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Author(s)	Molloy, M.; Comer, R.; Rogers, P.; Dowling, Maura; Meskell, P.; Asbury, K.; O'Leary, M.
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High Risk HPV testing following treatment for cervical intraepithelial neoplasia.

Maura MOLLOY
Registered Advanced Midwife Practitioner
Women's & Children's Directorate, University Hospital, Newcastle Rd, Galway, Ireland
Email Maura.molloy@hse.ie
353 91 893801
(First and corresponding author)

Rachael COMER
Clinical Nurse Manager
Women's & Children's Directorate, University Hospital, Newcastle Rd, Galway, Ireland
Email Rachael.comer@hse.ie

Pat ROGERS
Clinical Nurse Manager
Women's & Children's Directorate, University Hospital, Newcastle Rd, Galway, Ireland
Email patricia.rogers@hse.ie

Maura DOWLING
Lecturer
School of Nursing and Midwifery,
National University of Ireland, Galway, University Road, Galway, Ireland.
Email: Maura.dowling@nuigalway.ie

Pauline MESKELL
Lecturer
School of Nursing and Midwifery,
National University of Ireland, Galway, Ireland.
Email: Pauline.meskell@nuigalway.ie

Katharine ASTBURY
Consultant Obstetrician
Women's & Children's Directorate, University Hospital, Newcastle Rd, Galway, Ireland
Email katarine.asbury@hse.ie

Michael O'LEARY
Consultant Obstetrician and Gynaecological Oncologist
Women's & Children's Directorate, University Hospital, Newcastle Rd, Galway, Ireland
Email michael.oleary@hse.ie

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Introduction

One of the first Colposcopy clinics in Ireland was developed by a medical Consultant at University Hospital Galway in 1980. At that time laser ablation and knife cone biopsy were the available treatments for precancer (CIN) of the cervix. In 1994 Large Loop Excision of Transformation Zone (LLETZ) was introduced at the clinic replacing both laser and knife cone biopsy. From 1980 to 2008, follow up after treatment included Colposcopy and smear at 4 months with frequent cytology surveillance for 10 years.

Cervicalcheck, the Irish screening programme was launched in September 2008. In preparation for the national programme capacity at Colposcopy was increased. There are now fifteen colposcopy clinics in Ireland and most have registered nurse/midwife colposcopists delivering much of the service. There are three registered advanced nurse practitioners (RANPs) and two registered advanced midwife practitioners (RAMPs) employed in Irish colposcopy clinics. Most women referred to our Colposcopy service can be seen, examined and treated by the RAMP. Some women must be seen by a Medical Consultant, for instance, those with complex medical conditions and histologically confirmed cervical cancers.

National guidelines were introduced in 2009[1] and local guidelines were changed as a result. Colposcopy examination was removed from follow up after treatment and frequent cytology (smear test) without Colposcopy was the recommended local practice. On 1st January 2012 combined cytology and HR HPV (co-test) was introduced by cervicalcheck. Careful follow up of treated women is a vital element of

their care and this audit was performed by a RAMP to analyse the effectiveness of HPV testing after treatment.

Materials and Methods

The aim of this retrospective chart audit was to determine the results of combined cytology and HR HPV tests at six and eighteen months post colposcopy treatment. The audit was conducted at Galway University Hospital's colposcopy centre.

All women who attended the centre's colposcopy smear clinic for a post treatment co-test in the six months (initial test) from January 1st to June 30th 2012 were included (n=251). On arrival to the clinic, women were provided written information on the co-test. The initial HR HPV test was performed using HC2 High Risk HPV DNA (Digene) and the repeat HR HPV test at eighteen months after treatment was performed using Cobas 4800 PCR (Roche). The change of test was a policy decision by the Irish National Screening Programme.

HR HPV was reported as 'detected' or 'not detected' for fourteen high risk viruses at 6 months post treatment. However when positive, genotyping was not available to identify which of the 14 viruses was detected. Those women with HR HPV detected or cytology >ASCUS (atypical squamous cells of undetermined significance) had Colposcopy review. Those with HR HPV not detected and smear negative or ASCUS were informed of the result and booked for repeat smear and HR HPV test in one year.

