HPV Testing for Cervical Cancer Screening in Croatia

Grce, Magdalena; Grahovac, Blaženka; Rukavina, Tomislav; Vrdoljak-Mozetič, Danijela; Glavaš-Obrovac, Ljubica; Kaliterna, Vanja; Žele Starčević, Lidija

Coll. Antropol. **31** (2007) Suppl. 2: 67–71
Review

HPV Testing for Cervical Cancer Screening in Croatia

Magdalena Grce[1], Blaženka Grahovac[2], Tomislav Rukavina[3,4], Danijela Vrdoljak-Mozetič[5], Ljubica Glavaš-Obrovac[6,7], Vanja Kaliterna[8] and Lidija Žele-Starčević[9]

[1] Ruđer Bošković Institute, Zagreb, Croatia
[2] Department of Pathology, Rijeka University School of Medicine, Braće Branchetta 20, Rijeka, Croatia
[3] Department of Microbiology, Institute of Public Health, Rijeka, Croatia
[4] Department of Microbiology and Parasitology, Medical Faculty, University of Rijeka, Rijeka, Croatia
[5] Department of Gynecological Cytology, University Department of Gynecology and Obstetrics, University Hospital Centre Rijeka, Rijeka, Croatia
[6] Clinical Hospital Osijek, Department of Nuclear Medicine, Radiation Protection and Pathophysiology, Osijek, Croatia
[7] University in Osijek, School of Medicine Osijek, Department of Clinical Chemistry and Biochemistry, Osijek, Croatia
[8] Department of Medical Microbiology and Parasitology, Public Health Teaching Institute of Split and Dalmatian County, Split, Croatia
[9] Department of Medical and Molecular Microbiology, Clinical Hospital Centre Zagreb, Zagreb, Croatia

A B S T R A C T

Opportunistic screening based on the Pap smear has been undertaken in Croatia since 1953. However, cervical cancer remains an important health problem in Croatia when compared to European countries with organised screening programmes. In Croatia, in addition to screening based on well established cytology, Human papillomavirus (HPV) testing is widely used as secondary test as a triage to borderline cytology and as a follow-up after treatment of severe cervical lesions. Many different approaches for HPV testing arose in Croatia over the last decade depending on the needs of each medical institution involved. Presently, there is an urgent need for better networking between the laboratories, the implementation of quality assessment and the adaptation of a uniform system of referring to and reporting of HPV testing. In conclusion, the best possible organisation for HPV testing would be essential for implementation of HPV testing as primary screening test in Croatia, an thus ultimately and hopefully, the more successful cervical cancer control.

***Key words**: Cervical cancer, human papillomavirus testing, screening programme, quality assessment*

Background

Cervical cancer remains an important health problem in Croatia where the incidence rate of 14.4/100,000 women recorded in 2004[1] is slightly lower than the average world incidence (16.2/100,000 women-year) but much higher than rates recorded in European countries with organised screening programmes.

Cervical cancer is highly amenable to screening because it has a long pre-clinical phase with precursor lesions that can be identified by cervical cytology (Papanicolaou or Pap smears) and that can be easily treated using simple procedures if they are detected at an early stage. Opportunistic screening based on the Pap smear has been undertaken in Croatia since 1953 when the first laboratory for gynaecological cytology was established in Zagreb[2]. Since then, a network of more than 30 laboratories has developed and now provides good coverage of a large part of the target population. Opportunistic screening has produced a decrease in cervical cancer incidence from 26/100,000 to 15/100,000 women-years between 1970 and 1990, although it remained almost stable thereafter[1].

The European Code Against Cancer states that all women from 25 years of age should participate in organised cervical screening programmes, the Council of the

Received for publication January 31, 2007

European Union has recommended that all Member States should implement organised cervical cancer screening programmes and the new European Guidelines now state that cervical cancer screening should only be offered within the context of an organised programme[3,4]. In 2003, a panel of Croatian experts prepared a proposal for the implementation of an organised screening programme that was submitted to the Croatian Ministry of Health and Social Welfare[5]. This proposal recommended that the programme should start by offering conventional Pap smears to all women from 25 to 64 years of age (app. 1.2 million women) who should be screened annually for the first 3 years but with the screening interval subsequently extended to 3 years for all women who have had 3 consecutive normal Pap smears. The proposal also recommended the implementation of liquid-based cytology with the extension of the screening interval to 5 years and the addition of HPV testing for women over the age of 30[5].

