EVALUATION OF A VALIDATED METHYLATION TRIAGE SIGNATURE FOR HUMAN PAPILLOMAVIRUS POSITIVE WOMEN IN THE HPV FOCAL CERVICAL CANCER SCREENING TRIAL

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Abbreviations:

HPV: human papillomavirus
PPV: positive predictive value
CIN: cervical intraepithelial neoplasia
LBC: liquid-based cytology

NILM: negative for intraepithelial lesions and malignancy
RCT: randomized controlled trial
HC2: hybrid capture 2 high-risk HPV test
ASCUS: atypical squamous cells, undetermined significance
STM: specimen transport medium
ROC: receiver operating characteristic
RLU: relative light units
AUC: area under the ROC curve

Novelty and Impact

Human papillomavirus (HPV) screening requires triage of positive women. In a study of 257 HPV positive women from the HPV FOCAL trial, baseline S5 methylation testing had 93% sensitivity and 18% positive predictive value (PPV) for cervical intraepithelial neoplasia (CIN) grade 3, equivalent to combined cytology and HPV16/18 genotyping triage (86% sensitivity, 19% PPV), and no cancers were missed. Methylation triage of HPV positive women has performance comparable to current routine, more complex triage approaches.

Abstract

Human papillomavirus (HPV)-based cervical cancer screening requires triage of HPV positive women to identify those at risk of cervical intraepithelial neoplasia grade 2 (CIN2) or worse. We conducted a blinded case-control study within the HPV FOCAL randomized cervical cancer screening trial of women aged 25-65 to examine whether baseline methylation testing using the S5 classifier provided triage performance similar to an algorithm relying on cytology and HPV genotyping. Groups were randomly selected from 257 women with known HPV/cytology results and pathology outcomes. Group 1: 104 HPV positive (HPV+), abnormal cytology (54 CIN2/3; 50 <CIN2); Group 2: 103 HPV+, normal cytology with HPV persistence at 12 mo. (53 CIN2/3; 50 <CIN2); Group 3: 50 HPV+, normal cytology with HPV clearance at 12 mo. (assumed <CIN2). For the combined groups, S5 risk score CIN2/3 relative sensitivity, specificity and positive predictive value (PPV) were compared with other triage approaches. Methylation showed a highly significant increasing trend with disease severity. For CIN3, S5 relative sensitivity and specificity were: 93.2% (95%CI: 81.4-98.0) and 41.8% (35.2-48.8), compared to 86.4% (75.0-95.7) and 49.8% (43.1-56.6) respectively for combined abnormal cytology/HPV16/18 positivity (differences not significant); adjusted PPVs were 18.2% (16.2-20.4) and 19.3% (16.6-22.2) respectively. S5 was also positive in baseline specimens from eight cancers detected during or after trial participation. The S5 methylation score had high sensitivity and PPV for CIN3, compatible with US and European thresholds for colposcopy referral. Methylation signatures can identify most HPV positive women at increased risk of cervical cancer from their baseline screening specimens.

Introduction

Persistent high-risk human papillomavirus (HPV) infection is the primary cause of cervical cancer [1-3]. HPV-based cervical screening can identify >95% of pre-cancerous cervical lesions (cervical intraepithelial neoplasia [CIN] grade 2 or worse [CIN2+]) [4], but has a relatively low specificity for CIN2+ because most HPV positive women have transient infections which spontaneously clear [5], with few progressing to CIN3 and cancer [4].

Widespread adoption of primary HPV cervical screening has supported the search for a triage test which retains high sensitivity but increases specificity and positive predictive value (PPV), while accurately identifying women at high risk for CIN3+. Reflex liquid-based cytology (LBC) is commonly used [6], but its low sensitivity (~50-70%) [7] for CIN2+ limits its triage utility. Consequently, follow-up is usually required to monitor for HPV clearance or persistence in women with no intraepithelial lesions or malignancy (NILM) LBC diagnoses. Triage strategies can also include HPV16 and HPV18 (HPV16/18) genotyping together with LBC [8,9]. Immediate colposcopy referral is recommended in some countries for HPV16/18 positive women regardless of cytology diagnosis, and also for women with other HPV types who have abnormal LBC. HPV positive, LBC negative women are subsequently re-tested to identify persistent HPV infections with referral of these to colposcopy [8]. Another triage strategy is p16/Ki67 immunostaining which is more sensitive than standard LBC and identifies women at elevated risk of CIN2+ [10], but the interpretation still requires subjective microscopy. An objective triage strategy which could be automated and incorporated as a reflex molecular test following HPV screening would be advantageous. DNA methylation assays targeting host and/or HPV genes may meet this requirement as they have been shown to have higher sensitivity and similar specificity to LBC for identifying CIN2+ [11-13].

