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HPV immunisation and cervical screening—confirmation of changed performance of cytology as a screening test in immunised women: a retrospective population-based cohort study

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Background: To document the effect of bivalent HPV immunisation on cervical cytology as a screening test and assess the implications of any change, using a retrospective analysis of routinely collected data from the Scottish Cervical Screening Programme (SCSP).

Methods: Data were extracted from the Scottish Cervical Call Recall System (SCCRS), the Scottish Population Register and the Scottish Index of Multiple Deprivation. A total of 95 876 cytology records with 2226 linked histology records from women born between 1 January 1988 and 30 September 1993 were assessed. Women born in or after 1990 were eligible for the national catch-up programme of HPV immunisation. The performance of cervical cytology as a screening test was evaluated using the key performance indicators used routinely in the English and Scottish Cervical Screening Programmes (NHSCSP and SCSP), and related to vaccination status.

Results: Significant reductions in positive predictive value (16%) and abnormal predictive value (63%) for CIN2+ and the mean colposcopy score (18%) were observed. A significant increase (38%) in the number of women who had to be referred to colposcopy to detect one case of CIN2+ was shown. The negative predictive value of negative- or low-grade cytology for CIN2+ increased significantly (12%). Sensitivity and specificity, as used by the UK cervical screening programmes, were maintained.

Conclusions: The lower incidence of disease in vaccinated women alters the key performance indicators of cervical cytology used to monitor the quality of the screening programme. These findings have implications for screening, colposcopy referral criteria, colposcopy practice and histology reporting.

The UK cervical screening programme is a success and is estimated to have prevented the deaths from cervical cancer of 1 in 65 women born since 1950 (Peto *et al*, 2004). This success can be attributed to adherence to national protocols for regular screening and a strong commitment to regular quality assurance and monitoring. The clinical performance of any screening test is

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crucial and will be influenced not only by fundamental attributes of the test but also by the prevalence of the target disease in the population. This is particularly the case for tests that require subjective interpretation. The primary modality of screening in the UK is liquid-based cytology although primary screening using HPV testing is being piloted at six sites in England (http://www.cancerscreening.nhs.uk/cervical/hpv-primary-screening.html). In Scotland, cytological primary screening is supplemented by image assisted screening (ThinPrep Imaging System, HOLOGIC Inc, Marlborough, MA, USA.

The advent of immunisation against the two most common high-risk types of HPV (HPV16 and 18) is beginning to alter profoundly the prevalence of HPV16 and 18, as well as HPV 31, 33 and 45, in the population (Kavanagh et al, 2014; Pollock et al, 2014; Cameron et al, 2016). Immunisation with the bivalent vaccine began in Scotland in September 2008, with routine immunisation of girls aged 12–13 years in school and a catch-up programme for girls up to the age of 18 years. As a result, the amount of cervical intraepithelial neoplasia (CIN), the precursor of invasive cervical cancer, is also changing in young women attending for cervical screening. Scotland begins cervical screening at age 20 years, so girls immunised as part of catch-up have been screened since 2010. Significant reductions in type specific HPV infection and all grades of CIN, more pronounced with high-grade CIN, have already been demonstrated in Scotland and elsewhere (Kavanagh et al, 2014; Pollock et al, 2014; Drolet et al, 2015).

Much effort is invested in monitoring the effectiveness of cytology to detect high-grade cervical intraepithelial neoplasia (CIN) (ABC, 2013). Various measures have been devised to quantify this for various grades of cytology, including the positive predictive value (PPV) of high-grade dyskaryosis to detect high-grade CIN (HGCIN) and the ability of persistent low-grade cytology to predict HGCIN (abnormal predictive value, APV). Such quality monitoring is particularly relevant given predictions that the performance of cervical cytology may deteriorate as a consequence of immunisation. A reduction in disease in a screened population will directly reduce the PPV of any screening test (Franco et al, 2006). In cervical screening, the relative proportions of high- to low-grade disease influence the PPV for high-grade disease, the target of screening. With cytology as a screening test, this effect may be exaggerated by reader fatigue, with the possibility of missing rare positive events and of overcalling clinically insignificant reactive atypia. These forecasts are theoretical as no data from screening programmes have been published, given the time between vaccination and entry into cervical screening and the challenges of linking data between immunisation and screening databases robustly.