The implementation of an organised cervical screening programme in Croatia would be facilitated because many of the necessary elements are already in place:

- high quality cytology; gynaecological cytology is a mandatory sub-specialisation and cytotechnicians are required to participate in continuing education since 1967[2]. Also, gynaecological cytology is regularly reviewed and updated with the last improvement being a refinement of cytological classification to »Zagreb 2002« that was adapted from »Zagreb 1990« and the »National Cancer Institute Bethesda System 2001«[6].
- a well developed network of quality controlled gynaecologic cytology laboratories with extensive expertise in gynaecologic cytology
- a nationwide network of gynaecologists that currently offer screening to women,
- well established procedures for the diagnosis and treatment of women with abnormal smears[7],
- a tradition of expert colposcopy that has been in place since 1992,
- a network of laboratories with extensive experience in HPV testing for diagnostic purposes that has been in place since 1995, and
- the computerisation of the health system that is now in the process of being implemented.

However, opportunistic screening in Croatia does not include the entire target population and this is reflected in the mortality rate that has shown only a small decrease between 1970 and 2002 from 6/100,000 to 5/100,000 women-years[1] and the goal must be to reach the very low rate of 2–3/100,000 women-year that has been achieved by the Finnish screening programme[8]. As such, there is now an urgent need for the implementation of a properly organised, population-based screening programme that would effectively combine all the new technologies in a rational approach that would maximise the cost-effectiveness and equitably serve all the women of Croatia. Very importantly, this programme would also have to carefully consider the appropriate management of women who have been vaccinated against HPV and who would have a different risk profile from women who had not been vaccinated[9].

HPV Infections and Cervical Cancer

Persistent infection with HPV has now been confirmed as a necessary, although not sufficient cause of cervical cancer[10,11]. There are more than 130 well characterised HPV types, with approximately 40 types that can infect the genital mucosa. These have been classified into high-risk (hr) and low-risk (lr) HPV genotypes according to their association with cervical cancer and its precursor lesions; HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 were classified as high-risk or carcinogenic types, HPV 26, 53 and 66 as probably carcinogenic, while HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and CP6108 were classified as low-risk types[12].

The genital HPV types are primarily transmitted by sexual contact and all women who have had a sexual relationship are at risk for both HPV infection and cervical cancer. Host factors as well as behavioural and environmental factors, may facilitate cancer development in women with a persistent HPV infection and the risk of cervical cancer increases with immunodeficiency, higher parity, tobacco smoking, co-infection with other sexually transmitted agents (human immunodeficiency virus [HIV], herpes simplex virus 2 [HSV 2], *Chlamydia trachomatis* and *Neisseria gonorrhoeae*) and long-term (>5 years) use of oral contraceptives[10]. However, the association between hrHPVs and cervical cancer is the strongest ever found for a human cancer with a relative risks for the development of cervical cancer ranging from 50–500 depending on the HPV genotypes, single or multiple HPV infections and the amount of virus (virus load)[10,11]. Also, cervical cancer will not occur in the absence of a persistent infection with one of the oncogenic HPV types[13].

Worldwide, HPV 16 is the most common hrHPV type as it is found in 60% of cervical cancer cases, while HPV type 18 is found in about 10%, types 45 and 31 in 4% each, and types 33, 52 and 58 each in another 2%[10]. HPV 16 and 18 are also associated with about 25% of the low grade cervical lesions (LSIL) and 50 to 60% of the high grade cervical lesions (HSIL). HPV 6 and 11 are responsible for about 10% of LSIL and about 90% of genital warts that do not represent additional risk for cancer developement[10].

