ROLE OF HPV GENOTYPE, MULTIPLE INFECTIONS AND VIRAL LOAD ON THE RISK OF HIGH-GRADE CERVICAL NEOPLASIA

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ABSTRACT

Background: HPV testing provides a much more sensitive method of detection for high-grade lesions than cytology, but specificity is low. Here we explore the extent to which full HPV genotyping, viral load and multiplicity of types can be used to improve specificity.

Methods: A population-based sample of 47,120 women undergoing cervical screening were tested for 13 high-risk HPV genotypes. Positive predictive values (PPV) for CIN grade 2 or worse (CIN2+; N=3449) and CIN3 or worse (CIN3+; N=1475) over three years of follow-up were estimated for HPV genotype and viral load. Weighted multivariate logistic regression models were used to estimate the odds of CIN2+ or CIN3+ according to genotype, multiplicity of types and viral load.

Results: High-risk HPV was detected in 15.4% of women. A hierarchy of HPV genotypes based on sequentially maximizing PPVs for CIN3+ found HPV16>33>31 to be the most predictive, followed sequentially by HPV18>35>58>45>52>59>51>39>56>68. After adjusting for higher ranked genotypes, multiple HPV infections added little to risk prediction. High viral loads for HPV18, 35, 52 and 58 carried more risk than low viral loads for HPV16, 31 and 33. High viral load for HPV16 was significantly more associated with CIN3+ than low viral load.

Conclusion: HPV genotype and viral load, but not multiplicity of HPV infections, are important predictors of CIN2+ and CIN3+.

Impact: The ability to identify women at higher risk of CIN2+ and CIN3+ based on both HPV genotype and viral load could be important for individualising triage plans, particularly as HPV becomes the primary screening test.

INTRODUCTION

Cervical cancer is caused by infection from one or more of at least thirteen high-risk human papillomavirus (HPV) genotypes.(1,2) HPV testing provides a more sensitive method of detection for high-grade lesions than cytology.(3,4) Different HPV genotypes have different natural histories and it has become increasingly important to identify which genotypes are most indicative of an increased risk of developing a high-grade cervical lesion. Advances in HPV-based testing have enabled infections with individual HPV genotypes to be routinely determined, but currently only types 16 and 18 are widely reported and used to guide clinical management.(5,6) Previous reports have indicated differing risks associated with different genotypes.(7-9) Other HPV related factors associated with high-grade disease include viral load,(10-13) multiplicity of types(14-16) and methylation status,(17-20) as well as cytology and $p16^{Ink4a}$ which both require intact cellular preparations.

High viral load has been shown to be important for HPV16,(11,12,21-24) but more recently, Xi *et al*.(25) reported an association between cervical intraepithelial neoplasia grades 2 and 3 (CIN2/3) and high viral loads for other alpha-9 HPV species. Women with high viral loads have also been found to have more persistent infections, with longer clearance times.(26,27) However, Sherman *et al*.(28) showed that although viral loads were higher in women with CIN diagnoses than women with negative histology, there was no trend correlating viral load and severity of CIN grade. Current evidence about the effect of multiple HPV infections is conflicting; some studies have shown that co-infections increase a woman's risk of cervical pre-cancer and cancer,(29) whilst others show no impact.(14,30) Further, it has been shown that HPV positive women with elevated methylation levels of both human and viral genes have an increased risk of pre-cancerous lesions and cancer,(17,19,31,32) but this, and cytology and p16 which require cellular preparations, will not be explored in the study we report on here.

We examined the risk of CIN2+ and CIN3+ associated with different HPV genotypes and assess whether there is additional risk associated with genotype-specific high viral load and multiple HPV infections.

MATERIALS AND METHODS

Study Population

A population-based stratified sample of all women who underwent cervical screening in the state of New Mexico between December 2007 and April 2009 was used for this study. Data was obtained from the New Mexico HPV Pap Registry (NMHPVPR). The NMHPVPR is a state-wide public health surveillance program established in 2006 to assess all aspects of cervical cancer preventive care. It includes records of all cervical cytology and HPV tests, and all cervical, vaginal and vulvar pathology. Laboratories performing cervical cytology, pathology, and HPV tests on individuals residing in New Mexico are required to report all results to the NMHPVPR under NMAC 7.4.3.(33) Specimens were collected from selected laboratories under research protocols approved by the University of New Mexico Human Research Review Committee.

Residual material from liquid-based cytology (LBC) samples in 7 in-state laboratories were collected, stratified by age (≤30 years versus >30 years) and cytology outcome (negative or abnormal). The sampling plan targeted all specimens with abnormal cytology, along with 45% of specimens from women aged ≤30 years with negative cytology, and 8% of specimens with negative cytology from women aged >30 years. A total of 59,644 specimens were included for genotyping. The sample was further restricted to 'screening cytology' defined as LBC samples from women with no previous cytology in the past 300 days, and women <15 years or >75 years were excluded. This resulted in a sample of 47,120 women (Supplementary Figure 1). Although samples were chosen based on proportions of available samples, sampling weights that were applied in this report were based on the first screening sample per woman (Supplementary Table 1). Full details of this cohort have been described previously.(34-36) Follow-up was for 3 years after the collection of their screening specimen, and the worst histopathologic diagnosis identified in this period was used as the endpoint.