The IT system (Scottish Cervical Call-Recall System, SCCRS) which manages the cervical screening call and recall programme in Scotland holds cytology results, associated histology reports and also immunisation status including the number of doses. Scotland is well placed to assess the impact of immunisation on the performance of cervical cytology. In this paper, we consider the impact of immunisation on the performance of cytology for the detection of CIN.

MATERIALS AND METHODS

Scottish cervical screening programme (SCSP). Cervical screening starts at age 20 years in Scotland, with women being called within 4–6 weeks of their 20th birthday. All of Scotland uses Thinprep liquid based cytology with image assisted screening (ThinPrep Imaging System, HOLOGIC Inc). A total of eight NHS cytology laboratories serve the programme and process ∼400 000 samples a year (http://www.sccrs.scot.nhs.uk/lab.html). Cytology

and histology classification is performed according to British Association for Cytology and NHS Cervical Screening Programme (NHSCSP) criteria (Denton et al, 2008; NHSCSP, 2010). A comparison between the various reporting systems in use is given in Figure 1.

Referral for colposcopy is made after one instance of high-grade disease or borderline, query high-grade (ASC-H) and for persistent low-grade disease. Persistent low-grade disease is defined as two instances of low-grade dyskaryosis or three instances of borderline change during an episode of abnormal follow-up, or three abnormal smears in the last 10 years. In addition, referral for colposcopy is made following three consecutive unsatisfactory smears (SCSP, 2013).

Selection of analysis cohort. The screening records of women resident in Scotland and born between 1 January 1990 and 30 September 1993 who had cytology tests taken after the age of 20 years and before age 21 years, in their first year of eligibility for screening, were examined in this analysis. Data were extracted from SCCRS at the end of September 2014, giving a minimum of 12 months follow-up for each woman. The following data were extracted:

- Result of cytology tests taken in the first year of screening
- Histology results taken at colposcopy as a consequence of the cytology result
- Immunisation status by number of doses received (0, 1, 2 or 3 doses)
- Year of birth
- Postcode of residence

For most women, the results corresponded to their first smear or first colposcopy examination. For the relatively few women with more than one smear or biopsy the most severe result was used for analysis.

The extract criteria were chosen to obviate bias due to age at time of screening, and due to opportunity for disease detection.

NHSCSP	Two-tier	The Bethesda system
Borderline squamous and glandular changes without HPV		ASC-US/ASC-H/AGUS
HPV changes (koilocytosis) +/– low grade dyskaryosis	Low-grade	LSIL
High grade dyskaryosis (moderate)		HSIL
High grade dyskaryosis (severe)		
High grade dyskaryosis/ invasive		Cancer
Glandular neoplasia	High-grade	AGC favour neoplasia
		AIS, adenocarcinoma

Figure 1. Cytology classification according to British Association for Cytology (BAC) and NHS Cervical Screening Programme (NHSCSP) criteria (Denton et al, 2008).

The records extracted were compared with the population register to eliminate women who were not resident in Scotland at the time of immunisation. Duplicate records were identified and the cytology and histology results amalgamated into a single patient record. Following these two steps, the postcode of residence was used to derive the SIMD quintile and rurality index. The records were anonymised with preservation of linkage between immunisation, cytology, and histology where appropriate. Caldicott Guardian approval was obtained for the use of patient-identifiable data.

Statistical methods

Impact of immunisation on cytological abnormalities and on histological diagnosis. In order to be able to measure the performance of cytology and place it in context, the impact of immunisation on cytological and histological abnormalities was assessed. The cytology result was recorded as Inadequate, Negative (no evidence of disease), Borderline (including borderline glandular changes), and Low-grade, Moderate or Severe dyskaryosis (including glandular abnormalities). Histology was coded as Normal (no CIN detected), CIN1, CIN2, and CIN3 + (CIN 3 or cancer). The associations between the outcomes of colposcopy and cytology and the demographic variables were estimated using multinomial logistic regression models. Odds ratios (ORs) for the various response levels compared with the baseline or normal levels are reported with 95% confidence intervals (CIs). Univariate and multivariable models were used. In addition to the number of doses of vaccine, we investigated birth cohort, Scottish Index of Multiple Deprivation, (with level 1 corresponding to the most deprived), and an Urban Rural indicator, derived from the Scottish Government 8 level indicator and with three levels: Urban, Accessible Rural (30–60 min drive to a settlement of 10 000 or more), and Remote Rural (>60-min drive to a settlement of 10 000 or more). Two-level interactions were investigated using a Bonferroni correction to the P-value for multiple testing.