The mediscan computer system, designed specifically for colposcopy with an image capturing facility, stores all patients' clinical details. Patients' details were retrieved

electronically for the audit. Patient files were also retrieved in some cases (n=16) to verify results.

Ethical approval is not required for audit from the centre's ethics committee. Women who partake in the screening programme give written consent to have their information used to compile figures and reports. The centre's governance structure is adhered to for all audits and no women's names or other identifiers are included.

Results

A total of 251 women with a median age of 35 years (range 21-61 years) had co-test performed in the first six months of 2012. Of these 97% (n=244) had LLETZ procedure and 3% (n=7) had diathermy ablation. The RAMP performed 67% of LLETZ treatments (n=167). At six months after treatment high risk HPV was detected in 21% (n=53) and not detected in 79% (n=198). Histology of LLETZ specimens by HR HPV status and cytology result at six months post treatment is presented in table 1. High grade CIN (CIN2 and CIN3) was reported in 74% (n= 180), CIN1 in 16% (n=39), CGIN in 1% (n=3), micro-invasion in 1% (n=2), normal 3% (n=9) and cervicitis/viral in 5 % (n=15).

Age, excision margins and smoking by HR HPV status are presented in table 2. Excision margins were available on 214 of 244 women who underwent LLETZ. Involved excision margins were more likely to have positive HR HPV 6 months after treatment than clear margins (49% vs. 34%). A higher percentage of smokers had a positive HR HPV (49% vs. 38%), however an independent-samples t-test did not reach statistical significance ($t(248)=1.378$, $p=0.17$), so smokers (M=1.25, SD.388) were statistically no more likely to have a positive HR HPV than non smokers

(M=1.18, SD .438). Women aged 35 and over had a higher percentage of positive HR HPV test (57% VS 51%) however an independent-samples t-test did not reach statistical significance (*t*(249)=.983, p=0.327), and scores for women 35 years and over (M=1.24, SD.426) were not statistically significant than scores for women younger than 35years (M=1.19, SD .390). A Pearson's product-moment correlation coefficient was computed to assess the relationship between age and LLETZ histology. Results highlighted a weak positive correlation between age and histology (r =0.175, n=251, p=.006).

Of the women who were HPV negative at six months (78.9%, n=198), cytology results were negative (n=185), ASCUS (n=12), and low-grade squamous intraepithelial (LSIL), (n=1) (Table 2). None of the women with negative HR HPV test had high grade cytology result at the six or eighteen month test. Analysis showed a significant positive correlation (r= .439, n=225, p<0.01) between HPV status at six and eighteen months. Of HR HPV negative women at six months post treatment (n=198), most have had repeat co- test twelve months later (n=177). Analysis indicated a moderate positive correlation between HPV result and Cytology result (r=.510, n=250, p<0.01) suggesting that higher grades of cytology were more likely in the positive HPV status group.

At eighteen months post treatment the women HR HPV negative and cytology negative or ASCUS numbered one hundred and seventy three (Table 3) and they were discharged to three yearly smears. This figure represents 69% of the total (n=251) originally treated.

Fourteen women who tested HR HPV negative at six months were positive at eighteen months post treatment. Of these women, thirteen have been reviewed at

colposcopy, four had CIN1 biopsies (one aged 21), six had no abnormality detected and one had CIN3 on punch biopsy but repeat LLETZ reported CIN1. Two women who were HR HPV negative at six months were again HR HPV negative at eighteen months but had LSIL cytology at the eighteen month visit. These two women are under colposcopy review, one is immunosuppressed and one had diathermy ablation for low grade changes. Of the women who have not had their second test (n=21), the reasons are varied, from moving away for follow-up elsewhere (n=8), awaiting appointments (n=4) and non-attendance (n=9).