Laboratory Methods

Broad spectrum HPV genotyping was performed using the Roche LINEAR ARRAY (LA) HPV GENOTYPING test (Roche Diagnostics, Indianapolis, Indiana USA) on residual LBC specimens. The Roche LA genotyping test, which has been described in detail previously,(35) identifies 37 genotypes, of which 13 are high-risk (hr) HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and the remaining 24 are low-risk (lr) types.

Viral load was visually determined as low, intermediate or high by two independent reviewers for each genotype detected based on intensity of staining on the test strips. Before undertaking this task readers calibrated their semi-quantitative interpretation of viral load on a sample of readings to densitometry values and reference standards. Any discrepant results between the two readers for either HPV genotype or viral load were reviewed by a third independent reader and the determination of the third reader was final.

Statistical Analysis

Prevalence and positive predictive values (PPV) were calculated for hrHPV types, both overall and for each genotype in single and multiple infections. All analyses were weighted to reflect the number of first screening samples in the state-wide population (Supplementary Table 1). Hierarchical rankings of HPV genotypes for CIN2+ and CIN3+ were formed based on sequentially maximizing the PPV for the new genotype when infections which also contained HPV types higher in the hierarchy were omitted. Hierarchies were created both overall and within two age strata (≤30y and >30y). Cumulative sensitivity and specificity for increasing numbers of genotypes ordered by the hierarchy was plotted as a receiver operator characteristic curve (ROC). PPVs were also calculated for each of the thirteen hrHPV types stratified by viral load (low, intermediate, high). Viral load was determined separately for each genotype when more than one type was present, and hierarchies were created using genotype only and also using both genotype and viral load. Weighted multiple

logistic regression models were also fit to estimate the odd ratios (OR) of CIN2+ and CIN3+ for the joint effects of HPV genotype and viral load.

All analyses were conducted in STATA 13.1.

RESULTS

Cervical screening data and residual LBC samples were used for 47,120 women and estimated to represent 328,427 women in New Mexico who had a screening test between December 2007 and April 2009. The mean age of the weighted population was 40.3 years. 1,893 (0.6%) women had high-grade cytology (high-grade squamous intraepithelial lesion (HSIL), atypical squamous cells – cannot exclude HSIL (ASC-H), adenocarcinoma *in situ* (AIS) or cancer), and a further 19,946 (6.1%) had low-grade cytological abnormalities (low grade squamous intraepithelial lesion (LSIL) or atypical squamous cells of undetermined significance (ASC-US)). We estimated 27.4% women had at least one hr- or lrHPV infection and 15.4% had at least one hrHPV infection. Of those with at least one hrHPV infection, 25.1% had multiple hrHPV infections. In women aged ≤30 years, the prevalence of hrHPV infections was 28.1% compared to 9.2% in women aged >30 years ($p<0.001$). The prevalence of HPV16 was 3.5% overall and 1.4% (39.6% of all HPV16 infections) were single infections (Table 1). HPV16 was present in 22.7% of all hrHPV infections. On a population basis 1.1% of women were diagnosed with CIN2+ and 0.5% with CIN3+. The sensitivity for CIN2+ and CIN3+ in the 3 years after being positive for any hrHPV type was 79.6% and 88.1% respectively. The specificity for <CIN2 was 85.3%.

HPV Type Hierarchy

HPV genotypes were selected sequentially to maximize the PPV for CIN3+ endpoints and separately for CIN2+ endpoints among women who did not have HPV infections from higher risk types. This resulted in similar hierarchies for both endpoints. The resulting overall rank orders were HPV16>33>31>18>35>58>45>52>59>51>39>56>68 for CIN3+ and HPV16>33>31>35>18>58>51>45>39>52>59>56>68 for CIN2+ (Table 2 & Supplementary Table 2). Genotypes 16, 33 and 31 had the highest PPVs for CIN3+ and provided a cumulative sensitivity of 66.5% and specificity of 94.8%. Cumulative sensitivities and specificities for CIN2+ and CIN3+ as the number of HPV types included from the hierarchy

were sequentially increased are plotted in Figure 1. Types 56 and 68 had the lowest PPVs (<0.2% for CIN3+) and should probably not be considered 'high-risk' types, but either omitted or called 'intermediate-risk' types. HPV positivity was higher for women aged ≤30 years versus those aged >30 years and the PPV for CIN3+ was also higher in younger women (2.9% vs 2.1%, p<0.001). The CIN3+ hierarchy for women aged ≤30 years was similar to the overall rank order, except HPV18 ranked lower (8^{th} vs 4^{th} place). (Supplementary Table 3a&3b). For woman aged >30 years, HPV18 was ranked third, and HPV35 moved down in the hierarchy, although there were no significant differences in the PPV values. The PPV for CIN2+ for all hrHPV types combined was also greater for women aged ≤30 years compared to women aged >30 years (6.6% vs 3.7%, p<0.001).

The PPV for CIN3+ was greatest for HPV16, being 7.0% (95% Confidence Interval (CI) (6.5, 7.5)). The PPV for HPV33 was slightly lower at 4.9% (95% CI (3.9, 6.0)) univariately, and when multiple infections with HPV16 were excluded it was 3.3% (95% CI (2.4, 4.4)) (Table 2). Similar results were seen for CIN2+ (Supplementary Table 2).

Using the highest ranking HPV type within the hierarchy for each woman when multiple infections were present, weighted logistic regression models were fit. After excluding individuals with multiple infections with types higher in the hierarchy, the odds of having CIN3+ were statistically significant for all hrHPV types except HPV56 and 68 (OR=2.5, 95% CI (0.6, 10.8) and OR=1.7, 95% CI (0.2, 12.9), respectively) (Table 3).

