Medical Journal Of Montenegro
DOI: 10.5937/cma1.4672

The importance of HPV testing in cervical cancer prevention

Vuksanovic-Božaric Aleksandra[1], Jovanović Milovan[2], Stevović Željka[2], Abramović, Marija[1], Radunovic, Miroslav[1]

1 Medical Faculty, University of Montenegro, Podgorica, Montenegro
2 Primary health care for women, Podgorica, Montenegro

CORRESPONDING:
Abramovic Marija
Medical Faculty, University of Montenegro
Ljubljanska bb Crna Gora, 20000 Podgorica
Tel +382 68 407 540
marija.abramovic86@live.com

Abstract

Introduction: Cervical cancer is second most common malignant tumor of female reproductive system in the world. HPV infection is present in 99% of cases, therefore very important in its development.

Material and method: Our research was conducted in Primary health care center for women in Podgorica and included 100 women aged 20-60. During the examination PAP testing and colposcopy were performed to every participant. HPV mRNA detection test with differentiation of types 16, 18, 31, 35, 55 was done in cases of abnormal PAP test result, or suspected clinical or colposcopic examination accompanied by normal PAP test.

Results: PAP testing showed II group in 72 patients, IIIa in 19 and IIIb in 3. HPV testing was performed in 26 patients (in which 4 with PAP II result). It was positive for presence of high-oncogenic types in 15 patients (57,69%) and negative in 11 (42,31%). In patients with detected lesion by examination or colposcopy, we performed biopsy. Results showed CIN I in 1 patient, CIN II in 2, CIN III in 5 and invasive carcinoma in 1 patient.

Conclusion: Our research showed great importance of HPV testing in patients with false negative PAP tests; its importance as diagnostic marker in prognosis of illness, as well as the fact that HPV testing, as basic one, without examination and PAP testing, doesn't make sense as it would increase the number of false negative results.

Key words: Papillomavirus; Papillomavirus Infections; DNA Virus Infections; Uterine Cervical Neoplasms

Introduction

Cervical cancer is a global health care issue, especially in developing countries. It is considered to be a preventable illness, cause of its long pre-invasive period, possibility of conducting screening tests, and the most import cause of successful treatment of early stages of this disease. (1-4)

Cancer begins its development in the transformation zone, in which the process of metaplasia is continuous. The highest risk of HPV infection is in age period of 18 to 30, in which the metaplasia process is the most active. After that period, the risk is reducing.

The average age in which the invasive stage of cervical cancer is diagnosed is 48 to 52 years of age, and for in-situ carcinoma it is 35. This high age difference is considered to because of long latency in which the cancer progresses from intraepithelial lesion to invasive stage.

After introducing the PaP test as a screening method, just in USA, the cervical cancer incidence has been reduced to 12710 new cases in 2010, and mortality has dropped to 4290. Never the less, the test has its limitations. Studies conducted in recent years have shown that the test sensitivity ranges from 44% to 65% for CIN II or worse for women aged 30 and more. (5) Also, even though the introduction of this test has led to mortality reduction from cervical carcinoma, which accounts for 80 to 90% of all cervical cancers, it has been shown that the test is not efficient enough in adenocarcinoma prevention.(5,6)

For the last 20 years it is known that more than 97 to 99% of cervical cancer is in correlation with HPV infection. (5,7-9) The HPV type 16 shows the highest carcinogenic potential and is present in 55-60% cases of cervical cancer, while the second highest carcinogenic potential has the HPV 18, which is present in approximately 10 to 15% of cases. (10-13) Also, 68% cases of squamous cell carcinoma and 85% of adenocarcinoma are caused by HPV 16 and 18 infections. The rest of 17,8% of squamous cell carcinoma cases develop in presence of HPV 31, 33, 45, 52 and 58, while these types are also present in 82% of all HSIL (high grade squamous intraepithelial lesion). (14)

It has been proved that HPV testing has higher sensitivity but lower specificity for diagnosing CIN III and worse, as well as CIN II and worse, comparing to PaP testing. (15-21)

Most of HPV infection, almost 90%, is transient and cannot be detected after 1-2 years. (22,23) Also, the high number of CIN I and CIN II lesion are transient as well and will not develop into CIN III or cancer. (24-30)

Considering recently published studies and meta analyses, the American Cancer society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology, published screening guidelines for cervical cancer prevention and

early detection, in which HPV testing is included, in form of co-testing alongside cytology, for women aged 30 to 65 years, every five years. For women aged 21 to 30, the only screening test recommended is cytology alone, every three years. (10)

Because of HPV infection relevance in cervical cancer and its early stages development, as well as its high epidemiological significance in Montenegro, by conducting a prospective study, we present the importance of HPV testing as a co-test in particular cases for more efficient screening in invasive cervical cancer prevention.

