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PII: S1386-6532(15)00693-9
DOI: http://dx.doi.org/doi:10.1016/j.jcv.2015.10.008
Reference: JCV 3452

To appear in: *Journal of Clinical Virology*

Received date: 14-8-2015
Revised date: 6-10-2015
Accepted date: 10-10-2015

Please cite this article as: Cuschieri Kate, Bhatia Ramya, Cruickshank Margaret, Hilemanns Peter, Arbyn Marc.HPV Testing in the context of post-treatment follow up (test of cure).*Journal of Clinical Virology* http://dx.doi.org/10.1016/j.jcv.2015.10.008

HPV Testing in the context of post-treatment follow up (test of cure)

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Highlights

- Application of molecular HPV testing to adjudicate treatment success is common
- Assay choice, number of tests and testing intervals vary widely across settings.
- Pooling longitudinal data will help inform safe and practical recall intervals.
- Data on the utility of genotyping and biomarkers in this context are few.

Abstract

Background: Women treated for cervical lesions are at higher risk of subsequent disease compared to the general population. Consequently, post treatment surveillance strategies are required to ensure the success of treatment, so called “test of cure”. The high sensitivity and negative predictive value of HPV assays can enhance post-treatment strategies

Objectives: To provide an overview of the current data on test of cure strategies with a particular focus on HPV testing and to identify knowledge gaps and areas for further research

Results: HPV testing is sensitive for the detection of residual or recurrent disease post treatment for CIN2+ and is more sensitive than cytology alone. Co-testing increases sensitivity, marginally and there is a lack of consensus regarding the efficiency and safety to release negative women. Most test of cure studies have applied HPV DNA tests and post treatment positivity rates vary widely depending on assay and potentially, treatment type.

Conclusions: Globally, an increasing number of test of cure algorithms now incorporate HPV testing although there is heterogeneity of practice with respect to assay, number of post treatment tests, testing intervals, follow up time. While type specific persistence identified through genotyping may identify those at greater risk of disease there is no consensus as to how this may be applied, clinically. Data on HPV testing in women treated for glandular lesions would be welcome as would the performance of different HPV assays and associated biomarkers in this context.

Abbreviations
HPV: Human Papillomavirus
HR: High risk
LBC: Liquid based cytology
TOC: Test of Cure
CIN: Cervical Intraepithelial neoplasia
AIS: Adenocarcinoma in situ

Keywords: human papillomavirus; post treatment; test of cure; cervical lesions; testing strategies

The challenge of post treatment management

The fact that cervical cancer is preceded by a well defined precursor phase, provides key justification for cervical screening programmes where identification of relevant precursors indicate treatment to reduce the likelihood of lesions developing into invasive cancer.[1] Consequently, treatment of precursors via excisional or destructive removal of the affected area is common practice, although admittedly the type of procedure varies depending on local and national policies and infrastructure, themselves affected by temporal changes in practice.[2]

Global evidence indicates that women diagnosed with high grade lesions have an increased risk of developing lesions and cancer in the future.[3-8] A recent analysis by Strander et al (2014) showed that Swedish women diagnosed and treated for CIN3 between 1958-2008, had a substantially increased incidence of and mortality from cervical or vaginal cancer compared to the general population with a standardised mortality ratio of 2.35 (95% CI 2.11-2.61), based on a follow up of more than 3 million women years.[9] The authors also observed that risk of cancer incidence was five times higher in women diagnosed and treated over the age of 60 compared to those treated aged 30-39.

This work was particularly notable given that it included mortality and cancer as outcomes after CIN treatment. Furthermore, it builds on previous work from the authors which indicated that the excess risk period covers a span of 25 years post treatment.[3] The findings also reconcile with the work of Rebolj et al who demonstrated a four-fold risk of cancer in Dutch women who had been treated for CIN (not restricted to CIN3) and who had 3 normal smears as part of a routinely indicated follow up protocol[10]

The reasons as to why women diagnosed and treated for lesions are at increased risk of morbidity and mortality from subsequent CIN and cancer are not well defined and are likely

to be multi-factorial including host susceptibility to development of CIN and/or persistence or re-infection of HrHPV in addition to external risk factors including smoking.[11-13] Furthermore, not all treatment procedures are successful in removing the affected area/lesions.[7 14]

All things considered, the evidence suggests that women treated for high-grade lesions require enhanced surveillance given their additional risk; this paper endeavours to outline the strategies for achieving this (and the knowledge gaps therein) with particular reference to HPV testing.

Test of cure strategies - Cytology, HPV testing or both?

A variety of protocols have been assessed and implemented for monitoring women post treatment which vary both in technology, and length/ frequency of follow-up. With respect to the latter, guidelines range from annual visits for up to 20 years post treatment, to 1-2 follow up appointments within 24 months of initial treatment, which if negative can indicate a return to "routine" recall. Table 1 provides an indication of the heterogeneity of practice across countries.