Linked histology records and cytology results were tabulated and correlated with the number of doses of vaccine administered. Comparison of the measures of test performance between fully vaccinated and unvaccinated individuals was conducted using a χ^2-test of association with a Bonferroni correction used to account for multiplicity of testing based on the different measures and the two end points, CIN2 + and CIN3 +. Confidence intervals for binomial proportions were calculated using Wilson's method. In a sensitivity analysis this comparison was adjusted for cohort as the 1988 and 1989 cohorts are unimmunised and only the later ones have some fully immunised individuals. Logistic regression was used here with analysis of deviance tests.

Impact of immunisation on performance of cytology. In women who had a satisfactory colposcopic examination the following measures were calculated according to the formulae in the UK Cervical Screening Programme guidance 'Achievable Benchmarks in Cytology', version 3 (ABC, 2013) (Figure 2). Both CIN2 + and CIN3 + were used as end points:

- Sensitivity of high-grade dyskaryosis for CIN2 + and CIN3 +;
- Specificity of negative, borderline or low-grade dyskaryosis for absence of high-grade CIN;
- Positive predictive value of high-grade dyskaryosis for HGCIN;
- Abnormal predictive value of persistent low-grade dyskaryosis and/or borderline changes for HGCIN;
- Referral value, which is the number of women who are referred to colposcopy to detect one case of HGCIN;
- Total predictive value of any cytological abnormality for HGCIN;
- Negative predictive value of low-grade or negative cytology for HGCIN;

Cytology result	Histology result			
	Negative	HPV only	CIN1	CIN2, CIN3, invasive cancer CGIN/adenocarcinoma *in situ*
Low-grade cytology (boderline/mild)	*a*			*b*
High-grade cytology (moderate or worse)	*c*			*d*

Figure 2. Formulae used for derivation of predictive values.
Positive predictive value = $d/(c+d)$
Abnormal predictive value = $b/(a+b)$
Total predictive value (TPV) = $(b+d)/(a+b+c+d)$
Negative predictive value = $a/(a+b)$
RV = $1/TPV$.
Abbreviations: CGIN = cervical glandular intraepithelial neoplasia; CIN = cervical intraepithelial neoplasia.

- Mean CIN Score—the (weighted) average amount of disease per case seen at colposcopy (MCS).

RESULTS

Data extract. The final data set contained a total of 95 876 cytology records from women aged between 20 years and 20 years and 364 days, of whom 2226 had attended colposcopy. These women with both cytology and histology records were used for the analysis of cytology performance. A total of 34 161 (35.6%) women had received three doses of vaccine (complete schedule of immunisation in the catch-up cohorts), whereas 57 140 (59.6%) were unvaccinated. Only 1475 (1.5%) and 3100 (3.2%) records were associated with women who had received one and two doses of vaccine, respectively (Table 1).

Impact of immunisation on cytological abnormalities. Complete immunisation with three doses was associated with a highly significant ($P<0.001$) reduction in all grades of cytological abnormality (Table 2). The reduction in high-grade dyskaryosis was greater than that observed for low-grade abnormalities. When compared with fully vaccinated women, unvaccinated women had an odds ratio of severe dyskaryosis of 2.95 (95% CI 2.17–4.02), for moderate dyskaryosis of 2.43 (95% CI 1.94–3.05), for low-grade dyskaryosis of 1.38 (95% CI 1.26–1.51), and for borderline changes of 1.27 (95% CI 1.19–1.35). Partial immunisation with two doses was also associated with a reduction in low-grade dyskaryosis (OR 1.13, 95% CI 0.95–1.34) compared with unvaccinated (OR 1.38, 95% CI 1.26–1.51) but this was not statistically significant. Immunisation with one dose of vaccine was not associated with a reduction of abnormal cytology when compared with no immunisation.

