

Cervical cancer screening based on human papilloma virus-DNA testing: a scientific heritage to microbiologists or to pathologists? An on-going dilemma

Simona Venturoli,¹ Vittorio Sambri^{2,3}

¹Microbiology and Virology Unit, Bologna University Hospital, Sant'Orsola-Malpighi Policlinic, Bologna;
²Microbiology Unit, Romagna Local Health Unit, Pievesestina; ³DIMES, University of Bologna, Italy

Non omnia possumus omnes
(Virgilio, Bucoliche, VIII, 63)

Among the 28 current Members of the European Union, approximately 34,000 new cases of cervical cancer occur annually, with 13,000 related deaths (10). In Italy, about 2900 cases of cervical cancer are diagnosed annually (crude incidence rate/100,000/year=9.4). As a direct consequence, about 1016 deaths related to cervical cancer are reported annually in Italy (crude mortality rate/100,000/year=3.3). Cervical cancer stands in the 15th rank among female cancers and is currently the 3rd most common cancer in women aged 15-44 years in Italy (5).

A persistent mucosal infection with oncogenic (high-risk, HR) human papillomavirus (HPV), genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68, is the most significant cause that leads to the development of cervical cancer. The HPV genotypes 16 and 18 are the most commonly associated with the disease, and are identified in 70% of the cases of squamous cervical cancer (9).

The primary measure to control cervical cancer is the prevention through vaccinations. The massive screening of women to detect and treat the cervical precancerous lesions is an additional preventive approach. It is nowadays well established that most cases of cervical cancer may be prevented by effective and early treatment of precancer-

ous lesions detected by cervical screening performed by cytology testing (Pap test) (11). For over a decade, detection of the aetiological agent HPV in cervical samples has been investigated in randomised controlled intervention trials as a possible alternative method to Pap test (6,12,14,16). It is now evident that the detection of HPV DNA is more effective than cytology in preventing invasive cancers of the uterine cervix.

Very recently, four different European randomised trials have demonstrated that the cumulative incidence of cervical cancer was lower five years after a negative HPV DNA test than the one detected after three years following a normal cytology result (18).

Italy and the Netherlands, as first in Europe, consequently decided to innovate their National Prevention Programs (for Italy see: Piano Nazionale della Prevenzione 2014-2018) by switching to the HPV DNA test performed on liquid cytology specimens as a primary test for cervical screening in the population of women aged 30 years and older (17). In this novel strategy, the HPV DNA-positive women are not directly referred to colposcopy, but the use of a triage system is essential. The currently recommended method is to perform the cytological evaluation only in the HPV DNA positive women.

This epochal modification, which is now occurring gradually in different areas of our country, represents a new and important challenge for the Italian Public Health system (IPHS) that is required to enlarge the efforts to make this possibility available to all the women. The switch from cytology alone to HPV DNA testing with triage cytology as the primary screening approach represents a substantial modification for the IPHS and will require a strong adaptive effort in order to keep the preventive efficiency of the strategy. Women will be tested for HPV DNA by highly automated molecular techniques and some of them will ultimately be notified they have got HPV infection. In the context of the primary screening procedure, it is expectable that many women will be HPV DNA positive but still totally normal at the cytological subsequent evaluation. These women will be at greater oncologic risk than those completely negative for the HPV DNA. So it will be extremely important that this HPV positive population follows the path to stay within the screening process. Meanwhile it must be underlined that this is a population that has elevated likelihood to spontaneously revert to HPV DNA negativity.

It is therefore of outmost importance to emphasise that this new strategy represents a radically change in the concept of screening for cervical cancer: from a merely morphological path (cytology-colposcopy-histology) to a preventive biomolecular model, targeted at the detection of specific genome sequences from the oncogenic papillomavirus genotypes, thus allowing the stratification of the risk to develop a cervical cancerous lesion.

The European guidelines document for the quality assurance in the cervical cancer screening (20) has recently highlighted the necessity

Correspondence: Simona Venturoli, U.O. Microbiologia e Virologia, Policlinico S. Orsola Malpighi, via G. Massarenti 9, 40138 Bologna, Italy.
Tel.: +39.051.2144512 - Fax: +39.051.307397.
E-mail: simona.venturoli@unibo.it

Key words: Papilloma virus; Pap test; cervical cancer screening.

Contributions: SV wrote the manuscript, VS revised the paper.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 26 February 2016.
Accepted for publication: 26 February 2016.

©Copyright S. Venturoli and V. Sambri, 2016
Licensee PAGEPress, Italy
Microbiologia Medica 2016; 31:5849
doi:10.4081/mm.2016.5849

This article is distributed under the terms of the Creative Commons Attribution Noncommercial License (by-nc 4.0) which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

of a clinical validation, performed in accordance to the international criteria (13), as a basic requirement for adopting a HPV DNA test to be used in the screening for cervical cancer. Furthermore, additional aspects related to the quality of the procedures must be taken into account: the participation of the laboratories performing HPV DNA testing to either internal and external quality assurance programs is among the most relevant.

