Use of HPV testing for cervical screening in vaccinated women - insights from the SHEVa (Scottish HPV Prevalence in Vaccinated Women) study.

Short Title: HPV Testing in Vaccinated Women

Authors: R Bhatia[1], K Kavanagh[2], H Cubie[1,7], I Serrano[1], H Wennington[1], M Hopkins[3], J Pan[2], KG Pollock[4], TJ Palmer[5] and K Cuschieri[6]

Affiliations:

[1] HPV Research Group, University of Edinburgh, Edinburgh, UK
[2] Department of Mathematics and Statistics, Strathclyde University, Glasgow, UK
[3] Liverpool Clinical Laboratories, Royal Liverpool University Hospital, Liverpool, UK
[4] Health Protection Scotland, Glasgow, UK
[5] Department of Pathology, University of Edinburgh, Edinburgh, UK
[6] Scottish HPV Reference laboratory, Royal Infirmary of Edinburgh, NHS Lothian, Edinburgh, UK
[7] Global Health Academy, University of Edinburgh, Edinburgh, UK

Corresponding author: Dr Ramya Bhatia, HPV Research Group,The University of Edinburgh,The Queen's Medical Research Institute,47 Little France Crescent, Edinburgh EH16 4TJ, Tel: 0131 242 6625, Fax: 0131 242 6441, Email: ramya.bhatia@ed.ac.uk.

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Novelty and Impact Statement: Scotland is uniquely placed to address the implications of HPV immunisation on cervical screening given (1) a national immunisation programme with very high uptake, (2) the ability to link immunisation with cervical screening data, (3) the existence of a national HPV sample archive. This study investigates how clinically validated HPV assays are affected by immunisation. This is both novel and timely, given the global move towards HPV based primary screening.

Abstract

The management of cervical disease is changing worldwide as a result of HPV vaccination and the increasing use of HPV testing for cervical screening. However, the impact of vaccination on the performance of HPV based screening strategies is unknown. The SHEVa (Scottish HPV Prevalence in Vaccinated women) projects are designed to gain insight into the impact of vaccination on the performance of clinically validated HPV assays. Samples collated from women attending for first cervical smear who had been vaccinated as part of a national "catch up" programme were tested with three clinically validated HPV assays (2 DNA and 1 RNA). Overall HR-HPV and type specific positivity was assessed in total population and according to underlying cytology and compared to a demographically equivalent group of unvaccinated women. HPV prevalence was significantly lower in vaccinated women and was influenced by assay-type, reducing by 23-25% for the DNA based assays and 32% for the RNA assay ($p=0.0008$). All assays showed over 75% reduction of HPV16 and/or 18 ($p<0.0001$) whereas the prevalence of non 16/18 HR-HPV was not significantly different in vaccinated vs unvaccinated women. In women with low grade abnormalities, the proportion associated with non 16/18 HR-HPV was significantly higher in vaccinated women ($p<0.0001$). Clinically validated HPV assays are affected differentially when applied to vaccinated women, dependent on assay chemistry. The increased proportion of non HPV16 /18 infections may have implications for clinical performance, consequently**,** longitudinal studies linking HPV status to disease outcomes in vaccinated women are warranted.

Introduction

HPV vaccination programmes have been implemented in several countries with success and their impact is now evident at the population level. Several ecological and direct linkage studies have shown significant associations between the reduction of vaccine-type HPV prevalence, associated disease and vaccination with both the bivalent and quadrivalent vaccines(1). Furthermore, there is encouraging evidence to suggest HPV-type cross-protective effect of the vaccines(2,3).

We are at a crucial time for cervical disease management. In addition to vaccination programmes, there is now international consensus that HPV testing should replace cytology as the primary screening modality. Primary screening with HPV testing is predicated on several randomised control trials which demonstrate its superior sensitivity for the detection of CIN2+ over a longer time frame when compared to a single cytology screen(4). The demand and requirement for HPV testing is evident in the increasing range of assays, with varied chemistry, platform and typing capabilities, that are considered clinically validated for use in cervical screening(5).

Epidemiology and surveillance studies designed to assess HPV type specific prevalence in vaccinated populations, provide limited insight into how clinically validated assays will perform in cervical screening. The assays used in surveillance studies typically have a high-analytical sensitivity and broad spectrum genotyping capability and are not routinely used for cervical disease management. Comparatively, most clinically validated assays have been calibrated to clinical end points (CIN2+) and detect high-risk types only. There are very few studies which have applied clinically validated HPV assays to vaccinated cohorts. However, given that the reduction of HPV16 and/or 18 may lead to other high-risk (HR) HPV types becoming unmasked(6), potentially affecting the performance of validated assays, this should be addressed.

Scotland has a population of 5.3 million people and an organised cervical screening programme for women aged 20-60 with an overall coverage of around 70%(7). Since 2008, there has also been a school-based vaccination programme targeting 12-13 year old girls, with an initial three year catch-up campaign for girls aged up to 18. Vaccination coverage has been high with sustained uptake of over 90% in the target population and 65% overall in the catch-up group (with variation according to age and whether in or out of school)(8),(9). Girls vaccinated as

part of catch-up have been entering the screening programme since 2010. Data linkage capabilities in Scotland, facilitated through a unique personal identifier, enable screening, cervical disease and vaccination data to be assessed. A national surveillance programme in Scotland was set up to assess the impact of HPV immunisation at a population level and has shown significant reductions amongst immunised women in HPV16 and 18, in HPV 31, 33 and 45 as a result of cross-protection from the bivalent vaccine[2], a reduction in high and low grade cervical abnormalities with immunisation[9] and most recently early evidence of herd immunity in non-immunised women(10).

