The impact of HPV type on colposcopy performance in women offered HPV immunisation in a catch-up vaccine programme: a two centre observational study

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Shortened Running Title:

Impact of HPV genotypes on colposcopy

Authors: Ami Munro[1], Collette Gillespie[1], Seonaidh Cotton[1], Camille Busby-Earle[2], Kim Kavanagh[3], Kate Cuschieri[4], Heather Cubie[4], Chris Robertson[3], Louise Smart[5], Kevin Pollock[6], Catherine Moore[4], Timothy Palmer[7]and Margaret E Cruickshank[1]

[1] University of Aberdeen, Aberdeen

[2] NHS Lothian, Edinburgh

[3] University of Strathclyde, Glasgow

[4] Scottish Human Papillomavirus Reference Laboratory, Edinburgh, Scotland

[5] NHS Lothian, Edinburgh

[6] Health Protection Scotland, Glasgow, Scotland

[7] University of Edinburgh, Edinburgh, Scotland

Corresponding author
Professor Margaret E Cruickshank
Department of Obstetrics and Gynaecology
Aberdeen Maternity Hospital
Aberdeen
AB25 2ZD

Email: m.e.cruickshank@abdn.ac.uk
Telephone: 01224 438434
Fax: 01224 553200

Abstract

Objective

To determine if HPV immunisation has affected the prevalence of HPV genotypes and colposcopic features of CIN in young women referred for colposcopy.

Design

A two-centre observational study including vaccinated and unvaccinated women.

Setting

Colposcopy clinics serving two health regions in Scotland, UK.

Population

361 women aged 20-25 years attending colposcopy following an abnormal cervical cytology result at routine cervical screening.

Methods

Cervical samples were obtained from women for HPV DNA genotyping and mRNA E6/E7 expression of HPV 16,18,31,33 and 45. Demographic data, cytology and histology results and colposcopic features were recorded. Chi squared analysis was conducted to identify associations between vaccine status, HPV genotypes and colposcopic features.

Main outcome measures

Colposcopic features, HPV genotypes, mRNA expression and cervical histology.

Results

The prevalence of HPV 16 was significantly lower in the vaccinated (8.6%) compared with the unvaccinated (46.7%) group (p=0.001). The number of cases of cervical intraepithelial neoplasia 2 or more (CIN2+) was significantly lower in vaccinated women (p=0.006).HPV vaccine did not have a statistically significant effect on commonly recognised colposcopic features but there was a slight reduction in the positive predictive value (PPV) of colposcopy for CIN2+ from 74% (unvaccinated) to 66.7% (vaccinated).

Conclusions

In this group of young women with abnormal cytology referred to colposcopy, HPV vaccination via a catch-up programme reduced the prevalence of CIN2+ and HPV 16 infection. The reduced PPV of colposcopy for the detection of CIN2+ in vaccinated women is at the lower acceptable level of the UK national cervical screening programme guidelines.

Word count 246

Keywords

HPV, cervical screening, HPV vaccine, CIN, colposcopy, HPV genotyping

Tweetable Abstract

Reduction of hrHPV positivity and CIN in immunised women consistent with lower PPV of colposcopy for CIN2+

Introduction

HPV immunisation has been a major advance in the prevention of cervical disease and cancer. In September 2008, the bivalent vaccine (which protects against HPV 16 and 18) was introduced in the UK as part of the school-based immunisation programme.[1] The vaccine is given to girls aged 12-13 years and current uptake rate in schools in Scotland is 90%.[2] When the vaccine was introduced, it was also offered to girls aged 14-17 as part of a catch up campaign: 65.5% of the eligible catch up group in Scotland received the full three doses.[2]Within the school vaccination programme the bivalent vaccine was used initially (2008-2010) but since 2011 it was changed to the quadrivalent vaccine.

While prophylactic HPV vaccines offer primary protection against the highest risk HPV types, as well as a level of cross protection for other high risk HPV types (HPV 31,33,45)[3]. However, there will still be a residual risk of disease conferred by other high risk HPV genotypes which are not covered by the currently licensed vaccine(s). Therefore, there is a continued need for secondary prevention using cervical screening and colposcopy.

In Scotland cervical screening, using liquid based cytology, is offered to all women aged 20-60 years with referral to colposcopy for further investigation if the cytology shows high grade dyskaryosis or repeated low grade dyskaryosis or borderline nuclear abnormalities (BNA).[4,5] HPV triage is not part of the screening programme in Scotland.