With respect to technologies the most commonly applied within organised screening programmes are cytology and/or HPV testing – where abnormality, positivity (or a combination of the two) influence subsequent management. While other systems for monitoring post treatment success have been evaluated such as excised specimen margin status[7,15], colposcopy and use of endocervical curettage; these have not gained the traction nor the consistent performance of cytology or HPV testing.[16-17]

The high sensitivity of molecular HPV testing, make it attractive in post treatment contexts where the relative value/requirements of the test are weighted towards sensitivity. As in

other contexts, the objectivity of HPV testing (compared to cytology) is perceived as an advantage. However, given the added risk of lesion development in this population, the performance of HPV and cytology measured via screening and triage studies cannot be extrapolated directly to this context and specific data are needed. To this end meta-analyses have been produced to assess the performance HPV testing, cytology and co-testing for the prediction of residual CIN2+ in post treatment settings.[18,19]

The most recent, by Arbyn et al (2012) incorporated a total of fifteen studies where women had been followed up for a minimum of 18 months. Pooled sensitivity and specificity of HPV testing was 93% (95%CI: 85-97%) and 81% (95%CI: 74-86%); whereas the equivalent values for cytology were 72% (95% CI: 66-78%) and 84% (95%CI: 80-87%) respectively. HPV testing was significantly more sensitive than cytology as a stand-alone test after treatment whereas co-testing was slightly more sensitive (although this did not reach significance) and was significantly less specific.[19] Table 2 presents an update on the meta-analysis although the findings are generally very similar; the sensitivity of co-testing and HPV testing (alone) for CIN2+ was 95% (95% CI: 88-98)and 94% (95% CI: 88-97). The relative sensitivity and specificity of co-testing vs HPV testing alone was 1.07 (95% CI: 0.97-1.17) and 0.93 (95% CI: 0.88-0.97). While the data would suggest that the marginally higher sensitivity of co-testing is not statistically significant compared to HPV testing, it is notable that post-treatment algorithms generally incorporate co-testing.

There are naturally limitations to the above analyses; variation in cytology, ie liquid based cytology (LBC) or conventional, may have had a bearing on the findings yet not all studies provided this detail. The type of HPV test may also be influential - as will be discussed later - although when the meta-analysis of Arbyn et al (2012) was stratified by HPV assay type (signal or target amplification), the conclusions were unchanged.[19] Furthermore, although a minimum of 12-18 months follow up was required for inclusion, few studies within the

analyses included long term (>5 year) follow up. This sentiment was articulated in the 2012 ASCCP consensus guidelines (for the management of abnormal cancer screening tests and cancer precursors) where the authors stated "Follow-up is insufficient to determine post-treatment outcomes or optimal long-term follow-up intervals for women with treated CIN 2 and CIN 3 managed with serial co-testing".[20]

A longitudinal analysis of 435 women (median age 33) treated for CIN2 during 1988-2004 was undertaken within the Dutch system, where the cumulative risk of CIN2+ was assessed up to 2009.[21] The authors reported 5 year risks of CIN2+ associated with negative cytology, negative HPV status and a negative co-test at 6 months post treatment as 5.8% (95%CI 3.6-9.3), 4.4% (95%CI 2.5-7.5) and 3.0% (95%CI: 1.5-6.1%). This and further work by this group indicated that that negative co-tests at 6 and 24 months resulted in a 5 year risk of CIN2+ similar to that of women within routine screening (0.0%, 95% CI=0.0%-3.0%) leading to a proposal for new guidelines based on 6 and 24 months.[22,23] In line with these observations Gosvig et al (2015) compared the risk of CIN2+ in 447 women who were HPV negative post conisation with over 13,000 age matched HPV negative women from the general screening population in Denmark; treated women who had a subsequent HPV negative test had similar 3-year and 5-year risks of CIN2+ when compared to the general population (0.7% and 0.9% vs 0.4% and 1.0% respectively) although a higher risk at 8 and 10 years (2.8% and 5.7% vs 1.9% and 2.7% respectively.[24] In a US study of women treated for CIN2+, Adenocarcinoma in situ (AIS) or atypical glandular cells (AGC) within the Kasier Permanente system, Katki et al (2013), calculated 5 year risks of CIN2+ associated with HPV, cytology and co-testing of 3.7%, 4.2% and 2.4% respectively – reducing to 1.5% for two negative co-tests post treatment.[25] The higher risks compared to those reported in the Dutch and Danish study are consistent with the comments of Katki and colleagues who concluded that by "benchmarking to implicit risk thresholds" no women achieved a risk that was sufficiently low to be returned to 5 year screening (based on a Negative Pap within the screening setting having a 5 year risk of

0.68%). The level of acceptable risk will vary according to setting and will of course be influenced by perceived loss to follow up. Variation between studies may also be due to relative differences in the performance of cytology between settings which has been known to vary widely.

