMX1A. A DNA methylation panel, the S5 classifier consisting of DNA methylation of EPB41L3 and the late (L1 and/or L2) regions of HPV16, HPV18, HPV31, and HPV33, is shown to be a prognostic marker to identify women with progressive CIN2 [62].

Methylation testing can be used as a triage test for hrHPV positive women to identify CIN2+ at a sensitivity of 90% equivalent to the referral population to colposcopy amongst HPV positive women and abnormal cytology [63]. A meta-analysis of pooled data from 43 studies reported that DNA methylation testing had a marginally lower relative sensitivity of 0.81 (CI: 0.63–1.04) but a higher specificity than cytology at a cut off of ASCUS+ (1.25 (0.99–1.59)) but a higher relative sensitivity of 1.22 (1.05–1.42) with equivalent specificity to HPV 16/18 genotyping (1.03 (0.94–1.13)) amongst hrHPV positive women [55]. A lower sensitivity compared to cytology (at ASCUS + or LSIL+) in women under 30 years of age is possibly due to more regressive lesions in this age group. From the Dutch POBASCAM trial of HPV-based screening in 1,040 women with 14 years of follow-up, negative methylation testing with FAM19A4 and mir-124-2 genes provides a low cervical cancer risk in women over 30 years of age [64]. Overall, there are presently less studies evaluating methylation as a triage option when compared to other methods [55]. The current body of evidence to support methylation is poor (acceptable: ASCCP strength C; quality I). Table 2 is a list of the cited reports of the performance of single triage tests.

Combined triage tests

Triage combinations may be more accurate than cytology alone for predicting HSIL+ in a triage setting. Sensitivity is not the priority here for women already hrHPV positive and considering referral to colposcopy due to the high sensitivity of HPV testing. Triage combinations may be more effective at providing reassurance for those at low risk by improving specificity. For instance, negative p16 and Ki-67 dual-stain with HPVO for hrHPV positive women could be retested in three years [44]. Various triage strategies were explored for 520 self-sampled hrHPV positive women from the Dutch PROHTECT-3B trial, and of 18 options considered, three were considered acceptable with an NPV for CIN3+ being at least 98%, combined with maintenance or improvement of sensitivity and an increase in specificity compared to triage with cytology alone [65]. They were HPV16+ and/ or at least LSIL cytology; HPV16+ and/ or at least HSIL; HPV16+ and/ or HPV18+ and/ or at least HSIL. The second option resulted in a decrease in referral rate to colposcopy from 52.2–39.5%. HPV typing in addition to low grade or normal cytology allows delayed referral to colposcopy for women if HPVO and hrHPV positive.

Macdonald et al. [23] reported retrospective data from 1076 women who were hrHPV positive and cytology negative and having further triage with HPV 16/18 typing as part of the Sentinel Sites Pilot. Management depended upon typing with HPV 16/18 seen in colposcopy after first repeat if unchanged at 12 months and with HPVO seen in colposcopy if still unchanged 12 months later. Amongst the HPV 16 and 18 cases, 10% had CIN2+ when seen at one year and amongst the HPVO cases, four percent had CIN2+ at two years. Ten-year follow-up of cases from the ARTISTIC trial cohort also reported that one year repeat of testing was safe for HPV 16/18 positive women with low grade cytology and two-year follow-up was safe for HPVO genotypes [66].

The three-year risk of CIN3+ for all hrHPV positive women with ASCUS was 5.2% in women over 21 years of age [67], risk being highest for HPV type 16 (16.0%), 7.4% for HPV18; 7.1% for HPV33 or 58 and 7.0% for HPV31. The remaining 40% of hrHPV cases may not need immediate referral for colposcopy but would encompass 10% of all CIN3+ cases. HPVO cases would be recommended for short term recall to avoid missing a subpopulation of non 16/18 CIN2+ cases. Similar outcomes were seen for those over 25 and over 30 years of age [67]. However, in the US, women who are over 30 years with an hrHPV positive test and are cytology negative with positive HPV 16/18 typing have a PPV of 11.4 % for CIN2+ (9.8 % for CIN3+) with immediate referral to colposcopy recommended [68]. Re-testing is suggested in 12 months for those who were HPVO from the ATHENA study [36]. A slight variation of this algorithm was suggested from the Kaiser Permanente screening programme co-testing with cytology and HPV-based screening, those with ASCUS and are HPV 16/18 positive could be referred for immediate colposcopy and types 31/33/58 may also benefit from this. Other hrHPV types could be delayed for retesting at 12 months [67].