The proportions of women with the different grades of abnormal cytology varied by birth cohort ($P<0.001$). However, once adjusted for number of doses of the vaccine, only the reduction in borderline changes in the 1993 cohort remained significant. There are trends associated with deprivation ($P<0.001$) with higher odds of disease for all outcomes among the most deprived individuals (Table 2).

Table 1. Number of women born between 1 January 1988 and 30 September 1993 who attended for cervical screening within 1 year of becoming 20 years, by year of birth: outcome of cytology, outcome of any colposcopy examination and number of doses of HPV vaccine received

Year of Birth	1988	1989	1990	1991	1992	1993	Total
Total number	17 139	16 451	17 040	17 125	16 382	11 739	95 876
Cytology							
Normal	82.1	82.6	81.6	83.5	83.6	84.5	82.9
Borderline	10.6	11.1	12.3	11.4	10.2	7.5	10.7
Low-grade dyskaryosis	5.3	4.7	4.5	4.0	5.3	7.0	5.0
High-grade dyskaryosis (Moderate)	1.2	1.0	1.1	0.8	0.6	0.7	0.9
High-grade dyskaryosis (severe) or worse	0.7	0.6	0.5	0.4	0.3	0.4	0.5
Colposcopy attendance (%) with outcome							
No colposcopy	97.09	97.22	97.32	97.83	98.38	98.50	97.68
Colposcopy	2.91	2.78	2.68	2.17	1.62	1.50	2.32
Normal	0.78	0.72	0.70	0.57	0.41	0.32	0.60
CIN1	0.67	0.81	0.70	0.61	0.48	0.45	0.62
CIN2	0.86	0.69	0.68	0.55	0.42	0.48	0.61
CIN3	0.61	0.57	0.59	0.44	0.32	0.25	0.47
Immunisation							
Unimmunised	99.94	99.68	80.32	27.45	17.49	20.10	59.60
Partially immunised 1 dose	0.02	0.05	1.29	3.09	2.53	2.56	1.54
Partially immunised 2 doses	0.01	0.09	2.77	6.80	5.20	5.07	3.23
Fully immunised 3 doses	0.03	0.18	15.62	62.65	74.78	72.28	35.63

Abbreviations: CIN = cervical intraepithelial neoplasia; HPV = human papilloma virus.

Impact of immunisation on performance of cytology. In women with a satisfactory colposcopy ($n = 2226$), the sensitivity of $\geqslant$ high-grade dyskaryosis for CIN2 + and the specificity of $\leqslant$ low-grade dyskaryosis for $< = $CIN1 was slightly higher in fully immunised vs non-immunised women although these differences were not significant (Tables 3a and 3b).

The PPVs of $\geqslant$ high-grade dyskaryosis for both CIN2 + and CIN3 + reduced in fully immunised women by 16% ($P = 0.002$) and 14% ($P = 0.25$, NS), respectively. The NPV of $\leqslant$ low-grade dyskaryosis was higher in immunised women than in non-immunised women for both CIN2 + ($P = 0.002$) and CIN3 + ($P = 0.033$ (NS)). The APV of low-grade and borderline changes for CIN2 + decreased in fully immunised women for CIN2 + by 63% ($P = 0.002$) and for CIN3 + by 97% ($P = 0.049$ (NS)). The number of women who had to be referred to colposcopy to detect one case of high-grade disease (referral value) was significantly increased for both CIN2 + and CIN3 + in fully immunised women ($P < 0.001$ and $P = 0.005$, respectively). There was a corresponding significant decrease ($P < 0.0001$) in the average amount of disease per case seen at colposcopy (MCS) in fully immunised women (MCS = 1.23 (s.d. = 1.04)) compared with unimmunised women (MCS = 1.46 (s.d. = 1.07)). In a sensitivity analysis the comparison of fully immunised women with unimmunised women was adjusted for cohort. There was no evidence of a temporal trend associated with cohort and the conclusions were unaffected (data not shown).