HPV detection strategies

As sensitivity is the key attribute of the post treatment test, those who test HPV negative can be monitored less intensively. Those who remain HPV positive are more challenging to manage Scottish data show that at 6 months post treatment, 22% of women are HPV positive compared to 11% who are cytologically abnormal.[26] In an Australian population-based analysis of over 11,000 women who were offered co-testing at 2 visits post treatment, of the non "cured" women (who were defined as having at least one positive/abnormal HPV, cytology or colposcopy result), 56% were attributable to negative cytology/HPV positive status. This compared to 9% who were considered "not cured" due to negative HPV results, in line with the authors' comment that "further studies are needed of the high proportion of women with negative cytology classed as not cured due to HPV positivity."[27]

While HPV positive, cytology negative women require closer follow up; the substantial majority will not have residual significant disease, particularly if the treated lesion was CIN2, preceded by low-grade cytology and/or the women was less than 40 when the treatment occurred.[9,25] The issue of HPV infection without presence or development of CIN2+ suggests a potential utility of biomarkers to aid clinical management. However use of biomarkers in post-treatment follow up is under-researched, particularly when compared to the primary screening and low-grade triage contexts.[28] Furthermore, studies where the performance of different HPV assays have been compared, including those considered clinically validated in screening contexts, are relatively rare. A head to head study of *digene* Hybrid Capture 2

(HC2) Test (Qiagen, Manchester, UK) vs a PCR-EIA in a post-treatment context revealed a kappa agreement of 0.70 and similar sensitivity and specificity for residual CIN2+.[29] In another study where 5 clinically validated HPV tests were compared [HC2, Abbott RealTi*m*e HR-HPV assay (Abbott Molecular, Des Plaines, IL, USA), the cobas 4800 HPV test (Roche Molecular Diagnostics, Pleasanton, CA, USA) the APTIMA HPV assay (Hologic Inc, Bedford, MA, USA) and Cervista HPV HR test (Hologic Inc, Bedford, MA, USA)], HPV positivity after treatment ranged from 18% to 27% depending on assay.[26] Using HC2 at manufacturers cut off as an analytical comparator, significant between-assay differences were observed with the Aptima HPV (AHPV) having fewer positives ($p<0.0001$) and the COBAS and Cervista assays showing higher positivity ($p=0.0001$ and $p=0.0009$, respectively). This study was limited in its ability to measure relative clinical performance by ascertainment bias, as only a positive HC2 result or an abnormal cytology result triggered colposcopy referral. Nevertheless, it did show that assay choice would have a significant impact on the number of colposcopy referrals should HPV positivity be the trigger. Between-assay concordance was also lower in samples associated with borderline or negative cytology samples - which has been described in multi-platform screening studies.[30] These data are also consistent with the findings of Inammaa et al (2014) who, when changing their test of cure HPV platform from HC2 (at a cut off of 2), to the COBAS HPV assay, observed an increase in post treatment HPV positivity in cytology negative samples; 13.9% and 27.8% respectively ($P = <0.0001$).[31] Although positivity increased significantly, the authors did not observe an increase in CIN detection within the time frame of the study. Further, follow up & longitudinal analysis associated with such studies will be helpful to determine whether assay-discordance is attributable to clinically irrelevant noise or indeed predictive of disease in the longer term.

The case for HPV genotyping

In studies which have assessed the performance of HPV genotyping in the test of cure population, the consensus is that women who show type-specific persistent infection are at increased risk of developing CIN2+ and that women who are HPV 16 positive have an increased risk of residual or recurrent disease compared to other HR-HPV types .[32-39] Soderlung Strand et al (2014) performed a study on 178 women who had received conisation and who provided samples for genotyping before treatment and at 3, 6, 12, 24 and 36 months post treatment. All women with a subsequent CIN2+ had type-specific HPV-persistence, with the authors reporting a sensitivity and specificity of genotyping of 100% [95% CI 63-100%] and 94.7% [89.8-97.4%]) respectively, although CIN2+ outcomes in this study were small (n=9).[36] In another relatively small study of 183 patients treated by LEEP, Ryu et al (2012) showed that while LEEP margin status was a significant predictive factor for recurrent CIN2+, age, viral load and persistence of HPV did not predict recurrence.[34] Discordance between these studies may be due to how persistence was defined; the study of Soderlung-Strand defined persistence at the type-specific level whereas Ryu et al applied an aggregated approach of persistence of 16 and/or 18 vs persistence of any other HR type.

The increased risk associated with HPV 16 and persistent HR-HPV infection in post-treatment settings entirely reconciles with data in primary screening and triage contexts.[19, 40,] However, how this knowledge can be applied to post treatment algorithms is uncertain. Testing women prior to treatment clearly involves additional resource, also the analytical and performance requirements for a genotyping test for clinical use are uncertain in this and indeed other contexts (Arbyn et al (2015) .[41]Any algorithm that involves typing also runs the risk of being overly complicated, particularly if a simplified protocol (eg 16/18 over two time points) is less effective than more detailed resolution. In a sub-analysis of the study by Kocken et al (2011), the authors found that despite the higher risk of post-treatment CIN2+ in HPV16-positive women, women infected with hrHPV types other than 16, 18, 31, 33, and

45 (the 5 most common types found in cervical cancer) still had a 5-year risk of CIN2+ of almost 30%.[18] The authors thus concluded that de-escalated management in those without these types common would not be justified.

