

The effect of high risk HPV but normal cytology at Test of cure on achieving colposcopy standards.

Running title: HPV positive and normal cytology at ToC

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Funding

None.

Declaration of Interests

The authors have no conflicts of interest to declare.

Word count: Précis:25; Abstract:240; Text:1946

Count of figures:1

Count of tables:3

IRB status

Permission to collect clinical data was given by NHS Grampian Clinical Effectiveness department as a clinical audit reference number 3691

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Précis

~~We support practice where w~~Women HPV+ and negative cytology at TOC, with a normal and adequate colposcopy post-treatment, ~~can be safely~~could be returned to routine recall.

Abstract

Objective: In U.K., Test of Cure (ToC) after treatment of any grade of CIN incorporates high risk-HPV (Hr-HPV) test and cytology at 6 months follow-up. Our aim was to determine the rate of recurrent Cervical Intraepithelial Neoplasia (CIN) in women who are Hr-HPV positive and cytology negative and explore possible associated risk factors.

Methods: A retrospective observational cohort study was performed of women treated for any grade CIN between 2010-2015 from a regional population who were Hr-HPV positive and cytology negative at first follow-up.

Results: 2729 women were identified as treated for any grade CIN and 213 (7.8%) were re-referred to colposcopy having Hr-HPV positive test and negative cytology at ToC. Their mean age was 31.56 years (range 19-62 years). The mean time of follow-up per woman was 30.50 months (2-63 months). At colposcopy, 171 (80.3%) had colposcopy examination only and 42 (19.7%) women had a biopsy. 24 (11.3%) cases of CIN were identified of which 4 (1.9%) were CIN2/3. 11 (5.2%) women in total had a repeat treatment. 5 (2.3%) women had biopsy proven CIN2/3 within 12 months post-treatment. No cases of CIN3+ after negative colposcopy were identified during the follow-up period.

Conclusions: The incorporation of Hr-HPV testing yielded a very small number of women with residual CIN within 12 months of treatment. Our results ~~support the practice where~~suggest that women who are Hr-HPV positive and cytology negative post-treatment of CIN with normal adequate colposcopy ~~are~~could be discharged to routine recall if confirmed by larger national data.

Keywords

Test of cure, HPV positive, cytology negative, follow-up

Introduction

In the UK, cervical cancer is the second most common cancer in women under 35 years¹. Prevention of the mainly, as yet, unvaccinated target population centers on cervical screening and the detection and treatment of cervical intraepithelial neoplasia (CIN) in the screen positive population. Yet women treated for CIN, remain at 5-fold risk of cervical cancer up to 20 years after treatment². Human Papillomavirus (HPV) infection is necessary for the development of CIN and cervical cancer³. The high sensitivity and negative predictive value of high risk HPV (Hr-HPV) testing has been exploited to improve post-treatment surveillances⁴. Therefore, individuals with Hr-HPV negative test and negative cytology can safely return to 3 yearly routine recall management screening⁵ and women who test positive undergo further evaluation.

In Scotland, post-treatment surveillance is standard practice in the National Health Service (NHS), known as “test of cure” (ToC)^{5,6}. ToC of any grade treated CIN incorporates Hr-HPV test and cytology at 6 months’ follow-up in primary care⁵. ToC was first introduced in our region in 2010 as an early implementation site in Scotland. In Scotland, women who fail ToC continue to have annual cytology for 5 years even if colposcopy is normal. In Scotland, the colposcopy Quality Assurance standard following treatment of CIN is to achieve 90% of women with negative cytology 6 months post-treatment and the rate of histologically confirmed residual CIN <5% at 12 months.

We would anticipate that with cytology negative women but Hr-HPV positive, we would increase the proportion of residual or recurrent CIN detected within 12 months of treatment. This should reduce the number of cases detected later. Therefore, we aimed to determine the

incidence of recurrent CIN in women Hr-HPV positive/ cytology negative after treatment for CIN and explore possible associated risk factors.

Population and Methods

Our clinic in Aberdeen Royal Infirmary (ARI) serves a geographically defined population with a single pathology and cytology laboratory. Women are referred through the Scottish Cervical Screening Programme (SCCRS) and we perform 450 treatments for CIN per year approximately. All HPV tests are performed at the national HPV reference laboratory in Edinburgh using the Abbott RealTime High-Risk HPV assay (Abbott Molecular, Illinois, U.S.A.). Colposcopy data are collected routinely for all referred individuals in NHS Scotland via the National Colposcopy Clinical Information and Audit System (NCCIAS). This data base is episode based and includes patient demographics, appointment details, clinical data (referral, clinical signs and symptoms), colposcopy assessment and findings, histology and cytology results, treatments and the follow-up management plan.