There is inconsistent evidence as to whether the appearance of the cervix during colposcopy is influenced by the HPV genotypes present.[6-9] A study by Jeronimo et al.

found that colposcopic features characteristic of high grade cervical intraepithelial neoplasia (CIN) imply infection with HPV 16 but not necessarily other HPV types.[6] It has also been shown that lesions missed during colposcopy are more likely to be HPV 16 negative than HPV 16 positive.[7,8] In contrast, van der Marel et al. showed that the visual appearance of high grade HPV16 lesions at colposcopy is not different from lesions associated with other high risk HPV genotypes.[9]However, these studies do not include women who had been vaccinated against HPV infection. If the appearance of the cervix is associated with HPV genotypes present, it would be anticipated that HPV vaccination might alter the range of features seen at colposcopy and thereby potentially affect the performance of colposcopy.

In this study, we investigated cervical abnormalities, HPV genotypes and performance of conventional colposcopic evaluation in both vaccinated and unvaccinated women aged 20-25 years attending colposcopy.

Methods

Study design and population: This two centre cross-sectional study was conducted with women aged 20-25 years routinely attending colposcopy clinics following an abnormal cervical cytology result in two Scottish teaching hospitals (Aberdeen Royal Infirmary and Edinburgh Royal Infirmary) serving regional populations. The first group (Group 1) of women was recruited between February 2010 and March 2011(before women vaccinated as part of the catch-up immunisation campaign had entered the cervical screening programme) and the second group (Group 2) of women was recruited from December 2012 to November 2014 (after women vaccinated as part of the catch up campaign had entered the screening programme).Some individuals

(2008-2010) will have received Gardasil, through private arrangement, out with the catch up programme.

Recruitment& Consent: Women were eligible if they attended colposcopy for the first time following an abnormal cytology result at routine cervical screening. Women were excluded if they were unable to understand the patient information leaflet (PIL), if they were pregnant at the time of colposcopy or if they were being referred as a consequence of symptoms. Eligible women were sent an invitation letter and information before attending for colposcopy. At their appointment, written consent was obtained if they wished to take part in the study.

Data collection: Participants were assigned a unique study number and data were collected on age, referral cytology, parity and vaccination status (including vaccine type, number of doses and age at last dose). Women were considered to be vaccinated if they received two or more doses of a HPV vaccine.[10]Information on vaccine status was obtained from the Scottish Cervical Call-Recall System (SCCRS). SCCRS is the national cervical screening database that contains cytology results, associated histopathology, recall and management data and also immunisation status.

*Colposcopy:*Colposcopy was performed by BSCCP-accredited colposcopists, who recorded their findings using standard reporting features.Colposcopists were blind to the HPV status of the patient. Samples for HPV genotyping were obtained using a broom sampler before the application of acetic acid and were stored in ThinPrep® PreservCyt®(©Hologic UK, Crawley, West Sussex, UK).Biopsies were taken if

features indicative of CIN were seen at colposcopy, including acetowhite changesand capillary vessel patterns A ‘see and treat’ approach was considered for women referred with high grade dyskaryosis, as per local protocols. If a punch biopsy or diathermy loop excision treatment was undertaken, these had a histological diagnosis within the local NHS pathology laboratory. Histology results were captured from pathology records.

HPV genotyping: Samples were tested at the Scottish HPV Reference Laboratory, Edinburgh for the presence of 37 HPV genotypes using QIAamp® Media MDx[11]followed by LINEAR ARRAY HPV Genotyping Test (Roche Molecular Systems).[12]High-risk HPV types were considered to be: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. Intermediate risk HPV types were: 26, 53, 64, 66, 67, 69, 70, 73, 82, IS39 and CP6108. All other HPV genotypes that were identified were considered to be low-risk.[13]

A sub-set of samples (N=319;88%), based on availability of samples, were also tested for mRNA expression using PreTect HPV-Proofer (Norchip AS, Klokkarstua, Norway) which detects E6/E7 mRNA from HPV 16, 18, 31, 33 and 45.[14]

Statistical analysis: All statistical analyses were performed using SPSS Version 20 (IBM SPSS Statistics for Windows, Version 20.0, Armonk, NY: IBM Corp.) Chi-squared analysis was used to test for associations between vaccine status and colposcopic features, colposcopic opinion, histology results and HPV genotypes. All p values were two sided and for the chi-squared analysis were considered significant if their value

was less than 0.05. Z-tests of two proportions were used to assess the difference in prevalence for each of the 35 types genotyped. As multiple statistical tests were conducted, the significance threshold for the z-tests was subject to the Bonferroni correction and therefore considered significant if their value was less than 0.00143 (=0.05/35).

Performance analysis of colposcopy was conducted using histology results as the gold standard for final diagnosis. In cases where no biopsy was indicated, women were assumed to have no significant disease. Sensitivity, specificity, PPV and NPV of colposcopy were calculated for detection of high grade disease (CIN2+); a positive test was considered to be a colposcopic opinion of “high grade”. Comparisons were made between vaccinated and unvaccinated women, and also between those who were positive and those who were negative for DNA HPV16. Differences in the performance of colposcopy between groups were assessed using z-tests.