Few studies have assessed the impact of non HPV-DNA biomarkers for test of cure. Frega et al (2014) showed a 5-type E6/E7 mRNA test (PreTect HPV-Proofer, Norchip, Klokkarstua, Norway) had a sensitivity of 52% for predicting residual disease post treatment compared to 100% sensitivity associated with a hrHPV DNA amplification assay.[42] This aligns with the data of Trope et al (2011) who showed the sensitivity of the PreTect HPV-Proofer at six months post treatment was 45.5% for detection of residual CIN2+ over 18 months (compared to 99.5% sensitivity for a DNA amplification test).[43] Interestingly, Frega et al contended that the mRNA test was more sensitive for the prediction of recurrent (rather than residual) disease over 36 months follow up, nevertheless, the low sensitivities would indicate that 5-type mRNA testing a is not sufficient as a measure of treatment failure at least as a stand-alone test. A comparison of the 14 type E6/E7 mRNA based Aptima HPV assay (Hologic) and the DNA based Linear Array HPV genotyping assay (Roche) was performed in a post-treatment setting of 143 women by Perssons et al (2012). The RNA and DNA assay (confined to 14 HR DNA types) showed sensitivities for residual disease of 57.1% (95%CI: 25.0-84.2) vs 100% (95%CI: 64.6-100) respectively, with (respective) specificities of 93.4% (95%CI: 87.9-96.5) and 80.9% (95%CI: 73.5-86.6).[44] As this study had a small number of outcomes, further assessment of broad-spectrum mRNA based tests for test of cure would be worthwhile.

Opportunities for further research

Although there is global variation in treatment modality for cervical disease, few test of cure studies have presented or adjusted data according to treatment type. Understandably, studies have usually involved a single type of treatment applied locally according to routine guidelines/practice. In the aforementioned study of Kocken et al (2011) the authors

presented a list of confounding potential risk factors (which included treatment-type) and found that the 5 year risks for CIN2+ after LLETZ and Conisation were similar at 15.0 (95%CI 11.4-19.5) and 15.9 (8.8-27.0) respectively, with a slightly wider difference at 10 years post treatment of 18.1% (95%CI: 13.1-24.5) and 15.9% (95% CI: 7.9, 29.4) although these confidence intervals clearly overlap.[18] While a 2006 Cochrane review concluded that different treatment types were broadly as effective as each other,[14] it is feasible that the performance of HPV based test of cure may differ with destructive or excisional treatment.

Furthermore, to date, the bulk of test of cure studies have involved the assessment of women treated for squamous rather than glandular lesions. The lack of data on women treated for glandular disease together with the knowledge that these lesions can be both more challenging to detect and manage has led to countries issuing separate, follow-up protocols which are more intense than those for CIN.[45] Katki et al computed post-treatment risk of CIN2+ according to severity of the initial lesion separated as CIN2 vs CIN3 & adenocarcinoma in situ (AIS). While those treated for CIN3/AIS were at higher risk of recurrent disease, data were not presented separately for AIS.[25] In an Italian study of 119 women treated conservatively for AIS and followed up every 6 months with HPV testing, cytology and colposcopy, over a mean follow up period of 40.9 months, 15 had subsequent disease (7 AIS, 8 adenocarcinoma). According to the multivariate analysis which included margins, cone volume, test results and age, HPV positive status was the strongest predictor of disease post-treatment. In addition co -testing was 90% sensitive for detecting persistent disease at 6 months, increasing to 100% if performed at 12 months.[46-48] Further longitudinal data to inform and optimise management of those treated for glandular lesions will be welcome.

The utility of self-sampling in a test of cure context has not been reported. There are now a plethora of different devices for vaginal sampling and the optimisation of urine for HPV detection is the focus of much research.[49] However validation and clinical studies have been orientated to the populations eligible for screening or those attending for colposcopy.[50] A recent meta-analysis showed that relative sensitivity and specificity of HPV testing on self- versus clinician-based samples to detect high-grade CIN were similar in screening as in colposcopy clinics. However, one study, conducted in South-Africa showed that the relative sensitivity (HC2 testing on self vs clinician samples) was considerably lower in treated (0.64) than in untreated women (0.84).and when the data were confined to the HIV negative women in this study (86%), the conclusions were unchanged[51] In a recent study by Stanczuk et al (2015), women were twice as likely to test positive on a self sample (urine or swab) taken at 6 months post treatment compared to an LBC sample in this context.[52] While the study was not designed to assess clinical outcomes relative to sample type - the preliminary data may suggest that self sampling for post-treatment monitoring may lack specificity, at least at a 6 month visit.