HPV infection is very common in young women, but most infections are transient and resolve spontaneously in 6 to 24 months. Only a very small percentage of HPV infections will lead to precursor cervical lesions and only those that persist long-term pose a risk for the development of cancer. Cervical precursor lesions persist longer and progress more quickly in women with hrHPV infections than in women with lrHPV infections or those without HPV. Approximately 60% or more of cases of mild dysplasia resolve spontaneously and only about 10% progress to moderate or severe dysplasia within 2 to 4 years. In some cases, moderate or severe dysplasia may

occur without an earlier detectable mild dysplasia stage. Less than 50% of cases of severe dysplasia progress to invasive carcinoma, with much lower rates seen in younger women. Usually it takes 10 to 20 year for precursor lesions caused by hrHPV to progress to carcinoma and this is what makes cervical cancer a relatively easily preventable disease and provides the rationale for screening[10].

Pap Smear and HPV Testing

Cervical cancer is rare before the age of 30 years. Screening younger women detects many lesions that will regress spontaneously and leads to considerable overtreatment. Also, screening by Pap smear every three years is nearly as effective as yearly screening. According to the WHO recommendation if the resources are limited, screening every 5–10 years – or even just once between the ages of 35 and 45 years – will significantly reduce deaths from cervical cancer[11]. On the other hand, negative HPV test virtually excludes any risk of having significant prevalent cervical disease and provides the same degree of protection over 5 years that the accepted standard of a negative Pap smear provides over 2 years. Therefore, it is likely that HPV testing could also provide substantial cost savings for most European countries by reducing the screening frequency with no increase in risk for the women being screened[14]. However, HPV testing-based screening should not begin before 30 years of age.

Testing for HPV could be a useful cervical cancer screening tool and its use has been proposed for primary screening, triage of equivocal Pap smears and for the follow-up of patients after treatment for severe cervical lesions. Women who test positive are at high risk of developing cervical precursor lesions and cancer and they should be referred to more extensive diagnostic procedures. About 15–30% of women with normal cytology who are hrHPV positive will develop high-grade precancerous lesions, cervical intraepithelial neoplasia (CIN) grade 2 or 3 within 4 years of detecting the HPV infection[15]. In contrast, women who test negative or are lrHPV positive have almost no risk of developing cervical precancer or cancer and it is thus justifiable to offer such women less frequent screening.

HPV testing has been extensively evaluated and major international reviews have concluded that it 1) is more efficient than repeated cytology in the triage of ambiguous cytological lesions, 2) is at least as efficient as cytology as a primary screening test, and 3) is more efficient than cytology as a test of follow-up for recurrence after treatment of severe cervical lesions[10]. HPV testing as primary screening, at this time, is recommended for use only in pilot projects or other closely monitored settings. Several large-scale randomised controlled trials for evaluation of HPV testing for primary screening are at the moment conducted in Europe[9].

A comparative analysis of different studies on HPV testing for primary screening showed that HPV testing was substantially more sensitive in detecting CIN2+ than cytology (96.1% vs. 53.0%) but slightly less specific (90.7% vs. 96.3%). The sensitivity of HPV testing was uniformly high at all ages, whereas the sensitivity of cytology was substantially better in women over the age of 50 than in younger women (79.3% vs. 59.6%), while the specificity of both tests increased with age. These results support the use of HPV testing as the sole primary screening test, with cytology reserved for women who test HPV positive[16].

A good screening test should be accurate, reproducible, inexpensive, easy to perform and easy to follow-up, acceptable and safe. HPV testing meets all of these criteria, except for its high price, and this would probably decrease if the test is used on large-scale. At present, there is only one HPV test approved by the United States Food and Drug Administration (US FDA) and European Agency for the Evaluation of Medical Products (EMEA), the Hybrid Capture 2 test (HC2; Digene Co.), which uses a cocktail of 13 hrHPV types that are included within the 15 hrHPV types noted above.

Many other tests, commercial or in-house are polymerase chain reaction (PCR) based HPV tests, which uses general (consensus) primers that recognise most hr- and lrHPV types. The analysis of the PCR amplicon generated by consensus PCR by different methods (hybridization with specific probes, restriction fragment length polymorphism analysis, and sequencing) enables determination of HPV types as well as direct type-specific primer directed PCR. These methods of HPV genotyping, while sensitive and specific are too costly and cumbersome to incorporate into large-scale screening programmes. In the future, clinicians might benefit from knowing the number and the identification of the specific types present in order to follow for persistent infections and/or to test for cure after therapy, and also to monitor vaccinated women.