Statistical power: Power analysis was conducted to calculate how many participants were necessary to reach adequate sample size using EPISTAT software. The proportion of high risk types was estimated from previously published research.[13] A 1:1 ratio for HPV 16/18 against all other HPV types was used. It was estimated 400 women would give 95% power to detect a reduction in PPV of colposcopy from 70% to 52.5% between 200 HPV16/18 positive women and 200 women who did not have HPV16/18. If only 200 women in total were recruited (100 with and 100 without HPV16/18) there would be an 86% power to detect a 30% reduction from 70% to 40%.

Results

Recruitment

Figure S1: Flow diagram of recruitment and study processes

A flow diagram of recruitment and study processes is included in supplementary information. In Group 1 (recruited before women eligible for the HPV vaccine in the catch up campaign entered the cervical screening programme) 208 women agreed to participate,10 were excluded because they did not have a sample taken for HPV testing. Of the 198 women included in the final analysis, 172 had both HPV mRNA and DNA tests. In Group 2 (recruited after women eligible for the HPV vaccine in the catch up campaign entered the cervical screening programme) 175 women agreed to take part, 12 were excluded because they did not have a sample for HPV testing or colposcopy data. Of the 163 included in analysis, 147 had both HPV mRNA and DNA tests.

Participant Demographics

Table S1shows the participant characteristics for each group. Vaccine status was self-reported in Group 1 (three women (2%) reported being vaccinated: two received the quadrivalent vaccine and one received the bivalent vaccine).As this could not be verified by SCCRS at the time, all women were considered unvaccinated. In Group 2 the vaccine status was verified by SCCRS and 67 (41%) women were vaccinated. The mean age at colposcopy in both groups was 22 years. For those vaccinated, the mean age at last dose was 17.3years (SD1.2).

Table 1: Participant Demographic data by group

Impact of vaccination on colposcopic features and histology

Table 2: Impact of vaccination status on colposcopic features and histology

As shown in Table 2, the proportions of women with acetowhite changes (79% vs 77%), mosaic (44% vs 43%), punctation (38% vs 39%)or atypical vessels(1% vs 1%) were similar in both unvaccinated and vaccinated groups respectively. There was no significant impact on non-iodine staining epithelium, which is noted in a higher proportion of vaccinated women (56%) compared to unvaccinated women (50%; p=0.44). However, the use of iodine was inconsistent between colposcopists, and was not applied in 100 cases limiting any conclusions. Colposcopists were significantly more likely to record their opinion as high grade in unvaccinated women (34%) compared to vaccinated women (20%; p=0.027), a difference of 14% (95% CI 2%, 26%). Unvaccinated women were also more likely to have high grade disease (CIN2+) 36%, compared to 19% in vaccinated women, p=0.006; a difference of 17% (95% CI 5%, 29%). Unvaccinated women were also more likely to have any grade of CIN (CIN1+); 63% compared to 46% in vaccinated p=0.044, a difference of 17% (95% CI 2%, 30%).

All eight cases of invasive squamous carcinoma or CGIN were identified in unvaccinated women. All three cases of CIN3 identified in vaccinated womenwere HPV 16 and 18 negative on cervical samples; two of these were associated with HPV 33 (mRNA and DNA positive) and one with HPV 52(DNA positive). A higher proportion of vaccinated women (40% compared with 28% unvaccinated) did not have a biopsy taken (i.e. the colposcopic appearance did not indicate any significant disease).

HPV Genotyping Results

Figure 1: HPV genotyping results

Figure 1 demonstrates the HPV genotypes that were present in vaccinated and unvaccinated women. Only six vaccinated women (9%) had HPV 16, a significantly lower proportion than the unvaccinated group (47%; p<0.001). Two (3%) of the vaccinated women had an HPV 18 infection, compared to 17% of the unvaccinated women (p=0.003). High risk HPV types 52, 56 and 58 were found to be present in a higher proportion of women in the vaccinated group than in the unvaccinated group(23% vs 13%;p = 0.039, 16% vs 6%;p=0.023 and 13% vs 6%;p=0.029 respectively).The changes in HPV 18, 52, 56 and 58 are not considered statistically significant when multiple statistical testing is accounted for. For all other high risk HPV types, there was no difference in prevalence between vaccinated and unvaccinated women.

319 samples were tested for HPV mRNA (HPV 16, 18, 31, 33 or 45), 172 in Group 1 and 147 in Group 2. Although 14 (25%) samples in the vaccinated group had a transcriptionally active HPV infection indicated by the mRNA results, there was a significantly higher proportion of women in the unvaccinated group (63%) with transcriptionally active HPV infections (p<0.001). Of the vaccinated group, four (7%) tested positive for HPV 16 mRNA compared to 101 (38%) of the unvaccinated group (p<0.001).