We performed a retrospective observational cohort study of women treated for any grade CIN, as per national guidelines by accredited colposcopists and trainees of British Society Cervical Cancer and Pathology, who had failed ToC (Hr-HPV positive but with negative cytology) between 2010-2015. Women with a final diagnosis at the time of initial treatment of invasive carcinomas and Cervical Glandular Intraepithelial Neoplasia (CGIN) were excluded. Follow-up data on cytology, colposcopy and histology was collected up to December 2016. Follow-up was measured until 31/12/2016 unless a woman was identified with CIN recurrence when further follow-up was censored from that date. Permission to collect clinical data was given by NHS Grampian Clinical Effectiveness department as a clinical audit reference number 3691.

Patients were classified based on age at surgery into groups: ≤ 25 years of age, 26-45 years, ≥ 46 years. Various studies have documented significant predictive determinants that help identifying individuals at high risk of CIN recurrence or persistence^{3,7-9}. Thus, based on the literature, we examined potential risk factors of post-treatment CIN recurrence and/ or Hr-HPV persistence. These most common include age, parity, smoking status, HPV vaccination status, referral cytology, definitive histology at treatment, and excision margins status.

Data analysis

Quantitative variables are presented as mean and ranges. Frequency Tables are used to present the results for categorical variables. Logistic regression was used to evaluate the relation between quantitative variables. All comparisons are two-sided and the significance level was set to 0.05. Statistical Software for Social Sciences (SPSS Inc. Released 2009. PASW Statistics for Windows, Version 20.0. Chicago: SPSS Inc) and programming software R (version 3.3.1) were used for all the analyses.

Results

During the study period, a total of 2729 women were treated for any grade CIN in ARI. 213 (7.8%) women had negative smears but Hr-HPV positive test at ToC and formed our study group. The mean age of our study group was 31.56 years (range 19-62 years). Mean time of follow-up per woman was 30.50 months (2-63 months). Demographic characteristics of the study cohort and the women with recurrent CIN are presented in Table 1.

The colposcopic impression at following failed ToC is presented in Table 2. Of the 42 women with a biopsy, 20 women (83.3%) had CIN1, 3 women (12.5%) had CIN2 and 1 woman (4.2%)

had CIN3. The management plan post-colposcopy is given in Table 2, with 155 (72.8%) women advised to have an annual colposcopic assessment.

The incidence of any grade of CIN was 11.3% and 1.9% CIN2/3 at 6 months post-treatment. 5 (2.3%) women had biopsy proven CIN2/3 within 12 months post-treatment. Women treated for high grade CIN had a negative cytology rate at 91.5% 6 months post-treatment and histologically confirmed CIN at 3.8% 12 months post-treatment. The survival curve analysis of women with normal and adequate colposcopy (n=196) is presented in Figure 1. Finally, a total of 11 (5.2%) women had repeat treatment and final pathology diagnosis at second Large Loop Excision of the transformation zone (LLETZ) was 1 (9.0%) CIN1, 5 (45.5%) CIN2, 5 (45.5%) CIN3 respectively. 6 (2.8%) women had persistent CIN1 during their follow-up visits and were managed conservatively. 1 (0.5%) woman had been diagnosed with high grade Vaginal Intraepithelial Neoplasia. No cases of CIN3+ were identified during the subsequent follow-up period.

There was no statistically significant difference between age, parity, smoking status, referral cytology, definitive histology at LLETZ, and excision margins status between women with CIN recurrence/ persistence and women with no CIN who were Hr-HPV positive/ cytology negative (Table 3).

Discussion

In this retrospective observational cohort study, we have measured the outcome of women who were HPV positive/cytology negative on the ToC pathway. We also assessed possible risk factors of residual or recurrent CIN in women who fail ToC with Hr-HPV positive/ negative

cytology in Northeast of Scotland. To our knowledge, this is one of the few population-based studies investigating the aforementioned factors.

Development of cervical cancer is an ongoing multifactorial process through a well-defined precursor phase where interpretation, diagnosis and treatment in pre-invasive phase is crucial^{5,10}. Surgical treatment by LLETZ or Loop electrical excision procedure (LEEP) is the 'gold standard' technique for treating CIN⁷. LLETZ/LEEP has a high curative rate, but women treated for CIN are susceptible to recurrence within 2 years post-treatment^{7,11}. Thus, it is recommended that women treated for CIN should be kept under post-treatment follow-up.

Different methods have been evaluated for post-treatment follow-up including cervical cytology, HPV-DNA genotyping and colposcopy^{12,13}. Each method vary in methodology, as well as length/ frequency of follow-up⁵. Combined cytology and HPV testing has been implemented by many screening programmes although the assays used and timing may vary⁵. Evaluation of the specificity and sensitivity of HPV testing and cytology demonstrated pooled sensitivity and specificity of 93% (95% CI: 85-97%) and 81% (95% CI: 74-86%) for HPV testing vs. 72% (95% CI: 66-78%) and 84% (95% CI: 80-87%) for the cytology respectively¹⁴. Therefore, individuals with Hr-HPV negative test and negative cytology can safely return to routine recall management screening protocol⁵.