Viral Load

Viral load was scored as high, intermediate or low as detailed in the methods. Overall 36.7% of hrHPV infections were scored as high, 26.5% as intermediate and 36.8% as low. When considering the highest ranked HPV type per women the odds of CIN3+ were 4.9 (95% CI (3.4, 7.3)) times higher for high compared to low levels. The PPV for CIN3+ was above 5% for high level infections of HPV16 and intermediate level infections for HPV33, and the PPV was <2% for low level infections of all genotypes except HPV16 (Table 4). When considering

only the highest rank ordered genotype in the hierarchy if there were multiple HPV types detected, there was significant heterogeneity in viral load level for all HPV types (P<0.001, Table 5). For HPV16, 53.9% of HPV infections were scored as high viral load whereas only 41.5%, 15.2% and 14.9% were high for HPV33, 31 and 35 respectively. For HPV16 the odds of a woman having CIN3+ was 5.5 (95% CI (2.6, 11.7)) times greater for high versus low viral load (Table 5). After omitting the lower level infections in the hierarchy when there were multiple types, the odds of a woman having CIN3+ was greater for high versus low viral loads for HPV16 and 33. For CIN2+ the odds were significant for the first six HPV types (HPV16, 33, 31, 35, 18 and 58; HPV58 OR=5.5, 95% CI (1.8, 17.4)) (Supplementary Table 4). However, for most genotypes, the odds of having CIN3+ or CIN2+ was still significantly higher for low level infections compared to women who were negative (Table 5 & Supplementary Table 4). When allowing for an intensity interaction with age, overall there was no statistical evidence for a difference in risk by viral load level between women aged ≤30 years versus >30 years. However, there was evidence that high viral load of HPV16 increased the risk significantly more for women >30 years (CIN3+ OR 30.7 vs 86.7, $p<10^{-5}$). Further, there was some suggestion that high viral load of HPV18 and 58 also increased the risk significantly more in older women (hierarchical CIN3+ increase of 5.5 for HPV18 [p=0.001], and 5.1 for HPV58 [p=0.004]), but no other types showed a clear age interaction with intensity. Hierarchically, HPV16, 18 and 45 all had over 50% of HPV infections classified as high viral load. There was no difference between low and intermediate viral load by age overall or for any individual HPV type.

PPVs based on a bivariate model for individual HPV genotypes and viral load are shown in Table 4. As anticipated by the logistic regression analyses, there was a trend for increasing PPV with viral load for most HPV types, but especially for HPV16 (CIN2+ PPVs increased from 3.9% to 6.6% to 17.6% for low, intermediate and high viral loads respectively; trend $\chi^2=401.7$, $p<10^{-72}$). The trend in PPVs for CIN2+ with increasing viral load level was significant for all hrHPV types except HPV39 and 68. High viral load infections with HPV16,

33, 31 and 35 posed the greatest risk for CIN2+. However, high viral loads of HPV18 and 58 posed the same risk as intermediate viral loads of HPV16, 33, 31 and 35.

Similarly, for detection of CIN3+, PPVs increased from 2.1% to 3.6% to 10.6% for low, intermediate and high viral loads of HPV16, respectively (trend χ^2=247.5, $p<10^{-56}$). For CIN3+ the increase was significant for the first six HPV types in the hierarchy (HPV16, 33, 31, 18, 35 and 58), but the number of cases was too limited further down the hierarchy to make reliable inferences.

Multiple HPV Types

The inclusion of multiple infections with HPV types lower in the hierarchy added little to the risk prediction for CIN2+ or CIN3+ overall or when restricted to hrHPV types. There was a borderline significant increased risk of CIN3+ for women when adding any other lower risk HPV types to HPV35 (OR=2.7, 95% CI (0.9, 8.0) p=0.07), but no other specific type had a significant increase (Table 3).

DISCUSSION

The ability to identify women at higher risk of CIN2+ and CIN3+ based on both HPV genotype and viral load will be important for individualising triage plans, particularly when HPV is the primary screening test. The NMHPVPR provides a unique opportunity to investigate the effect of HPV genotyping, multiple HPV infections and viral load on the risk of high-grade CIN and cancer in a large population-based screening cohort. Use of the Roche LA HPV genotyping test enabled analyses of different genotypes, both in individual and multiple HPV infections, an area where current research has produced conflicting findings. Consistent with previous research, we found HPV16 to be the most prevalent HPV type. Overall 11.4% of women had multiple HPV infections, consistent with findings from Monsonego *et al*.(37) who showed prevalence of multiple HPV infections in a US screening population after using a hierarchical ranking to be 13.4%.

Ranking of HPV types by PPV provided similar hierarchies for CIN2+ and CIN3+, with HPV16, 33 and 31 posing the greatest risk of pre-cancerous disease. Notably, HPV33 and 31 were both ranked above HPV18. Recent research has emphasized the importance of HPV genotypes that are phylogenetically similar to HPV16.(38) The thirteen hrHPV genotypes can be clustered into species with more similar DNA sequences. Notably species alpha-9, which includes HPV16, 31, 33, 35, 52 and 58, is most associated with high disease risk, and the top three ranking HPV types we observed were all within the alpha-9 species. Although HPV35 was not common in this population, as there are few African Americans in New Mexico (2.5%), there are populations with high levels of African lineage, thus its prominent position in the hierarchy indicates its importance more broadly. When only considering the top 3 HPV types (HPV16, 33 and 31), their cumulative sensitivity was 66.5% for CIN3+, indicating they are not sensitive enough to be the sole screening test. However, their combined PPV was 5.5% versus 0.2% for the remaining high-risk types, so they can be useful for deciding upon the need for immediate colposcopy versus repeat testing at a 6 or 12 month interval.