Impact of HPV 16 infection on Colposcopic Features and histology

Table S1 in supplementary information shows colposcopic features and histology results by HPV 16 status

There was no association between presence of HPV 16 DNA or HPV16 mRNA and any individual colposcopic features. Despite this, colposcopists were more likely to

record a colposcopic opinion of high grade if participants were HPV 16 DNA positive (57%;p=0.006) or HPV 16 mRNA positive (59%;p=0.03) than if the woman was HPV16 DNA/mRNA negative (37% and 43% respectively). Women were also more likely to have a high grade histology result if they were positive for HPV 16 DNA (71%;p<0.001) or HPV 16 mRNA (77%;p<0.001) than if they tested negative (38% and 43% respectively).

Performance of colposcopy

Table 3: Impact of HPV vaccine and HPV 16 on performance of colposcopy

Table 3summarises the performance of colposcopy in vaccinated and unvaccinated women in terms of sensitivity, specificity, PPV and NPV for the detection of CIN2+.The HPV vaccination status did not have a statistically significant impact on the performance of colposcopy. The PPV of colposcopy was 74.0 (95% CI: 63.8-82.1) in unvaccinated women and 66.7 (95% CI: 35.4-88.7)in vaccinated women although this difference was not statistically significant (p=0.591).

HPV 16 presence or absence had a significant impact on the specificity and NPV of colposcopy for detecting high grade disease (p<0.001). Colposcopy was found to have a higher specificity (92.4 (95% CI: 87.1-95.7) compared to 75.0 (95%CI: 62.3-84.6)) and NPV (94.6 (95% CI: 89.7-97.3) compared to 64.9 (95% CI: 52.8-75.4)) in women who were HPV 16 negative compared to HPV 16 positive.

Discussion

Main Findings

Vaccination in the catch-up cohort is associated with a significant reduction in the prevalence of HPV 16/18 and CIN2+ in women aged 20-25 years attending colposcopy in Scotland[3]. Our results show that colposcopic features were similar in vaccinated and unvaccinated women and differences were related to the incidence of cervical disease. Our results indicate that the performance of colposcopy in vaccinated women has not diminished substantially. However, the PPV for CIN2+ was lower in vaccinated women (albeit not at a statistically significant level).

Strengths and Limitations

To our knowledge, this study is the first to investigate the impact of HPV genotypes on colposcopic features associated with CIN in HPV immunised women. This is possible as cervical screening in Scotland starts earlier than in many countries, with vaccinated women entering our national programme in 2010. Scotland achieved high rates of vaccination in the catch up campaign (65.5%) and has reasonable3 yearly cervical screening uptake (70.7% overall, 50.9% in 20-24 year olds).[15]For Group 2 we were able to assign vaccine status using SCCRS to improve reliability.

To minimise bias, colposcopists and histopathologists were blinded to HPV results and staff undertaking the HPV genotyping tests were blinded to vaccine status.

As the aim of the immunisation is to reduce deaths from cervical cancer, it could be at least age 30 before this can be confidently measured. The long lead–time between

HPV infection and development of malignancy means that high grade CIN (as used in our study) is a justifiable surrogate marker for cervical cancer.[16]

Where the cervix appeared normal, biopsies were not taken (as per local protocols) so these women lacked a "gold standard diagnosis" and were classified as 'disease negative' for analysis. A high proportion of women who did not have a biopsy taken were subsequently found to be HPV 16 negative. This resulted in a high NPV of colposcopy for detecting high grade disease in HPV 16 negative women, despite there being no histological confirmation of disease status for them. The NPV of colposcopy has been previously been recorded as high (up to 96%), so we expect to miss very few cases of CIN.[17,18]

However, as Jeronimo et al.[6] suggested that high grade CIN is more likely to be missed by colposcopy in the absence of HPV 16, it may be that the HPV 16 negative women with normal colposcopy have disease lacking characteristic colposcopic features. Follow up of our cohort in the future will address this.

Interpretation

We believe this is the first study conducted with this primary aim in women who have received HPV vaccine.[6-9]Previousstudies reporting on the impact of HPV genotypes on colposcopy were conducted as *ad hoc* analyses of larger studies with inconsistent results. Jeronimo et al. found that HPV 16 was more likely to produce lesions with colposcopically identifiable features than other HPV types, regardless of histology.[6] Louwers et al. reported the presence of HPV 16 significantly improved the sensitivity of the Dynamic Spectral Imaging colposcopy for CIN and hypothesised that HPV 16

is associated with acetowhitening.[7]Using data from this same study, Zaal et al. found that HPV 16 did not impact the performance of standard colposcopy and suggested that effects were dependent on the underlying grade of disease, rather than HPV16 *per se*.[8]Similarly, van der Marel found that the visual appearance of high-grade HPV16 lesions did not differ from lesions associated with other high-risk HPV types.[9] Our results support this with no significant difference in relation to either vaccine status or presence of HPV16. Changes in PPV relate to the reduced incidence of high grade disease in immunised women as PPV is strongly influenced by disease prevalence and the reduction reflects the reduction in CIN.[19]With the emerging cohort of women who received HPV immunisation as part of routine vaccination, rather than catch up, it is important to clarify the effect of reducing or even eliminating HPV vaccine types from the screened population as we use colposcopy to identify and treat CIN.