Wentzensen et al. [44] compared the performance of triage combinations of HPV 16/18 typing and cytology (at ASCUS+), typing and dual-staining, but also single triage options of cytology alone and dual-staining alone in a prospective study of hrHPV positive women. Both the combination triage strategies were superior to the single triage tests. Dual-staining was significantly superior to cytology when both were combined with HPV typing (p=0.005), reduced unnecessary colposcopy referral by 16.1% and so reducing unnecessary biopsies and treatments.

Combined triage by MAL/miR-124-2 methylation analysis with HPV16/18 typing as a sub-study of the PROHTECT-3 trial had a sensitivity equivalent to methylation alone of 77.6% but with an increased specificity of 54.8 versus 47.2% (p=0.001) compared to methylation alone to identify women with CIN3+ among self-sampled HPV positive women. The referral rate to colposcopy was also 5.8% lower for the triage combination (p=0.03) [60]. Furthermore, this combined strategy may be objective as it is fully molecular [55,60]. Automatable cytology reading is also feasible using FocalPoint (BD Burlington, NC) with partial genotyping and may be comparable to standard cytology and genotyping reported in 1839 women who were HPV positive [69]. See Table 3 for the performance of triage test combinations.

Apart from the triage tools already discussed, further important modifiers of cervical cancer risk can be included to develop a personal risk assessment. For instance, screening history, HPV vaccination status and nicotine use can be incorporated with current screening status as part of prediction modelling to triage to early repeat testing, referral to colposcopy or biopsy based on risk. Five clinical responses to screening and triage have been described by Schiffman et al. [67] with treatment for extremely high risk of CIN3+, colposcopic biopsy for substantial risk, intensified retesting with HPV and no pre-cancer, routine screening if no HPV and finally, exit from screening at virtually no risk. A risk-based management algorithm can be devised according to the level of risk [70] and an example of colposcopic management according to combined triage tests is shown in Fig. 1.

A threshold for colposcopy has been suggested in the US at 11.1% as equivalent to the one-year risk of CIN2+ with an hrHPV positive test and ASCUS cytology and that for one-year retesting as 6.2% as equivalent to the one-year risk of CIN2+ with an hrHPV positive test and negative cytology [49]. Such thresholds appear to vary as a recommended threshold for colposcopy in Europe may be a 10% risk of CIN3+ [71]. Immediate testing with a combined triage can be designed so that both tests are offered simultaneously and appears preferable to sequential testing to reduce anxiety and avoid default of a second attendance [72]. Combinations of triage can be evaluated with a neural network and can still be compared if datasets are incomplete as triage outcomes can be concluded as a 'best effort basis' [73,74]. In a study of 2267 women prospectively assessed with cytology, DNA HPV typing, HPV RNA and dual-staining compared to cytology alone in a screening setting, a neural

Table 3
Triage studies reporting triage combinations in this review.