Our previous studies that have used hierarchical ranking methods have also found similar rank orders; Cuzick *et al.*(7) reported a ranking based on PPVs for CIN3+ in a referral population with HPV16 and 33 having the highest ranks. In a further sample of HPV positive women aged ≥30 years, Schiffman *et al.*(39) found the HPV types with the greatest 3-year risk of CIN2+ were HPV16, 52 and 31. However, their study was based on disease prevalence and did not adjust for genotype prevalence and therefore used a different measure than PPV as used here. PPV is particularly important for HPV33. This type has a low prevalence but high PPV, and when present should be managed similarly to HPV16. Several HPV tests offer individual genotyping(7,39-41) and an HPV hierarchy helps to identify specific genotypes that pose the greatest risk of high-grade CIN, and thus can assist in improving the triage process for clinical management of HPV positive women. Currently HPV tests approved by the US Food and Drug Administration (FDA) only offer individual genotyping for HPV16, 18 or 45, but findings from our data show the importance of HPV31 and 33 as high-risk genotypes, and the value of downgrading HPV types 39, 51 and 59 to 'intermediate risk' types, although HPV51 was considerably higher in the CIN2+ hierarchy. This emphases the need for more complete hrHPV genotyping assays if the principle of equal management for equal risk is to be applied. Of note, HPV52 did not exhibit substantial risk here, but has been seen to do so in other populations.(42) When using the LA HPV Genotyping test, HPV52 is only inferred if co-infections with HPV33, 35 or 58 are not detected, so it will be underestimated in our study. This effect will be small, and under the assumption that the prevalence of these types is independent, HPV52 prevalence would only increase from 1.92% to an estimated 1.96% (Table 1). We did not supplement the HPV genotyping in this large population-based evaluation using HPV52 type-specific PCR although this could be an area of future research as suggested by others.(43),(44)

In our study, co-infections with HPV types lower in the hierarchy did not significantly increase the risk of CIN2+ or CIN3+ beyond that for the highest risk type found. Similar findings have previously been reported. Schmitt *et al*.(15) found that the occurrence of multiple HPV

infections did not affect the risk of a lesion being high or low-grade and Wentzensen *et al*.[16] found no association between disease status and the number of genotypes detected in a woman. Previous studies showing increased risk of CIN with multiple HPV infections had few CIN2+ cases, and were restricted to younger women,(45) a subgroup known to harbour a larger number of HPV infections.(14)

HPV16 is the genotype with the highest PPV for high-grade precursor lesions, especially when the viral load is high. It's PPV of 17.6% overall is well above the 10% threshold for CIN2+ (PPV 10.6% for CIN3+) suggested for determining immediate referral for colposcopy.(5,46,47) However, much of this previous research has not found an association between viral load and CIN2+ for other genotypes, possibly due to small sample sizes. In our study, after adjusting for multiple infections with types higher in the hierarchy, we found the risk for CIN2+ was above 10% only for high viral load infections for HPV types 16, 33, 31 or 35 and above 5% only for high or intermediate level infections with these types or high level infections with HPV18 or 58 (Table 4). Our finding that viral load was relevant for HPV18 is not in agreement with previous studies which found quantification of HPV18 had little predictive power and thus the clinical utility of this finding requires confirmation.(42,48) Notably, even a high viral load of HPV18 only carried a CIN2+ PPV of 7.9% (PPV 2.5% for CIN3+), which is still below the conventional 10% threshold for immediate colposcopy.

Noticeably, the top ranked HPV types for CIN risk are the seven high-risk types in the nonavalent vaccine (HPV16, 18, 31, 33, 45, 52 and 58) and HPV35. However, a key finding from this paper is that the risk can be substantially modified by viral load and thus a full risk stratification policy needs to include both HPV genotype and viral load. High levels of HPV types lower in the hierarchy (e.g. HPV18 and 58) pose a similar risk of disease to intermediate levels of higher risk types (HPV16, 33, 31, 35). The importance of viral load compared to HPV genotype has not been widely appreciated, but when considered together can improve assessments of the risk of a high-grade CIN lesion.

A full triage strategy will require consideration of other measures beyond the scope of this investigation, including cytology and potentially p16 status and HPV methylation in addition to HPV genotype and viral load information to guide management. Of note as well are HPV18 and 45 which are not strong predictors of CIN2+ or CIN3+, but are more common in cancer. They are associated with endocervical cancers, and their precursor lesions are not so easily seen at colposcopy. If HPV18 and 45 infections remain persistent more careful exploration of the endocervical canal by new methods may be needed, especially in older women (>30yrs) where HPV18 poses a greater risk. Whilst these results were not used for clinical management, if age categories were modified to put 30 year olds in the older age group to align with current screening recommendations, the conclusions from this study remain unchanged.

Further work on viral load is warranted before it can be used routinely, especially to standardise viral load measurements. While semi-quantitative estimates of viral load were important in our analysis, the biology behind viral load is complex. Low-grade lesions with koilocytes can have thousands of copies of HPV per cell and one cell can contribute more DNA than a hundred CIN3 cells with 10 copies per cell. Thus, viral load is a complex correlate of the interplay of grade, lesion size, sampling of lesions etc. In addition, none of the platforms currently approved by the FDA allow for routine reporting of viral load.