We did not find any association between HPV 16 and acetowhitening in women attending colposcopy. Colposcopists were able to identify HPV 16 negative lesions during colposcopy which were confirmed on biopsy. The women included in our study were younger (mean age 22.3 years) compared with previous studies (mean age ranged from 26.2 to 36.7 years).[6-9]Given that the peak prevalence of HPV infection occurs in women beforethat of CIN, we anticipate that the impact of HPV genotypes on colposcopic features may also vary according to age.[20]

The vaccinated women in this study received the HPV immunisation as part of the catch up campaign. The mean age at last dose was 17.3 years. Women were not asked about sexual activity. It is likely that some women were sexually active and therefore not HPV naïve prior to vaccination.[3,21,22]

Our study suggests that, compared to unvaccinated women, lower proportions of vaccinated women had high grade cervical cytology. A similar observation has been made in Australia.[16] This study reported a significant decrease (38%; p=0.003) in high grade cervical abnormalities in young girls (under 18 years) following the introduction of the HPV vaccine but no significant decrease in the incidence of low grade cervical abnormalities in this age category, or in women aged 18-20 years. As the cohort vaccinated in the school programme at age 12 enters screening, in 2021 in the UK, we would expect to see a greater impact on PPV with lower disease rates if we do not review risk stratification of our screening policy.

Our results are consistent with those reported in the screened population in Scotland with a significant reduction in circulating HPV vaccine types and associated diseaseand provides further evidence of the success of the vaccination programme.[3,20,23] The prevalence of HPV16/18 in vaccinated women attending colposcopy is similar to that in young women attending cervical screening (11.5% at colposcopy compared to 11% and 13.6% at screening).[3,23] Kavanagh et al. found that HPV 51 and 56 were the most prevalent HPV genotypes in vaccinated women attending cervical screening (10.5% and 9.6% respectively).[3]The prevalence of HPV 51 and 56 was higher in the vaccinated women attending colposcopy compared to the unvaccinated women (15.7% for each compared to 12.7% and 5.8% respectively in unvaccinated women) in our study. In contrast to Kavanagh et al, we found that HPV 52 and 59 emerged as the most prevalent HPV genotypes in vaccinated women attending colposcopy with abnormal cytology (22.9% and 17.1% respectively). However different HPV assays were used in those studies which may influence HPV genotype detection.

Conclusion

We found no significant impact of vaccination on colposcopic features in women aged 20-25 with abnormal cervical cytology who had received the HPV 16/18 vaccine as part of a catch up campaign. Despite the lower prevalence of HPV 16 in vaccinated women, features considered characteristic of high grade CIN were still detectable**.** Cervical screening needs to continue to offer protection from disease from non-vaccine types. However, the reduction in prevalence of CIN has impacted on the PPV of colposcopy and this has implications for quality assurance of colposcopy in the cervical screening programme.

In order to assess the impact of the HPV vaccination on colposcopy performance further, studies should be conducted when the women who received the vaccine as part of the school based immunisation programme (in whom the coverage rates were 90%) enter the cervical screening programme.

Word count 3465

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Disclosure of Interests

HAC received an honorarium from GSK for participation in an expert panel and received an educational grant from GSK in 2009 in relation to the Scottish HPV archive.

KC's institution has received project funding from GeneFirst and Euroimmune in the last 2 years.

Contribution to Authorship

AM conducted the study, performed the analysis and drafted the manuscript.

CG conducted the study and performed the statistical analysis of women in Group 1.

SCC had oversight of study conduct and statistical analysis, interpretation of results and critical revision of the manuscript.

KK advised on the statistical analysis and contributed to the draft and revisions of the manuscript.

KC had input into design on HPV testing elements, organisation of laboratory work and contribution to manuscript revisions

CM had operational oversight of all HPV testing and delivery of HPV genotyping

HC had input into design of original study and contributed to the draft and revisions of the manuscript

CR advised on the statistical analysis, study design and contributed to the draft and revisions of the manuscript

LS contributed to the local study organisation and contributed to manuscript revisions.

KP contributed to the interpretation of results and the discussion

CBE contributed to the local study organisation and conduct as Principal Investigator at a contributing centre; as well as to manuscript revisions.

TP contributed to interpretation of results and revisions of the final manuscript.