Women with HR HPV positive results at initial test post treatment 21% (n=53) all underwent colposcopy. Nineteen women with positive HR HPV had normal colposcopy appearance and were reviewed for repeat cytology and HR HPV test twelve months later. At the second HR HPV test, seven of these women were both HR HPV negative and cytology negative and were discharged to annual cytology (smear). The remaining twelve women, HR HPV positive with normal colposcopy, had cytology negative (n=7), ASCUS (n=1) and LSIL (n=4), and they are under annual review. The remaining women who tested positive for HR HPV at 6 months post treatment (N=34) had colposcopy impression of CIN; fourteen had punch biopsies and are currently under review and twenty underwent further treatments. Treatments included LLETZ (n=16), diathermy ablation (n=1) and hysterectomy (n=3). One of the hysterectomy patients had a further positive HR HPV and ultimately required partial vaginectomy. Grades of histology detected after the six month HR HPV test are presented in Table 4. A Pearson's product-moment correlation coefficient was computed to assess the relationship between initial cytology result and post cytology result after 18 months. Results indicated positive significance ($r = 0.261$, $n=221$,

p<0.01) and highlighted a weak positive correlation between initial cytology result at 6 months and cytology result at 18 months.

There were seven cases of persistent dysplasia including four cases of high grade dysplasia identified in women with HR HPV positive and negative cytology. In addition two women with positive HR HPV and cytology ASCUS had persistent high grade disease (Table 4). These cases would not have been identified at this early stage prior to the introduction of HR HPV testing.

Discussion

Negative HR HPV and normal smear has 99% negative predictive value,[2] and is more reliable than excision margins in predicting residual disease.[3] In this audit, after the initial co-test, 79% of women had negative HR HPV; a result in line with the 81% reported in a large UK study.[3] However, in the UK study,[3] the 81% with negative tests were returned to routine screening whereas the Irish protocol advises a second test twelve months later. In Australia, it is recommended that HPV testing is undertaken 12 months after treatment, and then annually until the woman has tested negative by both tests on two consecutive occasions.[4]

The number returned to routine screening after two negative HR HPV tests and cytology was 65% (n=162); a figure substantially lower than in England and Scotland (81%) where only one co-test is performed. The HR HPV test for follow-up after treatment has been shown to be a cost effective policy option in the UK. [5] However the financial cost to the Irish programme will be greater than that in the UK as we

require two tests. When the co-test has been in use for several years a review of results will definitively show whether the second co-test is an effective use of resources.

The co-test helps to identify the women who are at high risk of cervical cancer (HR HPV positive) after treatment. The main advantage of HR HPV testing is that it allows surveillance to be targeted towards at risk women. Added to the 21% with HR HPV positive at 6 months, a further 5% of women in our audit tested HR HPV positive at second test. The second co-test has led to the detection of one case of CIN3 on punch biopsy but repeat LLETZ reported CIN1 only in a woman whose initial HR HPV test was negative. It is possible that the different sensitivity of the HPV tests is the reason for these different results but it is also possible that re infection with oncogenic HPV could have occurred.

HR HPV testing is more sensitive than cytology but rare cases of CIN2+ and cervical cancer have been found within five years of HR HPV negative tests.[6] Prior to the introduction of the test of cure co-test women had 2 smears in the year after treatment and annual smears thereafter for 9 years. The reduction in frequency of follow up smears caused some concern for colposcopists but findings of this audit inspire confidence in the sensitivity of the HR HPV. All high grade cytology results had positive HR HPV meaning that all residual disease detected by smear was also detected by HPV test. This finding also correlates elsewhere,[3] with a small number of women identified with non negative cytology (n=39, all low grade) out of HR HPV negative women (n=783) at six months post treatment. Moreover, a recent Australian audit concluded that PAP smears and HR HPV testing may be sufficient for follow-up at 12 months after LLETZ and reported colposcopy examination unsatisfactory for the detection of persisting HPV-related change following excision of high-grade CIN. [7]