Conclusions

HPV testing is used increasingly as way to measure the success of treatment for cervical lesions. While, international consensus as to number of tests and follow up time(s), vary, generally women who are HPV negative have the advantage of less intense management. Further work to refine management of those who remain HPV positive but who otherwise have no pathological or colposcopic evidence of disease would be welcome. One of the challenges in measuring the performance of test of cure strategies is the relatively low frequency of outcomes, given that most treatments are indeed successful; this becomes particularly challenging when particular variables of relevance are assessed such as lesion morphology, lesion grade, age, treatment type, depth of treatment and HPV assay type. In

the relatively large study of the Kaiser Permanente cohort described earlier, the authors bemoaned the imprecise estimates relating to 1 mill women and 8 years follow up![21] Updated meta-analysis which take into account the more recent studies will thus be welcome. Finally, while we have an increasing handle on which HPV tests are valid for HPV primary screening, given an internationally accepted and applied validation framework [53, 54-] we do not have equivalent metrics to adjudicate the validity of HPV tests in other settings including for post treatment surveillance, and for the triage of low-grade abnormalities International efforts to create such guidelines will be of benefit particularly as existing data shows significant variation between commercially available tests within post -treatment settings. Other international endeavours of value would be the pooling of longitudinal data on outcomes associated with test of cure protocols to inform safe and practical recall intervals

Acknowledgements

Many thanks go to Dr Jesper Bonde, Dr Mari Nygard, Prof Joakim Dillner and Prof René H.M. Verheijen and Dr Francesca Carozzi for advice on country specific post-treatment protocols and guidelines.

Declarations

Funding: M. Arbyn is supported by the seventh framework program of DG Research of the European Commission, through the COHEAHR Network (grant No 603019) and by the Joint Action CANCON which has received funding from the European Union in the framework of the Health Programme (2008-13). Other funding was received from the German Guideline Program in Oncology (German Cancer Aid project # 110163).

Competing interests: KC's institution has received project funding and/or consumables to carry out assay evaluations from HOLOGIC, Qiagen, Roche, NorChip,

GSK & Abbott Molecular, Cepheid and Becton Dickinson. No other authors declare a conflict of interest.

Ethical Approval: N/A

All authors have contributed to and approved the final manuscript.

References

1: http://www.who.int/cancer/detection/cervical_cancer_screening/en/

2: Jordan J, Martin-Hirsch P, Arbyn M, Schenck U, Baldauf JJ, Da Silva D, Anttila A, Nieminen P, Prendiville W. European guidelines for clinical management of abnormal cervical cytology, part 2. Cytopathology. 2009; 20, 5-16.

3: Strander B, Andersson-Ellström A, Milsom I, Sparén P. Long term risk of invasive cancer after treatment for cervical intraepithelial neoplasia grade 3: population based cohort study. BMJ. 2007; 335(7629):1077.

4: Mitchell H, Medley G, Carlin JB. Risk of subsequent cytological abnormality and cancer among women with a history of cervical intraepithelial neoplasia: a comparative study. Cancer Causes Control. 1990; 1, 143-8.

5: Pettersson F, Malker B. Invasive carcinoma of the uterine cervix following diagnosis and treatment of in situ carcinoma. Record linkage study within a National Cancer Registry. Radiother Oncol. 1989; 16, 115-20.

6: Rapiti E, Usel M, Neyroud-Caspar I, Merglen A, Verkooijen HM, Vlastos AT, Pache JC, Kumar N, Bouchardy C. Omission of excisional therapy is associated with an increased risk of invasive cervical cancer after cervical intraepithelial neoplasia III. Eur J Cancer. 2012; 48, 845-52.

7: Ghaem-Maghami S, Sagi S, Majeed G, Soutter WP. Incomplete excision of cervical intraepithelial neoplasia and risk of treatment failure: a meta-analysis. Lancet Oncol. 2007; 8, 985-93.

8: Soutter WP, Sasieni P, Panoskaltsis T. Long-term risk of invasive cervical cancer after treatment of squamous cervical intraepithelial neoplasia. Int J Cancer. 2006; 118, 2048-55.

9: Strander B, Hällgren J, Sparén P. Effect of ageing on cervical or vaginal cancer in Swedish women previously treated for cervical intraepithelial neoplasia grade 3: population based cohort study of long term incidence and mortality. BMJ. 2014; 348:f7361. doi: 10.1136/bmj.f7361.

10: Rebolj M, Helmerhorst T, Habbema D, Looman C, Boer R, van Rosmalen J, van Ballegooijen M. Risk of cervical cancer after completed post-treatment follow-up of cervical intraepithelial neoplasia: population based cohort study. BMJ. 2012;345:e6855. doi: 10.1136/bmj.e6855.

11: Mohanty G, Ghosh SN. Risk factors for cancer of cervix, status of screening

and methods for its detection. Arch Gynecol Obstet. 201;291, 247-9.

12: Schiffman M, Wentzensen N. Human papillomavirus infection and the multistage carcinogenesis of cervical cancer. Cancer Epidemiol Biomarkers Prev. 2013; 22, 553-60.

13: Dugué PA, Rebolj M, Garred P, Lynge E. Immunosuppression and risk of cervical cancer. Expert Rev Anticancer Ther. 2013; 13, 29-42.