Author	Triage type	Design	Study size	Findings	Conclusion
[60]	Genotyping, methylation	Sub-study of PROHTECT-3 trial of self-sampled HPV tests.	1019 HPV +ve women 33–63yrs.	Triage of methylation & genotyping sensitivity equivalent to methylation alone of 77.6% with specificity of 54.8 vs 47.2% (p=0.001) compared to methylation alone for CIN3+. Referral rate to colposcopy 5.8% lower for triage combination (p=0.03).	Methylation testing can be used as triage test for HPV+ve with genotyping to identify cervical precancer.
[55]	Methylation vs cytology vs genotyping	Meta-analysis 43 studies including 8 host and 2 viral genes.	16,336 women.	DNA methylation testing lower relative sensitivity of 0.81 (CI: 0.63–1.04); higher specificity than cytology at cut off ASCUS (1.25 (CI: 0.99–1.59)) or LSIL; higher relative sensitivity of 1.22 (CI: 1.05–1.42) with equivalent specificity to genotyping (1.03 (CI: 0.94–1.13)).	DNA methylation as a triage test has higher specificity than cytology (ASCUS+) and higher sensitivity than genotyping.
[44]	Cytology, genotyping, p16/Ki-67	Prospective, observational study.	3,225 HPV +ve women.	Combinations better at predicting risk of CIN3+ than single triage and p16/Ki-67 combined with genotyping better than cytology with genotyping.	Women -ve for genotyping and dual-staining can be re-tested in 3 yrs.
[65]	Cytology, genotyping	Sub-study of PROHTECT-3 trial of self-sampled HPV tests.	520 HPV +ve women.	18 baseline triage strategies were evaluated for cytology and hrHPV genotyping.	Combining HPV16+ and/or HSIL+ yielded the highest specificity (74.9%, 95% CI 70.5–78.9), with a sensitivity (94.4%, 89.0–97.7) similar to cytology (93.5%, 87.7–97.1), and decrease in referral rate from 52.2%-39.5%.
[23]	Cytology, genotyping	Retrospective cohort study HPV+ve, cytology -ve.	1076 women.	For HPV 16/18, 10% had CIN2+ when seen at 1yr; amongst non-16/18, 4% had CIN2+ @ 2yrs.	HPV type 16 significantly higher risk (1 in 9) of high grade CIN.
[66]	Cytology, genotyping	Prospective, cohort study. HPV + ve with negative/borderline or low grade cytology.	2,383 women.	10-yr cumulative risk of CIN3+ was higher with HPV16/18 infection (19.4%, 95 % CI 15.8–23.8% with borderline/low-grade cytology and 10.7%, 95 % CI 8.3–13.9% with normal cytology) than for those with HPVO (7.3%, 95 % CI 5.4–9.7% with borderline/low-grade cytology and 3.2%, 95 % CI 2.2–4.5% with normal cytology).	Recall intervals of 1 year for HPV16/18 and 2 years for HPVO is justified for women with normal cytology and might also be considered for women with borderline/low-grade cytology.
[67]	Cytology, genotyping	Observational HPV/cytology co-testing >21 yr. HPV + ve, ASCUS.	13,890 women.	3yr risk of CIN3+ for HPV 16 16.0%; 7.4–7% for types 18/31/ 33/58. Lower risk for other types.	ASCUS and HPV 16/18+ve for colposcopy. Types 31/33/58 also for colposcopy. Other types for re-testing @ 12 months.
[69]	Cytology, genotyping	Retrospective HPV+ve FocalPoint risk scoring algorithm compared to standard cytology reading.	1,839 women.	Among HPV+ve women, algorithm matched triage performance of abnormal cytology. Combined with HPV16/18/45 typing the automatable strategy referred 91.7% of HPV+ve CIN3/AIS cases to immediate colposcopy while deferring 38.4% of all HPV+ve women to 1-year retesting (compared with 89.1% and 37.4%, respectively, for typing and cytology triage).	High-quality cervical screening and triage performance is achievable using this completely automated approach.

network developed by the research group and incorporating all the listed triage options had a higher accuracy in predicting CIN2+. All had colposcopy but biopsy was not performed in every case [73]. A more complex test structure combining test outcomes with clinical parameters may provide an improved risk of cervical pre-cancer. A personalized risk-based approach to predict present or future CIN3 + based upon current test results, screening history and previous treatment is a new addition for the 2019 Consensus Guidelines from the ASCCP [75].

Cost-effectiveness of various options

Combinations that reduce colposcopy referral yet maintain a high PPV for HSIL/ CIN2+ and a high specificity to provide strong reassurance with negative triage testing would appear to be potentially the best use of healthcare resources. Triage with HPV 16/18 typing and dual-staining with p16 and Ki-67 in the context of an HPV-based screening programme appears cost effective compared to cytology-based screening in Belgium [76] and in Thailand [77]. In a further study HPV 16/18 typing with dual-staining and cytology appeared ideal in comparison to three alternatives including cytology as triage and also with co-testing and triage with partial genotyping and dual-staining in Mexico [78]. Yet co-testing with HPV 16/18 typing as triage appeared most cost effective of six options in the US with the lowest incremental cost-effectiveness ratio per quality-adjusted life year saved [79]. HPVO and LSIL cytology for HPV positive women appears most cost effective if re-tested in 12 months rather than with a direct referral

Low risk Must have all of the following: • ≤LSIL cytology • HPVO • Colposcopy ≤low grade • Type 1 or 2 TZ	**Intermediate risk** All the other cases	**High risk** Must have 2 of the following: • ≥HSIL cytology • HPV 16/18 • Colposcopy ≥high grade
Action Short term recall	**Action** Biopsy	**Action** Biopsy and treatment advised

Fig. 1. Risk of CIN2+ combining colposcopic opinion, HPV partial genotyping and cytology triage for HPV + ve women.
Abbreviation: TZ - transformation zone.
Modified with permission from Wentzensen et al. [70].