The Scottish HPV Prevalence in Vaccinated women (SHEVa) projects are complementary to the surveillance programme, which uses a highly sensitive epidemiologically orientated HPV assay, in that they have been designed to provide information on the performance of clinically validated HPV assays in vaccinated women. The primary objective of the present study was to assess and compare the prevalence of HR-HPV (overall and HPV16 and/or 18) in young vaccinated women (and a demographically comparable group of unvaccinated women), as detected by three clinically validated HPV assays and to determine whether assays are affected differentially by vaccination. Secondary objectives included gaining insights into performance of clinically validated assays by assessing the relative burden of HPV16 and/or 18 and other 'HR-HPV' and type specific prevalence according to cytological reporting category, and the effect of age at first dose of vaccine on HR-HPV detection by these assays.. Implications of the data for the provision of future cervical screening services are discussed.

Materials and Methods

Sample set and data linkage

A national HPV epidemiology and surveillance programme was set up since 2009, to monitor the impact of the vaccine in the Scottish population. One aspect of this involved the yearly collection of 1000 anonymised residual liquid based cytology (LBC) samples from young women aged 20–21 years attending their first screening appointment from all (9) NHS cytopathology laboratories that serve the cervical screening programme. Impact of vaccine on infection using this sample through application of a sensitive epidemiologically orientated assay has been reported previously(11). A subset of this wider surveillance collection was used for the SHEVa study. These were chosen by sequentially selecting all vaccinated samples available from the surveillance collection in 2012 (n=653) supplemented with samples from

the 2011 collection to make 1000. Similarly, for unvaccinated women, samples were chosen from 2012, 2011 and 2010 collections to make 1000. Assuming an overall HPV prevalence of 34% in young vaccinated women, 1000 samples from vaccinated women were tested with 1000 age matched samples from unvaccinated women to enable us to estimate prevalence of HPV16 and/or 18 to +/- 3%. All cytology laboratories were represented in the SHEVa collection to enable representativeness.

All samples had been genotyped previously and linked to screening records and vaccination status with residual material stored in the Scottish HPV Archive (www.shine.mvm.ed.ac.uk/archive). Genotyping was originally performed using an analytically sensitive luminex-based assay – Optiplex HPV Genotyping kit (DiaMex Gmbh, Heidelberg, Germany) which resolves 24 HPV types including all types in IARC Group 1, 2A & 2B(12). Data on age at vaccination, dosage and cytology status were obtained via the Information Services Division (ISD) and Health Protection Scotland (HPS). Routine cytology classification was as per British Society for Clinical Cytology criteria. Cytology results were classed as negative (for any abnormality), low grade (borderline squamous changes, koilocytosis, and mild/low grade dyskaryosis) and high grade (which includes moderate dyskaryosis and worse)(13),(14).

Ethics

The East of Scotland Research Ethics Service has given generic approval to the Scottish HPV Archive as a Research Tissue Bank (REC Ref 11/AL/0174) for HPV related research on anonymised archive samples. The Scottish HPV Archive is also registered with NHS Lothian Tissue Governance and comes under its 'safe haven' for research using clinical samples. Samples were made available for the present project though application to the Archive Steering Committee (HPV Archive Application Ref 0017).

HPV testing protocols

All samples were subject to testing by three clinically validated assays: 1) RealTime High Risk HPV (rtHPV) assay (Abbott Molecular, Illinois, U.S.A.); 2) Aptima HPV (AHPV) assay (Hologic, Bedford, U.S.A) and 3) Onclarity™ HPV Assay (Becton Dickinson, NJ, U.S.A) according to manufacturer's guidelines. rtHPV and Onclarity HPV assays are DNA based assays while AHPV is an RNA based assay. All three assays provide consensus results of positivity for HR-HPV if one of the following types is present: 16, 18, 31, 33, 35, 39, 45, 51,

52, 56, 58, 59, 66, 68. Furthermore, rtHPV provides concurrent genotyping of 16 and 18; Onclarity provides concurrent, individual typing of 16, 18, 31, 45, 51, 52, in addition to three groups of types: 33/58, 56/59/66, 35/39/68; and AHPV provides reflex testing for 16 and for 18/45 as a duplex. The impact of three doses of vaccination on overall HR-HPV and on HPV16 and/or 18 prevalence demonstrated by the assays was assessed. Samples negative for either HPV16 and/or 18 but positive for one of the other HR-HPVs were designated as 'other HR-HPV'.

Statistical Analysis

Prevalence and corresponding 95% confidence intervals (CI) were stratified by vaccination status and subsequently according to cytological reporting category. The differences in prevalence for each assay were assessed using a test of two proportions with an adjustment made for multiple comparisons, using a Bonferroni correction applied to the significance cut-off point, due to the multiplicity of tests conducted (36 tests of proportions and 12 interaction tests giving the significant level, alpha=0.05/48=0.00104).

Between-assay differences in HPV prevalence were conducted using a multi-level logistic regression model to account for repeated testing of the same sample with each assay. Comparison of the between-assay differences in vaccinated vs unvaccinated women were assessed by conducting a test of interaction. Possible effect modification linked with cytological status was also examined. Type specific prevalence beyond HPV16 and/or 18 was assessed via descriptive analysis of the five most prevalent types in vaccinated vs unvaccinated women stratified by underling cytology. Impact of age at first dose of vaccine on the odds of being infected with HR-HPV and HPV16 and/or 18 was assessed for all assays and according to underlying cytology. All statistical analysis was carried out using R version 3.1.