DISCUSSION

Scotland is almost uniquely placed to determine the impact of immunisation on the performance of cytology screening in young women using national data sets, which can be linked effectively. The results show preservation of sensitivity of high-grade cytology for CIN2 + and specificity of negative or low-grade cytology for the absence of CIN2 +, yet a deterioration overall in the predictive value of cytology for the detection of CIN2 +. These findings confirm the expectation of Franco et al (2006) who predicted a reduction in the overall performance of cytology as a consequence of vaccination. In their 2006 paper, Franco used estimates of

sensitivity (51%) and specificity (98%) based on the correlation of HSIL with CIN2 + taken from Nanda et al (2000). In their 2009 modelling, Franco et al, 2009 used a sensitivity of 70% and specificity of 98%, similar to those achieved by the SCSP. Furthermore, these authors examined the effect of variations of sensitivity and specificity on the predictive value at various levels of disease prevalence. When the sensitivity and specificity are maintained, PPV drops sharply and progressively at disease prevalence of < 10%. As sensitivity falls, PPV declines even further. The prevalence of high-grade disease in the fully immunised cohorts is significantly lower in younger women, and we expect it to fall still further when the routinely immunised women enter the screening programmes in the UK from September 2015. Positive predictive value is dependent on disease prevalence, but cytology is a subjective technique, relying on pattern recognition. Thus, the effect on PPV may be exaggerated with a knock on effect for colposcopy by failing to identify a population with sufficiently high-risk to warrant further intervention.

The significant reduction in PPV of high-grade dyskaryosis for the detection of CIN2 + contrasts with the maintained PPV for CIN3 +. This may reflect the small numbers of CIN3 + cases in the fully immunised women compared with CIN2 + cases, and hence wider confidence limits. Other reasons for the difference between the outcomes for CIN2 + and CIN3 + include difficulty in the interpretation of cytology and difficulty in correctly distinguishing CIN2 from reactive metaplasia on histology in immunised women. The cytological features usually interpreted as dyskaryosis may have different significance in immunised than non-immunised women, being more likely to represent reactive changes and metaplasia than significant disease. The diagnosis of CIN2 is less robust than CIN3, with a greater possibility of over diagnosing reactive viral changes (Robertson et al, 1989; Mesher et al, 2015). The difficulty in interpreting correctly the cytology and histology may be a result of the changing HPV distribution in immunised women. Most of the high-grade disease observed in the UK in non-immunised women has been driven by HPV 16 and 18 (Mesher et al, 2015). HPV 16 particularly is known to have a shorter natural history in the development of CIN3. Lesions related to non-vaccine types may be detected at an earlier stage of development than hitherto, when they are smaller and have less

Table 2. Adjusted multivariate (OR) and 95% confidence limits (LCL lower, UCL upper) for the cytology outcomes of borderline, low-, moderate- and severe-grade dyskaryosis