HPV Testing in Croatia

Laboratory network

In Croatia, several laboratories for molecular diagnostics offer detection and genotyping of HPVs[17–20]. These laboratories are located in Zagreb, Split, Rijeka and Osijek, and belong to public or to private health care system; only one laboratory is based in research setting. All laboratories are equipped with special clean room to avoid PCR amplicon contamination, specified equipment and reagents required for a specific test. The work is performed by highly trained technicians and supervised by medical doctors, molecular biologists or medical biochemists.

HPV testing methods

At present, there are several HPV testing systems that are used in the established laboratory services in Croatia. The first step in HPV testing is to collect an adequate sample for HPV-DNA determination. HPV testing can be performed using the same specimen collection me-

dium used for cytological examination (Thin-Prep), or dedicated collection medium specified by the manufacturer (Digene, Roche, and other). It should be noted that sampling error and processing of the collected sample could play an important role when using highly sensitive molecular assays.

PCR was traditionally the first implemented method for clinical use and is still used widely in most Croatian laboratories[17]. HC2 method was the first commercial method used in clinical laboratories but now, several other commercial assays (PCR-based methods) are also used: AMPLICOR HPV Test (Roche Co.) and TaKaRa PCR Human Papillomavirus Typing Set (TAKARA Mirus Bio Inc.). In addition, several laboratories offer in-house consensus and type- specific PCR, HPV detection and HPV genotyping by line blot assays (LiPA, Innogenetics; LA HPV genotyping; Roche). Sequencing of PCR amplicon is used in only research setting.

Standardization and quality assessment

Standardization, quality assessment and quality control are important issues in any routine diagnostic testing. They all serve to establish, maintain and guarantee a high level of quality in the performance of a laboratory to provide correct diagnoses that has a major influence on the management of disease. In the past, substantial efforts have been made by public and private organizations to established and assure a high quality of diagnostic procedures. Standardization in protocols and methods, together with regular participation in internal and external quality testing is essential for molecular diagnostic laboratories[21,22]. The challenge for a laboratory that wants to introduce this kind of testing is that it is very demanding because of two major reasons: first, the molecular diagnostic tests are technically demanding and require more expertise from the user compared to conventional tests and secondly, because of the extreme sensitivity of PCR and similar tests that can produce false positive results created by contamination[23]. Scientific, commercial or public institutions should provide panels with negative and positive control specimens which will be analysed on regular basis in diagnostic laboratories participating in a quality control scheme. Data processing and statistical analyses should be done by independent institutions that are responsible for supervising and licensing procedures.

The HPV test should be performed in clinical laboratories with a supervision of specialist of microbiology, cytology, pathology or a molecular biologist. Only tests that have been validated and standardized tests for use in clinical practice should be used and the laboratories should have a validated license to perform the specific test. Other, not clinically validated, HPV tests should be done only for research purposes[24]. Interpretation of the HPV test result should be done according to the standard »cut-off« point recommended by the test manufacturer.

The referral order for HPV testing should include, besides the general identification data, the date the sample was taken, the date of birth, the stage of the menstrual cycle, previous Pap smear findings and, if present, previous colposcopy, treatment and histological results.

The report of HPV test result should be composed of: 1) general patient data (name, date of birth), 2) referral diagnosis (from the referral order: Pap test result, other relevant colposcopic or histological results, 3) date of sample taking, 4) referral physician and medical institution, 5) method of HPV testing, 6) interpretation of HPV test result, and 7) optionally, recommendation of future procedures or follow-up. However, the recommendation should be given only by experts in the field of cervical lesions (gynecologic cytologists, pathologists and gynecologists) considering all clinical, cytological and pathological data available about a particular patient, respecting the accepted algorithms of follow-up or treatment of abnormal cytology and histology[7].