Results

Study population

A total of 2000 samples were included the study, of which 993 were from unvaccinated and 1007 from vaccinated women (the number of vaccinated samples increased by 7 due to subsequent updated linkage of vaccination records). Four samples were excluded due to the following reasons: <3 doses of vaccine (n=1), age >21 (n=2) or missing age information (n=1). A further 8 samples were excluded due to technically invalid test results. Thus, the final study

population included samples from 988 unvaccinated and 1000 vaccinated individuals. Characteristics of the population can be seen in Table 1. The median age of receipt of first dose of vaccine and age at screening, was 17 and 21 years respectively. Abnormal cytology was associated with samples from 26.7% unvaccinated and 19% vaccinated women. The proportions of non-negative cytology in the wider national surveillance sample set (n= 6918) was very similar (25.6%) although was slightly higher when compared to Scottish national screening data on females born in 1990-1992 (n= 54518) which was 19.8% (Palmer et al, submitted)

HPV positivity

Of the 1988 samples tested with the Optiplex HPV assay, 48.7% (95% CI: 46.5-50.9%) were positive for any HR-HPV. The clinically validated assays showed lower positivity, 39.1% (AHPV), 43.2% (Onclarity), 44.0% (rtHPV). No significant difference in positivity was found between rtHPV and Onclarity ($p=0.193$) but a significant difference between rtHPV and AHPV was observed ($p<0.0001$).

Impact of vaccination on HR-HPV and HPV16 and/or 18 detected by clinically validated assays

Overall HR-HPV prevalence was significantly lower in women who had been vaccinated ($p<0.0001$) with all assays, with positivity reducing by 11.6%, 12.5% and 15.1% for the rtHPV, Onclarity and AHPV, respectively. This equates to a reduction in positivity for any hr-HPV of 23.2% and 25.2% for the rtHPV and Onclarity DNA assays, respectively, and 32.2% for the AHPV RNA assay (Table 2). The AHPV assay showed the greatest reduction between the vaccinated and unvaccinated groups when compared with the other assays ($p<0.0001$).

As expected, reduction of HPV16 and/or 18 was highly significant for all assays ($p<0.0001$) with the prevalence in unvaccinated and vaccinated women being 21.4% and 5.0% for the rtHPV assay, 21.2% and 4.7% for the Onclarity assay and 19.8% and 4.5% for the AHPV assay (with no delineation between 18/45). No significant between-assay differences were observed for HPV 16 and/or 18 ($p=0.813$).

When considering other HR-HPV, a small but insignificant increase was observed in the vaccinated group with all three clinically validated assays. The AHPV assay demonstrated a smaller difference between the unvaccinated and vaccinated groups, as compared to the other

two DNA based assays for the detection of other HR-HPV and AHPV was significantly different from the other two assays ($p<0.0001$).

Impact of vaccination on HR-HPV and HPV16 and/or 18 according to underlying cytology

As the number of women with high grade cytology was very small (21 in the unvaccinated group and 6 in the vaccinated group), this analysis focuses on women with either negative (n=1507) or low grade cytology (n=427).

A total of 707 and 800 samples with negative cytology were present in the unvaccinated and vaccinated groups respectively. Significant reductions in HR-HPV and HPV16 and/or 18 prevalence were observed in vaccinated women ($p<0.0001$ for all assays, Figure 1). No significant differences between the assays for the detection of HR-HPV ($p=0.0044$) and HPV16 and/or 18 ($p=0.37$) were observed. Non-significant increases in other HR-HPV were observed for rtHPV ($p=0.17$) and Onclarity ($p=0.36$) whereas a non-significant reduction for other HR-HPV was observed with AHPV ($p=0.25$).

A total of 243 and 184 samples with low grade cytological abnormality were present in the unvaccinated and vaccinated groups respectively. The prevalence of HPV16 and/or 18 in unvaccinated and vaccinated groups reduced significantly from 36.1% to 9.8% for rtHPV, 37.0% to 8.7% for Onclarity and 39.5% to 9.2% for AHPV ($p<0.0001$ for all assays). A highly significant ($p<0.0001$) increase in other HR-HPV types was also observed for all assays (Figure 1). No significant difference between the assays for the detection of HR-HPV ($p=0.186$), HPV 16/18 ($p=0.122$) or other HR-HPV ($p=0.80$) was observed in the group with low grade cytology.

Impact of age at vaccination on prevalence of HR-HPV and HPV16 and/or 18

Odds ratios (ORs) for infection with HR-HPV and HPV16 and/or 18 according to age at first dose of vaccine (ages 15/16, 17 and 18+) were calculated for each assay in the total population and according to underlying cytology (Table 3). Detection of HR-HPV or other HR-HPV, according to all assays was not affected by age at 1st dose of vaccine, whereas HPV16 and/or 18 was less likely to be detected in girls vaccinated at a younger age (p for linear trend <0.00001 for all assays).