| | No. of women | Low grade | | | | | | High-grade | | | | | |
| | | Borderline | | | Low-grade dyskaryosis | | | Moderate dyskaryosis | | | ≥Severe Dyskaryosis | | |
		n	%	OR (CI)	n	%	OR (CI)	n	%	OR (CI)	n	%	OR (CI)
Year of birth													
1988	17 139	1823	10.64	1.23 (1.12, 1.36)	910	5.31	0.61 (0.55, 0.69)	206	1.20	0.99 (0.74, 1.33)	121	0.71	1.00 (0.68, 1.48)
1989	16 451	1830	11.12	1.28 (1.16, 1.41)	775	4.71	0.54 (0.48, 0.61)	164	1.00	0.82 (0.61, 1.11)	93	0.57	0.08 (0.54, 1.20)
1990	17 040	2098	12.31	1.49 (1.36, 1.63)	768	4.51	0.55 (0.49, 0.62)	179	1.05	0.98 (0.73, 1.30)	88	0.52	0.84 (0.57, 1.24)
1991	17 125	1945	11.36	1.50 (1.38, 1.63)	681	3.98	0.56 (0.50, 0.62)	132	0.77	1.02 (0.77, 1.34)	69	0.40	0.99 (0.68, 1.46)
1992	16 382	1672	10.21	1.39 (1.27, 1.51)	862	5.26	0.77 (0.69, 0.84)	91	0.56	0.82 (0.61, 1.11)	55	0.34	0.95 (0.64, 1.42)
1993	11 739	877	7.47	1.00	822	7.00	1.00	82	0.70	1.00	43	0.37	1.00
SIMD													
1 Most deprived	21 468	2459	11.45	1.13 (1.06, 1.20)	1168	5.44	1.16 (1.06, 1.27)	261	1.22	1.81 (1.46, 2.25)	146	0.68	2.21 (1.62, 3.03)
2	20 211	2156	10.67	1.05 (0.98, 1.12)	1035	5.12	1.08 (0.99, 1.19)	205	1.01	1.52 (1.21, 1.9)	133	0.66	2.13 (1.55, 2.92)
3	18 743	1994	10.64	1.05 (0.98, 1.12)	904	4.82	1.02 (0.92, 1.12)	154	0.82	1.21 (0.95, 1.55)	66	0.35	1.10 (0.76, 1.58)
4	16 957	1730	10.20	1.00 (0.93, 1.07)	827	4.88	1.03 (0.93, 1.13)	114	0.67	1 (0.78, 1.3)	70	0.41	1.31 (0.92, 1.88)
5 Least deprived	18 497	1906	10.30	1.00	884	4.78	1.00	120	0.65	1.00	54	0.29	1.00
Dose of vaccine													
0	57 140	6516	11.40	1.27 (1.19, 1.35)	2938	5.14	1.38 (1.26, 1.51)	644	1.13	2.43 (1.94, 3.05)	363	0.64	2.95 (2.17, 4.02)
1	1475	179	12.14	1.33 (1.13, 1.56)	89	6.03	1.38 (1.10, 1.72)	14	0.95	1.92 (1.11, 3.34)	11	0.75	3.07 (1.63, 5.80)
2	3100	353	11.39	1.22 (1.08, 1.37)	155	5.00	1.13 (0.95, 1.34)	35	1.13	2.28 (1.57, 3.3)	16	0.52	2.13 (1.24, 3.65)
3	34 161	3197	9.36	1.00	1636	4.79	1.00	161	0.47	1.00	79	0.23	1.00
Urban rural													
Urban	88 904	9589	10.79	1.21 (1.06, 1.39)	4460	5.02	1.07 (0.89, 1.29)	781	0.88	0.79 (0.54, 1.16)	419	0.47	0.51 (0.33, 0.77)
Accessible rural	4277	412	9.63	1.10 (0.93, 1.3)	231	5.40	1.18 (0.94, 1.47)	44	1.03	1.03 (0.64, 1.66)	26	0.61	0.75 (0.43, 1.32)
Very remote rural	2695	244	9.05	1.00	127	4.71	1.00	29	1.08	1.00	24	0.89	1.00

Abbreviations: CI = confidence interval; OR = odds ratio; SIMD = scottish index of multiple deprivation. *n* is the number of women with the event % is the percentage of women with the event.

specific features. Further, it may be that the non-vaccine related types have different cytological presentations (Bosch *et al*, 2008; Thomsen *et al*, 2015).

The reduction in the APV is also clinically important in cytology based screening, as current management protocols for low-grade cytology are centred on the likelihood of high-grade CIN being present (NHSCSP20, 2010). This reduction was only significant using CIN2 + as an outcome despite a much greater percentage reduction for CIN3 + (54%) than for CIN2 + (39%). The lack of significance at the CIN3 + level could be influenced by the small number of cases in this young age group. The higher negative predictive value (NPV) of low-grade cytology in immunised women is in keeping with the reduced APV. For CIN3 +, this offers considerable reassurance as < 1 in 20 immunised women with persistent low-grade abnormalities will have CIN3 + .

The strengths of this study include the use of routinely collected data from a nationally organised cervical screening programme that uses a single information system, SCCRS. The information in SCCRS is scrutinised regularly as it is used to monitor the performance and quality of the programme. The HPV immunisation programme is also organised at a population level, with high uptake and direct linkage to the cervical screening data. The histological diagnoses of women referred for colposcopy and who had a biopsy are comparable to those already reported from Scotland (Pollock *et al*, 2014).