The S5 DNA methylation classifier was developed in a London UK colposcopy referral population [14] and was later validated with cervical screening samples [15]. It is based on targeting late regions of HPV16, HPV18, HPV31 and HPV33 combined with the promoter region of the human tumour suppressor gene *EPB41L3*. **HPV FO**r Cervi**CAL** Cancer Screening (HPV FOCAL) is a population-based Canadian randomized controlled trial (RCT) comparing HPV versus LBC for primary cervical cancer screening [16,17]. The trial provided an ideal study for additional validation of "real-world" molecular triage test performance. We assess the S5 methylation classifier for detecting histopathologically confirmed CIN2/3 vs. <CIN2 among HPV positive HPV FOCAL trial women.

Materials and Methods

HPV FOCAL Trial Design

The HPV FOCAL RCT [16-18] (ISRCTN79347302) compared HPV (Hybrid Capture® 2 High-Risk HPV DNA Test® [HC2]; Qiagen Inc., Germantown, MD, USA) (Intervention and Safety [HPV] Arms) versus LBC screening (Control Arm) in women aged 25-65. HC2 positive (HC2+) women in the HPV Arms were triaged by LBC, with immediate colposcopy referral for abnormal cytological findings. Women with NILM cytology were re-screened 12 months later, with those who remained HC2+ and/or had abnormal cytology referred to colposcopy (Supplementary Figure S1). HPV genotyping was included in the trial as an adjunct study [19], which allowed modeling the performance of combination triage approaches using both cytology and HPV16/18 genotyping. Women were randomly enrolled into one of the three FOCAL Trial arms until closure of the Safety Arm, after which randomization continued to the Intervention and Control arms (final enrollment: Intervention Arm, 9,552; Control, 9,457; Safety, 6,214). Round 1 screening, follow-up and management were identical for the two HPV Arms, so these were combined for the present analysis. After excluding 22 women with invalid/incomplete baseline HC2 results, the HPV Arms included 15,744 women. Colposcopy examination included biopsy and/or endocervical curettage. CIN diagnoses were based on histopathology. Written informed consent was obtained from all trial participants. Both the RCT (H06-04032) and the nested methylation case-control study (H14-02974) were approved by the University of British Columbia/BC Cancer Agency Clinical Research Ethics Board.

Methylation Case-Control Study Population

We focused on baseline HPV positive women detected by the HC2 test. Women were classified into three groups based on their HC2 and reflex LBC results (Table 1). Group 1: HC2+, LBC ≥atypical squamous cells of undetermined significance (ASCUS; referred to colposcopy at baseline); Group 2: HC2+, LBC NILM at baseline, remained HC2+ and/or had LBC ≥ASCUS at the 12-month subsequent screen (referred to colposcopy at 12 months); Group 3: HC2+, LBC NILM at baseline with HPV clearance

at 12 months (not referred to colposcopy; assumed to have <CIN2 histopathology). At enrollment, a duplicate cervical sample collected in specimen transport medium (STM; Qiagen) was stored at −80°C for molecular studies. For groups 1 and 2, STM samples were randomly selected from all women with CIN2/3 and <CIN2 in each group to achieve approximately equal distribution of CIN2/3 and <CIN2. For group 3, STM samples were randomly selected from all samples meeting the group definition. The three groups were combined to estimate methylation test characteristics for HC2+ triage. In addition, samples were tested from eight women from any study arm who developed invasive cervical cancer during or after the trial; these women with malignancy were not included in Groups 1-3, nor the sensitivity, specificity, PPV or receiver operating characteristic (ROC) calculations. Personal identifying information was removed and a unique ID number was applied to each study sample prior to methylation analyses.

Sample Preparation, HPV genotyping, and Methylation Testing

HPV16/18 genotyping was done by the cobas® 4800 HPV test (Roche Molecular Systems, Pleasanton CA). The Linear Array HPV Genotyping Test (Roche) was used to genotype cobas "other high risk positive" specimens. HPV16/18 genotype was assigned if the specimen was cobas positive for one or both of HPV16 or HPV18, regardless of the detection of any other HPV type(s).