MEC designed the original study, participated in interpretation of results, provided critical revision of the manuscript.

All authors approved the final version.

Details of Ethics Approval

Ethical approval was granted by the North of Scotland Research Ethics Committee on 22/12/2009 prior to the start of recruitment (reference number 09/S0801/106).

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Figure 1: Flow diagram of recruitment

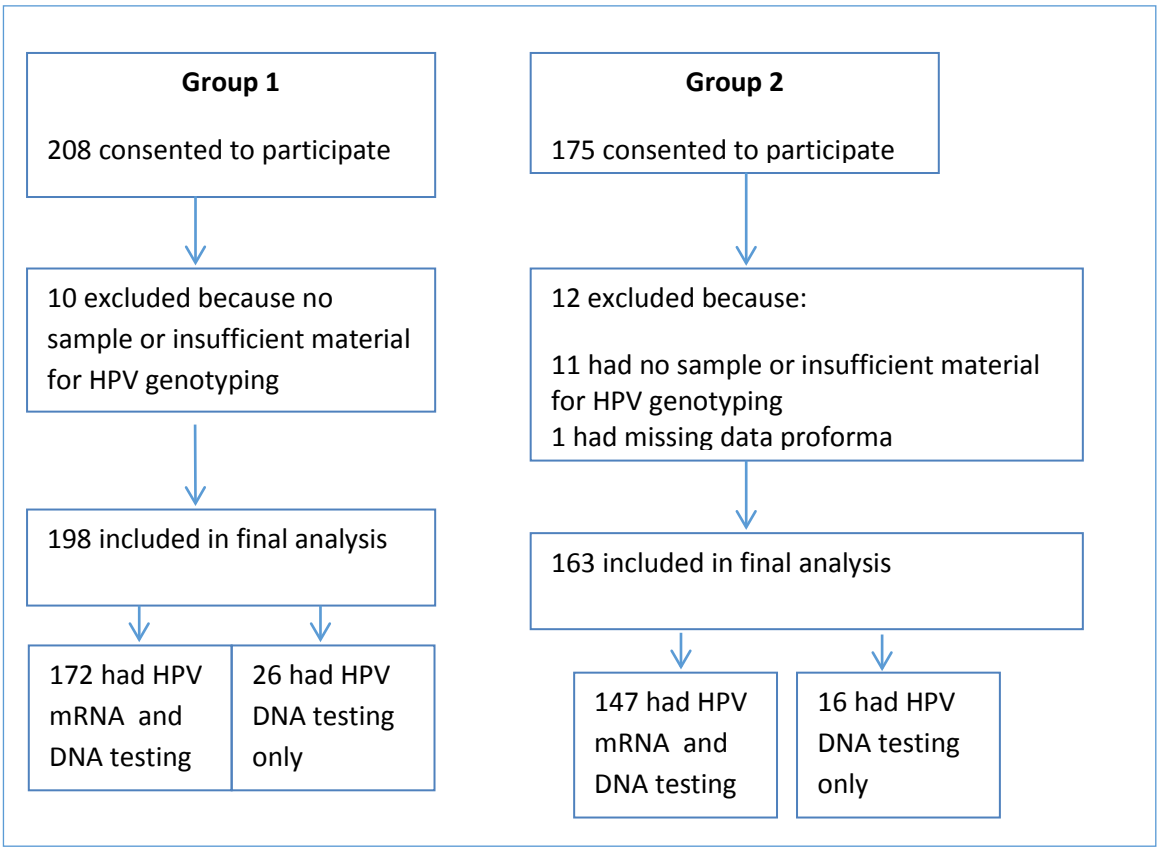

Figure 2: HPV genotyping results from samples collected at colposcopy

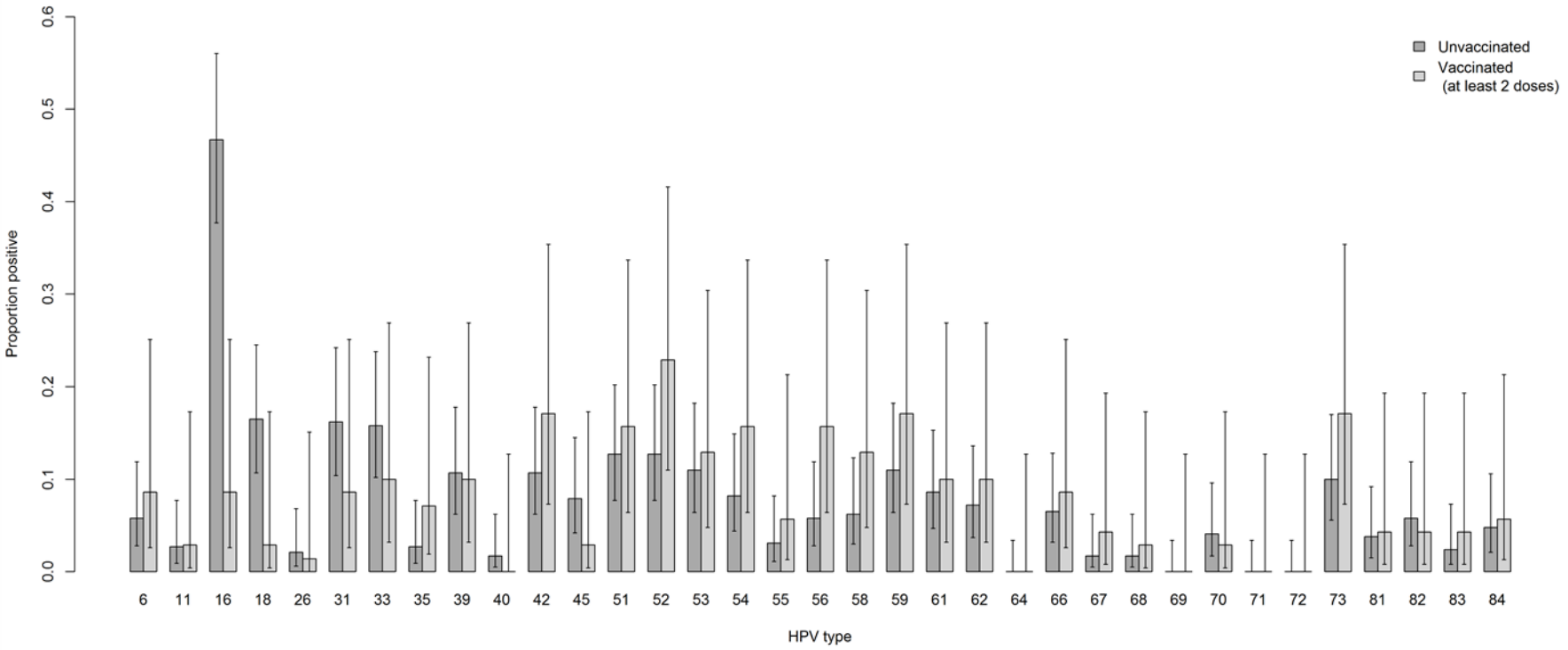

Table 1: Participant demographic data by group

	Group 1 N (column %) N=198*	Group 2 N (column %) N=163		Overall N=361
		Vaccinated 67 (41.1)	Unvaccinated 96 (58.9)	
Site				
Site 1	95 (48.0)	53 (79.1)	93 (96.9)	241 (66.8)
Site 2	103 (52.0)	14 (20.9)	3 (3.1)	120 (33.2)
Age at colposcopy				
20 years	42 (21.2)	17 (25.4)	5 (5.2)	64 (17.7)
21 years	33 (16.7)	31 (46.3)	5 (5.2)	69 (19.1)
22 years	29 (14.6)	14 (20.9)	18 (18.8)	61 (16.9)
23 years	39 (19.7)	3 (4.5)	31 (32.3)	73 (20.2)
24 years	40 (20.2)	1 (1.5)	17 (17.7)	58 (16.1)
