Established in 1871

Swiss Medical Weekly

Formerly: Schweizerische Medizinische Wochenschrift

An open access, online journal • www.smw.ch

Viewpoint | Published 20 November 2017 | doi:10.4410/smw.2017.14559
Cite this as: Swiss Med Wkly. 2017;147:w14559

HPV-negative CIN3 and cervical cancer in Switzerland: any evidence of impact on screening policies?

Vassilakos Pierre[ab], Tran Phuong Lien[b], Sahli Roland[c], Low Nicola[d], Petignat Patrick[b]

[a] Geneva Foundation for Medical Education and Research, Geneva, Switzerland
[b] Department of Gynaecology, Geneva University Hospitals, Switzerland
[c] Institute of Microbiology, Lausanne University Hospitals, Switzerland
[d] Institute of Social and Preventive Medicine, University of Bern, Switzerland

New practice guidelines for cervical cancer screening in Switzerland recommend testing for the presence of DNA from high-risk types of human papillomavirus (hrHPV) as the primary screening test for all women 30 years and older. However, the possibility that HPV testing might not detect some cervical precancerous high-grade lesions and cancers has raised questions about the performance of HPV testing as a primary screening method for two reasons. First, HPV-negative lesions (cervical intraepithelial neoplasia grade 3 [CIN3], adenocarcinoma *in situ* or invasive carcinoma [CIN3+]) have been found in studies that analysed archived paraffin-embedded cervical tissue [1] and in reviews of clinical records [2, 3]. Second, some authors have reported that the combined use of HPV testing with cytology (co-testing) identified CIN3+ in women who had tested negative for HPV within 1 year before their cervical biopsy [2]. Consequently, to maximise the detection of cervical cancer, co-testing has been recommended as the primary screening method instead of HPV-testing alone [4].

Recent data from Switzerland provide useful information about HPV-negative CIN3+ lesions [5]. The CIN3+ plus study was a cross-sectional study that provides baseline data for monitoring future HPV vaccine impact in Switzerland [5]. Ten pathology institutes from six cantons and three language regions participated in the study. Each laboratory conducted DNA extraction and HPV typing according to their standard practice and was requested to participate in the quality assurance system for HPV genotyping designed by the World Health Organization (WHO) HPV Laboratory Network [6, 7], to ensure comparable coverage of most important anogenital HPV genotypes by each laboratory. Seven hundred and sixty-eight formaldehyde-fixed, paraffin-embedded tissue specimens of histologically confirmed CIN3+ cases were tested for any HPV type, including the 12 genotypes that the International Agency for Research on Cancer (IARC) recognises as causes of cervical cancer (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59). Of these, 729 (95%) samples were hrHPV-positive and 39 samples were either hrHPV-negative (n = 20) or not evaluable (n = 19) owing to inhibitors of the polymerase chain reaction (PCR) or lack of material. Confirmatory testing of 20 available samples (18 initially negative samples and two initially nonevaluable samples) was performed by the WHO Global Reference Laboratory, which found 16 retested samples to be hrHPV positive. The final results found: 97.0% (745/768) hrHPV positive, 2.3% (18/768) nonevaluable and 0.7% (95% confidence interval [CI] 0.2–1.5%) (5/768) hrHPV negative. Only two of these five negative samples could be subjected to confirmatory testing. The hrHPV-positivity rate amongst all evaluable samples was therefore 99.3% (95% CI 98.5–99.8%, 745/750). The number of hrHPV-negative precancers and cancers in the CIN3+ study was low but shows that a small percentage of samples will have negative results with hrHPV testing. The findings from retrospective studies and archival material cannot be directly extrapolated to the performance of hrHPV testing in cervical samples in screening populations because these studies include samples from both women who have presented with clinical symptoms and asymptomatic women undergoing screening. Predictive values of hrHPV testing depend on the prevalence of the disease, which is not comparable between these two populations of women.

In this article, we summarise the rationale for hrHPV testing as the primary test for screening, explain potential reasons for false-negative hrHPV test results in the presence of CIN3+ lesions, describe true HPV-negative precancer and cancer, and comment on the relevance of the debate about co-testing versus hrHPV-only screening approaches in Switzerland.