14: Martin-Hirsch PL, Paraskevaidis E, Kitchener H. Surgery for cervical intraepithelial neoplasia. Cochrane Database Syst Rev. 2000;(2):CD001318. Review. Update in: Cochrane Database Syst Rev. 2010; CD001318.

15: Ghaem-Maghami S, De-Silva D, Tipples M, Lam S, Perryman K, Soutter W. Determinants of success in treating cervical intraepithelial neoplasia. BJOG;118, 679-84.

16: Paraskevaidis E, Arbyn M, Sotiriadis A, Diakomanolis E, Martin-Hirsch P, Koliopoulos G, Makrydimas G, Tofoski J, Roukos DH. The role of HPV DNA testing in the follow-up period after treatment for CIN: a systematic review of the literature. Cancer Treat Rev. 2004; 30, 205-11.

17: Arbyn M, Sasieni P, Meijer CJ, Clavel C, Koliopoulos G, Dillner J. Chapter 9: Clinical applications of HPV testing: a summary of meta-analyses. Vaccine 2006; 24 (SUPPL. 3): S78-S89.

18: Kocken M, Uijterwaal MH, de Vries AL, Berkhof J, Ket JC, Helmerhorst TJ, Meijer CJ. High-risk human papillomavirus testing versus cytology in predicting post-treatment disease in women treated for high-grade cervical disease: a systematic review and meta-analysis. Gynecol Oncol. 2012; 125, 500-7.

19: Arbyn M, Ronco G, Anttila A, Meijer CJ, Poljak M, Ogilvie G, Koliopoulos G, Naucler P, Sankaranarayanan R, Peto J. Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. Vaccine. 2012; 30 Suppl 5:F88-99.

20: Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, Solomon D, Wentzensen N, Lawson HW; 2012 ASCCP Consensus Guidelines Conference. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2013; Apr;17(5 Suppl 1):S1-S27. Erratum in: J Low Genit Tract Dis. 2013;17, 367.

21: Kocken M, Helmerhorst TJ, Berkhof J, Louwers JA, Nobbenhuis MA, Bais AG, Hogewoning CJ, Zaal A, Verheijen RH, Snijders PJ, Meijer CJ. Risk of recurrent high-grade cervical intraepithelial neoplasia after successful treatment: a long-term multi-cohort study. Lancet Oncol. 2011; 12, 441-50.

22: Uijterwaal MH, Kocken M, Berkhof J, Bekkers RL, Verheijen RH, Helmerhorst TJ,

Meijer CJ. Posttreatment assessment of women at risk of developing high-grade cervical disease: proposal for new guidelines based on data from the Netherlands. J Low Genit Tract Dis. 2014; 18, 338-43.

23: Uijterwaal MH, Verhoef VM, Snijders PJ, Meijer CJ. Arguments in favor of HPV testing for cervical screening and post-treatment CIN2+ monitoring. Expert Rev Mol Diagn. 2014; 14, 245-8.

24: Gosvig CF, Huusom LD, Andersen KK, Duun-Henriksen AK, Frederiksen K, Iftner A, Svare E, Iftner T, Kjaer SK. Long-term follow-up of the risk for cervical intraepithelial neoplasia grade 2 or worse in HPV-negative women after conization. Int J Cancer. 2015; Jul 2. doi: 10.1002/ijc.29673. [Epub ahead of print]

25: Katki HA, Schiffman M, Castle PE, Fetterman B, Poitras NE, Lorey T, Cheung LC, Raine-Bennett T, Gage JC, Kinney WK. Five-year risk of recurrence after treatment of CIN 2, CIN 3, or AIS: performance of HPV and Pap cotesting in posttreatment management. J Low Genit Tract Dis. 2013; 17(5 Suppl 1):S78-84.

26: Cubie HA, Canham M, Moore C, Pedraza J, Graham C, Cuschieri K. Evaluation of commercial HPV assays in the context of post-treatment follow-up: Scottish Test of Cure Study (STOCS-H). J Clin Pathol. 2014; 67, 458-63.

27: Morrell S, Qian L. A whole-population profile of HPV testing as a test of cure for high-grade cervical dysplasia in NSW, Australia. J Med Screen. 2014; 21, 151-62.

28: Cuzick J, Bergeron C, von Knebel Doeberitz M, Gravitt P, Jeronimo J, Lorincz AT, J L M Meijer C, Sankaranarayanan R, J F Snijders P, Szarewski A. New technologies and procedures for cervical cancer screening. Vaccine. 2012; 30 Suppl 5:F107-16.

29: Söderlund-Strand A, Rymark P, Andersson P, Dillner J, Dillner L. Comparison between the Hybrid Capture II test and a PCR-based human papillomavirus detection method for diagnosis and posttreatment follow-up of cervical intraepithelial neoplasia. J Clin Microbiol. 2005; 43, 3260-6.

30: Rebolj M, Preisler S, Ejegod DM, Rygaard C, Lynge E, Bonde J. Disagreement between human papillomavirus assays: an unexpected challenge for the choice of an assay in primary cervical screening. PLoS One. 2014;9, e86835.

