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The role of human papillomavirus (HPV) genotyping in cervical cancer screening: A large-scale evaluation of the cobas HPV test

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Abstract

Background—The cobas® HPV Test ("cobas", Roche Molecular Systems) detects HPV16 and HPV18 individually, and a pool of 12 other high-risk (HR) HPV types. The test is approved for 1) ASC-US triage to determine need for colposcopy, 2) combined screening with cytology ("co-testing"), and 3) primary HPV screening.

Methods—To assess the possible value of HPV16/18 typing, >17,000 specimens from a longitudinal cohort study of initially HPV-positive women (HC2, Qiagen) were retested with cobas. To study accuracy, cobas genotyping results were compared to those of an established method, the LINEAR ARRAY HPV Genotyping Test (LA, Roche Molecular Systems). Clinical value of the typing strategy was evaluated by linking the cobas results (supplemented by other available typing results) to 3-year cumulative risks of CIN3+.

Results—Grouped hierarchically (HPV16, else HPV18, else other HR types, else negative), the kappa statistic for agreement between cobas and LA was 0.86 (95%CI=0.86-0.87). In all 3 scenarios, HPV16-positive women were at much higher 3-year risk of CIN3+ than HPV16-negative women: women aged 21 and older with ASC-US (14.5%, 95%CI=13.5%-15.5% versus 3.5%, 95%CI=3.3%-3.6%); women aged 30 and older that were HPV-positive cytology-negative

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Conflicts of Interest Statement: The NCI authors have conducted studies in which HPV testing was performed at no cost by Roche Molecular Systems or BD. The Roche Molecular Systems authors are or were employees of the company during the investigation. The analyses were conducted and the manuscript was written by the corresponding author; all authors suggested editorial changes but the final decisions were made by the corresponding author. The manuscript was approved by all authors.

(10.3%, 95%CI=9.6%-11.1% versus 2.3%, 95%CI=2.2%-2.4%); and all women 25 and older that were HPV-positive (18.5%, 95%CI=17.8%-19.2% versus 4.3%, 95%CI=4.2%-4.4%).

Conclusion—The cobas and LA results show excellent agreement. The data support HPV16 typing.

Impact—HPV16 typing is useful in the management of HPV- positive/cytology-negative women in co-testing, of all HPV-positive women in primary HPV testing, and perhaps in the management of HPV-positive women with ASC-US.

Introduction

Human papillomavirus (HPV) testing is an increasingly important part of cervical screening(1). Effective implementation of HPV testing requires the use of thoroughly validated assays, and restricts their use to clinical indications supported by data (2, 3).

The Roche cobas® HPV test (Roche Molecular Systems, Pleasanton CA) is one of 4 HPV tests currently approved by the US Food and Drug Administration (4). Its indications include: 1) to triage patients 21 and older with ASC-US (atypical squamous cells of undetermined significance) cervical cytology results to determine the need for referral to colposcopy, 2) to test women 30 years and older, adjunctively with cytology, in the context of general screening ("co-testing") and 3) for primary HPV screening alone (without cytology) among women 25 and older.

The cobas assay targets DNA of 14 HPV types. It simultaneously provides HPV type-specific results for HPV16 and HPV18, the 2 most important high-risk (HR) genotypes as well as a pooled result for 12 other HR types. The 12 other HR group includes the 10 other established carcinogenic types (HPV31, HPV33, HPV35, HPV39, HPV45, HPV52, HPV56, HPV58, and HPV59), a probably carcinogenic HPV type (HPV68) and a possibly carcinogenic type (HPV66) (5).

The clinical value of HPV16 and HPV18 genotyping in each of three approved test settings is not clearly established. Current U.S. consensus guidelines do not recommend use of HPV16 and HPV18 typing among women with HPV-positive ASC-US, who are uniformly referred to colposcopy instead (6, 7). Guidelines do recommend use of HPV16/18 genotyping for women with co-testing results of HPV-positive, cytology-negative; those with HPV16/18 are immediately referred to colposcopy while those with other types are re-tested at 1 year (6, 7). Formal guidelines for the use of genotyping as part of primary HPV testing are not yet established; interim guidance (Huh, Obstet Gynecol, 2015) recommends colposcopy for HPV16/18 positive women, with cytology reserved for women positive for one or more infections with the 12 other HR HPV types.