25 years	15 (7.6)	1 (1.5)	20 (20.8)	36 (10.0)
Mean Age (years)	22.2 (SD 1.6)	21.2(SD 1.0)	23.2 (SD 1.4)	22.3 (SD 1.6)
Referral Cytology				
Borderline	46 (23.2)	19 (28.4)	27 (28.1)	92 (25.5)
Mild dyskaryosis	86 (43.4)	34 (50.7)	28 (29.2)	148 (41.0)
Moderate dyskaryosis	36 (18.2)	12 (17.9)	28 (29.2)	76 (21.1)
Severe dyskaryosis	24 (12.1)	2 (3.0)	11 (11.5)	37 (10.2)
Glandular neoplasia	1 (0.5)	-	2 (2.1)	3 (0.8)
Invasive cancer	1 (0.5)	-	-	1 (0.3)
Missing	4 (2)	-	-	4 (1.1)
Histology				
Biopsy not taken[±]	61 (30.8)	27 (40.3)	20 (20.8)	108 (29.9)
Normal (No CIN)	19 (9.6)	9 (13.4)	10 (10.4)	38 (10.5)
CIN1	53 (26.8)	18 (26.9)	24 (25.0)	95 (26.3)
CIN2	35 (17.7)	9 (13.4)	23 (24.0)	67 (18.6)
CIN3	24 (12.1)	3 (4.5)	14 (14.6)	41 (11.4)
Invasive squamous 1a1	1 (0.5)	-	1 (1.0)	2 (0.6)
CGIN	2 (1.0)	-	4 (4.2)	6 (1.7)
Unsatisfactory	3 (1.5)	1 (1.5)	-	4 (1.1)

Table 1: Comparison of participant demographics between groups."Vaccinated" women refer to women who had received 2 or more doses of the HPV vaccination. *Group 1 includes 3 women who reported they had received the HPV vaccine. [±]All cases where biopsy was not taken were because colposcopic appearances were normal.