DNA was extracted from 100µL of each STM sample (MagMAX™ Total Nucleic Acid Isolation Kit; Life Technologies, Burlington ON, Canada), eluted into 100µL, and used for methylation testing. DNA concentrations were estimated and DNA was shipped on dry ice to the Wolfson Institute laboratory where methylation testing was done as previously described [14]. Lab personnel were blinded to the sample group assignment, HPV genotype and CIN outcomes.

Statistical Analysis

All analyses were based on a pre-specified statistical analysis plan. The main hypothesis was that S5 methylation triage at baseline had equivalent sensitivity and PPV to triage by baseline LBC ≥ASCUS or

LBC NILM and HPV16/18 positivity (LBC ≥ASCUS/HPV16/18). Histopathologically confirmed CIN2/3 versus <CIN2 was used as the reference standard.

The S5 risk score is based on methylation levels of the human gene *EPB41L3* together with HPV16L1, HPV16L2, HPV18L2, HPV31L1 and HPV33L2. The PCR-based multiplex assay was followed by quantitative pyrosequencing to measure methylation levels of each assay component. The S5 risk score was calculated as: S5 = 30.9(*EPB41L3*) + 13.7(HPV16L1) + 4.3(HPV16L2) + 8.4(HPV18L2) + 22.4(HPV31L1) + 20.3(HPV33L2); a score of ≥0.8 indicated a positive methylation test [14].

HC2 relative light unit (RLU)/cutoff ratios, where a positive test was ≥1.0, were used as a surrogate for HPV viral load; a higher ratio indicated higher viral load.

Relative sensitivity and specificity (i.e., relative to the FOCAL Trial triage for HC2+ women as described in the trial design) for cumulative round 1 CIN2/3 and CIN3 during the trial were calculated for S5 performed at baseline; 95% non-parametric bootstrapped CIs were obtained from 10,000 bootstrap replicates. Unadjusted PPVs were calculated by dividing the number of women with CIN2/3 or CIN3 cervical lesions (true positive screens) by the number with a positive triage test in the methylation study subset [17]. PPVs were also adjusted for CIN2/3 and CIN3 prevalence estimates (26.8% and 12.2% respectively) for the trial HPV arms (Table 1), using the following formula: PPV= (Sn*Pr)/((Sn*Pr)+(1-Sp)*(1-Pr)), where Sn is sensitivity, Sp is specificity, and Pr is the CIN2/3 or CIN3 prevalence. To place S5 triage in context, the same parameters were calculated at baseline for triage by: 1) LBC ≥ASCUS/HPV16/18 (the main comparison); 2) HPV16/18 positive; and 3) LBC ≥ASCUS. The S5 colposcopy referral rate was estimated using the S5 positive rates for CIN2/3 versus <CIN2 in the case-control study, and extrapolating to the distribution of CIN2/3 and <CIN2 for all HC2+ women in the HPV arms of the

trial by re-weighting the sampling groups according to the trial population data (Table 1). Colposcopy referral rates for the other three triage strategies were calculated from round 1 trial data for the HPV arms. Wilson's method was used to calculate 95%CI. Cuzick's test [20] was used to test for trend in S5 scores by disease category (ordered <CIN2, CIN2, CIN3 and cancer) and by HPV viral load. McNemar's test was used to explore differences in paired nominal data.

S5 ROC curves were generated for CIN2/3 and CIN3 by re-weighting the sampled groups as described above, from which area under the ROC curve (AUC) with 95%CI was calculated from a non-parametric empirical bootstrap. The combined ROC estimated the classification performance of S5 and its components for all HC2+ women in the HPV arms of the trial.

Statistical calculations were performed using R version 3.3.1.

Results

Relative to the HPV FOCAL triage algorithm (Supplementary Figure S1), which was used as the reference standard, the S5 classifier had sensitivities for CIN2/3 and CIN3 of 75.7% (95%CI: 67.3-83.7) and 93.2% (95%CI: 84.8-100.0) respectively (Table 2). S5 sensitivity was significantly greater than either cytology or HPV16/18 genotyping (Table 2) but was not significantly different (CIN2/3: p=0.170; CIN3: p=0.248) than the sensitivity of combination triage by LBC ≥ASCUS/HPV16/18. S5 relative specificities for <CIN2 and <CIN3 [44.0% (95%CI: 36.1-52.2) and 41.8% (95%CI: 35.3-48.4) respectively] were similar to LBC ≥ASCUS/HPV16/18 triage, but were lower than both LBC ≥ASCUS and HPV16/18 triage (Table 2). The adjusted PPVs of S5 for CIN2/3 (33.1%) and CIN3 (18.2%) were similar to the corresponding PPVs for triage by LBC ≥ASCUS/HPV16/18 (34.2% for CIN2/3 and 19.3% for CIN3), and for LBC ≥ASCUS alone (35.7% and 19.1% respectively), but lower than for HPV16/18 triage alone (44.4% and 28.1% respectively).