By performing a large-scale study in a cohort of clinical specimens, we assessed the accuracy of HPV16/18 genotyping by cobas and the clinical utility of its typing strategy in the three possible clinical scenarios. To assess typing accuracy, we compared the genotype-specific clinical performance of cobas to that of an established research-use-only HPV typing method, LINEAR ARRAY HPV Genotyping Test (LA, Roche Molecular Systems). To assess utility, we examined the risk stratification provided by type-specific HPV16 and

HPV18 results for women aged 21 and older with ASC-US; HPV positive, cytology-negative women aged 30 and above; and all HPV positive women aged 25 and above. Specifically, we assessed 3-year risk of CIN3 as the measure of risk; using 3-year risk rather than cross-sectional risk helped to maximize ascertainment of outcomes regardless of immediate management.

Material and Methods

Study Population

To achieve the study objectives, *a posteriori* re-testing of a large population of known HPV-positive women was performed. To assess typing accuracy, we analyzed paired cobas and LA test data on more than 17,000 specimens from a cohort study of initially Hybrid Capture 2 (HC2)-positive (Qiagen, Germantown, MD) women being conducted collaboratively by the US National Cancer Institute (NCI) and Kaiser Permanente Northern California (KPNC) (8, 9).

The NCI-KPNC cohort study from which the specimens were drawn is called the Persistence and Progression (PaP) Cohort (8). The PaP Cohort is designed to explore viral and host determinants of cervical precancer among HPV-positive women; as the major part of the study, participants were enrolled after they tested positive during routine screening for HPV (as part of HPV and cytology co-testing) using HC2.

For the comparison of cobas and LA, a very large convenience sample of the initially HC-positive cohort was re-tested, namely all available paired cobas/LA test results from nested (completed and ongoing) case-control studies of prevalent and incident precancer. Women in the cohort can contribute many specimens in longitudinal fashion, as they return for follow-up. Thus, the tested specimens included both HC2-positive enrollment specimens and subsequently collected follow-up specimens from the same women (both HC2-positive and HC2-negative). In contrast, for the assessment of clinical utility of HPV typing among HPV-positive women, data from case-control analyses were used among HC2-positive specimens only from enrollment without consideration of longitudinal results from the same women (see details on Definitions of Cases and Controls below).

The PaP cohort was nested within routine practice at KPNC, where HPV testing using HC2 has long been used to triage ASC-US cytology. KPNC introduced cytology co-testing in 2003 for cervical screening at 3-year intervals for women 30 and older(10). Pap tests are interpreted at KPNC regional and facility laboratories; HPV testing of a co-collected second cervical specimen is performed at the single region laboratory. In most but not all KPNC laboratories, cytology is interpreted with knowledge of the concurrent HPV result. Until 2009, conventional Pap slides were manually reviewed following processing by the BD FocalPoint Slide Profiler (BD Diagnostics, Burlington, NC, USA), in accordance with FDA-approved protocols. Starting in 2009, KPNC transitioned to liquid-based cytology using BD SurePath (BD Diagnostics, Burlington, NC, USA). Conventional or liquid-based Pap tests are reported according to the 2001 Bethesda System(11). HC2 is used to test for high-risk HPV types as a pool according to manufacturer’s instructions.

Starting in 2007, the The PaP Cohort was created by banking residual ("waste") cervical specimens, collected into specimen transport medium (STM; Qiagen), from a randomly selected majority of women who tested HC2-positive. From 2007-2011, 45,302 HC2-positive women were enrolled. An additional small percentage (~7%) of women opted-out in response to a letter informing them of the PaP study. NCI and KPNC institutional review boards have approved the study yearly.

The present study within the PaP cohort is based on Roche Molecular Systems research testing of 17,262 selected archived specimens from the PaP Cohort. Clinical follow-up cytology and histology were obtained on the cohort as described elsewhere (12). NCI selected the samples for Roche testing, which was performed masked to all other data. LA and cobas results were generated in tandem on the same aliquot of specimens.

As detailed below, to evaluate the performance of the cobas typing strategy for clinical risk stratification, we estimated cumulative 3-year risk of CIN3+ by typing result. However, a group of PaP cohort specimens from enrollment (those from the prevalent cases and controls who at that time had not developed CIN2+) had been tested only in another laboratory (RD Burk, Albert Einstein College of Medicine), using another (MY09/11) L1 PCR-based method (13). To generate cumulative 3-year risk estimate, we included those results as well, as described below. Of note, the two assays varied in several details including specimen input (cobas used more than MY09/11), extraction efficiency, and PCR efficiency. Nonetheless, this pooling of typing data was justified by good comparability of the additional data to cobas typing results (HPV16, else HPV18, else HR, else negative) as indicated by a kappa of 0.65 (95%CI=0.57-0.73) for 233 specimens tested by both assays. As the main difference, the cobas assay was more likely to detect HPV16 than the MY09/11 assay.