A weakness of the study is that the immunised women attending for screening were vaccinated as part of the catch-up programme, and some will have been sexually active before immunisation (Kavanagh *et al*, 2014; Pollock *et al*, 2014). This would reduce the effectiveness of immunisation, and also influence the effect of immunisation on cytology performance. The data presented in this paper may therefore underestimate the true effect of immunisation on cytology performance in women immunised as part of the routine programme. In addition, the true immunisation status is only known for women immunised as part of the national programme. Women moving into Scotland and those immunised in the private sector are recorded on SCCRS as unvaccinated. Although relatively few women come into either of these categories, this also leads to a slight underestimate of the true effect of immunisation.

The measures of cytology performance may be confounded by colposcopy performance and disease ascertainment, and by non-attendance at colposcopy. Knowledge of referral cytology is recognised as influencing the colposcopic impression (Shafi *et al*, 1993). Given that colposcopy performance relies on pattern recognition by the practitioner, unfamiliarity with this new referral population may result in the colposcopist missing disease or, alternatively, increasing the number of interventions, biopsies or treatment with negative histology. This may not be a significant problem at present because there are relatively few fully immunised women in the screening programme, but it will be become increasingly important. This will have to be addressed through colposcopy training and quality assurance.

The key performance indicators have been calculated using known histology outcomes only. An alternative methodology is to presume that all women who attend colposcopy and are not biopsied, and all those who do not attend colposcopy, do not have disease (called 'predictive value of referral'). Although the use of only histological outcomes will overestimate the predictive values, the use of the referral population, irrespective of attendance, will underestimate

Table 3a. Sensitivity, specificity, PPV, NPV, APV, TPV and RV of cytology for colposcopy outcomes (CIN2+) among women attending for a colposcopy within 12 months of their first invitation for screening

Measure	Vaccination	N	R	Estimate (95% CI)	P-value
Sensitivity high-grade dyskaryosis CIN2+	Unimmunised	807	604	74.85 (71.74, 77.72)	0.793
	Fully immunised	176	134	76.14 (69.32, 81.83)	
Specificity Neg/Border/LG CIN2+	Unimmunised	815	630	77.30 (74.30, 80.04)	0.950
	Fully immunised	303	233	76.90 (71.83, 81.29)	
PPV of high-grade dyskaryosis for CIN2+	Unimmunised	789	604	76.55 (73.47, 79.38)	0.002
	Fully immunised	204	134	65.69 (58.94, 71.86)	
NPV Neg/Border/LG for CIN2+	Unimmunised	833	630	75.63 (72.60, 78.42)	0.002
	Fully immunised	275	233	84.73 (80.00, 88.50)	
APV of Bl/LG for CIN2+	Unimmunised	759	179	23.58 (20.70, 26.73)	0.003
	Fully immunised	256	37	14.45 (10.67, 19.29)	
TPV of all colp for CIN2+	Unimmunised	1622	807	49.75 (47.32, 52.18)	0.000
	Fully immunised	479	176	36.74 (32.55, 41.15)	
RV of all colp for CIN2+	Unimmunised	1622	807	2.01 (1.92, 2.11)	0.000
	Fully immunised	479	176	2.72 (2.43, 3.07)	

Abbrevaitions: APV = abnormal predictive value; CI = confidence interval; CIN = cervical intraepithelial neoplasia; colp = colposcopy; NPV = negative predictive value; PPV = positive predictive value; RV = referral value; TPV = total predictive value.

Table 3b. Sensitivity, specificity, PPV, NPV, APV, TPV and RV of cytology for colposcopy outcomes (CIN3+) among women attending for a colposcopy within 12 months of their first invitation for screening

