Clinical Performance of Triage Strategies for Hr-HPV–Positive Women; A Longitudinal Evaluation of Cytology, p16/K-67 Dual Stain Cytology, and HPV16/18 Genotyping

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ABSTRACT

Background: We evaluated the longitudinal performance of three options: HPV16/18 genotyping (HPV16/18), cytology (LBC), and p16/Ki-67 dual stain cytology (DS) for the triage of high-risk Human Papillomavirus–positive (Hr-HPV$^+$) women within the cervical screening program in Scotland.

Methods: Data were derived from a cohort of Hr-HPV$^+$ women ($n = 385$) who participated in PaVDaG (Papillomavirus Dumfries and Galloway) study. Performance of triage strategies for detecting high-grade disease was assessed at 3 (in women <50 years) or 5 years (in women >50 years). Sensitivity, specificity, PPV, and cNPV of each triage test were calculated for CIN2$^+$ and CIN3$^+$ when used singly or sequentially.

Results: The sensitivity of LBC (≥ borderline), DS, and HPV 16/18 genotyping for the detection of CIN2$^+$ was 62.7% (50.7–73.3), 77.7% (63.1–83.7), and 62.7% (50.7–73.3) with corresponding cNPVs of 10.9%, 8.4%, and 11.9%. The option with the highest sensitivity and lowest cNPV was HPV 16/18 genotyping followed by LBC of Hr-HPV other$^+$ and then DS of the LBC negatives. This yielded sensitivity of 94.7% (86.2–98.3) and cNPV 2.7% for CIN2$^+$. Triage performance was similar if women had tested Hr-HPV$^+$ positive by vaginal self-sampling.

Conclusions: Two-step triage with HPV 16/18 genotyping before LBC (or DS) for Hr-HPV other$^+$ women was associated with a lower risk of significant disease at follow-up compared with single triage approaches.

Impact: This study provides longitudinal performance data on triage strategies in Hr-HPV$^+$ women and will be informative for the evolution of cervical screening programs that increasingly rely on molecular technologies.

Introduction

Cervical screening programs based on primary high-risk Human Papillomavirus (Hr-HPV) testing as opposed to primary cytology have increased, globally in recent years. Key drivers for this approach include the enhanced sensitivity of Hr-HPV testing compared with cytology for the detection of existing or future disease, the non-requirement for highly skilled cytotechnologists and the feasibility of using Hr-HPV testing for self-taken samples (1–5).

Hr-HPV–based screening programs generally recall women who test screen Hr-HPV negative for routine screening in 5 years and this group represents the majority (3). However, there is greater variation with respect to the recommended management for women who test Hr-HPV–positive (Hr-HPV$^+$). The challenge of managing Hr-HPV$^+$ women lies in separating those with transient infection versus those who have an infection associated with significant underlying or incipient disease. Current Hr-HPV–screening tests are not able to do this, so additional risk stratification or "triage" is required. The triage technologies most commonly used at present are cytology (alone), cytology with adjunctive biomarker staining (incorporating p16INK4a), or limited genotyping for HPV types 16 and/or 18 (6, 7). However, none of these approaches are entirely satisfactory. Cytology still demands a level of subjectivity and requires a highly specialist workforce for it to function optimally. Although adjunctive staining can reduce the subjectivity, judgment is still required for the interpretation of the p16/Ki-67 Dual Stain (DS). The DS is also subject to technical constraints (8) and the clinical performance of triage that requires cytology, even within the same screening program, has been shown to vary (9). HPV16/18 genotyping is operationally straightforward, particularly as some (though not all) HPV assays approved for screening offer this output routinely. This said even though there is robust evidence to suggest that non-16/18 types, that is, "Hr-HPV other" pose a lower risk of subsequent high-grade disease (6, 7), the optimal management of these infections still needs to be determined. Furthermore, the performance and usefulness of genotyping in increasingly immunized populations is uncertain (10).

In addition, these single-step triage approaches, when applied, do not generally offer a binary outcome: colposcopy if positive and routine screening if negative. Rather, they leave a group of Hr-HPV$^+$ women who require re-testing, resulting in a longer screening cycle. Furthermore, defining triage strategies, which allow safe return to routine screening, requires longitudinal data from population-based studies.