The estimated colposcopy referral rate for S5 methylation classifier positive women (4.3%) was higher than for HPV16/18 positive and LBC ≥ASCUS triage, but was similar to the combined strategy of LBC ≥ASCUS/HPV16/18 triage (4.2%), which was our most sensitive and main comparison. The highest referral rate was for the full FOCAL trial triage approach (5.9%) which detected all 107 CIN2+ cases.

Of the 107 CIN2/3 cases, 81 (76%) were S5 positive at baseline. FOCAL triage identified 54 (50%) at baseline and the remaining 53 (50%) cases after 12 month re-screening. For the 44 CIN3 cases, 41 (93%) were S5 positive at baseline. FOCAL triage identified 27 (61%) at baseline and the remaining 17 (39%) cases at 12 months (Table 3).

Figure 1 illustrates the median S5 scores, stratified by CIN diagnosis, for women in groups 1-3 combined and those diagnosed with cervical cancers. Median S5 scores showed a significantly increasing trend

with both lesion severity (Supplementary Table 1; Cuzick $p_{trend}<0.0001$) and with HPV viral load (Supplementary Table 2; $p_{trend}=0.0001$). Women with <CIN2 and LBC ≥ASCUS, LBC NILM or non-HPV16 positivity had median scores near the S5 cutoff, while HPV16 positive women had a higher median S5 score, similar to some of the women with high-grade disease and cancer (Supplementary Table 1).

S5 ROC curves for CIN2/3 and CIN3 are shown in Figure 2; for CIN2/3 the AUC was 0.70 (95%CI: 0.64-0.77) and for CIN3 was 0.83 (95%CI: 0.75-0.90). Figure 2 also shows CIN2/3 and CIN3 ROC point estimates for women based on HPV genotype and reflex cytology triage combinations.

All baseline specimens from the eight invasive cervical cancer cases were S5 positive and all cases were HPV16 or HPV18 positive on the baseline or 12-month subsequent-to-baseline sample (Table 4). Of these cancers, six were adenocarcinomas and two were squamous cell carcinomas. For the four individuals who had another sample post-baseline but prior to diagnosis of the cancer, the S5 scores had increased. All trial-detected cancers underwent secondary review by a senior trial pathologist and all were confirmed to be of cervical origin.

Details of the S5 negative CIN2 and CIN3 cases are shown in Supplementary Table 3. For CIN3, one case was HPV58 positive and another was both HPV52 and HPV68 positive; these are HPV types not included in the S5 classifier. The third CIN3 case was associated with HPV67 which was detected only in the 12-month subsequent-to-baseline specimen. HPV67 has been designated as possibly carcinogenic to humans [21], but is not included in most commercial high-risk HPV screening assays. For CIN2, most S5 negative cases were also associated with HPV types not included in the S5 classifier, but one S5 negative CIN2 case was HPV18 positive at baseline, another was HPV33 positive and two additional cases had HPV16 detected only in the 12-month subsequent-to-baseline specimens.

Discussion

We observed a moderate baseline sensitivity of the S5 DNA methylation classifier for CIN2 and a high sensitivity (>90%) for CIN3 and cancer among HPV positive women. S5 specificities were lower but PPVs were comparable to other accepted triage methods. Compared to the FOCAL trial triage of colposcopy referral for HPV positive women with baseline abnormal reflex cytology or NILM baseline cytology with 12-month HPV persistence, methylation triage can provide objective and more timely identification of most women with high-grade cervical lesions at baseline screening. Of women with CIN3, S5 detected 93% of cases at baseline, compared to 61% for the FOCAL trial baseline triage. For CIN2/3 the percentages were 76% for S5 triage and 50% for FOCAL triage, respectively.