HPV Testing

Laboratory methods have been described fully elsewhere (12). In brief, denatured STM specimens were neutralized within 14 hours to minimize DNA damage.

For the cobas HPV testing, the x480 sample preparation module was used to prepare and aliquot the master mix and to perform sample addition. Twenty-five microliters of sample was added to 25 μl of master mix in a 96-well PCR plate. This plate was then manually sealed and transferred to the z480 real-time amplification and detection module of the cobas 4800 system as per the manufacturer's protocol, using spectrally unique fluorescent dyes to label TaqMan probes for HPV16, HPV18, and the 12 other HR-HPV genotypes.

The LA assay targets the 14 HR types included in the cobas assay and also HPV6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39(82 subtype), and 89 (CP6108). In brief, automated sample extraction was performed on the neutralized STM sample using the x480 sample extraction module of the cobas 4800 system. The HPV LA test was carried out according to the manufacturer's protocol available within the package insert of the kit with minor modifications (12).

For the subset of specimens from prevalent cases and controls, whose results were needed to complete 3-year risk estimates, genotyping results generated with another PCR-based method in the Burk laboratory were included. The MY09/M11 L1 degenerate primer PCR (MY09/11 PCR) system used by the Burk laboratory to test prevalent cases and controls has been described previously (13).

Definitions of Cases and Controls for Risk Estimation

In the comparison of cobas with LA, all specimens with paired results were considered, disregarding case-control status. For analyses assessing clinical utility, 3-year cumulative risk of CIN3+ or CIN2+ were calculated based on results from enrollment specimens. Cases diagnosed prior to a repeat screening-type visit (i.e., a second cotest) were classified as prevalent, while those cases diagnosed after a second screening visit were considered incident; admittedly, this division is arbitrary. The analyses focused on CIN3+ as the main case group and surrogate of cancer risk, but also considered CIN2+, despite lack of diagnostic reproducibility (14), because CIN2 lesions are commonly treated. The CIN3+ or CIN2+ cases were compared with a random sample of HC2-positive control women that had not been diagnosed with CIN2+ at the time of selection, and had returned at least for one subsequent screening at approximately 1 year post-enrollment (9). As shown below, this analysis was conducted separately for all selected women 21 and older with ASC-US to address triage, all selected women aged 30 and above that were HC2+ and cytology negative to address co-testing, and all selected women aged 25 and older that were HC2+ to address primary HPV screening. The testing fractions are shown in Table 1. As shown in the table, the supplementary testing by MY09/11 PCR applied to prevalent cases and controls diagnosed during the enrollment period. However, some prevalent cases developed more severe diagnoses during follow-up (e.g., CIN2 was followed by CIN3), and some prevalent controls developed CIN2+. For analysis, these individuals were categorized as incident cases instead according to their worst diagnosis.

Statistical Analyses

To assess typing accuracy, the LA and cobas assays were compared at the level of HPV16, HPV18, 12 other HR types, or negative for the 14 targeted types, using the kappa statistic and asymmetry chi-square. This analysis was hierarchical, using “else if” logic. Thus, any specimen with HPV16 detected was grouped as HPV16, only specimens without HPV16 could be grouped as HPV18, and only specimens negative for both HPV16 and HPV18 could be grouped as 12 HR. (This analysis was confirmed with non-hierarchical analyses, in which a specimen concurrently could have HPV16, and/or HPV18, and/or other HR types, but we do not present those very similar results here.) The comparison was repeated, stratified by correlates of HPV viral load, i.e., concurrent HC2 result (positive/negative), cytologic result (negative; ASC-US; LSIL; and high-grade including AGC, ASC-H, HSIL, AIS, and cancer), and also by case status (worst histopathologic result).

Results were also examined at the HPV type-specific level, with type determined by LA. For clarity, this analysis was restricted to specimens that were positive for only a single one of the 14 cobas-targeted types (whether or not other, e.g., low-risk types, were also present). To address a limitation of LA (15), HPV52 was called positive only when no other type in the

LA pooled probe used to define HPV52 was individually positive. As there was no attempt to adjudicate absolute truth, discrepancies between the 2 assays could be described only in relative terms (e.g., in the case of additional positives by LA, it was not possible to distinguish between true and false positives). Moreover, the inter-assay comparison was conducted at the level of specimens; some women contributed more than 1 specimen (enrollment plus 1 or more follow-up specimens) to the analysis. We did not adjust for the remote possibility of statistical auto-correlation.