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We previously published work where cross-sectional accuracy of three triage strategies; HPV16/18 genotyping, liquid-based cytology (LBC), p16/Ki-67 DS cytology and combinations thereof, were assessed in the context of a primary Hr-HPV screening study in Scotland—Papillomavirus Dumfries and Galloway (PaVDaG) study (11). The present study updates this analysis with 3 to 5 years of follow-up data, including the offer of a second screen with LBC to all original participants. These data provide insight into the longitudinal performance of triage strategies in their ability to predict disease over a minimum of 3 years.

Materials and Methods

Parent PaVDaG cohort/study

Full details of the study recruitment and testing can be found in Stanczuk and colleagues (11, 12). Women who attended for routine cervical screening within a single Scottish territorial health board (Dumfries and Galloway) were invited to participate in the study through provision of a cervical LBC sample (as standard) in addition to a self-taken vaginal swab and random void urine specimen. Hr-HPV testing was performed using the Cobas 4800 DNA test (Roche Diagnostics) that detects 14 Hr-HPV types with separate identification of HPV 16 and 18. Cervical screening information, including cytopathology results, was accessed through the national IT system, the Scottish Cervical Call-Recall System (SCCRS). The British Association for Cytopathology (BAC) reporting guidelines and CIN nomenclature were used to describe cytological and histological findings. Management of women with abnormal cytology results was performed according to national guidelines, although additional colposcopy for Hr-HPV$^+$ women who were LBC$^-$ was performed as described in earlier work (11, 12).

Description of Hr-HPV$^+$ cohort used to assess triage performance for the detection of high-grade disease

Data from 385 Hr-HPV$^+$ women from the original PaVDaG cohort formed the basis of this analysis. Sixty-six had CIN2$^+$ (26 CIN2, 39 CIN3, and 1 cervical cancer) diagnosed as a result of the initial screen. Most of the lesions detected in the first round were diagnosed and treated within a year of the initial screen with 30% diagnosed within 2 months, and 71% and 94% within 1 and 2 years, respectively. 319/385 women had ≤CIN1 in the first round and were invited for a second round of screening, following completion of any follow-up, at either 3 years if aged 49 years or under (89% of participants), or at 5 years if ages 50 years or over (11%), as per routine screening guidelines of the time.

A total of 288 (out of a possible 319) women completed a second round of screening. 279 were had ≤CIN1 whereas 9 had CIN2$^+$ (5 CIN2 and 4 CIN3). The final cohort therefore represented 279 women classed as having "no disease," 75 women with CIN2$^+$ and 44 women with CIN3$^+$. Recruitment and attrition is summarized in **Fig. 1**. The primary analysis of triage performance was based on the clinician-taken sample, although the performance of triage strategies in women who tested Hr-HPV$^+$ ($n = 446$) on a self-taken vaginal sample was also analyzed.

Triage testing (historic)

The present analysis extends the earlier assessment described previously in Stanczuk and colleagues (11) by providing longer-term clinical outcomes associated with the baseline triage test. HPV16/18 status was determined using the output from the Cobas 4800 DNA test. Cytology was performed according to BAC guidelines and p16//Ki-67 DS cytology was performed using the CINtec PLUS cytology kit (Roche Diagnostics). Cytotechnologists performing DS cytology were blind to the original LBC and Hr-HPV result and a number of Hr-HPV$^-$ LBC samples were randomly included in the DS cytology study group.

Data analysis

The final cohort represented 279 women classed as having "no disease" and 75 women with CIN2$^+$. The "No disease" category was defined as a biopsy result of ≤CIN1, or a normal colposcopy with no clinically indicated biopsy, or having a history of at least three negative cytology-screening results and no previous history of CIN2$^+$.

The longitudinal sensitivity, specificity, positive predictive value, and complement of the negative predictive value (1-NPV; cNPV) for the detection of CIN2$^+$ and CIN3$^+$ for LBC at a threshold of borderline nuclear abnormalities (≥BNA/≥ASCUS), DS, and HPV 16/18 genotyping as single triage approaches were assessed 3 to 5 years after baseline testing. In addition to triage using a single test, combinations involving two triage tests (HPV 16/18 before LBC or HPV 16/18 before DS) or three triage tests (HPV16/18, LBC and DS) were also estimated.

Governance and ethics statement

The present analysis represents a passive study of follow-up data. The baseline PavDag study for which written informed patient consent was obtained was approved by an independent regulatory body; the West of Scotland Research Ethics Committee Ref. 12/WS/0085 in accordance with Declaration of Helsinki guidelines. Follow-up analysis obtained institutional approval by the NHS Dumfries and Galloway Caldicott Guardian.