Table 4
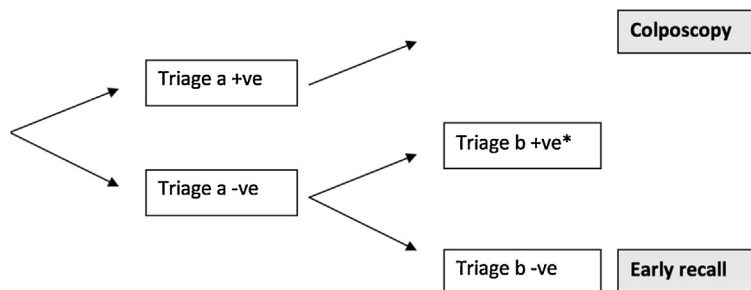Triage studies reporting cost effectiveness in this review.

Author	Triage type	Design	Study	Findings	Conclusion
[76]	Genotyping, p16/Ki-67 in HPV arm	25–65yrs Budget-based impact model.	Cytology-based vs HPV-based screening.	After 2 cycles of screening 21% costing saving due to drop of prevalence of CIN.	Combination appears cost effective.
[77]	Genotyping, p16/Ki-67 in HPV arm	30–65 yrs Budget-based impact model.	Cytology-based vs HPV-based screening.	Comparison with cytology and HPV-based screening with genotyping and dual-staining.	Combination with dual-staining and genotyping appears more cost effective than cytology-based screening.
[78]	Genotyping, cytology, p16/Ki67	Calculation of total cost of each option to treat each case of CIN2+.	Cytology vs HPV as primary screening with cytology triage vs HPV as primary screening with genotyping, cytology and p16/Ki67 triage vs co-testing with hrHPV and cytology with genotyping and p16/Ki67 triage.	Triage with genotyping, cytology with p16/Ki67 best alternative because is least expensive with acceptable number of missed cases.	Combining multiple tests may be cost-effective.
[79]	Genotyping, cytology	Lifetime model for 6 screening strategies.	Cytology vs cytology + HPV triage vs HPV cytology triage vs co-testing vs co-testing + HPV genotyping, vs HPV only + HPV genotyping.	HPV genotyping with co-testing most effective strategy with ICER of $33,807/ QALY compared to HPV genotyping for all hrHPV-positive women.	Addition of HPV-16 and -18 genotype triage to co-testing was cost-effective.
[80]	Cytology, genotyping	Dynamic model.	Referral to colposcopy vs 12-month follow-up for HPVO and low grade cytology.	Immediate colposcopy referral increases the number of colposcopies/ cancer.	12-month follow-up for women with HPVO and low-grade cytology associated with low risk of developing cervical cancer.

Abbreviations: ICER - incremental cost-effectiveness ratio, QALY - quality-adjusted life year.

to colposcopy [80] for the national screening programme in Australia. Cost effectiveness calculations would vary for differing populations depending on local HPV vaccination uptake, local care costs and what would be considered a suitable cost-effectiveness threshold per year of life saved for that population. Overall, there is a lack of consistent data to clarify cost effectiveness for triage strategies. Despite these caveats, a contemporary cost benefit analysis of various triage options within a national HPV-based screening programme and additional calculations for the impact of HPV vaccination would be a welcome addition to current published clinical data. See Table 4 for details of the reported cost effectiveness studies.
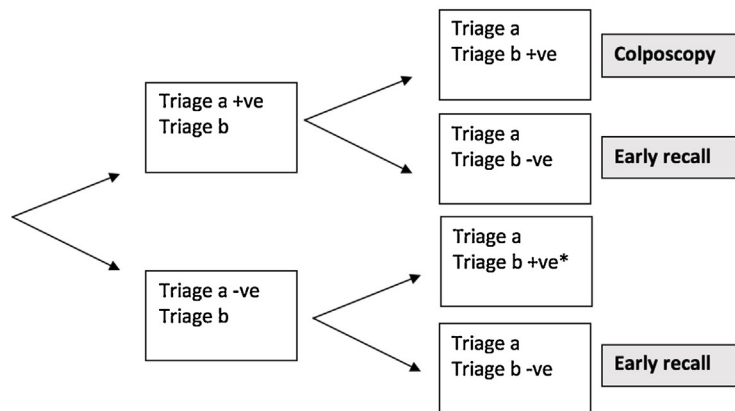
Fig. 2. Examples of triage test combinations.
*If cytology is triage b, management may be graded: e.g. colposcopy for ASCUS+ with early recall for NILM or alternatively colposcopy for HSIL+ with early recall for ≤LSIL. For other methods of triage then triage b +ve are all referred to colposcopy.
Abbreviation: NILM - negative for intraepithelial lesion or malignancy.