S5 methylation testing had similar triage performance for detection of CIN2/3 at baseline compared to a triage approach based on immediate colposcopy referral for women with LBC ≥ASCUS, or LBC NILM with HPV16/18 positivity, a triage approach used predominantly in the US [22]. Our trial did not include an option for colposcopy referral of baseline LBC NILM, HPV16/18 positive women, as this was not recommended in Canada when the FOCAL trial was designed. In addition, baseline LBC NILM, HPV16/18 positive women would not have been referred to colposcopy unless the 12 month subsequent specimen was HC2 positive or LBC ≥ASCUS. Thus, we were not able to determine how many additional CIN2/3 would have been detected among baseline HPV 16/18 positive women in the trial by the US approach. However, that approach would have increased colposcopy referral rates, which goes against our search for triage strategies that can reduce over-treatment [23]. In the HPV arms of the trial, S5 triage would have reduced clinician visits and screen tests as more high-grade disease would have been detected at baseline, thus simplifying the screening algorithm and potentially reducing loss to follow-up. In future, methylation markers may be shown to preferentially detect advanced lesions with a high short term risk of cervical cancer; indeed, a recent study from the POBASCAM trial showed that women negative for DNA methylation had a low future risk of cervical cancer over the subsequent 14 years [24].

An earlier study of S5 [15] among women in the Predictors 3 (P3) trial, whose initial screen was cytology with subsequent HPV testing, reported S5 CIN2+ and CIN3+ sensitivities of 74% and 84% respectively for HPV positive women, similar to our study (75.7% and 93.2% respectively). However, <CIN2 and <CIN3 specificities for S5 in the P3 study (65% and 63%) vs. FOCAL (44.0% and 41.8%) were higher. The lower S5 specificity in our study may partly be related to the relatively high S5 scores obtained for HPV16 positive women with <CIN2. Furthermore, women in the FOCAL HPV arms underwent HPV primary screening rather than cytology. It seems plausible that primary cytology screening may preferentially detect later stage disease because HPV screening detects more transient HPV infections in addition to the persistent HPV infections responsible for CIN2+, and thus, S5 triage might be expected to have lower specificity among women screened for HPV. A review of studies of host gene methylation in cervical cancer [13] revealed wide methylation variations in the same gene between different studies, some of which may be related to population differences and/or the methylation testing methodology.

Performance characteristics for methylation studies (not including those with self-collected samples) using a variety of genes [25] reported CIN2+ sensitivities ranging from 48%-89% in populations initially screened by either HPV or cytology, and 44%-90% in colposcopy referral populations. Specificities ranged from 50%-81% and 49%-95% respectively. The S5 sensitivity in the FOCAL case-control study is consistent with the upper range of results of these studies, whereas specificity is within the lower range. Of note, the areas under the ROC curve for FOCAL (CIN2/3: 0.70; CIN3: 0.83) are consistent with other studies of both screening (CIN2+ 0.72-0.80; CIN3+ 0.84) and colposcopy referral (CIN2+ 0.82; CIN3+ 0.77-0.97) populations [25].

Sensitivity and specificity was not reported for the FOCAL trial as there was no verification performed for negative screens. We used the FOCAL triage approach as the reference method; thus, the sensitivities for other single and combination triage approaches reported in this paper are relative to those based on the FOCAL trial, which were assumed for comparison purposes to be ~100%. The relative CIN3 sensitivity for the S5 classifier (93.2%) was similar to FOCAL while that for CIN2/3 was lower (75.7%). This might be expected given that most of the S5 negative CIN2+ cases were associated with non-HPV16/18/31/33 genotypes. Targeting additional HPV genotypes in the S5 classifier might improve sensitivity, but could result in lower specificity. Methylation triage including the *EPB41L3* or other host genes has been reported to have comparable performance to cytology for HPV positive women [26], although cytology performed slightly better, especially when attempting to maximize the sensitivity of methylation triage [25]. S5 methylation triage has also been shown to be more sensitive for CIN2+ than HPV16/18 genotyping and displayed similar specificity [15]. Triage based on HPV16/18 positivity in our study (CIN2/3 sensitivity: 49.5%%; CIN3: 72.7%) compared to S5 (CIN2/3 sensitivity: 75.7%; CIN3: 93.2%) is consistent with this observation.

Of eight women who developed cervical cancers during or after FOCAL trial participation, two were HC2 negative on the baseline specimen. All eight cancers were S5 positive at the baseline screen, but the median S5 score for the baseline samples for women with cancers was lower than for women with CIN3 (5.8 vs. 9.3). Some of the tested samples from subjects with cancer were obtained several years prior to the cancer diagnosis, which could have resulted in lower S5 scores than if samples had been tested closer to their cancer diagnoses. This is likely the case, as the four women who had a subsequent sample tested had substantially higher S5 scores than for their baseline samples. Moreover, six of the cancers tested were adenocarcinomas and it has been reported that these tend to display lower methylation levels compared to squamous cell carcinomas [25,27].