Data accessibility

Data in anonymized form can be made available upon reasonable request to the senior author and in line with governance requirements

Results

Longitudinal absolute accuracy of triage tests to detect CIN2$^+$

The longitudinal accuracy for CIN2$^+$ of HPV16/18 genotyping, LBC or DS cytology as individual triage approaches for Hr-HPV positive, clinician taken samples is presented in **Table 1**.

At the CIN2$^+$ endpoint, DS cytology sensitivity was highest at 77.7% [95% confidence interval (CI), 63.1–83.7] with an associated specificity of 74.2% (95% CI, 68.6–79.1) whereas LBC and HPV 16/18 genotyping showed the same sensitivity at 62.7% (95% CI, 50.7–73.3) and had respective specificities of 82.4% (95% CI, 77.3–86.6) and 74.6% (95% CI, 68.9–79.5). The performance of sequential triage options are summarized in **Table 2**; sensitivities for HPV16/18 genotyping followed by LBC or DS triage of Hr-HPV other$^+$ were both 89.3% (95% CI, 79.5–95.0) with respective specificities of 60.6 (95% CI, 54.6–66.3) and 56.6 (95% CI, 50.6–62.5). The three-step approach of HPV16/18 genotyping with LBC of Hr-HPV other$^+$ and then DS of LBC negatives yielded the highest longitudinal sensitivity at 94.7% (95% CI, 86.2–98.3), with a specificity of 51.3% (95% CI, 45.2–57.2) and cNPV of 2.7%.

Longitudinal absolute accuracy of triage tests to detect CIN3$^+$

At the level of CIN3$^+$, when considering individual triages, DS triage had the highest sensitivity: 81.8% (95% CI, 66.8–91.3) followed by HPV16/18 genotyping: 70.5% (95% CI, 54.6–82.8) and LBC: 61.4% (95% CI, 45.5–75.3). LBC was the most specific triage with respect to absolute values 77.7%, (95% CI, 72.6–82.2); **Table 1**.

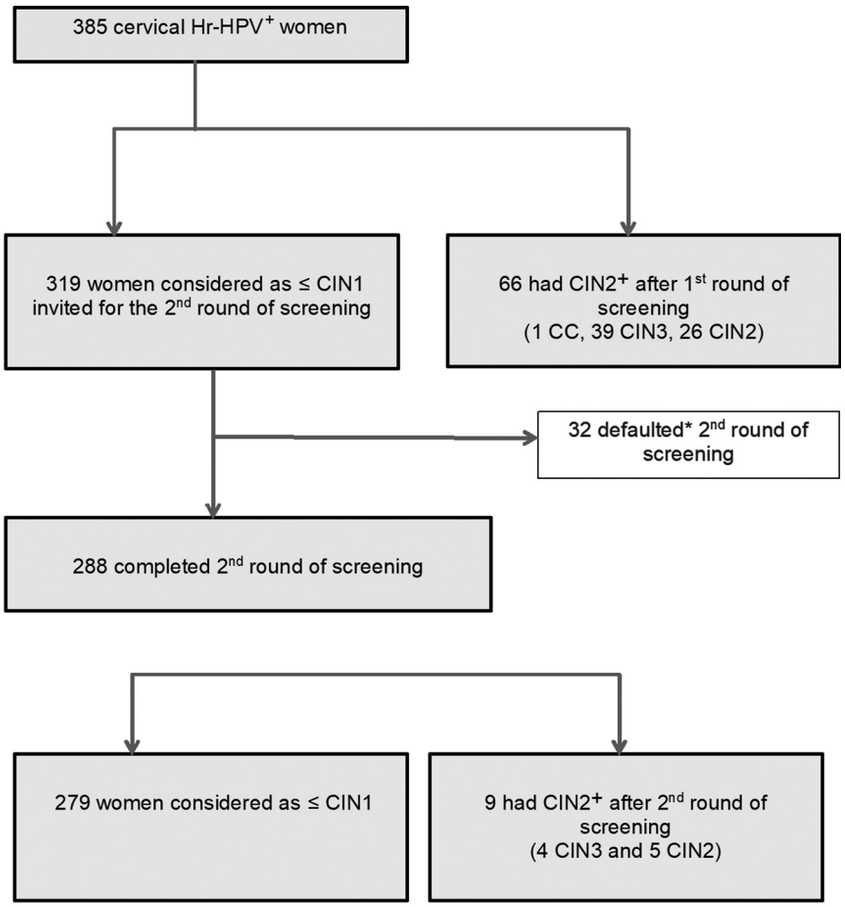

Figure 1.
Study flow and attrition; includes distribution of outcomes after 1st and 2nd round of screening. CIN2, cervical intraepithelial neoplasia grade 2; CIN3, cervical intraepithelial neoplasia grade 3; and CC, cervical cancer. Data collection was completed by the end of January 2020. *, A defaulter is the term used to describe women who have not taken up an invitation to have a cervical screening test carried out after receiving reminders.