Little variation was seen in the hierarchy for different genotypes when the PPVs were based on both genotype and viral load, (Supplementary Table 5) compared with using only genotype (Table 2). In particular, the top ranking HPV types (HPV16, 33 and 31) did not change. However viral load was important e.g. low viral loads of HPV16, 31 and 33 were lower in ranking than high levels of HPV18, 35, 52 and 58. This emphasises the management benefits which could be gained if HPV genotyping and viral load were both used.

One of the strengths of this study was the access to a large population-based stratified screening sample. However, the study had some limitations; histology outcomes were only

available for women who were referred to colposcopy because of cytological abnormalities. Thus CIN2+ lesions arising from infections not producing a detectable cytological abnormality would have been missed. CIN determination was based on routine clinical practice and while all cases diagnosed within 3 years were included, a closeout visit at 3 years, as would be common for a clinical trial, would result in higher disease detection rates. Analyses of viral load were based on a visual 3-level cut off criteria - high, intermediate and low, and further work is needed to determine if its assessment would benefit from more precise quantitation. Additionally, different HPV tests assess viral load in different ways e.g. many PCR based methods use cycle threshold (CT) values, Hybrid Capture 2 uses signal-amplified luminescence levels (relative light units) to establish cut-off values, and our method based on LA genotyping uses visual assessment based on colorimetric precipitate observed as lines or “bands” on a solid-phase matrix. However, semi-quantitation via the LA genotyping test has been supported by its correlation with a gold standard of quantitative PCR.(49) As noted above, the importance of viral load appears to be genotype-specific, so an overall combined result for any hrHPV type may be less informative than the type-specific viral load, as provided here.

To conclude, in a large population-based stratified screening sample of women we found the risk of high-grade CIN was dependent on both the HPV genotype and viral load, with no added risk associated with co-infections from other HPV types lower in the hierarchy. Algorithms based on both HPV genotype and viral load in combination show promise for refining clinical management of hrHPV positive women, and reducing the number of women who are currently recommended to have immediate colposcopy.

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Table 1: Prevalence of 13 high-risk (hr) HPV genotypes, weighted to the state-wide population of women. For all, single, and multiple hrHPV genotypes.

hrHPV type	Prevalence (%)		
	All	Single	Multiple – with other hrHPV types
Any hrHPV	*15.37*	*6.59*	*8.78*
16	3.48	1.38	2.11
18	1.21	0.32	0.89
31	1.82	0.60	1.21
33	0.48	0.14	0.34
35	0.89	0.31	0.58
39	2.11	0.69	1.42
45	1.06	0.31	0.75
51	2.30	0.65	1.65
52	1.92	0.63	1.29
56	1.26	0.27	0.99
58	1.20	0.33	0.87
59	2.11	0.70	1.40
68	0.82	0.26	0.56

Table 2: High-risk (hr) HPV genotype-specific Positive Predictive Values (PPV) and hierarchical ranking by PPV for CIN3+ detected within 3 years of the enrolment cytology, weighted to the state-wide population.

	Individual hrHPV type	For the new hrHPV type				Cumulative				
hrHPV type	PPV (%) (95% CI)	N at risk	Positive		PPV (%)	Positive		PPV (%)	Sensitivity	Specificity
			CIN3+	HPV		CIN3+	HPV			
16	6.98 (6.51, 7.45)	328 427	799	11 445	6.98	799	11 445	6.98	54.17	96.74
33	4.94 (3.87, 6.01)	316 981	45	1360	3.31	844	12 805	6.59	57.22	96.34
31	3.47 (3.01, 3.94)	315 622	136	5139	2.65	980	17 944	5.46	66.47	94.81
18	2.10 (1.65, 2.54)	310 483	56	3084	1.82	1 036	21 027	4.93	70.28	93.89
35	2.10 (1.58, 2.62)	307 399	34	2211	1.53	1 070	23 238	4.61	72.57	93.22
58	2.25 (1.78, 2.71)	305 189	36	2860	1.26	1 106	26 098	4.24	75.01	92.36
45	2.49 (1.97, 3.00)	302 329	31	2595	1.18	1 137	28 693	3.96	77.09	91.57
52	2.22 (1.85, 2.58)	299 734	53	4737	1.11	1 190	33 431	3.56	80.66	90.14
59	1.64 (1.34, 1.94)	294 996	46	4668	0.98	1 236	38 099	3.24	83.78	88.73
51	1.73 (1.44, 2.02)	290 328	42	4634	0.90	1 277	42 732	2.99	86.61	87.32
39	1.71 (1.40, 2.01)	285 694	17	4108	0.41	1 294	46 840	2.76	87.76	86.07
56	1.00 (0.69, 1.30)	281 587	3	2171	0.16	1 298	49 011	2.65	87.99	85.41
68	0.98 (0.61, 1.35)	279 416	2	1462	0.11	1 299	50 473	2.57	88.09	84.96

CI: Confidence Interval

For example, the highest ranked HPV type was the genotype with the highest univariate PPV. All co-infections with this HPV type were then excluded from the subsequent analysis, and the HPV type with the next highest PPV was identified. This was repeated for all 13 hrHPV types.