Measure	Vaccination	N	R	Estimate (95% CI)	P-value
Sensitivity high-grade dyskaryosis CIN3+	Unimmunised	351	288	82.05 (77.70, 85.71)	0.427
	Fully immunised	75	65	86.67 (77.17, 92.59)	
Specificity Neg/Border/LG CIN3+	Unimmunised	1271	770	60.58 (57.87, 63.23)	0.081
	Fully immunised	404	265	65.59 (60.83, 70.06)	
PPV of high-grade dyskaryosis for CIN3+	Unimmunised	789	288	36.50 (33.22, 39.92)	0.249
	Fully immunised	204	65	31.86 (25.85, 38.54)	
NPV Neg/Border/LG for CIN3+	Unimmunised	833	770	92.44 (90.44, 94.04)	0.033
	Fully immunised	275	265	96.36 (93.44, 98.01)	
APV of Bl/LG for CIN3+	Unimmunised	759	51	6.72 (5.15, 8.73)	0.049
	Fully immunised	256	8	3.13 (1.59, 6.04)	
TPV of all colp for CIN3+	Unimmunised	1622	351	21.64 (19.70, 23.71)	0.005
	Fully immunised	479	75	15.66 (12.68, 19.18)	
RV of all colp for CIN3+	Unimmunised	1622	351	4.62 (4.22, 5.08)	0.005
	Fully immunised	479	75	6.39 (5.21, 7.89)	

Abbrevaitions: APV = abnormal predictive value; CI = confidence interval; CIN = cervical intraepithelial neoplasia; colp = colposcopy; NPV = negative predictive value; PPV = positive predictive value; RV = referral value; TPV = total predictive value.

predictive values as some of the non-attenders will have significant disease. Referral urgency is graduated according to the cytology and it is possible, for example, that women with persistent low-grade disease, who wait longer for colposcopy, are less likely to attend than women with high-grade disease; similar biases may exist for taking coloposcopic biopsies. It is not possible to account for these factors with the data available in SCCRS but ongoing studies using the national clinical colposcopy database will address this issue.

The findings have implications for colposcopy, for cervical cytology, and for histology reporting. There are clear implications for colposcopy services. The prevalence of significant disease in immunised women seen at colposcopy will reduce and the number of women who need to be referred to detect one case of CIN2+ will increase significantly. At the level of CIN3+, 38% (CIN2+, 35%) more immunised women than non-immunised women have to be referred following abnormal cytology to detect one case. The criteria for referral for colposcopy need revision for immunised women with persistent low-grade disease to avoid over

investigation. Colposcopy with or without associated diagnostic and therapeutic interventions brings its own physical and psychological effects (Sharp et al, 2009).

The changing environment in which cytology is practiced is having an adverse effect on its utility as a screening test. Performance is likely to reduce further as the proportion of immunised women in the screened population rises, particularly in routinely immunised women, where a greater reduction in HPV prevalence and associated disease is anticipated. This reduction in performance will be accentuated if the sensitivity of high-grade cytology for CIN2+ declines. There will come a point where the balance of benefit and harm reverses, and cytology will no longer be the screening test of choice. With regards to histology, cervical biopsies from immunised women may be more difficult to interpret, and adjunctive tests may need to be used for accurate classification (Galgano et al, 2010).

Testing for the presence of high-risk HPV is the obvious alternative to cytology as a screening test, although we

acknowledge that the performance of HPV-based primary screening has been assessed almost entirely in unimmunised women to date (Dijkstra *et al*, 2014; Ronco *et al*, 2014). Consequently, endeavours to assess the impact of immunisation on HPV primary screening are underway in Scotland (Bhatia *et al*, 2014; Cruickshank *et al*, 2014). Women positive for high-risk HPV are likely have a much higher prevalence of high-grade disease than the current primary screening population. In these circumstances, cytology could serve as an effective triage test (Franco *et al*, 2009; Rijkart et al, 2012). Our results should be generalisable to other populations with high vaccine coverage and organised screening. The effect of cytology performance is likely to be less noticeable in populations with low immunisation rates, but may be relevant to forward planning if uptake is expected to increase.

In conclusion, the performance of cervical cytology as a screening test is adversely affected by immunisation, particularly the ability of low-grade cytology to predict clinically significant disease, with consequences for referral criteria and colposcopy practice. Implications for colposcopy services include the challenge of managing a higher proportion of referred women who have no (or clinically insignificant) disease. Continued monitoring of cervical screening performance in immunised women and the assessment of new models of screening (e.g. HPV testing) more adapted to the immunisation era are essential so that the quality of what is arguably the most successful cancer screening programme to date can be maintained.

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CONFLICT OF INTEREST

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