Comparison of type specific prevalence using clinically validated and epidemiological assay

Among the women with HR-HPV infection, type-specific HPV data (beyond HPV16 and/or 18) was available via the Onclarity and epidemiological Optiplex tests, although the Onclarity can only provide resolution of 16, 18, 31, 45, 51, 52 with the remaining types detected as three groups: 56/59/66, 35/39/68 and 33/58. According to the Optiplex assay, HPV16 remained within the top 5 HPV types in vaccinated women overall irrespective of underlying cytology. However, this was not observed using the Onclarity assay where HPV16 did not feature in the top 5 for any cytology category in vaccinated women (Table 4a and b). Further, the most common type detected by the Optiplex and Onclarity assays differ when stratified by age at first dose of vaccination (Table 4c). For the Optiplex assay, HPV16, HPV59 and HPV56 were the most frequently detected types in girls vaccinated at 18, 17 and 15/16 respectively. Comparatively, application of the Onclarity assay showed the most common group 56/59/66 as unchanged with age at first dose.

Discussion

HR-HPV infection reduced significantly in vaccinated women attending for cervical screening as measured by three clinically validated HPV assays which target both viral DNA and RNA. The reduction in HR-HPV in vaccinated women as measured by the AHPV assay was significantly higher than the reduction measured by the DNA-based assays ($p<0.001$). The AHPV has shown higher clinical specificity compared to DNA based assays in low grade triage and more recently, primary screening contexts(15),(16). It is feasible that the greater reduction associated with AHPV is due to exclusion of transient or clinically insignificant infection through amplification of an mRNA target. However, longitudinal follow-up relative to disease end points is required to demonstrate this. The data also show that the most common types in vaccinated women detected by Optiplex (epidemiologically orientated)assay were different to those detected by Onclarity (clinically validated) assay. This may reflect the difference in assay design (consensus PCR versus gene-specific type detection) which influences the assay's ability to accurately detect multiple infections(17) and the fact that the epidemiological assay cut off is not calibrated to disease end-points. This re-emphasises the point that extrapolation of surveillance data to inform HPV based screening strategies should be performed with caution.

Given the move to HPV primary screening, an understanding of the prevalence of infection at clinically relevant levels in vaccinated women will help inform the design of screening services. Figure 2 shows an overview of HPV positivity in the vaccinated (A) and unvaccinated

groups (B) according to each test with further stratification into the cytology categories. This provides preliminary insight into the management challenges of HPV primary screening in vaccinated populations. Using the rtHPV assay as an example, approximately 50% of the HPV positive unvaccinated women were associated with no cytological abnormality, whereas in vaccinated women this was approximately 60%.

The optimal management of HPV positive infections in the era of primary HPV screening remains a challenge. There is consensus that triage of HPV positive women is required for management, but the method of triage is still a matter of debate. Cytology, HPV testing and/or partial genotyping for HPV16 and/or 18 alone or in combination have all been suggested(18). Our data indicate that the burden of HPV positive samples for any triage will be lower in vaccinated women using clinically validated assays (a reduction of 23-25% for the DNA based assays and 32% for the RNA based assay). Furthermore of the 32-38% of HR-HPV infections detected in vaccinated women only 4.5-5% were HPV16 and/or 18 positive, compared to around 20% which were HPV16 and/or 18 positive in unvaccinated women. As the impact on HPV16 and/or 18 in the present analysis was observed in the catch-up population, reduction of these types in the girls vaccinated as part of the routine programme will be greater. Indeed, even within the catch-up group, girls vaccinated at a younger age were significantly less likely to be infected with HPV16 and/or 18 ($p<0.00001$ for all assays). Therefore, the practicalities and success of triage of HPV positive women using HPV16 and/or 18 typing will be affected by the increasing number of vaccinated women who enter cervical screening programmes.

When women with low-grade cytology results were assessed separately, all assays showed a significantly higher proportion of other HR-HPV in vaccinated compared with unvaccinated women ($p<0.0001$). This has implications when considering HPV triage of low grade smears in vaccinated women within existing cytology-based programmes. Given the established higher risk of HPV16 and/or 18, compared to other high-risk types for CIN2+ development, the positive predictive value of low grade triage following HPV testing may reduce(19),(20). One might speculate that the positive predictive value of abnormal cytology (alone) might also reduce, given that a greater proportion of low grade smears will be associated with lower risk types. This speculation is consistent with a recent analysis of Scottish national data which indicated that the abnormal predictive value and positive predictive value of cytology in vaccinated women has reduced(21).

Whether the aforementioned observations represent a true increase in other HR-HPV is debatable as the number of abnormal samples was relatively small and there was an overall reduction in the number of low grade abnormalities in vaccinated women, resulting in different denominators. Our observations are complementary to the findings in a similar age-matched cohort of 13-26 year-old women from two US primary care clinics by Kahn *et al* (2014)(22), where the prevalence of non-vaccine HPV types increased by 14.0% (60.8%–74.8%) for all participants and that this increase was significant in vaccinated (but not unvaccinated) participants. A recent ecological modelling study suggested that co-existence of multiple HPV types is possible due to 'patchy distribution' of the more virulent types with less virulent types(23). The introduction of a vaccine with modest degree of cross-protection might result in eradication of non-vaccine types. However, we observed a higher level of non-vaccine types in women with low-grade abnormality and this would be consistent with the 'competitive exclusion equilibrium' described by Waters (2012), rather than a competitive co-existence equilibrium which was observed in the overall population.