Conclusion

The higher sensitivity of HPV testing over cytology offers a number of advantages, including, most importantly, the potential of reducing cervical cancer rates while reducing the number of screens in a lifetime necessary to achieve this goal. In Croatia, in addition to screening based on well established cytology[2], HPV testing is widely used as secondary test as a triage to borderline cytology and as a follow-up after treatment of severe cervical lesions[7]. At present, screening is done only with the conventional Pap test, and HPV testing therefore requires an additional visit to gynaecologist which wastes both time and money. Moreover, many different approaches for HPV testing arose in Croatia over the last decade depending on the needs of each medical institution involved. Consequently, the implementation of HPV testing in Croatia is still very heterogeneous and uncontrolled. So, there is an urgent need for better networking between the laboratories, the implementation of quality assessment and the adaptation of a uniform system of referring to and reporting of HPV testing. In conclusion, the best possible organisation for HPV testing would be essential for implementation of HPV testing as primary screening test in Croatia, an thus ultimately and hopefully, the more successful cervical cancer control.

REFERENCES

1. CROATIAN NATIONAL CANCER REGISTRY, Cancer Incidence in Croatia, Bulletin No 24–28. (Croatian National Institute of Public Health, Zagreb, 2001–2005). — 2. PAJTLER M, AUDY-JURKOVIĆ S, KARDUM-SKELIN I, MAHOVLIĆ V, MOZETIĆ-VRDOLJAK D, OVANIN-RAKIĆ A, Coll Antropol 31 (2007) Suppl 2 (in press). — 3. OFFICIAL JOURNAL OF THE EUROPEAN UNION. Council Recommendation of 2 December 2003 on cancer screening (2003/878/EC). — 4. BOYLE P, AUTIER P, BARTELINK H, BASELGA J, BOFFETTA P, BURN J, BURNS HJ, CHRISTENSEN L, DENIS L, DICATO M, DIEHL V, DOLL R, FRANCESCHI S, GILLIS CR, GRAY N, GRICIUTE L, HACKSHAW