Primary hrHPV screening for cervical cancer

Randomised controlled trials and cohort studies have consistently demonstrated the high sensitivity of clinically validated hrHPV tests for the detection of clinical lesions. The high negative predictive value allows longer screening intervals than cytology alone [8]. Furthermore, randomised controlled trials [9–15] showed that hrHPV testing detects

Correspondence:
Phuong Lien Tran, Department of Gynaecology and Obstetrics, Geneva University Hospitals, Boulevard de la Cluse 30, CH-1205 Geneva, phuong_lien_tran[at]yahoo.com

more CIN3 lesions than cytology at the first screening round and leads to an overall reduction in the incidence of these lesions and of invasive cervical cancer in the following years among the screened population. The protective effect of HPV testing for CIN3 and invasive cancer was not observed in the first 2.5 years after screening but increased significantly thereafter.

The results of most randomised controlled trials also showed that primary HPV screening applies only to women from 30 years. In younger women, HPV testing has a lower specificity for underlying precancer and cancer.

Based on these data and on cost-effectiveness modelling analyses [16], several European countries (Italy, Denmark, The Netherlands, UK, Estonia, Germany, Portugal, Spain, Sweden, Turkey), as well as the USA, Australia, New Zealand, and 10 other countries have now adopted or plan to implement primary hrHPV testing as a stand-alone screening modality without cytology.

Possible reasons for a negative hrHPV test result in a CIN3+ lesion

1. *Reasons related to specimens*. A false-negative hrHPV test in a cervical sample could result from problems with: the specimen, such as low hrHPV DNA copy number owing to inadequate cellularity in the specimen or insufficient specimen collected from a clinically overt cancer with necrosis, interfering substances such as lubricants in the specimen, or suboptimal specimen types. Tests for hrHPV were developed primarily for use on fresh cytology specimens. When applied to formaldehyde-fixed, paraffin-embedded tissues, technical artefacts resulting from poor DNA extraction and quality and/or low viral load can result in negative test results [17–19].
2. *Reasons related to hrHPV tests*. There are many different hrHPV tests. A negative test result can occur if the test does not detect a specific rare hrHPV type. Other tests such as Hybrid Capture 2 [20] have limited analytic sensitivity. To minimise these deficiencies, clinicians are advised to use sufficiently sensitive and clinically validated hrHPV tests for primary screening [21] in qualified laboratories accredited by authorised accreditation bodies and in compliance with international standards. However, even very good HPV tests may miss CIN3 lesions associated with hrHPV types in 1–3% of cases [22].
3. *True hrHPV-negative CIN3 and cervical cancer*. Although hrHPV is a necessary causal factor in the pathway to cervical cancers [23], hrHPV is not always detected in tumour specimens from women diagnosed with CIN3 or invasive cervical cancer. Nearly all CIN3 lesions are associated with hrHPV types, with a very small proportion associated only with low risk HPV types [24].

True primary cervical cancers not linked to HPV account for a small portion of hrHPV-negative testing [25, 26]. They include endocervical adenocarcinomas of the gastric type, minimal deviation adenocarcinomas, as well as clear cell and mesonephric adenocarcinomas [27, 28]. A small group of hrHPV-negative endocervical carcinomas have features of endometrial adenocarcinomas. A recent study systematically evaluated 777 cervical cancer tissues from several US-based cancer registries and found carcinogenic HPV in 91% of the cases. Nearly 60% of the HPV-negative cases could not be distinguished histologically from endometrial primary adenocarcinomas, suggesting that these tumours may not be of cervical origin [29].

Histological findings of cervical adenocarcinoma with hrHPV-negative testing should be verified using immunostaining for p16 and additional biomarkers to exclude an origin from other sites.

There are limited data regarding the performance of the Pap test for hrHPV-negative adenocarcinomas. Because false negative Pap results are common with endocervical adenocarcinomas [30], it is unclear how accurate cytology is for the detection of these rare tumours.