The number of CE and IVD marked technical platforms available for HPV DNA testing is increasing day after day and the participation of each individual laboratory to an appropriate Quality Assurance Programs, which can monitor the longitudinal robustness and the clinical performance over time, are of paramount importance. It is noteworthy that only clinically validated tests for the detection of the HPV genome must be used in Screening Programs (1), as it is customary and requested since a long time for molecular tests employed to identify the genome of other viruses (e.g. HIV, HCV, HBV) in diagnostic Microbiology laboratories. This approach is now at the bottom line to achieve the accreditation of the diagnostic workflow by authorized accreditation bodies in compliance with the international standards.

All the above reported issues imposes the need for a correct and continuous monitoring of the on going process that is translating from Pap test to the detection of HPV DNA as the primary screening procedure. We must also consider that the knowledge about the efficacy and safety of the screening for cervical cancer with HPV DNA testing is rapidly evolving and it is therefore acceptable that in the coming years some changes in the screening protocols would likely occur, in particular the management of HPV DNA positive women and of the population of women previously vaccinated against HPV in their adolescence.

In this frame of evolutionary knowledge, many other additional diagnostic approaches and methods have been described as holding a potential use in triaging the HPV DNA positive women in order to increase the specificity of the procedures for the detection of cervical precancerous lesions. These new methods include DNA genotyping for HPV 16 or HPV 16/18, HPV mRNA testing, detection of p16 (INK4a) and/or detection of other non-HPV biomarkers (biomarkers of cellular proliferation and detection of the host epigenetic changes) (4).

Moreover it is true that persistent positivity for HR HPV DNA is considered a prognostic index of recurrent disease in patients treated for high-grade cervical intraepithelial neoplasia (CIN2/3) or adenocarcinoma *in situ* (HPV as *test of cure*) (7). HPV genotyping methods, as biological indicators of persistent disease, could be more suitable for a predictive role and risk stratification (particularly in the case of persistence of HPV 16/18) than pooled HPV DNA based testing (19). Recent studies suggest that the oncogenic activity of HR HPV-mRNA transcripts may be a better indicator of women at risk for persistent disease or relapse showing a higher specificity and negative predictive value (NPV) than just DNA based assay. On the same time other scientific reports pointed out that HPV E6/E7 mRNA is not useful for the detection of relapses. As a consequence of the above enlisted discrepant data it will be necessary to collect further data in order to assess the usefulness of HR HPV-mRNA related findings in the clinical practice.

A clinical validation is still lacking for most of these new biomarkers that could be used in the diagnosis of high-grade lesions and in the follow-up post treatment. In addition, a wide assessment of their inter-laboratory reproducibility and longitudinal studies to ensure the safety for negatives, are not yet available. Within this evolving scenario it is clearly perceived that the Virologist actually holds the necessary expertise to generate the scientific evidences required for the introduction of new screening protocols into the routine procedures and so to improve the follow-up management of women treated conservatively for high-grade cervical intraepithelial neoplasia or adenocarcinoma *in situ*.

Talking about the history of the screening for cervical cancer, at least in Italy, it must not be forgotten the important role played so far by the Pathologists. This professional category has mainly performed the

workflow based on Pap test as the primary assay, but they have also been deeply involved in the change about the role of cytology (triage) in the Screening Program. So nowadays a question clearly arises: who could play better the pivotal role in running the screening procedure for cervical cancer? Should this be done by Microbiologists and Virologists that have the knowledge about the pathogenic mechanisms of HPV and that hold large experience in running other screening oriented procedures for largely diffuse viral infections such as hepatitis and HIV? Or should this process be run by the Pathologists that have a long lasting experience in the Pap test use and in all the subsequently derived investigations for those women that are affected by pre-cancer or truly cancerous cervical lesions? We do believe that the answer could be only one: these two professional categories must constantly collaborate and interact constructively. The best answer to this new and multi faceted screening workflow is the implementation of a multidisciplinary network of Microbiologists-Virologists, Pathologists, Epidemiologists and Gynaecologists that act all together in the frame of the IPHs in order to provide the best attitudes to the different stages of the whole screening process (1st, 2nd and 3rd level). As a final and overall outcome an increased protection against cervical cancer and a reduced burden of high-grade cervical intraepithelial neoplasia (CIN2+) would be achieved in a few years. This model has been already implemented in the frame of the local Health Authority of Romagna, in Italy, (AUSL della Romagna) where virologists and pathologists are sitting together to provide epidemiologists and gynaecologists with the results obtained by an integrated screening workflow based on massive HPV DNA testing and triage derived Pap tests.