The observations are more likely to reconcile with the notion of unmasking, i.e. non HPV16 and/or 18 types become more readily detected as a consequence of less competition for molecular resources within the assay. DNA amplification approaches which incorporate consensus primers, rather than type specific primers, might be expected to be particularly affected by this phenomenon and it was of note that the assays associated with higher increases in the overall population were the Optiplex and rtHPV assay, the two DNA assays that utilise consensus PCR. Moreover, this unmasking phenomenon has been observed in an Australian post-vaccine surveillance study using simulated samples tested with a consensus primer approach(24). Recent data from another study within the Scottish primary screening population (PaVDaG) showed no overall decrease in the prevalence of HR-HPV associated with vaccination in women aged 20-24 using the cobas® HPV Assay (Roche, Basel, Switzerland)(25). In PaVDaG, where Cobas HPV is based on consensus primers, HPV positivity was detected in 45.3% and 47.6% of unvaccinated and vaccinated women respectively, although the prevalence of HPV16 and/or 18 was 15.2% and 4.7% respectively. Interestingly, the distribution of the other HR-HPV types is different depending on the assay used (Table 4c). HPV type 16 was the most common type detected by the Optiplex assay in the overall population whereas 56/59/66 was the most common grouping detected by Onclarity. This re-emphasizes the fact that assays maybe affected differentially by vaccination. If the proportion of HPV16 and/or 18 infections in women attending for primary screening reduces, while other

types become more prevalent through unmasking, the PPV of an HPV positive result for significant disease may also reduce. Clearly, further studies are needed and this should be addressed through longitudinal follow-up of HPV infection in vaccinated women relative to disease.

There are limitations to this study. Firstly, women were vaccinated as part of catch-up. An earlier Scottish epidemiological study of unvaccinated 11-18 years olds showed 12.6% of girls aged 15-18 were infected with HR- HPV, so some may have been exposed to HPV before vaccination(26). Consequently the impact of vaccination when girls vaccinated aged 12-13 enter the screening system will be different to the observations reported here. Secondly, we did not include all clinically validated assays (Arbyn et al(5)) and no signal (as opposed to target) amplification assays were assessed. Finally, we did not directly evaluate clinical performance of the assays in vaccinated women relative to disease endpoints in this study. Future plans to address this are in process, through linkage to clinical follow-up data as this is important for assessing clinical performance of these assays in vaccinated women.

To conclude, while there will be less HR-HPV infection to manage in vaccinated women using clinically validated assays, the amount remaining may differ significantly according to assay chemistry. Potential unmasking of other HPV types, given the substantial reductions in HPV16 and/or 18 and dominance of other HR-HPV, may have implications for both HPV and cytology based screening programmes. Clinical validation metrics designed to investigate the performance of established and new HPV assays which take into account the increasing influence of vaccination will be welcome, as will longitudinal data series which link HPV status in vaccinated women to long term disease outcomes.

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		Cytology grade (N)				
Age at cytology	**Vaccination status**	**High grade**	**Low grade**	**Negative**	**Unsatisfactory**	**Total**
<20 or 20	**Unvaccinated**	12	138	494	12	656
	vaccinated- age 15		3	20	0	23
	vaccinated- age 16	3	98	336	4	441
	vaccinated- age 17		26	197	2	225
	vaccinated- age 18	1	24	141	1	167
	vaccinated- age 19		5	13		18
21	**Unvaccinated**	9	105	213	5	332
	vaccinated- age 16		2	10		12
	vaccinated- age 17	1	23	65	3	92
	vaccinated- age 18	1	3	12		16
	vaccinated- age 19			6		6

Table 1: Overview of study population.

	Assay	% positive Unvaccinated (95% CI) N= 988	% positive Vaccinated (95% CI) N=1000	% difference	p-value
Overall HR-HPV	rtHPV	49.8 (46.7-52.9)	38.2 (35.2-41.3)	-11.6	**< 0.0001**
	Onclarity	49.5 (46.4-52.6)	37.0 (34.1-40.0)	-12.5	**< 0.0001**
	AHPV	46.7(43.6- 49.8)	31.6 (28.8-34.5)	-15.1	**< 0.0001**
HPV16 and/or 18	rtHPV	21.4 (18.9-24.0)	5.0 (3.8- 6.5)	-16.4	**< 0.0001**
	Onclarity	21.1 (18.6-23.7)	4.7 (3.6-6.2)	-16.4	**< 0.0001**
	AHPV	19.8 (17.5-22.4)	4.5 (3.4-6.0)	-15.3	**< 0.0001**
Other HR-HPV	rtHPV	28.4 (25.7-31.3)	33.2 (30.4-36.2)	+4.8	0.024
	Onclarity	28.4 (25.7-31.3)	32.3 (29.5-35.3)	+3.9	0.068
	AHPV	26.8 (24.2-29.7)	27.1 (24.4-29.9)	+0.3	0.929

Table 2: HPV prevalence in women receiving no and three doses of vaccine according to HPV assays.

HPV outcome	Assay	Age at 1st dose of vaccine	Odds Ratio	95% CI lower	95% CI upper	Linear trend p-value
16 and/or 18	rtHPV	15/16	1	-	-	**<0.0001**
		17	2.716	**1.166**	**6.654**	
		18+	7.538	**3.483**	**17.742**	
		Cytology Negative	1	-	-	
		Cytology Low	3.62	1.887	6.819	
16 and/or 18	Onclarity	15/16	1	-	-	**<0.0001**
		17	3.257	**1.386**	**8.24**	
		18+	7.111	**3.151**	**17.627**	
		Cytology Negative	1	-	-	
		Cytology Low	3.262	1.657	6.254	
16 and/or 18	AHPV	15/16	1	-	-	**<0.0001**
		17	2.521	**1.065**	**6.236**	
		18+	6.449	**2.922**	**15.365**	
		Cytology Negative	1	-	-	
		Cytology Low	3.75	1.916	7.208	
Other HR-HPV	rtHPV	15/16	1	-	-	0.143
		17	0.983	0.708	1.362	
		18+	0.748	0.505	1.099	
		Cytology Negative	1	-	-	
		Cytology Low	6.992	4.932	10.025	
Other HR-HPV	Onclarity	15/16	1	-	-	0.108
		17	0.943	0.676	1.313	
		18+	0.724	0.485	1.069	
		Cytology Negative	1	-	-	
		Cytology Low	7.612	5.356	10.945	
Other HR-HPV	AHPV	15/16	1	-	-	0.395
		17	1.089	0.766	1.545	
		18+	0.835	0.547	1.26	
		Cytology Negative	1	-	-	
		Cytology Low	8.079	5.699	11.552	