Co-testing versus hrHPV-only primary screening

Prospective European randomised screening trials have shown that adding cytology to hrHPV primary testing (co-testing) offers minimal increased protection against the subsequent development of cervical disease, at the expense of a considerable loss in specificity (colposcopy is performed if positive by either test), compared with hrHPV-only primary screening [12, 14, 31–33]. Similar results have been found in a large prospectively conducted US FDA registration trial of primary hrHPV screening [15]. Another US study, which included approximately one million women aged 30–65 years, demonstrated that co-testing confers only a very slight marginal gain (0.003%) in reassurance (lower cancer risk) of safety against cancer over 5 years. Most of the reassurance provided by co-testing was derived from the hrHPV test component. The study also demonstrated that the 5-year reassurance after a negative co-test is approximately the same as the 4-year reassurance after a negative HPV test alone [14].

From a population management perspective, screening modalities must be projected over the screening lifetime to estimate what strategy is best for women in terms of benefits, harms and costs. Primary screening with two tests, especially in places like Switzerland where the prevalence of disease in screened populations is very low, may lead to over referral and overtreatment. Another consequence is that two test results obtained simultaneously are difficult to interpret resulting in significant algorithmic complexity for the management of screened women [34]. Furthermore, combined cytological and hrHPV screening is not cost-effective, since there is an increased cost without significantly increasing sensitivity [35].

Conclusion

Testing for hrHPV DNA, like other cancer screening tests, cannot detect all cases of prevalent or incipient cervical cancer. Most missed cases are a small subset of adenocarcinomas that are not linked to HPV. The evidence base demonstrated that a validated hrHPV test is sufficiently accurate for clinical use and could reduce the complexities of interpretation and management of co-test results as well as resource expenditure inherent in screening with two tests.

Disclosure statement

No financial support and no other potential conflict of interest relevant to this article was reported.

References

1 Pirog EC, Lloveras B, Molijn A, Tous S, Guimerà N, Alejo M, et al.; RIS HPV TT study group. HPV prevalence and genotypes in different histological subtypes of cervical adenocarcinoma, a worldwide analysis of 760 cases. Mod Pathol. 2014;27(12):1559–67. doi: http://dx.doi.org/10.1038/modpathol.2014.55. PubMed.

2 Blatt AJ, Kennedy R, Luff RD, Austin RM, Rabin DS. Comparison of cervical cancer screening results among 256,648 women in multiple clinical practices. Cancer Cytopathol. 2015;123(5):282–8. doi: http://dx.doi.org/10.1002/cncy.21544. PubMed.

3 Zhao C, Li Z, Nayar R, Levi AW, Winkler BA, Moriarty AT, et al. Prior high-risk human papillomavirus testing and Papanicolaou test results of 70 invasive cervical carcinomas diagnosed in 2012: results of a retrospective multicenter study. Arch Pathol Lab Med. 2015;139(2):184–8. doi: http://dx.doi.org/10.5858/arpa.2014-0028-OA. PubMed.

4 Zhao C, Yang H, Li Z. Cytopathology and more: evidence emerging for HPV-negative cervical cancer [serial online]. CAP Today. Available at http://www.captodayonline.com/cytopathology-and-more-evidence-emerging-for-hpv-negative-cervical-cancer/. Accessed February 10, 2017.

5 Genotypen des humanen Papillomavirus in Krebs-vorstufen und Krebserkrankungen am Gebärmutterhals in der Schweiz zu Beginn der kantonalen Impfprogramme: die CIN3+plus Studie BAG-Bulletin 2017; Nr 6:12-18 available from: For the German version: https://www.bag.admin.ch/dam/bag/de/dokumente/mt/infektionskrankheiten/hpv/hpv-cin3plusplus-studie.pdf.download.pdf/hpv-cin3plusplus-studie-de.pdf. For the French version: https://www.bag.admin.ch/dam/bag/fr/dokumente/mt/infektionskrankheiten/hpv/hpv-cin3plusplus-studie.pdf.download.pdf/hpv-cin3plusplus-studie-fr.pdf

6 Available from. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/003852/human_med_001863.jsp&mid=WC0b01ac058001d124

7 Available from. http://www.nordicehealth.se/hpvcenter/

8 Ronco G, Dillner J, Elfström KM, Tunesi S, Snijders PJ, Arbyn M, et al.; International HPV screening working group. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet. 2014;383(9916):524–32. doi: http://dx.doi.org/10.1016/S0140-6736(13)62218-7. PubMed.