Table 3: Hierarchical odds ratios for CIN3+ among 13 high-risk (hr) HPV genotypes, both alone and with adjustment term for multiple hrHPV types ranked lower in the hierarchy, weighted to the state-wide population of women.

hrHPV type	Univariate Model	P-value	Multivariate Model	P-value
	OR (95% CI)		OR (95% CI)	(other types)
16	35.12 (28.21, 43.71)	2.5E-220	40.62 (30.76, 53.66)	
other types			0.78 (0.60, 1.01)	0.06
33	17.10 (10.27, 28.47)	1.1E-27	27.50 (13.50, 56.00)	
other types			0.43 (0.17, 1.11)	0.08
31	17.09 (11.96, 24.44)	1.8E-54	20.60 (12.84, 33.03)	
other types			0.72 (0.42, 1.26)	0.25
18	12.99 (8.29, 20.33)	4.0E-29	18.87 (10.60, 33.59)	
other types			0.53 (0.25, 1.11)	0.09
35	11.69 (6.67, 20.50)	9.6E-18	6.07 (2.36, 15.61)	
other types			2.72 (0.93, 7.96)	0.07
58	10.47 (6.19, 17.71)	2.0E-18	15.05 (7.73, 29.31)	
other types			0.51 (0.21, 1.22)	0.13
45	10.59 (5.40, 20.74)	6.2E-12	12.11 (4.94, 29.68)	
other types			0.79 (0.24, 2.57)	0.70
52	11.62 (6.73, 20.05)	1.3E-18	11.20 (5.42, 23.17)	
other types			1.07 (0.48, 2.38)	0.88
59	12.06 (4.29, 33.89)	2.3E-06	6.88 (2.38, 19.87)	
other types			2.50 (0.50, 12.39)	0.26
51	13.14 (4.30, 40.19)	6.3E-06	6.08 (1.86, 19.87)	
other types			3.17 (0.59, 16.96)	0.18
39	6.45 (2.72, 15.29)	2.3E-05	6.65 (2.58, 17.13)	
other types			0.93 (0.21, 4.20)	0.93
56	2.47 (0.56, 10.83)	0.23	3.04 (0.40, 23.22)	
other types			0.68 (0.04, 11.10)	0.79
68	1.70 (0.22, 12.94)	0.61	2.96 (0.39, 22.75)	
other types			-	-

OR: Odds Ratio, CI: Confidence Interval

Table 4: Hierarchical Positive Predictive Values (PPV) for 13 high-risk (hr) HPV genotypes stratified by viral load groups (high, intermediate [Inter], low) for CIN2+ and CIN3+, weighted to the state-wide population of women.

HPV type	PPV (%) by viral load			P-value for trend[a]
	CIN2+			
	Low	Inter	High	
Any hrHPV[b]	2.38	4.46	9.28	5.3E-169
16	3.90	6.61	17.62	1.2E-72
33	4.43	9.41	10.29	1.1E-03
31	4.08	8.93	10.76	1.2E-12
35	3.62	6.03	10.10	1.2E-05
18	2.07	2.83	7.70	2.5E-09
58	1.18	2.22	6.20	4.0E-09
51	2.31	4.40	4.50	1.2E-04
45	1.32	2.35	3.38	0.01
39	1.99	2.78	2.11	0.63
52	0.40	1.16	4.57	6.4E-15
59	0.93	1.09	2.80	9.4E-05
56	0.17	2.45	1.32	0.01
68	1.02	0	0.82	0.74

HPV type	PPV (%) by viral load			P-value for trend[a]
	CIN3+			
	Low	Inter	High	
Any hrHPV[b]	0.98	1.90	4.66	5.3E-105
16	2.11	3.58	10.56	9.2E-56
33	1.00	6.20	3.93	0.01
31	1.75	4.32	1.77	0.05
18	1.25	0.95	2.52	0.01
35	0.85	1.97	2.50	0.01
58	0.22	0.29	2.20	1.5E-05
45	1.25	1.23	1.07	0.71
52	0.26	0.84	2.55	1.4E-09
59	0.42	0.65	1.51	8.7E-04
51	1.11	0.78	0.55	0.11
39	0.15	0.56	0.83	4.1E-03
56	-	-	0.45	
68	-	-	0.27	

CIN2+	<2%	2-5%	5-10%	>10%
CIN3+	<1%	1-2%	2-5%	>5%

Colour codes give categories of 3-year risk (<2% (green), 2-5% (yellow), 5-10% (orange) and >10% (red) for CIN2+; <1% (green), 1-2% (yellow), 2-5% (orange) and >5% (red) for CIN3+).

[a] P-values for trend in PPV by increasing viral load category.
[b] Highest ranked hrHPV type per woman

Table 5: Hierarchical odds ratios for CIN3+ for high-risk (hr) HPV genotypes and for different viral load groups (high, intermediate [Inter], low), ordered by the genotype hierarchy and weighted to the state-wide population of women.