In the end it is important to evaluate the use of protocols and methods for the identification of HR-HPV infections in the prevention and diagnostic laboratory process of vulvar, vaginal (15), penile (8), anal (2) pre-neoplastic lesions and in the clear cancers of the head and neck region, especially the oropharynx (3). The major issues that Virologists have nowadays to face is related to the lack of CE IVD methods for the molecular detection of HPV DNA to be used with specimens other than cervical cells. As a consequence a huge effort is required to define the possibility to use methods that hold validation for cervical derived samples on other biological specimens. For all the HPV-related diseases that do not involve the cervix, the most appropriate HPV tests still need to be evaluated. Unfortunately, there is no current standard for testing or interpretation of the HPV detection assays, and each assay has variable technical limitations. Only a close integration, in randomized controlled trials, including Microbiologists-Virologists, which are responsible for the selection, implementation and validation of diagnostic tests for HPV, Pathologists that identify and characterize the lesion/tumour and clinicians (Urologists, Otolaryngologists, Infectious Disease specialists, Dermatologists, and so on...) managing the patient, will in the future be able to validate univocal diagnostic protocols that could be inserted in the frame of certified laboratory diagnostics.

References

1. Arbyn M, Snijders PJ, Meijer CJ, et al. Which high-risk HPV assays fulfil criteria for use in primary cervical cancer screening? Clin Microbiol Infect 2015;21:817-26.
2. Bernardi MP, Ngan SY, Michael M, et al. Molecular biology of anal squamous cell carcinoma: implications for future research and clinical intervention. Lancet Oncol 2015;16:e611-21.
3. Bhatia A, Burtness B. Human papillomavirus-associated oropharyngeal cancer: defining risk groups and clinical trials. J Clin Oncol 2015;33:3243-50.
4. Brown AJ, Trimble CL. New technologies for cervical cancer screening. Best Pract Res Clin Obstet Gynaecol 2012;26:233-42.

5. Bruni L, Barrionuevo-Rosas L, Albero G et al. ICO information centre on HPV and cancer (HPV Information Centre). Human papillomavirus and related diseases in Italy. Summary Report 2015-12-23. Available from: <http://www.hpvcentre.net/statistics/reports/XWX.pdf>
6. Bulkmand NW, Berkhof J, Rozendaal L et al. Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. *Lancet* 2007;370:1764-72.
7. Costa S, Venturoli S, Origoni M, et al. Performance of HPV DNA testing in the follow-up after treatment of high-grade cervical lesions, adenocarcinoma in situ (AIS) and microinvasive carcinoma. *Ecancermedalscience* 2015;9:528.
8. Djajadiningrat RS, Jordanova ES, Kroon BK, et al. Human papillomavirus prevalence in invasive penile cancer and association with clinical outcome. *J Urol* 2015;193:526-31.
9. Doorbar J, Quint W, Banks L, et al. The biology and life-cycle of human papillomaviruses. *Vaccine* 2012;30:F55-70.
10. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. *Eur J Cancer* 2013;49:1374-403.
11. International Agency for Research on Cancer, WHO. Cervix cancer screening. IARC press; 2015. Available from: <https://www.iarc.fr/en/publications/pdfs-online/prev/handbook10/HANDBOOK10.pdf>
12. Kitchener HC, Almonte M, Gilham C et al. ARTISTIC: a randomised trial of human papillomavirus (HPV) testing in primary cervical screening. *Health Technol Assess* 2009;13:1-150.
13. Meijer CJ, Berkhof J, Castle PE et al. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. *Int J Cancer* 2009;124:516-20.
14. Naucler P, Ryd W, Tornberg S et al. Human papillomavirus and Papanicolaou tests to screen for cervical cancer. *N Engl J Med* 2007;357:1589-97.
15. Nygard M, Hansen BT, Dillner J, et al. Targeting human papillomavirus to reduce the burden of cervical, vulvar and vaginal cancer and pre-invasive neoplasia: establishing the baseline for surveillance. *PLoSOne* 2014;9:e88323.
16. Ronco G, Giorgi-Rossi P, Carozzi F et al. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. *Lancet Oncol* 2010;11:249-57.
17. Ronco G, Biggeri A, Confortini M, et al. Ricerca del DNA di Papillomavirus Umano (HPV) come test primario per lo screening dei precursori del cancro del collo dell'utero. *Epidemiol Prev* 2012;36: e1-72.
18. Ronco G, Dillner J, Elfstrom KM et al. Efficacy of HPV based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. *Lancet* 2014;383:524-32.
19. Venturoli S, Ambretti S, Cricca M, et al. 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.
20. von Karsa L, Arbyn A, De Vuyst H, et al. Executive summary. In: Anttila A, Arbyn A, De Vuyst H, et al. (eds.). *European guidelines for quality assurance in cervical cancer screening*. 2nd ed. Luxembourg: Office for Official Publications of the European Union; 2015. pp. XIII-XXIV.