9 Naucler P, Ryd W, Törnberg S, Strand A, Wadell G, Elfgren K, et al. Human papillomavirus and Papanicolaou tests to screen for cervical cancer. N Engl J Med. 2007;357(16):1589–97. doi: http://dx.doi.org/10.1056/NEJMoa073204. PubMed.

10 Rijkaart DC, Berkhof J, Rozendaal L, van Kemenade FJ, Bulkmans NW, Heideman DA, et al. Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial. Lancet Oncol. 2012;13(1):78–88. doi: http://dx.doi.org/10.1016/S1470-2045(11)70296-0. PubMed.

11 Kitchener HC, Almonte M, Thomson C, Wheeler P, Sargent A, Stoykova B, et al. HPV testing in combination with liquid-based cytology in primary cervical screening (ARTISTIC): a randomised controlled trial. Lancet Oncol. 2009;10(7):672–82. doi: http://dx.doi.org/10.1016/S1470-2045(09)70156-1. PubMed.

12 Ronco G, Giorgi-Rossi P, Carozzi F, Confortini M, Dalla Palma P, Del Mistro A, et al., New Technologies for Cervical Cancer screening (NTCC) Working Group. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. Lancet Oncol. 2010;11(3):249–57. doi: http://dx.doi.org/10.1016/S1470-2045(09)70360-2. PubMed.

13 Ronco G, Dillner J, Elfström KM, Tunesi S, Snijders PJ, Arbyn M, et al.; International HPV screening working group. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet. 2014;383(9916):524–32. doi: http://dx.doi.org/10.1016/S0140-6736(13)62218-7. PubMed.

14 Gage JC, Schiffman M, Katki HA, Castle PE, Fetterman B, Wentzensen N, et al. Reassurance against future risk of precancer and cancer conferred by a negative human papillomavirus test. J Natl Cancer Inst. 2014;106(8):dju153. doi: http://dx.doi.org/10.1093/jnci/dju153. PubMed.

15 Wright TC, Stoler MH, Behrens CM, Sharma A, Zhang G, Wright TL. Primary cervical cancer screening with human papillomavirus: end of study results from the ATHENA study using HPV as the first-line screening test. Gynecol Oncol. 2015;136(2):189–97. doi: http://dx.doi.org/10.1016/j.ygyno.2014.11.076. PubMed.

16 Lew JB, Simms KT, Smith MA, Hall M, Kang YJ, Xu XM, et al. Primary HPV testing versus cytology-based cervical screening in women in Australia vaccinated for HPV and unvaccinated: effectiveness and economic assessment for the National Cervical Screening Program. Available from www.thelancet.com/public-health, Vol 2 February 2017.

17 de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al.; Retrospective International Survey and HPV Time Trends Study Group. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol. 2010;11(11):1048–56. doi: http://dx.doi.org/10.1016/S1470-2045(10)70230-8. PubMed.

18 Steinau M, Patel SS, Unger ER. Efficient DNA extraction for HPV genotyping in formalin-fixed, paraffin-embedded tissues. J Mol Diagn. 2011;13(4):377–81. doi: http://dx.doi.org/10.1016/j.jmoldx.2011.03.007. PubMed.

19 Lagheden C, Eklund C, Kleppe SN, Unger ER, Dillner J, Sundström K. Validation of a standardized extraction method for formalin-fixed paraffin-embedded tissue samples. J Clin Virol. 2016;80:36–9. doi: http://dx.doi.org/10.1016/j.jcv.2016.04.016. PubMed.

20 Rodríguez-Carunchio L, Soveral I, Steenbergen RDM, Torné A, Martinez S, Fusté P, et al. HPV-negative carcinoma of the uterine cervix: a distinct type of cervical cancer with poor prognosis. BJOG. 2015;122(1):119–27. doi: http://dx.doi.org/10.1111/1471-0528.13071. PubMed.

21 Meijer CJLM, Berkhof J, Castle PE, Hesselink AT, Franco EL, Ronco G, et al. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. Int J Cancer. 2009;124(3):516–20. doi: http://dx.doi.org/10.1002/ijc.24010. PubMed.

22 Petry KU, Cox JT, Johnson K, Quint W, Ridder R, Sideri M, et al. Evaluating HPV-negative CIN2+ in the ATHENA trial. Int J Cancer. 2016;138(12):2932–9. doi: http://dx.doi.org/10.1002/ijc.30032. PubMed.