HPV type	Viral load (N, %)	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Any hrHPV[a]	*High (18 541, 0.6)*	77.31 (46.48, 128.61)	*9.4E-63*	*4.94 (3.35, 7.28)*	*8.1E-16*	*2.53 (2.00, 3.19)*	*6E-15*
	Inter (13 374, 0.4)	*30.57 (17.87, 52.30)*	*1.0E-35*	*1.95 (1.28, 2.98)*	*2.0E-03*	*ref*	
	Low (18 558, 0.6)	*15.66 (8.42, 29.11)*	*3.6E-18*	*ref*			
	Negative (277 954, 84.6)	*ref*					
	P-value (trend)	*2.6E-123*		*3.0E-20*			
16	High (6171, 1.9)	55.28 (44.27, 69.03)	5.3E-271	5.48 (2.57, 11.67)	1.1E-05	3.18 (2.16, 4.69)	5.1E-09
	Inter (2431, 0.7)	17.36 (11.56, 26.08)	6.1E-43	1.72 (0.75, 3.94)	0.20	*ref*	
	Low (2844, 0.9)	10.09 (4.70, 21.67)	3.1E-09	*ref*			
	Negative (316 981, 96.5)	*ref*					
	P-value (trend)	2.8E-286		4.7E-08			
33	High (564, 0.2)	20.42 (10.45, 39.92)	1.2E-18	4.04 (1.07, 15.32)	0.04	0.62 (0.21, 1.83)	0.39
	Inter (287, 0.1)	32.97 (13.56, 80.19)	1.3E-14	6.53 (1.53, 27.84)	0.01	*ref*	
	Low (509, 0.2)	5.05 (1.55, 16.46)	7.2E-03	*ref*			
	Negative (315 622, 96.1)	*ref*					
	P-value (trend)	1.6E-31		0.04			
31	High (781, 0.2)	11.27 (4.76, 26.65)	3.5E-08	1.01 (0.39, 2.61)	0.98	0.40 (0.16, 0.99)	0.05
	Inter (1807, 0.6)	28.28 (18.23, 43.88)	3.6E-50	2.53 (1.40, 4.59)	2.2E-03	*ref*	
	Low (2551 0.8)	11.16 (6.65, 18.72)	6.7E-20	*ref*			
	Negative (310 483, 94.5)	*ref*					
	P-value (trend)	1.2E-65		0.14			
18	High (1553, 0.5)	18.07 (11.03, 29.61)	1.5E-30	2.05 (0.70, 6.02)	0.19	2.69 (0.90, 8.09)	0.08
	Inter (675, 0.2)	6.71 (2.36, 19.08)	3.6E-04	0.76 (0.18, 3.14)	0.70	*ref*	
	Low (855, 0.3)	8.83 (3.17, 24.57)	3.0E-05	*ref*			
	Negative (307 399, 93.6)	*ref*					
	P-value (trend)	2.8E-34		0.14			

Table 5: Continued.

HPV type	Viral load (N, %)	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
35	High (330, 0.1)	19.30 (7.36, 50.60)	1.8E-09	2.97 (0.70, 12.70)	0.14	1.27 (0.41, 3.97)	0.68
	Inter (848, 0.3)	15.16 (7.43, 30.91)	7.7E-14	2.34 (0.64, 8.52)	0.20	*ref*	
	Low (1032, 0.3)	6.49 (2.06, 20.43)	1.4E-03	*ref*			
	Negative (305 189, 92.9)	*ref*					
	P-value (trend)	2.8E-23		0.09			
58	High (1483, 0.5)	18.46 (10.69, 31.89)	1.5E-25	10.33 (1.36, 78.49)	0.02	7.87 (1.03, 60.04)	0.05
	Inter (595, 0.2)	2.35 (0.32, 17.34)	0.40	1.31 (0.08, 21.63)	0.85	*ref*	
	Low (781, 0.2)	1.79 (0.24, 13.15)	0.57	*ref*			
	Negative (302 329, 92.1)	*ref*					
	P-value (trend)	1.8E-23		0.01			
45	High (919, 0.3)	9.56 (3.94, 23.17)	5.8E-07	0.85 (0.22, 3.32)	0.82	0.87 (0.22, 3.44)	0.84
	Inter (749, 0.2)	11.03 (3.51, 34.68)	4.0E-05	0.98 (0.21, 4.62)	0.98	*ref*	
	Low (928, 0.3)	11.24 (3.62, 34.90)	2.8E-05	*ref*			
	Negative (299 734, 91.3)	*ref*					
	P-value (trend)	1.4E-13		0.82			
52	High (1391, 0.4)	27.06 (14.70, 49.82)	3.6E-26	9.93 (2.86, 34.39)	3.0E-04	3.10 (1.12, 8.57)	0.03
	Inter (1458, 0.4)	8.73 (3.31, 23.01)	1.2E-05	3.20 (0.75, 13.70)	0.12	*ref*	
	Low (1888, 0.6)	2.73 (0.82, 9.08)	0.10	*ref*			
	Negative (294 996 89.8)	*ref*					
	P-value (trend)	3.7E-26		9.9E-05			
59	High (2164, 0.7)	18.65 (4.91, 70.94)	1.8E-05	3.62 (0.50, 26.11)	0.20	2.34 (0.39, 14.08)	0.35
	Inter (1129, 0.3)	7.98 (2.10, 30.27)	2.3E-03	1.55 (0.21, 11.20)	0.66	*ref*	
	Low (1375, 0.4)	5.15 (1.08, 24.64)	0.04	*ref*			
	Negative (290 328, 88.4)	*ref*					
	P-value (trend)	1.7E-06		0.20			
51	High (876, 0.3)	8.03 (2.36, 27.36)	8.7E-04	0.50 (0.07, 3.56)	0.48	0.70 (0.15, 3.38)	0.66
	Inter (1466, 0.4)	11.42 (3.56, 36.65)	4.3E-05	0.70 (0.10, 4.86)	0.72	*ref*	
	Low (2292, 0.7)	16.21 (3.08, 85.18)	1.0E-03	*ref*			
	Negative (285 694, 87.0)	*ref*					
	P-value (trend)	3.5E-10		0.55			

Table 5: Continued.