Table 3: Impact of age at vaccination on prevalence of HR-HPV and HPV16 and/or 18.

Total population		**Cytology Negative**		**Cytology Low grade**	
Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
16	52	16	16	16	52
52	56	51	59	56	56
56	51	52	51	52	51
51	16	56	56	59	66
66	59	18	52	66	16

A. Optiplex HPV

Total population		**Cytology Negative**		**Cytology Low grade**	
Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
56/59/66	56/59/66	16	56/59/66	56/59/66	56/59/66
16	51	56/59/66	33/58	16	51
35/39/68	35/39/68	33/58	35/39/68	35/39/68	35/39/68
33/58	33/58	35/39/68	52	51	52
31	52	31	51	52	33/58

B. Onclarity HPV

Age at 1st dose of vaccine	**Total population**		**Cytology Negative**		**Cytology Low grade**	
	Optiplex	Onclarity	Optiplex	Onclarity	Optiplex	Onclarity
15-16	56	56/59/66	59	56/59/66	56	56/59/66
17	16	56/59/66	16	56/59/66	51	56/59/66
18	16	56/59/66	16	56/59/66	16	56/59/66

C. Age at 1st dose of vaccine

Table 4: Five most common types in unvaccinated and vaccinated women stratified by cytology category using the Optiplex (A) and Onclarity assays (B). The most common HR-HPV types detected according to age at first dose of vaccine is shown in C.

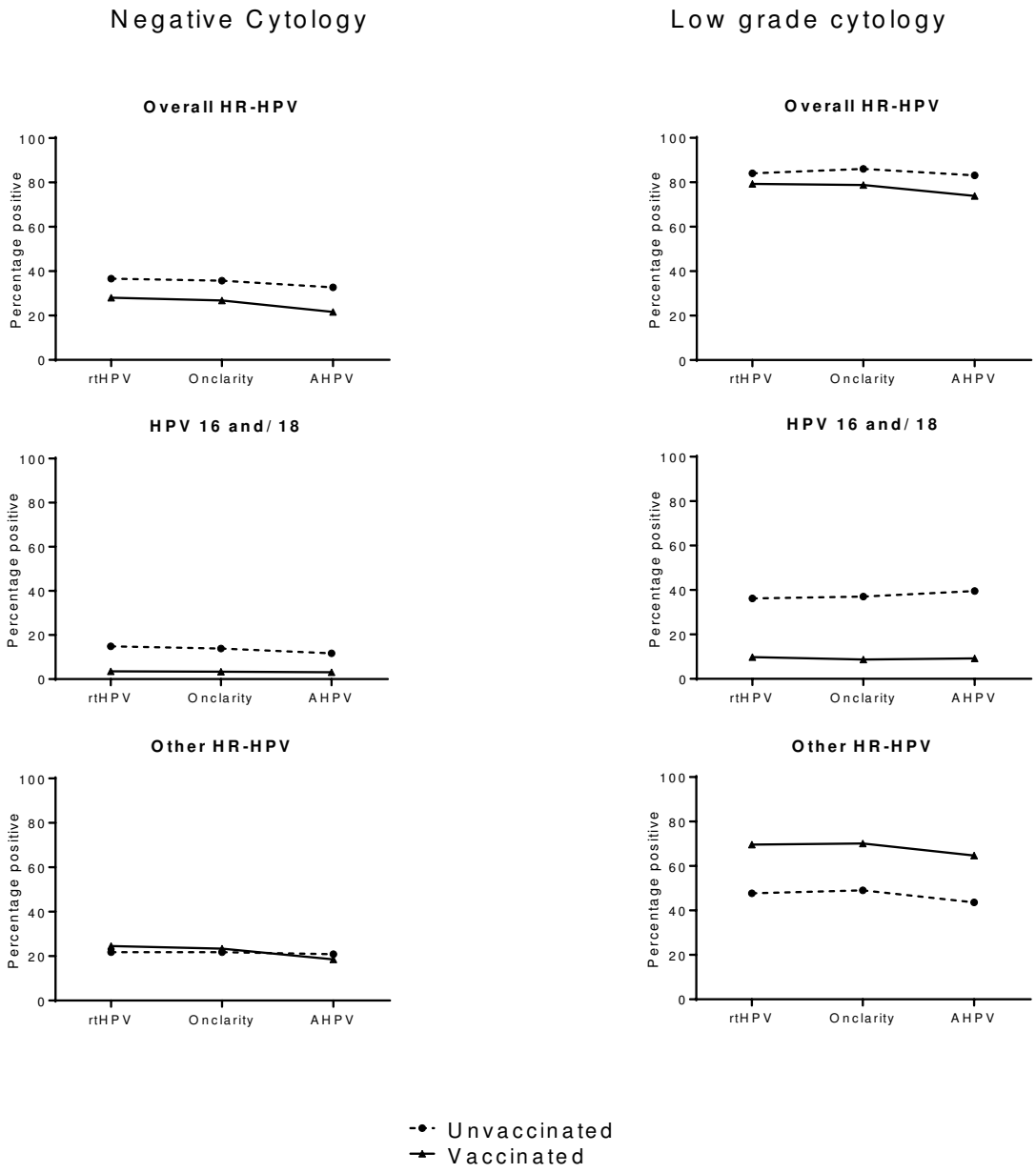

Figure 1: Positivity for Overall HR-HPV (top), HPV16 and/or 18 (middle) and other HR-HPV (bottom) stratified by vaccination status, assay type and cytological result (negative/low grade).