31: Innamaa A, Dudding N, Ellis K, Crossley J, Smith JH, Tidy JA, Palmer JE. High-risk HPV platforms and test of cure: should specific HPV platforms more suited to screening in a 'test of cure' scenario be recommended? Cytopathology. 2014; doi: 10.1111/cyt.12223. [Epub ahead of print]

32: Heymans J, Benoy IH, Poppe W, Depuydt CE. Type-specific HPV geno-typing improves detection of recurrent high-grade cervical neoplasia after conisation. Int J Cancer. 2011; 129, 903-9.

33: Jones J, Saleem A, Rai N, Shylasree TS, Ashman S, Gregory K, Powell N, Tristram A, Fiander A, Hibbitts S. Human Papillomavirus genotype testing combined with cytology as a 'test of cure' post treatment: the importance of a persistent viral infection. J Clin Virol. 2011; 52, 88-92.

34: Ryu A, Nam K, Kwak J, Kim J, Jeon S. Early human papillomavirus testing predicts residual/recurrent disease after LEEP. J Gynecol Oncol. 2012; 23, 217-25.

35: Venturoli S, Ambretti S, Cricca M, Leo E, Costa S, Musiani M, Zerbini M. Correlation of high-risk human papillomavirus genotypes persistence and risk of residual or recurrent cervical disease after surgical treatment. J Med Virol. 2008; 80, 1434-40.

36: Söderlund-Strand A, Kjellberg L, Dillner J. Human papillomavirus type-specific persistence and recurrence after treatment for cervical dysplasia. J Med Virol. 2014; 86, 634-41.

37. Gosvig CF, Huusom LD, Andersen KK, Iftner A, Cederkvist L, Svare E, Iftner T, Kjaer SK. Persistence and reappearance of high-risk human papillomavirus after conization. Gynecol Oncol. 2013, 131, 661-6.

38: Gök M, Coupé VM, Berkhof J, Verheijen RH, Helmerhorst TJ, Hogewoning CJ, Snijders PJ, Meijer CJ. HPV16 and increased risk of recurrence after treatment for CIN. Gynecol Oncol. 2007; 104, 273-5.

39: Cruickshank ME, Sharp L, Chambers G, Smart L, Murray G. Persistent infection with human papillomavirus following the successful treatment of high grade cervical intraepithelial neoplasia. BJOG. 2002 109, 579-81.

40: Thomsen LT, Frederiksen K, Munk C, Junge J, Iftner T, Kjaer SK. Long-term risk of cervical intraepithelial neoplasia grade 3 or worse according to high-risk human papillomavirus genotype and semi-quantitative viral load among 33,288 women with normal cervical cytology. Int J Cancer. 2015; 137, 193-203.

41: Arbyn A, Depuydt C, Benoy I, Bogers J, Cuschieri K, Schmitt M, Pawlita M, Geraets D, Herd I, Gheit T, Tommasino M, Poljak M, Bonde J, Quint W . VALGENT: a protocol for clinical validation of human papillomavirus assays. J Clin Virol 2015;

42: Frega A, Sesti F, Lombardi D, Votano S, Sopracordevole F, Catalano A, Milazzo GN, Lombardo R, Assorgi C, Olivola S, Chiusuri V, Ricciardi E, French D, Moscarini M. Assessment of HPV-mRNA test to predict recurrent disease in patients previously treated for CIN 2/3. J Clin Virol. 2014; 60, 39-43.

43: Tropé A, Jonassen CM, Sjøborg KD, Nygård M, Dahl FA, Alfsen GC, Lie AK. Role of high-risk human papillomavirus (HPV) mRNA testing in the prediction of residual disease after conisation for high-grade cervical intraepithelial neoplasia. Gynecol Oncol. 2011; 123, 257-62.

44: Persson M, Brismar Wendel S, Ljungblad L, Johansson B, Weiderpass E, Andersson S. High-risk human papillomavirus E6/E7 mRNA and L1 DNA as markers of residual/recurrent cervical intraepithelial neoplasia. Oncol Rep. 2012; 28, 346-52.

45: http://www.cancerscreening.nhs.uk/cervical/hpv-triage-test-flowchart-201407.pdf

46: Costa S, Venturoli S, Origoni M, Preti M, Mariani L, Cristoforoni P, Sandri MT. Performance of HPV DNA testing in the follow-up after treatment of high-grade cervical lesions, adenocarcinoma in situ (AIS) and microinvasive carcinoma. Ecancermedicalscience. 2015; 9, 528.

47: Costa S, Venturoli S, Negri G, Sideri M, Preti M, Pesaresi M, Falasca A, Barbieri D, Zerbini M, Santini D, Sandri MT, Ghiringhello B, Caroppo Venturini N, Syrjänen S, Syrjänen K. Factors predicting the outcome of conservatively treated adenocarcinoma in situ of the uterine cervix: an analysis of 166 cases. Gynecol Oncol. 2012; 124, 490-5.