HPV type	Viral load (N, %)	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
39	High (978, 0.3)	13.03 (4.72, 36.00)	7.4E-07	5.65 (1.07, 29.71)	0.04	1.48 (0.25, 8.68)	0.67
	Inter (1008, 0.3)	8.83 (1.78, 43.85)	7.7E-03	3.83 (0.48, 30.33)	0.20	*ref*	
	Low (2122, 0.6)	2.31 (0.53, 10.09)	0.27	*ref*			
	Negative (281 587, 85.7)	*ref*					
	P-value (trend)	9.6E-08		0.03			
56	High (750, 0.2)	7.18 (1.62, 31.72)	9.3E-03				
	Inter (446, 0.1)						
	Low (975, 0.3)						
	Negative (279 416, 85.1)	*ref*					
	P-value (trend)	0.10					
68	High (591, 0.2)	4.21 (0.55, 32.39)	1.7E-01				
	Inter (469, 0.1)						
	Low (402, 0.1)						
	Negative (277 953, 84.6)	*ref*					
	P-value (trend)	0.39					

Multiple HPV infections with types ranked higher in the hierarchy are excluded.

Separate analyses are shown with low and intermediate viral loads as the reference category.

[a] Highest ranked hrHPV type per woman

OR: Odds Ratio, CI: Confidence Interval

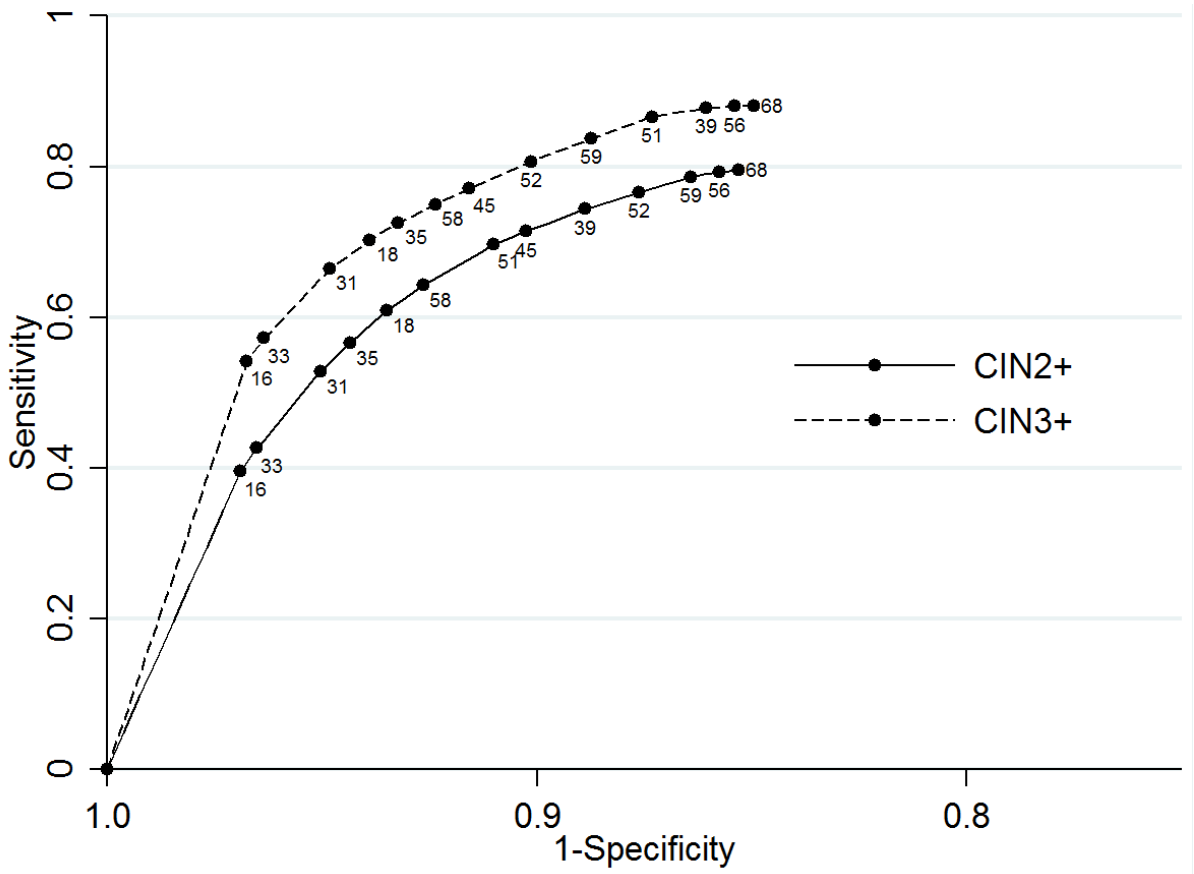

Figure 1: ROC curve of cumulative sensitivity and specificity for CIN2+ and CIN3 according to hierarchical ordering of hrHPV genotypes.

A receiver operator characteristic curve (ROC) showing the cumulative diagnostic ability of 13 high risk HPV (hrHPV) types is shown for outcomes CIN2+ and CIN3+ separately. Sensitivity and specificity for HPV types, in the order determined by sequentially maximizing the positive predictive values for both outcomes, and plotted against each other. Each hrHPV type is labeled on graph. (Exact values in Table 2 & Supplementary Table 2)