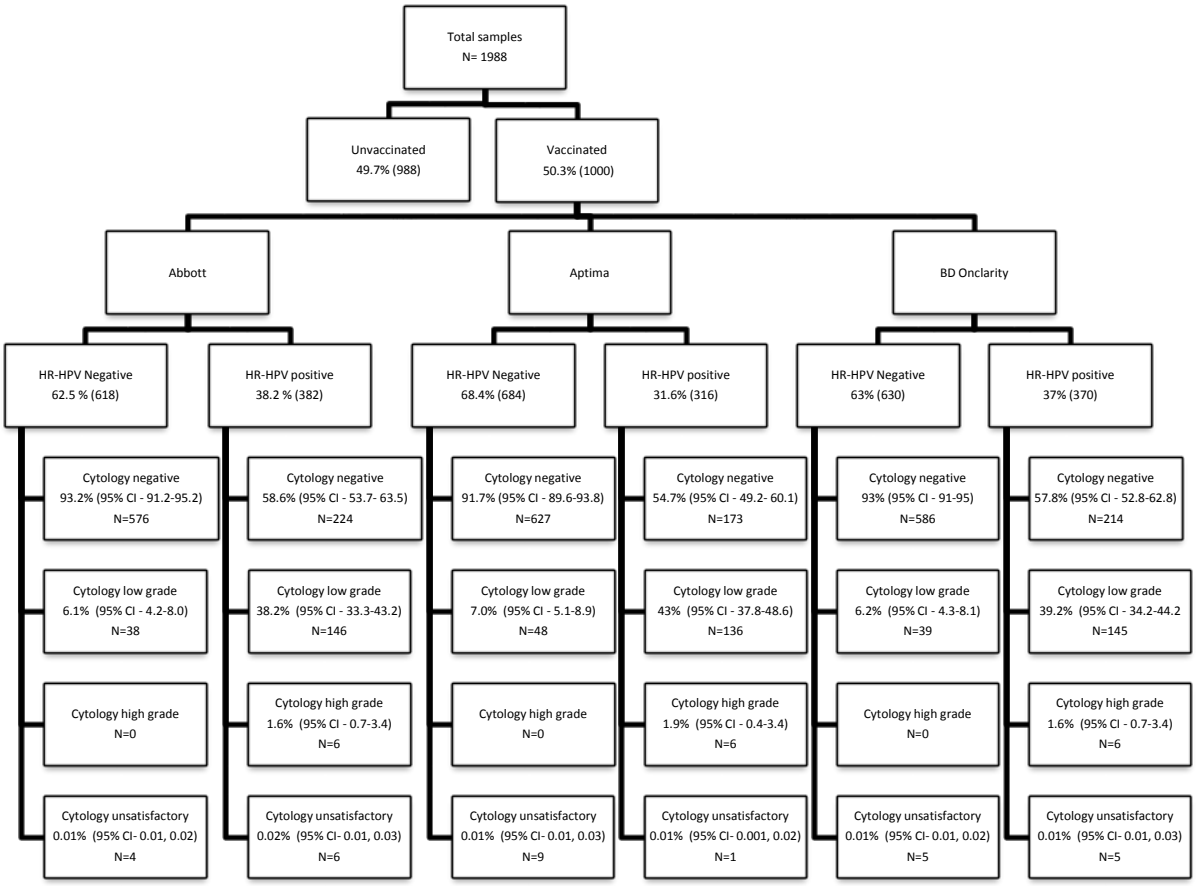

Figure 2A: Overview of HPV status in vaccinated women according to assay stratified by underlying cytology.