Poor specificity of HR HPV tests has been highlighted in a number of studies with false positives of 3-10%.[8] This means that not all women who test positive for HR HPV will have persistent CIN. An Australian study found that women who tested positive for HPV 16, 18, 33, 44 or multiple HPV types pre treatment were more likely to have residual disease even when excision margins were clear.[9] Genotyping may offer improved specificity in the future as HPV 16 and 18 account for 71% of all cervical cancers.[10]

Based on the results (Tables 2 and 3), the most useful predictive factor for persistent CIN six months after treatment is the HPV test. Learning from the audit includes the need for caution in the management of women with positive HR HPV results post LLETZ. The presence of HR HPV indicates a risk of residual disease but is not diagnostic and these women need surveillance, but do not always need further treatment. In addition, repeat LLETZ leaves women at increased risk of preterm delivery,[11] so should be avoided if possible.

In this audit, 36% (19 of 53) with positive HR HPV initial test had normal colposcopy. At eighteen months post treatment, 13% (7 of 53) had negative HR HPV, which represents 3% of the total group (n=251). When the co-test was introduced in Ireland, Cervicalcheck recommended that if HR HPV was detected at 6 or 18 months, women should have annual cytology. However, guidelines recommend that women with positive initial HR HPV with no residual disease detected and negative HR HPV at eighteen months can be discharged to routine cytology.[12] Although this is a small percentage of the overall number it will add further to the number of women that will be reassured by their results and returned to routine screening after treatment.

High grade smear results with positive HR HPV were a strong indicator of residual disease, and these cases would have been detected with cytology alone. But additional cases of residual high grade disease were detected by the HR HPV test where cytology was reported normal and ASCUS. Two women with HR HPV positive and normal cytology had CIN2 on repeat LLETZ, and one woman had CGIN on repeat treatment after ASCUS smear and positive HR HPV (first LLETZ was CIN3). These findings confirm that co-test has increased sensitivity over cytology alone. Cytology alone could have eventually detected these but it is preferable that they were detected early.

Developments in cervical screening is likely to consist of HR HPV testing as the initial test followed by cytology on HR HPV positive results. HPV-based screening provides 60–70% greater protection against invasive cervical carcinomas compared with cytology.[13] In addition, follow up of treated women is an essential aspect of the cervical screening programme. Treated women are a different group to the screening population and are at increased risk of pre-cancer and cancer compared to the general public. Recurrence has been shown to be due to persistence of HR HPV infection.[14] Previously, follow up at our colposcopy clinic included colposcopy and cytology at 4 months with 2 further smears in the first year and annual cytology for 9 years thereafter. Colposcopy did not add to the effectiveness of follow up and added increased strain on resources as well as putting women through the emotional and physical discomfort of the procedure. Guidelines for the NHS screening programme do not recommend colposcopy post treatment.[15] The authors acknowledge that treated women are at increased risk of disease but found no clear evidence that colposcopy combined with cytology is superior to cytology alone for follow up. This means that colposcopy after treatment which is a subjective test, is not as sensitive as

we would like, probably due to distortion and scarring of the squamous columnar junction. However HR HPV testing post treatment is an objective test that this study has shown to improve sensitivity for persistent disease. The co-test provides analysis of the cellular component of the smear (cytology) and the HPV test is a genetic predictor of future risk. Therefore the HR HPV test must be the cornerstone of post treatment follow up and cytology should also be performed to ensure that cases of CIN2 in HR HPV negative women [6] are detected.

Limitations of this study include the use of a different HR HPV test at six months and at eighteen months. There are two possible explanations for women testing positive for HR HPV at 18 months who had tested negative at six months; i.e. re-infection or different sensitivity of the tests.

Conclusions

This audit is significant as it is the first review of HR HPV post treatment tests in an Irish population and it demonstrates that the addition of HR HPV testing improves outcomes for women. A cost benefit analysis is recommended because large numbers are needed to determine the value of the second co-test.