A, KASLER M, KOGEVINAS M, KVINNSLAND S, LA VECCHIA C, LEVI F, MCVIE JG, MAISONNEUVE P, MARTIN-MORENO JM, BISHOP JN, OLEARI F, PERRIN P, QUINN M, RICHARDS M, RINGBORG U, SCULLY C, SIRACKA E, STORM H, TUBIANA M, TURSZ T, VERONESI U, WALD N, WEBER W, ZARIDZE DG, ZATONSKI W, ZUR HAUSEN H. European Code Against Cancer and scientific justification: third version, Ann Oncol, 14 (2003) 973. — 5. ZNAOR A, BABIC D, CORUŠIĆ A, GRCE M, MAHOVLIĆ V, PAJTLER M, ŠERMAN A, Liječnički Vjesnik, 2007 [in Croatian] (in press). — 6. OVANIN-RAKIĆ A, PAJTLER M, STANKOVIĆ T, AUDY-JURKOVIĆ S. Gynecol Perinatol 12 (2003) 148. [In Croatian]. — 7. LJUBOJEVIC N, BABIC S, AUDY-JURKOVIC S, OVANIN-RAKIC A, JUKIC S, BABIC D, GRUBISIC G, RADAKOVIC B, LJUBOJEVIC-GRGEC D. Coll Antropol, 25 (2001) 467. — 8. FERLAY J, BRAY F, PISANI P, PARKIN DM (Eds), GLOBOCAN 2002, Cancer Incidence, Mortality and Prevalence Worldwide (IARC Cancer Base No. 5, version 2.0., IARC Press, Lyon 2004). — 9. DAVIES P, BOGDANOVIC GUILLION A, GRCE M, SANCHO-GARNIER H, Coll Antropol 31 (2007) Suppl 2 (in press) — 10. INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC). IARC Handbook of Cancer Prevention. Cervical Cancer Screening, Vol.10, (IARC Press, Lyon, France, 2005). — 11. WHO, Comprehensive Cervical Cancer Control: a guide to essential practice, WHO Library Cataloguing-in-Publication Data, 2006. — 12. MUNOZ N, BOSCH FX, DE SANJOSE S, HERRERO R, CASTELLSAGUE X, SHAH KV, SNIJDERS PJF, MEIJER CJLM, N Engl J Med, 384 (2003) 518. — 13. WALBOOMERS JMM, JACOBS MV, MANOS MM, BOSCH FX, KUMMER JA, SHAH KV, SNIJDERS PJF, PETO J, MEIJER CJLM, MUNOZ N, J Pathol, 189 (1999) 12. — 14. SCHLECHT NF, PLATT RW, DUARTE-FRANCO E, COSTA MC, SOBRINHO JP, PRADO JC, FERENCZY A, ROHAN TE, VILLA LL, FRANCO EL, J Natl Cancer Inst, 95 (2003) 1336. — 15. GOLDIE SJ, KIM JJ, WRIGHT TC, Obstet Gynecol, 103 (2004) 619. — 16. CUZICK J, CLAVEL C, PETRY KU, MEIJER CJ, HOYER H, RATNAM S, SZAREWSKI A, BIREMBAUT P, KULASINGAM S, SASIENI P, IFTNER T, Int J Cancer, 119 (2006) 1095. — 17. GRCE M, HUSNJAK K, MAGDIĆ L, ILIJAŠ M, ZLAČKI M, LEPUŠIĆ D, LUKAČ J, HODEK B, GRIZELJ V, KURJAK A, KUSIĆ Z, PAVELIĆ K. Eur J Epidemiol 13 (1997) 645. — 18. GRCE M, HUSNJAK K, BOZIKOV J, MAGDIC L, ZLACKI M, LUKAC, J, FISTONIC I, SIKANIC-DUGIC N, PAVELIC K, Anticancer Res, 21 (2001) 579. — 19. VINCE A, IVANICEVIC M, HARNI V, SKALKO D, JEREN T, J Clin Virol, 20 (2001) 91. — 20. HADZISEJDIC I, SIMAT M, BOSAK A, KRASEVIC M, GRAHOVAC B, Coll Antropol, 30(4) (2006) 315. — 21. ROSENSTRAUS M, WANG Z, CHANG S-Y, De BONVILLE D, SPADORO J-P, J Clin Microbiol, 36 (1998) 191. — 22. BURKARDT HJ, Clin Chem Lab Med, 38(2) (2000) 87. — 23. PERSING DH, J Clin Microbiol, 29 (1990) 1281. — 24. COX T, CUZIC J, Gynecol Oncol 103 (2006) 8.

M. Grce

Ruđer Bošković Institute, Division of Molecular Medicine, Bijenička 54, HR-10002 Zagreb, Croatia
e-mail: grce@irb.hr

HPV TESTIRANJE U PROBIRU RAKA VRATA MATERNICE U HRVATSKOJ – DANAS I SUTRA

S A Ž E T A K

Oportunistički probir temeljen na citološkom Papa-testu provodi se u Hrvatskoj od 1953. godine. Unatoč tome, u usporedbi s Europskim državama s organiziranim programima probira, rak vrata maternice u Hrvatskoj još uvijek predstavlja značajni zdravstveni problem. U Hrvatskoj, uz probir temeljen na priznatoj i dobro uhodanoj citologiji, HPV testiranje sve se više koristi kao sekundarni test razvrstavanja nakon graničnog citološkog nalaza te kao kontrolni test nakon liječenja težih promjena vrata maternice. Tijekom posljednjih deset godina u Hrvatskoj se pojavljuju brojni različiti pristupi HPV testiranju, ovisno o potrebama i stavovima pojedinih zdravstvenih ustanova koje ga primjenjuju. Stoga se danas javlja neophodna potreba za boljom povezanošću laboratorija, uspostavljanjem sustava kontrole kvalitete i zauzimanjem ujednačenog stava o indikacijama za HPV testiranje i načinu izdavanja nalaza HPV testa. U zaključku smatramo da će najbolje moguće uređen sustav HPV testiranja biti preduvjet za uvođenje HPV testa u primarni probir u Hrvatskoj, kao i uspješniju borbu za suzbijanje raka vrata maternice.