Good practice in colposcopy comprises the distinction of normal from the abnormal transformation zone¹⁵. A normal examination is vital to minimize the risk of undetected disease and development of cancer and is essential to sustain confidence and compliance within a quality assured cervical screening programme¹⁵. In our study group, we found that the incidence of any grade of CIN was 11.3% 6 months post-treatment. Only 5 (2.3%) women had biopsy proven CIN2/3 within 12 months post-treatment. The overall cure rate of CIN from our unit meets the

quality performance indicator¹⁶. It is also important to mention that in our group no cases of CIN3+ were detected after negative colposcopy during the follow-up period. Concerns have been raised about the performance and accuracy of colposcopy¹⁷. However, 2 large studies from the UK, in the context of women referred with low grade cytology, both found very low rates of CIN3+ by the next round of screening^{15,18}. A normal colposcopic examination can reassure women that, even with a positive HPV test, the risk of developing CIN2 or worse is sufficiently low to return to the routine recall^{15,18}. Thus, our results with annual cytology for 5 years, also confirm the importance of normal colposcopy in a different colposcopy population. Moreover, our results support the practice where women who are Hr-HPV positive and cytology negative post-treatment of CIN with normal adequate colposcopy are discharged to routine recall¹⁶ in the context of a quality assured screening programme and colposcopy.

The Abbott RealTime Hr-HPV assay, which is used for ToC in Scotland¹⁹, is an DNA based assay which provides 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, 68¹⁹. It also provides concurrent genotyping of 16 and 18¹⁹ but these data are not provided to smear taker. Thus, we cannot comment on limited genotyping and the importance of type specific persistent HPV infections.

HPV vaccination should reduce the risk of cervical cancer by at least 70%²⁰. The UK HPV immunisation programme started in 2008 and uptake in Scotland, has been high (>91%)²⁰. Vaccinated women attending for cervical screening have a significant lower Hr-HPV infection incidence¹⁹. In our study group, the vaccination uptake was very low, as only 5 (2.3%) women have been fully vaccinated which supports the aforementioned evidence.

In other countries is now common practice to follow-up patients 6-12 months post-treatment^{5,21,22}; in Ireland, HPV DNA test is performed at 6 months in colposcopy clinic and then

12 months in primary care²¹ whereas in Sweden HPV DNA test and cytology is done 6 months post-treatment²². The great majority of women in our group diagnosed with CIN 6 months post-treatment were found to regress to normal within 12 months. Therefore, whether ToC is performed prematurely at 6 months post-treatment needs to be further evaluated since most women would clear from HPV in 12 months. In that way, false positive results which may be seen in UK when we test at 6 months may reduce. However, this has to be balanced against women having to wait longer to find out if their treatment was successful and they can return to routine recall.

In the era of ToC, this cohort includes a moderate number of treatments for CIN in a relatively stable population with the colposcopy system capturing smears on a national basis²³. Taking all into consideration, we can be reassured that this cohort is representative of the UK. Our study results add to the current literature on the usefulness of ToC as a post-treatment curative success screening method.

There are a number of limitations in this study with the retrospective study design and lack of follow-up data because of patients' non-attendance being potential biases of this study. Furthermore, homogeneity of treatment and cytopathological accuracy may depend on quality control policies and experience.

Conclusion

The incorporation of Hr-HPV testing found a very small number of women with residual CIN detected within 12 months of treatment. Our results support the practice in England where women who are Hr-HPV positive and cytology negative post-treatment of CIN with normal

adequate colposcopy are discharged to routine recall. If our findings are confirmed with national Scottish colposcopy data, this could supports a change to our programme guidance.

Acknowledgements

We thank Sister Christine Godley for helping with data collection.

Declaration of Interests

The authors have no conflicts of interest to declare.

Funding

None.

Abbreviations

Cervical Intraepithelial Neoplasia: CIN

Human Papillomavirus: HPV

High risk-HPV: Hr-HPV

National Health Service: NHS

Test of Cure: ToC

Aberdeen Royal Infirmary: ARI

Scottish Cervical Screening Programme: SCCRS

National Colposcopy Clinical Information and Audit System: NCCIAS

Cervical Glandular Intraepithelial Neoplasia: CGIN

Large Loop Excision of Transformation Zone: LLETZ

Loop electrical excision procedure: LEEP

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Table legends

Table 1.

Demographic and clinical characteristics of Hr-HPV positive/ cytology negative women and women with CIN 6 months post-treatment. Type of treatment, final histology and excision margins status also presented.

Table 2.

Colposcopic outcomes, histopathological results at first visit after ToC failure and patients' management plan.

Table 3.

Logistic regression between patients with no CIN and these with recurrent/ persistent CIN 6 months post treatment. Odds Ratio (OR), 95% Confidence Interval (95% CI) and p-values also presented.