Conflict of interest

None declared

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Table 1: Histopathology of lesions treated 6 months before first HPV test

	HPV negative		HPV positive	
Total (n=251)	**Cytology Negative (n=185)**	**Cytology Borderline (n=12) LSIL(n=1)**	**Cytology Negative (n=25)**	**Cytology ≥Borderline (n=28)**
Normal	7 (2.7%)	1(0.4%)	1 (0.4%)	2 (0.8%)
Cervicitis	7 (2.7%)			
Viral	5 (2.0%)			1 (0.4%)
CIN1	28 (11.1%)	4(1.6%)	2 (0.8%)	7 (2.7%)
CIN2	41(16.3%)	4(1.6%)	10 (4%)	6 (2.4%)
CIN3	93 (37%)	4(1.6%)	12 (4.8%)	11 (4.38%)
CGIN	3 (1.2%)			
Microinvasion	1 (0.4%)			1 (0.4%)

Table 2: Cytology and HR HPV tests at six months post treatment

	HPV negative	**HPV positive**
	n=198 (79%)	**n=53 (21%)**
Age		
<35	97 (49%)	23 (43%)
>35	101 (51%)	30 (57%)
Smoker		
Yes	75 (38%)	26 (49%)
No	123 (62%)	27 (51%)
Cytology		
Negative	185 (93%)	25 (47%)
ASCUS	12 (6%)	13 (24%)
LSIL	1 (0.5%)	10 (19%)
HSIL		4 (8%)
ASCH		1 (2%)
Treatment		
LLETZ excision	193 (97%)	51 (96%)
Diathermy ablation	5 (3%)	2 (4%)
Excision Margins		
Clear margins	97 (49%)	20 (39%)
Endocervical margin inv.	44 (25%)	17 (33%)
Ectocervical margin inv.	18 (9%)	8 (16%)
Not available/uncertain	23 (12%)	6 (12%)

ASCUS: atypical squamous cells of undetermined significance
LSIL: low-grade squamous intraepithelial lesions
HSIL: high-grade squamous intraepithelial lesions
ASCH: atypical squamous cells- high grade
LLETZ: Large Loop Excision of Transformation Zone

Table 3: Grades of cytology at 6 months (baseline), and 18 months of follow up

	HPV negative		HPV positive	
Total (n=251)	**Cytology Negative (n=185)**	**Cytology Borderline (n=13)**	**Cytology Negative (n=25)**	**Cytology ≥Borderline (n=28)**
6 months				
Negative	185 (73.7%)		25 (10%)	
ASCUS		12 (4.8%)		13 (5.2%)
LSIL		1(0.4%)		10 (4.0%)
HSIL Moderate				3 (1.2%)
HSIL Severe				1 (0.4%)
ASCH				1 (0.4%)
Inadequate				
Not tested				
18 months				
Negative	156 (62.1%)	11(4.4%)	17(6.8%)	19 (7.6%)
ASCUS			1 (0.4%)	1 (0.4%)
LSIL	1(0.4%)	1(0.4%)	1 (0.4%)	5 (2.0%)
HSIL Moderate				1 (0.4%)
HSIL Severe				
ASCH				
Inadequate	1 (0.4%)	1 (0.4%)		
Not tested	27 (10.8%)		6(2.4%)	2(0.8%)

Table 4: Grades of histopathology of lesions detected after first HPV test according to baseline cytology and HPV status

	HPV negative		HPV positive	
Total (n=251)	**Cytology Negative (n=185)**	**Cytology Borderline (n=13)**	**Cytology Negative (n=25)**	**Cytology ≥Borderline (n=28)**
Normal	-	1(0.4%)	1 (0.4%)	9 (3.6%)
Cervicitis	-	-	-	-
Viral	-	-	-	-
CIN1	-	-	3 (1.2%)	6 (2.4%)
CIN2	-	-	3 (1.2%)	5 (2.0%)
CIN3	-	-	-	1 (0.4%)
CGIN	-	-	-	1 (0.4%)
Micro-invasion	-	-	-	-
VAIN1	-	-	-	-
VAIN2/3	-	-	1 (0.4%)	1 (0.4%)