At least two methylation assays based on human genes are commercially available for HPV positive triage. The GynTect® assay is based on ASTN1, DLX1, ITGA4, RXFP3, SOX17 and ZNF671 [28], while the QIAsure Methylation Test Kit is based on promoter hypermethylation of FAM19A4 and hsa-mir-124-2 [29]. The S5 classifier utilizes the *EPB41L3* human gene, which was found to have the best performance in an earlier credentialing study of a number of human genes in the Predictors 1 and 2 studies [30]. S5 triage sensitivity for CIN3 was higher than for the GynTect® assay (93% vs. 65%) but GynTect® had higher specificity (42% vs. 89%) [31]. Using two types of self-collected samples tested by the same methylation components as the QIAsure assay, De Strooper et al. [32] reported CIN3+ sensitivities of 68%-71% and specificities of 68%-76%. Sensitivity improved to 85%-89%, but specificity was lower at 46%-55%, when methylation was combined with HPV16/18 genotyping. Further research will be needed to optimize the sensitivity and specificity of methylation assays for triage.

A strength of our study is that the samples were obtained from a RCT embedded within an organized cervical screening program, with high compliance to colposcopy recommendations, standardized colposcopic examinations with biopsy, and centralized blinded pathology review. An important limitation of our case-control study is that it was retrospective because the trial was not designed specifically to assess prospectively additional molecular triage methods in HPV positive women. In addition, although women with CIN2/3 and <CIN2 were randomly selected from the population of women meeting those criteria, it is possible that the methylation-tested sub-population is not representative of all women in the trial with CIN2/3 and <CIN2. Optimal ethnic and geographically representative validation of S5 triage will require additional studies designed to directly compare S5 with established strategies, preferably with colposcopy referral for all women with a positive triage test. An intriguing question is whether S5 classifier negative CIN2+ reflects lesions destined to regress

spontaneously, or result from the S5 classifier not including targets for some high-risk genotypes. To understand this phenomenon would require systematic follow up of CIN2+ women who are undergoing assessment for CIN progression or regression.

In conclusion, DNA methylation assessed by the S5 classifier correlates strongly with aggressive cervical disease, showing high sensitivity for CIN3 and cancer, the raison d'être for a cervical screening program. S5 PPV for CIN3 is compatible with both US and European colposcopy referral thresholds [33,34]. Methylation tests have the potential to simplify triage by more quickly identifying HPV-infected women in need of colposcopy. Of the 107 CIN2/3 in our follow-up study, 81 cases were identified at baseline by S5 as compared to 73 by combination LBC ≥ASCUS/HPV16/18 triage; the remaining 34 women were diagnosed only after 12 months of follow-up. Thus, S5 can detect a greater proportion of high-grade disease with a high short-term risk of cervical cancer at the baseline screen than the other approaches, which can lessen concerns of losing women during follow-up.

Figure 1. S5 score distributions by CIN diagnosis

CIN: cervical intraepithelial neoplasia

Note: The middle line is the median; the box shows the inter-quartile range (IQR) and the whiskers extend to at most 1.5 times the IQR. Cancer S5 scores include only those for the baseline samples taken between 4 and 67 months before cancer diagnosis.

Figure 2. S5 receiver operating characteristic curves, CIN2/3 and CIN3

HC2: hybrid capture 2 HPV test; CIN: cervical intraepithelial neoplasia; ASCUS: atypical squamous cells, undetermined significance; AUC: area under the curve; CI: confidence interval

The markings shown in the legend illustrate CIN2/3 and CIN3 point estimates for HC2+ women, and for each modeled triage option.

Supplementary Figure 1. HPV FOCAL Trial Schematic

The yellow highlighted area illustrates the trial subset used for the methylation case-control study.

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Conflicts of Interest

DAC has received speaker honoraria and travel expense reimbursement from Hologic, Inc. MK has received research grant funding via his institution from Roche Molecular Systems, Boehringer Ingelheim, Merck, Siemens Healthcare Diagnostics and Hologic Inc. AJC has received funding via his institution from Roche Molecular Systems to compare the accuracy of different HPV assays but has received no personal financial reimbursement. LWS has worked as a paid consultant to Roche Molecular Systems. All other authors report no conflicts of interest.

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