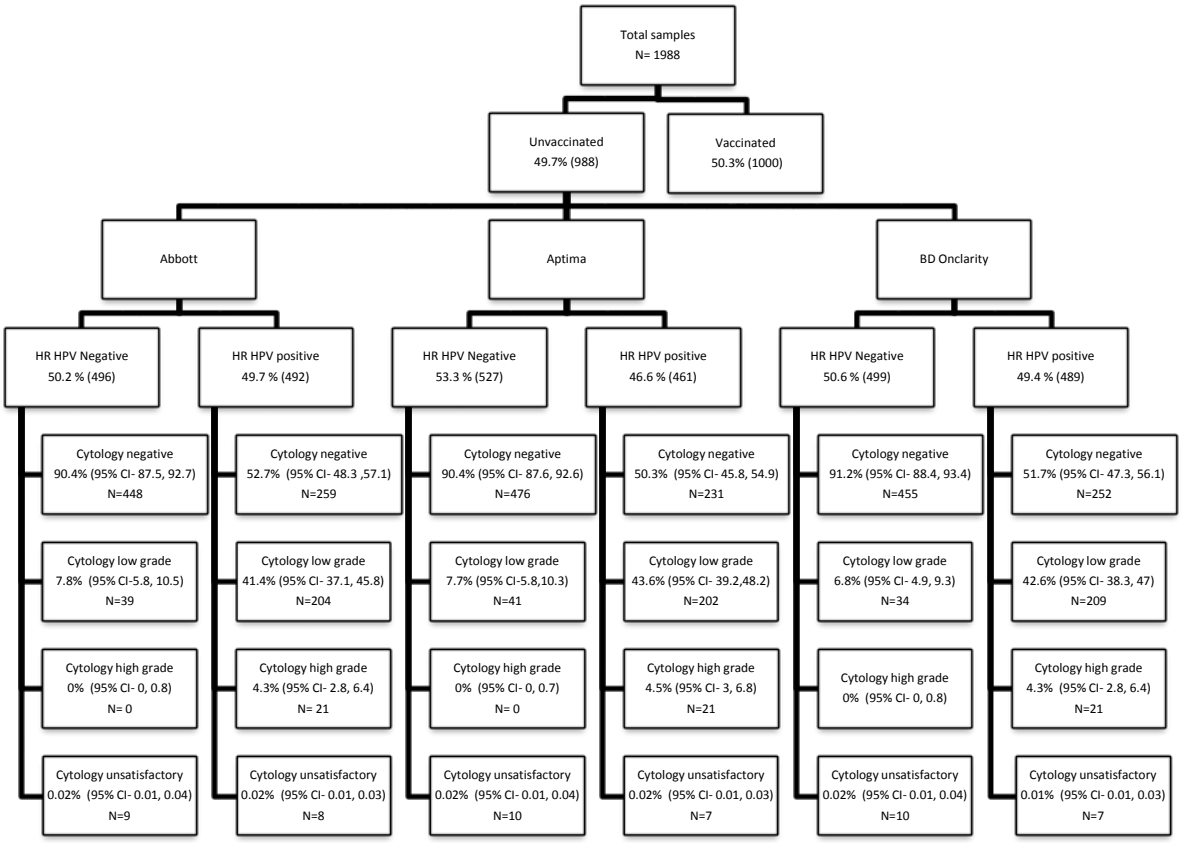

Figure 2B: Overview of HPV status in unvaccinated women according to assay, stratified by underlying cytology. (B) Stratification of the unvaccinated group.

			Unvaccinated	Vaccinated		
	Cytology Grade	**Assay**	**Prevalence 95% CI**	**Prevalence 95% CI**	**% difference**	**p-value**
Overall HR-HPV	Negative	Optiplex	43.2 (39.7-47.0)	33.4 (30.2-36.7)	-9.8	**<0.0001**
		rtHPV	36.6 (33.1–40.3)	28 (25.0-31.2)	-3.7	**0.0004**
		Onclarity	35.7 (32.2-39.2)	26.8 (23.8-29.9)	-8.9	**0.0002**
		AHPV	32.7 (29.3-36.2)	21.6 (18.9-24.6)	-11.1	**<0.0001**
	Low	Optiplex	84.8 (79.7-88.7)	80.4 (74.1-85.5)	-4.4	0.294
		rtHPV	84 (78.8-88)	79.3 (72.9-84.6)	-4.7	0.272
		Onclarity	86 (81.0-89.8)	78.8 (72.3-84.1)	-7.2	0.067
		AHPV	83.1(77.9-87.3)	73.9 (67.1-79.7)	-9.2	0.027
HPV16 and/or 18	Negative	Optiplex	25.7 (22.7-29.1)	10.1 (8.2-12.4)	-15.6	**<0.0001**
		rtHPV	14.9 (12.4- 17.7)	3.5 (2.4-5.0)	-11.4	**<0.0001**
		Onclarity	13.9 (11.5-16.6)	3.4 (2.3-4.9)	-10.5	**<0.0001**
		AHPV	11.7 (9.6- 14.3)	3.1 (2.1-4.6)	-8.6	**<0.0001**
	Low	Optiplex	49 (42.8-55.2)	19.0 (14.0-25.3)	-30	**<0.0001**
		rtHPV	36.2 (30.4- 42.4)	9.8 (6.3-14.9)	-26.4	**<0.0001**
		Onclarity	37.0 (31.2-43.3)	8.7 (5.4-13.7)	-28.3	**<0.0001**
		AHPV	39.5 (33.6-45.8)	9.2 (5.8-14.3)	-30.3	**<0.0001**
Other HR-HPV	Negative	Optiplex	17.5 (14.9-20.5)	23.25 (20.5-26.3)	10.8	0.007
		rtHPV	21.8 (18.9-25.0)	24.5 (21.6-27.6)	2.7	0.236
		Onclarity	21.8 (18.9-25.0)	23.4 (20.6-26.4)	1.6	0.499
		AHPV	20.9 (18.1-24.1)	18.5 (16.0-21.3)	-2.4	0.262
	Low	Optiplex	35.8 (30.0-42.0)	61.4 (54.2-68.1)	25.6	**<0.0001**
		rtHPV	47.7 (41.5-54.0)	69.6 (62.6-75.8)	21.8	**<0.0001**
		Onclarity	49.0 (42.8-55.2)	70.1 (63.1-76.3)	21.1	**<0.0001**
		AHPV	43.6 (37.5-49.9)	64.7 (57.5-71.2)	21.1	**<0.0001**

Table S1: HPV prevalence in unvaccinated and vaccinated women detected by HPV assays stratified by cytology grade.