In line with the observations for CIN2, multistep approaches conferred a higher sensitivity for the detection of CIN3$^+$. An initial triage of HPV16/18 genotyping followed by a second triage of Hr-HPV other$^+$ to either LBC or DS led to sensitivities of 90.0% (95% CI, 77.4–97.0) and 93.2% (95% CI, 80.3–98.2) and specificities of 55.8% (95% CI, 50.1–61.4) and 52.6% (95% CI, 46.9–58.2). The cNPV for CIN3 was 1.8% in the scenario where HPV16/18$^+$ women were offered direct colposcopy and women with Hr-HPV other$^+$ were triaged by DS—and was 2.3% if LBC was used instead of DS for the triage of Hr-HPV other$^+$ women. The three-step approach of HPV16/18 genotyping with LBC of Hr-HPV other$^+$ and then DS of LBC negatives provided the highest longitudinal sensitivity at 95.5% (95% CI, 83.3–99.2), with a specificity of 46.8 (95% CI, 41.1–52.5) and a cNPV of 1.4%.

Analysis of single and combination triages was also performed on women who were Hr-HPV$^+$ on self-taken samples. Very similar findings were observed as with clinician-taken samples. DS exhibited the highest longitudinal sensitivity as a single triage approach (compared with HPV16/18 genotyping and LBC) and combination triages starting with HPV16/18 genotyping before LBC or DS of Hr-HPV others$^+$ showed the higher sensitivity than any single approach with

Table 1. Longitudinal accuracy of triage tests in women with Hr-HPV$^+$ in clinician-taken samples for the cumulative detection of CIN2$^+$ and CIN3$^+$ over two screening rounds that were 3 years apart for women 49 years and younger (89% of the study cohort) and 5 years apart for women 50 years and older (11%).

Triage option	Sensitivity	Specificity	PPV	cNPV
Single triage test				
@CIN2$^+$				
LBC ≥BNA	62.7% (50.7%–73.3%)	82.4% (77.3%–86.6%)	49%	10.9%
Dual Stain$^+$	77.7% (63.1%–83.7%)	74.2% (68.6%–79.1%)	43.8%	8.4%
HPV16/18$^+$	62.7% (50.7%–73.3%)	74.6% (68.9%–79.5%)	39.8%	11.9%
@CIN3$^+$				
LBC ≥BNA	61.4% (45.5%–75.3%)	77.7% (72.6%–82.2%)	28.1%	6.6%
Dual Stain$^+$	81.8% (66.8%–91.3%)	70.3% (64.8%–75.3%)	28.1%	3.5%
HPV16/18$^+$	70.5% (54.6%–82.8%)	71.9% (66.5%–76.8%)	26.3%	5.5%

Table 2. Longitudinal accuracy of triage combinations when applied as a two-step or three-step process.

Triage option	Sensitivity	Specificity	PPV	cNPV
Two-step approach				
@CIN2$^+$				
HPV16/18 and if HPV other$^+$/LBC$^+$	89.3% (79.5%–95.0%)	60.6% (54.6%–66.3%)	37.9%	4.5%
HPV16/18 and if HPV other$^+$/DS$^+$	89.3% (79.5%–95.0%)	56.6% (50.6%–62.5%)	35.6%	4.8%
@ CIN3$^+$				
HPV16/18 and if HPV other$^+$/LBC$^+$	90.0% (77.4%–97.0%)	55.8% (50.1%–61.4%)	22.6%	2.3%
HPV16/18 and if HPV other$^+$/DS$^+$	93.2% (80.3%–98.2%)	52.6% (46.9%–58.2%)	21.8%	1.8%
Three-step approach				
@CIN2$^+$				
HPV16/18 and if HPV other$^+$/LBC$^+$ and DS$^+$ if LBC$^-$	94.7% (86.2%–98.3%)	51.3% (45.2%–57.2%)	34.3%	2.7%
@ CIN3$^+$				
HPV16/18 and if HPV other$^+$/LBC$^+$ and DS$^+$ if LBC$^-$	95.5 (83.3%–99.2%)	46.8 (41.1%–52.5%)	20.3%	1.4%