The second major part of the statistical analysis was to determine whether detecting the presence of HPV16 or HPV18 at enrollment, as provided by cobas, would meaningfully alter the risk of CIN3+ (or secondarily, CIN2+), in a clinically actionable manner, i.e., changing management from colposcopy to 1-year follow-up in the case of HPV-positive ASC-US or, conversely, changing management to immediate colposcopy for co-testing or primary HPV screening. Because, in the course of the nested case-control studies, only a random sample of 10-15% of the non-cases (and most of the cases) were tested by cobas, with the supplementation of Burk lab PCR, it was necessary to use the sampling fractions to weight up estimates from the tested group to properly represent the full PaP cohort (Table 1). The tested group was multiplied by the inverse of the sampling fractions to estimate the distributions for the whole population, and the cumulative 3-year risks and 95% confidence intervals were calculated using weighted Kaplan-Meir methods (using SAS-callable SUDAAN version 11.0.1) to adjust for censoring and incomplete follow-up. The consideration of HPV types was hierarchical. To determine this order, an iterative approach was used in which each of the preceding higher-risk channels was excluded from consideration for the analyses of the remaining HPV channels. We looked at each HPV channel individually and chose the one with the highest positive predictive value for the 3-year risk of CIN3+, i.e., HPV16 (see Results).

We then sought to determine, given an HPV16-negative status, whether testing positive for any other type would indicate the need for colposcopic referral. Consequently, we excluded all women testing positive for HPV16 and repeated the risk calculations among the remaining women to determine the next highest risk channel.

Results

Agreement Between cobas and LA

Overall, 17,262 specimens from the PaP study were tested by both cobas and LA. The specimens were collected from 8,451 women, whose median age was 37 at enrollment (range 21 to 87; interquartile range 30-47). There was a close concordance between cobas and grouped LA results. As shown in Table 2, when the results were grouped hierarchically (HPV16, else HPV18, else other HR types, else negative), the kappa statistic for agreement between the assays was 0.86 (95% CI = 0.86-0.87), indicating excellent agreement. As inter-assay agreement was not perfect, the discrepancies were further examined. There was a steady trend for agreement to be higher in subgroups of the cohort with presumed higher HPV viral load (i.e., when HC2 was positive and/or cytology was abnormal). Thus, the lowest but still good agreement (kappa = 0.65, 95% CI 0.62-0.69) was observed among specimens that were concurrently HC2-negative and cytology-negative; at the other extreme, agreement among HC2-positive cases with CIN3+ diagnoses was very high (kappa = 0.90, 95% CI 0.88-0.92). The cobas assay tended to classify 1.5% more specimens as HPV16-positive than did LA (kappa for agreement on HPV16 =0.92, 20.8% vs. 19.3%, respectively, p<0.001 from McNemar's test). Most HPV16 cobas-positive, LA-negative specimens were classified by LA as having other HR HPV types; no particular type predominated (data not shown). The HPV16 cobas-positive, LA-negative specimens (n=344) were as likely as HPV16 LA-positive, cobas-negative specimens (n=91) to come from women with CIN3+ (~15% in both groups), but either set of discrepant results was less likely to derive from women with CIN3+ than concordantly positive results (~30% of 3229, data not shown).

Further type-specific analyses of the positivity of cobas relative to LA are shown in Table 3; in this analysis, we were particularly interested in exploring LA-positive specimens that were negative by cobas. LA results were used to classify single-type infections for each of the 14 types targeted by cobas. Multiple concurrent infections with one of the 14 cobas-targeted types were excluded for the comparison shown in Table 3. Each type by LA was compared to the corresponding cobas result (HPV16, HPV18, or each of the other 12 HR types). The results indicate that, with LA as the reference-standard typing assay, cobas detected a very high (>95%) percentage of all single-type infections for almost all targeted types. As the exceptions, the cobas HR was negative for 19.9% of 1223 specimens that LA classified as single HPV52, 16.2% of 648 specimens that LA classified as single HPV58, and 9.8% of 594 specimens that LA classified as single HPV51. When multiple infections were included rather than excluded from the analysis, cobas detection of LA-positive HPV16 or HPV18 slightly declined (data not shown).

To explore the fraction of specimens that tested positive by cobas but negative by LA for the 14 types targeted by both assays, we examined whether cobas cross-reacted with additional (e.g., low-risk) types detected by LA. There was no evidence of cross-reactivity for this group. In fact, the cobas assay was less likely to be positive (for at least one HPV channel) when LA detected only 1 or more types not targeted by cobas (9.8% cobas-positive), compared with completely LA-negative specimens (15.6% cobas-positive).