23 Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999;189(1):12–9. doi: http://dx.doi.org/10.1002/(SICI)1096-9896(199909)189:1<12::AID-PATH431>3.0.CO;2-F. PubMed.

24 Böhmer G, van den Brule AJ, Brummer O, Meijer CL, Petry KU. No confirmed case of human papillomavirus DNA-negative cervical intraepithelial neoplasia grade 3 or invasive primary cancer of the uterine cervix among 511 patients. Am J Obstet Gynecol. 2003;189(1):118–20. doi: http://dx.doi.org/10.1067/mob.2003.439. PubMed.

25 Liebrich C, Brummer O, Von Wasielewski R, Wegener G, Meijer C, Iftner T, et al. Primary cervical cancer truly negative for high-risk human papillomavirus is a rare but distinct entity that can affect virgins and young adolescents. Eur J Gynaecol Oncol. 2009;30(1):45–8. PubMed.

26 Li N, Franceschi S, Howell-Jones R, Snijders PJ, Clifford GM. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. Int J Cancer. 2011;128(4):927–35. doi: http://dx.doi.org/10.1002/ijc.25396. PubMed.

27 Holl K, Nowakowski AM, Powell N, McCluggage WG, Pirog EC, Collas De Souza S, et al. Human papillomavirus prevalence and type-distribution in cervical glandular neoplasias: Results from a European multinational epidemiological study. Int J Cancer. 2015;137(12):2858–68. doi: http://dx.doi.org/10.1002/ijc.29651. PubMed.

28 Kurman RJ, Carcangiu ML, Herrington CS, et al., eds. WHO classification of tumors of female reproductive organs, 4th edn., vol. 6. Geneva: World Health Organization, 2014

29 Hopenhayn C, Christian A, Christian WJ, Watson M, Unger ER, Lynch CF, et al. Prevalence of human papillomavirus types in invasive cervical cancers from 7 US cancer registries before vaccine introduction. J Low Genit Tract Dis. 2014;18(2):182–9. doi: http://dx.doi.org/10.1097/LGT.0b013e3182a577c7. PubMed.

30 Ault KA, Joura EA, Kjaer SK, Iversen OE, Wheeler CM, Perez G, et al.; FUTURE I and II Study Group. Adenocarcinoma in situ and associated human papillomavirus type distribution observed in two clinical trials of a quadrivalent human papillomavirus vaccine. Int J Cancer. 2011;128(6):1344–53. doi: http://dx.doi.org/10.1002/ijc.25723. PubMed.

31 Kitchener HC, Gilham C, Sargent A, Bailey A, Albrow R, Roberts C, et al. A comparison of HPV DNA testing and liquid based cytology over three rounds of primary cervical screening: extended follow up in the ARTISTIC trial. Eur J Cancer. 2011;47(6):864–71. doi: http://dx.doi.org/10.1016/j.ejca.2011.01.008. PubMed.

32 Arbyn M, Ronco G, Anttila A, Meijer CJLM, Poljak M, Ogilvie G, et al. Evidence Regarding Human Papillomavirus Testing in Secondary Prevention of Cervical Cancer. Vaccine. 2012;30(Suppl.5):F88–99. doi: . Erratum in: Vaccine. 2013;52:6266. . http://dx.doi.org/10.1016/j.vaccine.2012.06.095. PubMed.

33 Rijkaart DC, Berkhof J, van Kemenade FJ, Coupe VM, Rozendaal L, Heideman DA, et al. HPV DNA testing in population-based cervical screening (VUSA-Screen study): results and implications. Br J Cancer. 2012;106(5):975–81. doi: http://dx.doi.org/10.1038/bjc.2011.581. PubMed.

34 Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, et al.; 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;17(5, Suppl 1):S1–27. doi: http://dx.doi.org/10.1097/LGT.0b013e318287d329. PubMed.

35 Naucler P, Ryd W, Törnberg S, Strand A, Wadell G, Elfgren K, et al. Efficacy of HPV DNA testing with cytology triage and/or repeat HPV DNA testing in primary cervical cancer screening. J Natl Cancer Inst. 2009;101(2):88–99. doi: http://dx.doi.org/10.1093/jnci/djn444. PubMed.