Note: In the two-step approach, those who are HPV 16/18 positive are referred to colposcopy, whereas those with "HPV other" are reflexed to LBC—data row 1 or Dual Stain (DS)—data row 2 with positivity, indicating referral. For the three-step approach, those who are HPV 16/18 positive are referred to colposcopy and those who are HPV other$^+$ are reflexed to LBC, if this were negative there would be a further reflex to Dual Stain. LBC is set at a level of borderline changes or above (≥BNA/≥ASCUS).

cNPV for CIN3$^+$ at less than 2.4% and 1.4%, respectively. Full details can be seen in **Table 3**.

Discussion

In the baseline cross-sectional analysis related to this work (11), we reported that DS conferred an observably higher sensitivity as a single triage test compared with LBC (85% vs. 68.3%) but was also associated with lower specificity (76.7% vs. 89.1%) and an increase in the number of colposcopy referrals required to find one case of CIN2$^+$ (number needed referred, 2.4 vs. 1.8; ref. 11). The present data are consistent with this initial observation in that they indicate that the longitudinal sensitivity of DS when used as a single triage approach remains higher compared with LBC. Although we accept that we did not perform a formal analysis to infer superiority of a particular approach, we would suggest that the data are valuable as there is still a relative paucity of longitudinal information related to triage performance on which to base appropriate recall intervals. The data partially reconcile with two recent US observational studies, also set in the context of population screening, where the authors concluded that risk of high-grade CIN was lower in DS negative than in cytology negative women (13, 14).

In a recent Italian study of over 3,000 women who tested HPV$^+$ in a screening setting, triage by LBC or DS cytology was associated with sensitivities of 61% (95% CI; 53.6–68.0) and 75.2% (95% CI, 68.1–81.6), respectively, for the detection of CIN2$^+$ during 24 months of follow-up (15). These observations are very similar to the sensitivities described in the present study (where follow-up was available for a minimum of 3 years) with respective values of 62.7% (95% CI, 50.7–73.3) and 77.7% (95% CI, 63.1–83.7). A criticism that has been leveled against DS is between site variability in performance (9). However, the

Table 3. Longitudinal accuracy of triage tests in women with Hr-HPV$^+$ in self-taken samples for the cumulative detection of CIN2$^+$ and CIN3$^+$ over two screening rounds that were 3 years apart for women 49 years and younger (89% of the study cohort) and 5 years apart for women 50 years and older (11%).

Triage option	Sensitivity	Specificity	PPV	cNPV
Single triage test				
@CIN2$^+$				
LBC ≥BNA	66.7% (54.2%–77.3%)	84.1% (79.7%–87.8%)	46.5%	7.6%
Dual stain$^+$	78.3% (66.4%–86.9%)	78.7% (73.9%–82.9%)	43.2%	5.4%
HPV16/18$^+$	60.9% (48.4%–72.2%)	71.6% (66.3%–76.3%)	30.7%	10.2%
@ CIN3$^+$				
LBC ≥BNA	65.9% (49.3%–79.4%)	80.1% (75.5%–84.0%)	27.3%	4.6%
Dual Stain$^+$	87.8% (73.0%–95.4%)	75.4% (70.6%–79.7%)	28.8%	1.8%
HPV16/18$^+$	65.0% (49.3%–79.4%)	69.6% (64.5%–74.3%)	19.7%	5.3%
Two-step approach				
@CIN2$^+$				
HPV16/18 and if HPV other$^+$/LBC$^+$	89.9% (79.6%–95.5%)	59.3% (53.8%–64.6%)	31.3%	3.4%
HPV16/18 and if HPV other$^+$/DS$^+$	88.4% (77.9%–94.5%)	58.1% (52.6%–63.4%)	30.3%	4.0%
@ CIN3$^+$				
HPV16/18 and if HPV other$^+$/LBC$^+$	87.8% (73.0%–95.4%)	55.2% (50.0%–60.4%)	18.2%	2.4%
HPV16/18 and if HPV other$^+$/DS$^+$	92.7% (79.0%–98.1%)	55.0% (49.7%–60.2%)	18.9%	1.4%
Three-step approach				
@CIN2$^+$				
HPV16/18 and if HPV other$^+$ LBC$^+$ and DS$^+$ if LBC$^-$	94.2% (85.1%–98.1%)	53% (47.5%–58.4%)	29.3%	2.2%
@ CIN3$^+$				
HPV16/18 and if HPV other$^+$ LBC$^+$ and DS$^+$ if LBC$^-$	95.1% (82.2%–99.2%)	49.4% (44.2%–54.7%)	17.6%	1.1%

data from these two totally separate evaluations nested within population-based programs, show good consistency.