48: Costa S, Negri G, Sideri M, Santini D, Martinelli G, Venturoli S, Pelusi C, Syrjanen S, Syrjanen K, Pelusi G. Human papillomavirus (HPV) test and PAP smear as predictors of outcome in conservatively treated adenocarcinoma in situ (AIS) of the uterine cervix. Gynecol Oncol. 2007; 106, 170-6.

49: Pathak N, Dodds J, Zamora J, Khan K. Accuracy of urinary human papillomavirus testing for presence of cervical HPV: systematic review and meta-analysis. BMJ. 2014 349:g5264. doi: 10.1136/bmj.g5264.

50: Arbyn M, Verdoodt F, Snijders PJ, Verhoef VM, Suonio E, Dillner L, Minozzi S, Bellisario C, Banzi R, Zhao FH, Hillemanns P, Anttila A. Accuracy of human papillomavirus testing on self-collected versus clinician-collected samples: a meta-analysis. Lancet Oncol. 2014; 15, 172-83.

51: Taylor S, Wang C, Wright TC, Denny L, Kuhn L. A comparison of human papillomavirus testing of clinician-collected and self-collected samples during follow-up after screen-and-treat. Int J Cancer. 2011; 129, 879-86.

52: Stanczuk GA, Currie H, Baxter G, Foster A, Gibson L, Graham C, Cuschieri K.

Cobas 4800 HPV detection in the cervical, vaginal and urine samples of women with high-grade CIN before and after treatment. J Clin Pathol. 2015; 68, 567-70.

53: Meijer CJ, Berkhof J, Castle PE, Hesselink AT, Franco EL, Ronco G, Arbyn M, Bosch FX, Cuzick J, Dillner J, Heideman DA, Snijders PJ. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. Int J Cancer. 2009; 124, 516-20.

54: Arbyn M, Snijders PJ, Meijer CJ, Berkhof J, Cuschieri K, Kocjan BJ, Poljak M. Which high-risk HPV assays fulfil criteria for use in primary cervical cancer screening? Clin Microbiol Infect. 2015pii: S1198-743X(15)00426-7.
[Epub ahead of print]

55: Arbyn M, Anttila A, Jordan J, Ronco G, Schenck U, Segnan N, Wiener H, Herbert A, von Karsa L. European Guidelines for Quality Assurance in Cervical Cancer Screening. Second edition--summary document. Ann Oncol. 2010; 21, 448-58.

Tables

Table 1: International algorithms for post treatment surveillance of CIN+ lesions

Country	Post treatment recommendation	Reference
US (ASCCP)	Co-testing at 12 and 24 m	Massad et al 2013[16]
UK	Co-testing - between 1 to 2 time-points depending on country	http://www.cancerscreening.nhs.uk/cervical/hpv-triage-test-flowchart-201407.pdf
The Netherlands	3 Paps at 6,12, 24 months Proposal cotesting at 6 and 24 months	Uijterwaal et al 2013[18]
Denmark	Co-testing and margin assessment at (6 m) and 12 m *if* any of initial test(s) positive)	(http://sundhedsstyrelsen.dk) Cervical cancer screening recommendation, version 2012 (Appendix 10, pp. 139).
Sweden	One HPV test or two cytology tests	J Dillner – personal communication
Norway	Co-testing at 3-6 and 12 months	http://legeforeningen.no/Fagmed/Norsk-gynekologisk-forening/Veiledere/veileder-i-gynekologisk-onkologi-2009/Premaligne-lidelser-i-cervix-uteri/
Belgium	Co-testing at two time points	Marc Arbyn - personal communication
Australia	Co-testing at 12 and 24 m	National Health and Medical Research Council. Screening to Prevent Cervical Cancer: Guidelines for the Management of Women with Screen Detected Abnormalities. Canberra: NHRMC, 2005.
EU guidelines	3 Paps at 6,12, 24 months cotesting with HPV recommended (no evidence regarding interval)	Jordan 2009 Cytopathology[2]; Arbyn AnnOncol 2010[55]

Table 2. (A) Pooled absolute sensitivity and specificity of HPV testing, of cytology, or co-testing to predict residual or recurrent CIN2+ after treatment of high-grade CIN. (B) Relative sensitivity and specificity of cytology versus HPV testing, and of cotesting vs HPV testing alone to predict residual or recurrent CIN2+ after treatment of high-grade CIN.

A: Test	Absolute sensitivity (95% CI)	Absolute specificity (95% CI)
HPV testing (HC2 or PCR)	94% (88-97)	80 (74-85)
Cytology (ASC-US+)	72% (66-78)	85% (81-88)
Cotesting	95% (88-98)	69 (62-77)

B: Test comparison	Relative sensitivity (95% CI)	Relative specificity (95% CI)
HPV vs Cytology (ASC-US+) vs HPV (HC2 or PCR)	1.29 (1.18-1.40)	0.94 (0.90-0.99)
Cotesting vs HPV	1.07 (0.97-1.17)	0.93 (0.88-0.97)

Updated from Arbyn et al Vaccine 2012.