Table 2: Impact of HPV vaccine on colposcopic features and histology.

	Unvaccinated n/N (%)	Vaccinated n/N (%)	chi squared p-value* (Pearson unless indicated)
Colposcopic Features			
Acetowhite	231/291 (79.4)	54/70 (77.1)	0.623
Mosaic	129/291 (44.3)	30/70 (42.9)	0.791
Punctation	111/291 (38.1)	27/70 (38.6)	1.00
Atypical Vessels	3/291 (1.0)	1/70 (1.4)	0.589†
Iodine Negative**	101/202 (50.0)	33/59 (55.9)	0.442
Colposcopic Opinion			
High Grade***	99/290 (34.1)	13/66 (19.7)	0.027
Histology****			
CIN2+	103/286 (36.0)	13/69 (18.8)	0.006
CIN1+	179/286 (62.6)	32/69 (46.3)	0.044†

Table 2 compares the features seen at colposcopy between all participants regardless of disease status who were vaccinated against HPV 16 and 18, and women who were not. It also compares the colposcopic opinion and histology results between these groups. In patients where biopsies were not taken, they were considered to have no disease.*Pearson's test used unless otherwise indicated. †Fisher's exact test used. **in 100 cases, iodine was not used. This was for a variety of reasons including patient allergy or colposcopist preference. ***High grade colposcopic opinion was appearance suggestive of CIN2+. ****Histology results were "unsatisfactory" for 5 unvaccinated and 1 vaccinated therefore were excluded from histology analysis.

Table 3: Impact of the vaccine and of HPV 16 on the performance of colposcopy

	Unvaccinated (95% CI) N=294	**Vaccinated** (95% CI) N=67	**z-test for difference**		**HPV 16+** (95% CI) N=142	**HPV 16 -** (95% CI) N=219	**z-test for difference**
Sensitivity	69.6 (59.6-78.1)	66.7 (35.4-88.7)	p=0.835		65.8 (53.9-76.0)	76.3 (59.4-88.0)	p=0.251
Specificity	86.3 (80.2-90.7)	92.5 (80.9-97.6)	p=0.228		75.0 (62.3-84.6)	92.4 (87.1-95.7)	p<0.001
PPV	74.0 (63.8-82.1)	66.7 (35.4-88.7)	p=0.591		75.8 (63.4-85.1)	69.0 (52.8-81.9)	p=0.443
NPV	83.5 (77.3-88.4)	92.5 (80.9-97.6)	p=0.103		64.9 (52.8-75.4)	94.6 (89.7-97.3)	p<0.001

Table 3: Predictive values of colposcopy for detecting high grade disease where histology results were considered "gold standard" and the test was colposcopic opinion. This has been done to compare predictive values between vaccinated and unvaccinated participants and between participants who are HPV 16 positive and negative.

3: Impact of HPV 16 on colposcopic features and histology

	HPV 16 DNA + n/N (%)	HPV 16 DNA - n/N (%)	chi squared p-value*	HPV 16 mRNA + n/N (%)	HPV 16 mRNA - n/N (%)	chi squared p-value*
Colposcopic Features						
Acetowhite	105/109 (96.3)	104/107 (97.2)	1.00†	85/87 (97.7)	103/107 (96.3)	0.693†
Mosaic	69/109 (63.3)	63/107 (58.9)	0.58	56/87 (64.4)	64/107 (59.8)	0.554
Punctation	61/107 (57.0)	55/107 (51.4)	0.49	50/86 (58.1)	55/106 (51.9)	0.466
Atypical Vessels	2/107 (1.9)	1/106 (0.9)	1.00†	2/85 (2.4)	1/106 (0.9)	0.586†
Iodine Negative	46/109 (42.2)	44/108 (40.7)	0.41	37/87 (42.5)	49/108 (45.4)	0.853
Colposcopic Opinion						
High Grade	61/108 (56.5)	40/107 (37.4)	0.006	51/86 (59.3)	46/107 (43.0)	0.03
Histology						
CIN2+	77/108 (71.3)	39/103 (37.9)	<0.001	67/87 (77.0)	45/104 (43.3)	<0.001

Table 3 compares colposcopic features, colposcopic opinion and histology results between participants with cervical disease (CIN1+) by HPV 16 DNA status, and by HPV 16 mRNA status. Iodine was not used in 31 participants who were HPV 16 DNA+, 24 HPV 16 DNA-, 24 mRNA+, 26mRNA-. *Pearson's test used unless otherwise indicated. †Fisher's exact test used.