ASC-US Triage: HPV Typing and Risk of CIN3+, among Women with Positive HC2 Results and ASC-US Cytology

A possible use of cobas typing (HPV16 and HPV18) not recommended by current guidelines is among women with HPV-positive ASC-US, who are routinely referred to colposcopy. The question is whether women with ASC-US who are negative for HPV16 and HPV18 might be at sufficiently low risk not to need immediate colposcopy; if so, perhaps they could be asked to return in 1 year instead. Thus, 3-year cumulative risk of CIN3+ was estimated by HPV16 and HPV18 typing results among women with HPV-positive ASC-US.

As shown in Table 4, overall, women 21 and older with ASC-US who tested HC2+ had a 5.2% 3-year risk of CIN3+ overall, but those negative for HPV16 were at much lower risk of CIN3+ (3.5%, 95% CI 3.3%-3.6%) than HPV16-positive women (14.5%, 95% CI 13.5%-15.5%). Thus, testing negative for HPV16 yielded a risk that was lower than the current colposcopy threshold, which is benchmarked in guidelines to the risk (here, 5.2%) of all HPV+ ASC-US (6,7). Among HPV16-negative women, HPV18 or other HR detection only slightly stratified risk in this group of HC2-positive women with ASC-US. Repeating the analysis using CIN2+ as the disease definition yielded similar conclusions (Supplemental Table 1). An ancillary analysis restricting to cobas data (excluding the MY09/11 PCR data) resulted in falsely low cumulative risk estimates (because many prevalent cases were excluded) but the pattern of risk stratification was unchanged (data not shown). There was no meaningful change in the pattern of results when age was stratified into tertiles (data not shown).

Co-testing: HPV Typing and Risk of CIN3+ in Co-testing, among Women Aged 30 an Above with Positive HC2 Results and Negative Cytology

In order to evaluate clinical use of the HPV16 and HPV18 typing in co-testing, specifically in the triage of HPV-positive/Pap-negative results, the analysis was restricted to HPV-positive (by HC2), cytology-negative enrollment specimens. Again, 3-year cumulative risk of CIN3+ was calculated by typing result. In the absence of HPV HPV16 and HPV18 typing results, guidelines recommend that such women return in year (6, 7). The question was whether HPV16 and HPV18 typing by cobas would stratify risk in such a way that it might justify immediate colposcopy.

As shown in Table 5, the 3-year cumulative risk of CIN3+ among women 30 and older who tested HC2-positive but cytology-negative was 3.5% overall; as expected, this risk is lower than the colposcopy threshold according to current guidelines as applied to this group, which is set by the risk for LSIL (5.9%) or all HPV+ ASC-US (5.5%). HPV-positive, cytology-negative women that were positive for HPV16 were at much higher cumulative risk of CIN3+ (10.3%, 95% CI 9.6%-11.1%), compared with HPV16-negative women (2.3%, 95% CI 2.2%-2.4%). Thus, the magnitude of risk among HPV16-positive women exceeded the threshold of risk at which current guidelines recommend referral to colposcopy. Among HPV16-negative women, HPV18 detection only slightly stratified risk (Table 5). In the absence of either HPV16 or HPV18, positivity for the other HR types did not meaningfully change risk of CIN3+ in this group of HC2-positive women. Repeating the analysis using CIN2+ as the disease definition yielded similar conclusions (Supplemental Table 2), as did restricting to cobas data (data not shown).

Primary HPV Testing: HPV Typing and Risk of CIN3+ in Primary HPV Testing, among Women Aged 25 and Older with Positive HC2 Results

In order to evaluate clinical use of the HPV16 and HPV18 typing in primary HPV testing, the analysis was restricted to HPV-positive (by HC2) specimens among women aged 25 and older, i.e., the age group for which the FDA recently approved primary HPV testing by cobas. Again, 3-year cumulative risk of CIN3+ by typing result was calculated. The question was whether HPV16 and HPV18 typing would stratify risk in such a way that it would justify immediate colposcopy rather than repeat testing in 1 year. Of note, the 25-29 year old group tested by HC2 in KPNC was dominated by ASC-US triage (approximately half had ASC-US); thus, this population had a higher intrinsic risk than a true population sample of all women aged 25 and older.