In our original cross-sectional study (11), we demonstrated the risk in triage-negative Hr-HPV$^+$ women for CIN2$^+$ using the pre-test-post-test probability plots associated with various triage options. We defined 3 benchmarks for CIN2$^+$ risk, with a 20% risk, indicating colposcopy; ≥2% and <20%, indicating surveillance and a <2% risk, indicating return to 3 yearly recall as proposed in a meta-analysis on the accuracy in women with minor abnormal cytology (16). Although this summary/benchmark approach is attractive in its clarity, it is notable that there is no International consensus on "acceptable risk" and thus where these benchmarks should be set. For example, the US has a low threshold of risk to indicate immediate colposcopy compared with the figures described above, whereas in most other countries decision thresholds are higher (17–19). Perhaps the search for a universal benchmark will remain elusive given that population-specific incidence of cancer may have a bearing on benchmarks. We also accept that how assiduously any benchmark may be followed will depend on the level of opportunistic versus organized screening. In practice, the thresholds may arrive from a mixture of scientific precepts, existing practice, manpower and infrastructure, prevalence of cervical pathology, and the abilities of given country screening system to adhere to the agreed thresholds.

Historically, cytology screening in Scotland would refer women to colposcopy with a risk of CIN2$^+$ and CIN3$^+$ of 45.5% and 26.6%, respectively (20). This would translate to a risk of CIN2$^+$ and CIN3$^+$ of cytology negative women 3 to 5 years later of 1.6% and 0.8%, respectively (20), which compares well with the risk reported in populations from six European countries of 0.97% for CIN3$^+$ 6 years after testing (21). Furthermore, this translates to risk of CIN2$^+$ and CIN3$^+$ in our Hr-HPV$^+$ and LBC$^-$ women, 3 to 5 years later of 10.9% and 6.6%, respectively. This would be an accepted risk in historic cytology screening.

Our data suggest that none of the initial triage approaches (from single to three-step) conferred a risk of CIN2$^+$ of <2% at 3 to 5 years if negative. If the benchmark for routine referral is set at <2% CIN3$^+$ risk, offering two-step triage with HPV16/18 genotyping before LBC or DS of HPV other$^+$ confers risks close to (2.3%) or within this (1.8%), respectively, at 3 to 5 years after primary testing. This aligns with the observations of Wentzensen and colleagues (14) who suggested, "retesting intervals in HPV16/18-negative women with negative DS results can be safely extended to 3 years."

Our data indicate that when a three-step approach of HPV 16/18 genotyping with LBC of HPV other$^+$ followed by DS for the remaining HPV other$^+$/LBC$^-$ is used; the risk of CIN3$^+$ remains below 2% (1.4%) for 3 to 5 years after testing. The two- to three-step approaches were associated with between 2.6 and 3.5 colposcopies to detect a single case of CIN2$^+$ in the PaVDaG cohort (11). However, in the current screening population in Scotland, which will incorporate a greater number of immunized women, the rate of colposcopy referrals is expected to be lower. Interestingly, Jiang and colleagues (22) observed that extending HPV genotyping could reduce the number of colposcopies in Chinese women. The combination of HPV 16/18 genotyping and DS triage of HPV 31/33/58/52/45/59/56/and 66 positive women instead of all 12 other HPV type positive was described as promising for use in low and middle income countries if low cost products for DS and HPV genotyping were developed. Two-step triage strategy (HPV16/18 and LBC) was examined in a large cross-sectional study in Mexico. The sensitivity of two-step triage versus LBC triage of HPV$^+$ women for detection of CIN2$^+$ was 86.6% and 42.9%, respectively. The number of colposcopies needed to diagnose one CIN2$^+$ was increased from 5.9 to 7.2 by using two-step triage (23). This may demonstrate the varying quality of cytology, between settings, on the number of colposcopies. Despite the high number of colposcopies, the authors concluded that in resource-poor setting with poor access to care, using more sensitive methods with higher number of colposcopies "will produce a better outcome for women's overall health. . ." (23).