Implications for colposcopy

After a positive screen, a single positive triage prompts referral to colposcopy, and those negative to early recall; with an option of colposcopy if HPV positivity persists for at least 12 months. Colposcopy appears to perform well when the referral group is accurately selected on basis of risk of underlying HSIL. With a low rate of disease, the risk is of over-investigating and treating women with all the associated adverse effects. However, it is difficult to assess the comparative performance of cytology, partial genotyping, dual-staining and methylation as triage options as no published studies have compared all four options. The sensitivity for CIN3+ may be better for partial genotyping than for cytology [28]. The five-year cumulative risk for CIN2+ and CIN3+ may be higher for dual-stain positive cases than for cytology (threshold ASCUS+) and lower if dual-stain negative than for negative cytology [49]. Methylation may have a lower sensitivity for CIN2 + than cytology (ASCUS+) but a higher sensitivity than partial genotyping [55].

Combination triages can be performed sequentially and would recommend short term recall if the first and second triages are negative (see first example in Fig. 2). Assuming the triages are performed sequentially and assuming the first triage is partial genotyping, then HPV 16/18 positive women would be referred to colposcopy. HPVO cases would be offered a second triage with cytology [81], dual-staining or methylation. Colposcopy would be recommended also if the second triage is positive. But for cytology as the second triage, the grade may be relevant with colposcopy recommended for ASCUS+, LSIL+ or for HSIL+. Alternatively, tests can be performed simultaneously, for example, with the first triage as partial genotyping being HPV 16 or 18 positive; the second triage can be negative leading to short term recall rather than referral to colposcopy (see second example in Fig. 2). If either partial genotyping or cytology is positive then sensitivity is improved compared to LSIL+ only for triage [28] as two triage tests are used instead of one. The risk of CIN2+ has been reported as greater than 20% for HPV 16/18 positive and with dual-stain or cytology positive as the second triage [44] and the combination of cytology (ASCUS+) and dual-stain positive cases [49]. Specificity was improved with an equivalent sensitivity for combined partial genotyping and methylation [60].

A sequential combined triage would refer more cases to colposcopy than a single triage and simultaneous combined triage (see Fig. 2). Simultaneous combined triage requires more tests so is more expensive but may reduce colposcopy referrals and so may be cost effective. Overall a simultaneous combined triage algorithm with any two of cytology, partial genotyping or dual-staining is preferred. Combined triage options would seem ideal from the available evidence as they have improved performance compared to single triage tests (preferred: ASCCP strength A; quality I).

Conclusions

We have explored different options for triage of HPV positive women in the context of a primary HPV-based screening programme. With HPV-based screening compared to cytology-based screening more women will be referred with only HPV infection or LSIL. A lower prevalence of HSIL+/CIN2+ in the low grade group will result in a drop in the PPV for the colposcopic impression of high grade lesions with the risk of over investigation of women. Effective triage should improve selection of women at high risk of HSIL+/CIN2 + to colposcopy thereby maintaining colposcopy performance and avoidance of excessive referral rates. Data will be needed from vaccinated populations to guide practice which is expected to alter with an anticipated reduced incidence of HPV types 16, 18 and possibly other oncogenic genotypes over several years. Triage combinations appear more accurate than algorithms using a single triage test. The ideal option or combination of options is likely to differ for different national programmes. This will be dependent upon national HPV prevalence and relative importance of high risk HPV types, particularly of types 16 and 18 with reference to differing national vaccination uptake rates, as well as the referral rate to colposcopy with a positive triage. Quality assurance of screening programmes, including colposcopy and laboratory services need to guide and validate programme change. Cost effectiveness for the local population as well as acceptability of triage algorithms for colposcopists are also important considerations. Our conclusions are consensus opinion only because, except for a few studies, the reported outcomes are difficult to compare as they differ in terms of risk of high grade cervical disease, cost effectiveness, or other outcomes. This highlights a need to benchmark various HPV positive outcomes within each cervical screening programme to use as a template to modify triage schedules and guide future practice.

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