As shown in Table 6, the 3-year cumulative risk of CIN3+ among women 25 and older who tested HC2-positive was 6.7% overall. Formal guidelines do not yet exist for colposcopy referral following primary HPV testing (Huh, Obstet Gynecol, 2015); of note, the risks in this group were very similar when comparing all HC2-positive women, to those with LSIL (6.0%) and those with HPV+ ASC-US (5.5%). However, those positive for HPV16 were at much higher cumulative risk of CIN3+ (18.5%, 95% CI 17.8%-19.2%), compared with HPV16-negative women (4.3%, 95% CI 4.2%-4.4%). Among HPV16-negative women, HPV18 detection only slightly stratified risk (Table 6) in these HC2-positive women aged 25 and above. In the absence of either HPV16 or HPV18, positivity for the other HR types did not meaningfully change risk of CIN3+ in this group of HC2-positive women. Repeating the analysis using CIN2+ as the disease definition yielded similar conclusions (Supplemental Table 3), as did restricting to cobas results (data not shown).

Discussion

Agreement between different HPV assays is rarely if ever perfect(16). As expected from previous work (17), we observed that agreement between cobas and LA (at the level of HPV16, else HPV18, else 12 other HR types, else negative) was at least good in all subgroups, but tended to be stronger when viral load was higher (as indicated by HC2 positivity, abnormal cytology, or diagnosis of CIN3+ compared with controls). Also, cobas positivity for HPV16 or HPV18 was slightly higher when LA indicated the presence of a single HPV type rather than multiple HR infections. The cobas assay tended to yield HPV16-positive results slightly more often than LA, but the association with CIN3+ was equal for the two assays. Among the targeted, 12 other HR types, cobas sensitivity relative to LA was somewhat lowered for HPV52, HPV58, and HPV51. We used a stringent definition for HPV52 (calling it positive only when none of the other types in the mixed probe were present) to avoid false LA HPV52 positivity. No cancers were missed by cobas that were detected by HC2 and/or LA but the sample of cancers was small. There was no indication of cobas cross-reactivity with non-HR types detected by LA.

The clinical performance demonstrated among women with HPV-positive enrollment results supports the recent interim clinical guidance (Huh, Obstet Gynecol, 2015) regarding primary HPV testing among women 25 and older, which recommend colposcopy for those with HPV16. The estimates of 3-year cumulative risk were quite similar to those generated in the ATHENA trial, which was presented to support FDA-approval of primary HPV testing. Specifically, for the 3 cobas channels, the KPNC versus ATHENA estimates were quite close, as follows: HPV16 (18.5% vs. 25.2%, respectively), else HPV18 (7.8% vs. 11.0%) else HR (4.3% vs. 5.4%). (18)

Clinical performance among women with HPV-positive, Pap-negative results among women aged 30 and older supports recent consensus guidelines regarding co-testing, which recommend colposcopy for those with HPV16 (6, 7). In both the primary HPV testing and co-testing scenarios, the 3-year cumulative risk of CIN3+ (or CI for women with HPV16 was substantially greater than the benchmark risk thresholds for colposcopy, i.e., higher than the risks associated with LSIL or HPV-positive ASC-US in the same populations.

In contrast, in both cases (primary HPV testing or co-testing), the 3-year risk of CIN3+ (or CIN2+) was not particularly elevated (for all HPV-positive women aged 25 and older or those 30 and above with negative cytology, respectively) among HPV18-positive women in the absence of HPV16. It is not surprising that our 3-year analyses based on CIN3+ or CIN2+ did not demonstrate increased risk linked to HPV18. The clinical concern of HPV18 infection is not based on short-term risk of precancer, rather it is based mainly on the link between HPV18 and hard-to-detect precancerous and invasive lesions (particularly glandular lesions) that are observed in prospective studies with longer follow-up periods (19, 20).

The 2012 guidelines do not recommend HPV typing of women with HPV-positive ASC-US; such women all are currently referred to colposcopy (7). However, our findings, in contrast to the scant data considered for the management guidelines, suggest that the absence of HPV16 might lower risk below the threshold for colposcopy. If confirmed and found to be cost-effective, this finding could lead to consideration of HPV typing for HPV16 (and HPV18, for the aforementioned separate reason) as part of ASC-US triage.