Given the issues with discharging women to routine recall according to single triage approaches—the majority of Hr-HPV$^+$ women will remain in the "surveillance cohort." When considering the dimensions of (and HPV prevalence within) the PaVDaG cohort, this surveillance cohort represents 8.1% or 9.8% of the population of women 25 years and older if testing is performed on cervical or self-collected vaginal samples, respectively. As discussed earlier, since the start of the PaVDaG study (2014), more women eligible for screening have been vaccinated, which will reduce the number of women under surveillance, but most importantly the number of HPV16/18$^+$ women making two-step triage (16/18 genotyping and LBC or DS of Hr-HPV other$^+$ women), potentially more manageable. We do accept, however, that before any change can occur a full cost utility analysis would be essential as would a comprehensive assessment of the implications on workforce, skills, and laboratory infrastructure.

Agreeing on thresholds of risk for immediate colposcopy, surveillance and return to routine screening will be a key in the design and implementation of triage strategies and clinical pathways for cervical screening. Ensuring that these risks are managed consistently across all parts of the program is important. Furthermore, the risk-based system should take account of changes in infection and disease levels as a result of vaccination. It is notable that the recently published fourth iteration of the American Society of Colposcopy and Cervical Pathology ASCCP guidelines/recommendations for the management of cervical cancer screening are fundamentally predicated on risk and include the statement "Recommendations of colposcopy, treatment, or surveillance will be based on a patient's risk of CIN3$^+$ determined by a combination of current results and history (including unknown history)" (17).

Our study, although relatively small has some important strengths. It reflects real-life screening scenarios in homogenous population of one Scottish Health Board with high disease ascertainment at baseline, which is reflected in few CIN2$^+$ cases diagnosed at the second-screening round. A similarly designed longitudinal study of a large Italian population showed improved triage performance using two and three-step triage, including partial genotyping p16 immunostaining and cytology at baseline, then cytology only at 3 years follow-up. The amount of CIN2$^+$ was lower than ours at 12.6% compared with 19.5%, which is most likely a reflection of higher HPV prevalence in PaVDaG versus the Italian population (14.7% vs. 8%; refs. 12, 24) and potentially, a lower threshold for obtaining cervical biopsy in colposcopy. Notwithstanding the argument that this could represent diagnosis and treatment of potentially self-limiting lesions, the prevalence of CIN2$^+$ diagnosed during the 3 years follow-up was lower in our population than the one reported in the Italian population (ref. 24; 2.3% vs. 4.2% of Hr-HPV$^+$ women at baseline).

Although the cost and resource implications of additional colposcopy should not be trivialized, the evidence of potential performance benefit of HPV16/18 genotyping as a triage strategy with LBC/DS reserved for women Hr-HPV other$^+$ is attested to by reports from both high- and middle-income countries (14, 18, 22–26). A one-time triage approach may also obviate reduction of HPV-screening sensitivity through loss of engagement in downstream re-testing and referral protocols (27, 28). Scotland has been engaged in national

HPV-based primary screening since March 2020, and has a cervical screening IT system that accommodates full screening and treatment history (where indicated) as well as vaccination status. It is therefore well placed to gather the data that will inform risk-based screening protocols that reflect local infection and disease levels, levels of vaccine uptake and clinical practice. Although any change in practice and protocol would need to underpinned by a robust financial and operational case as well as a detailed exercise in workforce implications, Scotland is arguably well positioned to examine the benefits of contemporary triage approaches for both unvaccinated and vaccinated women.

Authors' Disclosures

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Authors' Contributions

G. Stanczuk: Conceptualization, resources, data curation, supervision, validation, investigation, methodology, writing–original draft, writing–review and editing. **H. Currie:** Supervision, investigation, methodology, writing–review and editing. **W. Forson:** Investigation, writing–review and editing. **G. Baxter:** Conceptualization, resources, data curation, supervision, investigation, methodology, writing–review and editing. **J. Lawrence:** Conceptualization, supervision, methodology, writing–review and editing. **A. Wilson:** Investigation, writing–review and editing. **T. Palmer:** Formal analysis, writing–original draft. **M. Arbyn:** Formal analysis, writing–review and editing. **K. Cuschieri:** Conceptualization, formal analysis, supervision, writing–original draft.

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