There are limitations to our large performance study of the cobas HPV test. The study was limited to a large convenience sample of longitudinal specimens from women that initially tested positive by HC2, using the leftover routine cotest specimen collected after cytology. An analysis in which cobas was used to determine HPV positivity might have yielded slightly different results, despite the fact that the two HPV tests have similar performance (16). Our cobas-LA comparison included only Roche testing; however, to permit 3-year cumulative risks necessitated including some MY09/11 PCR results from another laboratory; therefore, our risk stratification estimates are not based solely on cobas data. Restricting to cobas data led to the same conclusions, despite artificial lowering of the cumulative risk estimates (data not shown). Our HC2-negative specimens were derived from follow-up of women who were initially positive for HPV by the HC2 test, and cannot be generalized to the larger HPV-negative population. Also, both cobas and LA testing were performed out of a remaining aliquot of the HC2 test specimen, collected into the Qiagen STM buffer, denatured and neutralized. This is not an FDA-approved process for cobas testing. These factors limit, albeit to an unknown extent, the generalizability of our findings to cobas performed in the FDA-approved manner. Finally, it is worth noting that this study was conducted at a large integrated health system in the US, not in a population-based screening program like those in some other countries. The optimal screening and management strategies could differ when organized screening with wide coverage and high compliance with follow-up intervals are present. Specifically, reliance on longer periods of follow-up to differentiate benign HPV infections from those leading to precancer is more practical in an organized national program.

The strength of the study is its large size, which permitted a statistically powerful, detailed examination of cobas performance relative to LA typing and of typing relative to CIN3+ outcomes.

In conclusion, the results demonstrate that cobas and LA results show excellent but not perfect agreement. The data support the current clinical guideline recommending HPV16 typing in the management of HPV-positive women, among HPV-positive/Pap-negative women, and suggest that knowing HPV16 is absent might be useful in the management of HPV-positive women with ASC-US.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Sampling and testing methods for 3 subcohorts for which 3-year cumulative risks of CIN3+ and CIN2+ were estimated. HC2+ enrollment specimens were tested for HPV16, else HPV18, else 12 other high-risk types.

a. ASC-US TRIAGE: Sample size of women with HC2+ ASC-US, aged 21 and older (median 33), tested by cobas or MY09/MY11 PCR

		Tested by cobas		**Tested by MY09/MY11 PCR or cobas**	
Case-control status	**Total N in Subcohort**	**N**	**% of Total**	**N**	**% of Total**
Control	11966	1244	10.4%	1670	14.0%
Prevalent CIN2+	1300	528	40.6%	1171	90.1%
Incident CIN2+	624	426	68.3%	450	72.1%

b. COTESTING: Sample size of women with HC2+/negative cytology, aged 30 or older (median 40), tested by cobas or MY09/MY11 PCR

		Tested by cobas		Tested by MY09/MY11 PCR or cobas	
Case-control status	Total N in Subcohort	N	% of Total	N	% of Total
Control	17425	2773	15.9%	3490	20.0%
Prevalent CIN2+	204	25	12.3%	194	95.1%
Incident CIN2+	1176	897	76.3%	970	82.5%

c. PRIMARY HPV TESTING: Sample size of women with HC2+, aged 25 or older (median 38), tested by cobas or MY09/MY11 PCR

		Tested by cobas		Tested by MY09/MY11 PCR or cobas	
Case-control status	Total N in Subcohort	N	% of Total	N	% of Total
Control	35134	4708	13.4%	6047	17.2%
Prevalent CIN2+	3290	761	23.1%	2989	90.9%
Incident CIN2+	2315	1683	72.7%	1827	78.9%

A control was Hybrid Capture 2 (HC2) positive at enrollment but, during observation, never developed CIN2+. A prevalent case of CIN2+ (or CIN3+) was diagnosed after enrollment (HC2+) screening, prior to a second screen. An incident case of CIN2+ (or CIN3+) was HC2+ at enrollment, but diagnosed after at least the second screen. The table demonstrates that cobas data were supplemented by MY09/MY11 PCR data mainly for prevalent cases of CIN2+. Combining both testing methods, a high percentage of cases of CIN2+ were typed, permitting reasonably precise estimation of cumulative risk. A smaller fraction of HC2+ controls was tested.

Table 2

Comparison of hierarchical HPV results from cobas and LINEAR ARRAY (LA), grouped as HPV16 else HPV18 else 12 high-risk (HR) HPV else negative for 14 targeted types.

	Cobas HPV16	**Cobas HPV18**	**Cobas High risk HPV**	**Cobas HPV negative**	**Total**
Linear Array Result	**#**	**#**	**#**	**#**	**#**
LA HPV16	**3229**	5	46	40	3320
LA HPV18	40	**904**	21	7	972
LA High risk HPV	230	57	7767	537	8591
LA HPV Negative	74	45	446	**3728**	4293
Total	3573	1011	8280	4312	17176

Kappa statistic = 0.86 (95% CI 0.86-0.87, indicating excellent overall agreement), test of symmetry $p < 0.0001$ (indicating relatively increased HPV16 and HPV18 positivity of cobas relative to LA). Of note, all specimens with both LA and cobas paired results were tested, including enrollment specimens (all HC2-positive) and subsequent longitudinal specimens from the same women (many of which were HC2-negative)

Frequency Missing = 86

Table 3

Individual type % positivity by cobas, restricted to samples positive by LA for only one of 14 cobas-targeted high-risk types. Multiple infection with other, i.e., low-risk types, was not reason for exclusion. HPV16 positivity by LA was compared with the cobas HPV16 channel, HPV18 positivity by LA was compared with the cobas HPV18 channel, and 12 other high-risk types by LA were compared with the cobas HR12 channel.

LINEAR ARRAY (LA) Single HPV Type	Total Number LA Single-Type Positive	% cobas Positive on Corresponding Channel
HPV16	2143	98.4%[*]
HPV18	603	98.5%[*]
HPV31	1108	98.9%
HPV33	258	100.0%
HPV35	407	96.8%
HPV39	626	96.7%
HPV45	450	97.8%
HPV51	594	90.2%
HPV52	1223	80.1%
HPV56	516	99.0%
HPV58	648	83.8%
HPV59	506	95.3%
HPV66	391	98.0%
HPV68	292	96.6%

[*] 1.5% of 7622 LA negative for HPV16 (among this group with single-type infections) were cobas-positive for HPV16, and 0.6% of 9162 LA negative for HPV18 were cobas positive for HPV18.

Table 4

Use of cobas-like partial HPV typing in triage of ASC-US. 3-year cumulative risk of CIN3 or worse (CIN3+) among women with Hybrid Capture 2–positive (HC2+) ASC-US, aged 21 and older, stratified by HPV type status (HPV16, else HPV18, else other 12 high-risk (HR) types targeted by cobas).

	HPV-type positive			**HPV-type negative**		
	3-yr risk of CIN3+	**Lower CI**	**Upper CI**	**3-yr risk of CIN3+**	**Lower CI**	**Upper CI**
HPV16	14.5%	13.5%	15.5%	3.5%	3.3%	3.6%
HPV18	4.8%	4.1%	5.7%	3.3%	3.2%	3.5%
HPV HR	3.5%	3.3%	3.6%	2.8%	2.5%	3.2%

The estimated CIN3+ risk among all women with ASC-US testing HC2+, prior to typing, was 5.2%; by current US guidelines all would be referred to colposcopy.

Table 5

Use of cobas-like partial HPV typing in co-testing. 3-year cumulative risk of CIN3 or worse (CIN3+) among women with Hybrid Capture 2–positive (HC2+) negative cytology, aged 30 and older, stratified by HPV type status (HPV16, else HPV18, else other 12 high-risk (HR) types targeted by cobas).

	HPV-type positive			HPV-type negative		
	3-yr risk of CIN3+	**Lower CI**	**Upper CI**	**3-yr risk of CIN3+**	**Lower CI**	**Upper CI**
HPV16	10.3%	9.6%	11.1%	2.3%	2.2%	2.4%
HPV18	5.0%	4.3%	5.8%	2.1%	2.0%	2.2%
HPV HR	2.3%	2.2%	2.5%	1.4%	1.4%	1.6%

The estimated CIN3+ risk among all women aged 30 and older testing HC2+ with negative cytology, prior to typing, was 3.5%; by current US guidelines all would be recommended to return for retesting in one year.

Table 6

Use of cobas-like partial HPV typing in primary HPV testing. 3-year cumulative risk of CIN3 or worse (CIN3+) among women with Hybrid Capture 2–positive (HC2+) results, aged 25 and older, stratified by HPV type status (HPV16, else HPV18, else other 12 high-risk (HR) types targeted by cobas).

	HPV-type positive			**HPV-type negative**		
	3-yr risk of CIN3+	**Lower CI**	**Upper CI**	**3-yr risk of CIN3+**	**Lower CI**	**Upper CI**
HPV16	18.5%	17.8%	19.2%	4.3%	4.2%	4.4%
HPV18	7.8%	7.2%	8.5%	4.0%	3.9%	4.1%
HPV HR	4.3%	4.2%	4.4%	3.0%	2.8%	3.2%

The estimated CIN3+ risk among all women aged 25 and older testing HC2+, prior to typing, was 6.7%; the current US interim guidance would recommend, in the absence of typing, return for retesting in one year. Note that there is no true HPV-negative group in this table; also, roughly half of the women aged 25-29 had ASC-US being tested for triage. Thus, this is not a complete, general screening population.