Material and method

The research has been conducted in Primary health care center for women in Podgorica. Study included 100 women aged 20 to 60. The exclusion factors were pregnancy and previous visit to gynecologist in past two years which included the clinical examination. Patients were divided, according to their age, in for groups: 20 to 30, 31 to 40, 41 to 50 and 51 to 60 years of age. Every group included 25 women.

In every patient, during the preventive examination, cervical swab for cytology analysis was taken according to standards. Also, alongside the PaP testing, colposcopy was performed in every participant. In cases of abnormal PaP test result (Pap IIIa and worse), HPV testing was done, using the mRNA detection method, with differentiation of 16, 18, 31, 35 and 55 types. In cases of other PaP test results (PaP I and PaP II), HPV testing for performed if clinical examination of colposcopy have shown suspected changes.

After the HPV testing, women with positive results for high carcinogenic potential HPV, as well as those with suspected clinical and colposcopic results, underwent biopsy and the histopathology analysis, regardless the PaP testing results.

The aim of study

To present the importance of HPV testing in patients with suspected clinical and colposcopic results, and normal PaP testing results, in early detection of cervical cancer

To present the significance of HPV testing in cases of abnormal PaP testing results, as the prognostic marker for disease development.

Results

From 100 tested women, PaP testing showed normal result – PaP II – in 72 women. In 19 cases result was PaP IIIa, in 3 it was PaP IIIb, while in 6 cases PaP smear wasn't eligible for analysis, which excluded these women from further testing. (Tables No 1 and No 2)

PaP testing results	PaP II	PaP III A	PaP III B	PaP IV	Failed PaP smears	Total
Number of cases	72	19	3	0	6	100

Table No 1. Results of PaP testing

Age of participants	20-30	31-40	41-50	51-60	Total
PaP II	18	14	17	23	72
PaP III A	5	8	5	1	19
PaP III B	1	1	1	0	3
PaP IV	0	0	0	0	0
Failed PaP smears	1	2	2	1	6
Total	25	25	25	25	100

Table No 2. Results of PaP testing in relation to age groups of participants

Out of 72 participants with normal PaP testing result (PaP II), clinical and colposcopic examination, in 4 women (5,55%) have shown suspected changes on cervix. In all of these patients HPV testing was performed. In one of these four testing showed presence of HPV 18. Patient was 22 years old. Afterwards, histopathology analysis showed CIN I (LSIL). This patient had no children. In other three women, testing reviled the presence of HPV 16, which also led to histopathology testing and it showed CIN III (HSIL) in all of them. Patients were in 20 to 30 years group. Results are shown in table No 3.

Results of HPV testing	HPV16	HPV18	Total
Number of participants	3 (75%)	1 (25%)	4 (100%)

Results of histopathology analyses	CIN III	CIN I	Total
Number of participants	3 (75%)	1 (25%)	4 (100%)

Results HPV / Results HP	HPV16	HPV18	Total
	0	1	1
CIN III	3	0	3
Total	3	1	4

Table No 3. Results of HPV testing and histopathology analyses in patients with normal PaP testing, and their relation

Special case was the patient, from age group 20 to 30, in who PaP testing showed PaP II. During our research, this patient got pregnant, and during her pregnancy, in the first trimester, in routine checkup, clinical examination was done, and it revealed the presence of suspected change on cervix. PaP test was repeated. Pregnancy was 11 gestation week old, and time difference from the previous PaP testing was 3 months. The new result was IIIb, and biopsy showed CIN III. HPV testing wasn't performed. She was excluded from result interpretation because of pregnancy.

Nineteen participants with PaP results IIIa underwent microbiological testing in Institute for public health, and antibiotic treatment according to results. After the treatment, repeated test in 12 women showed normal results; in eight of them colposcopy indicated presence of unspecific cervicitis and in four the colposcopy and clinical examination revealed normal cervix surface, whilst in seven patients repeated PaP was IIIa. All nineteen women were tested for HPV. Eleven (57,89%) of them were negative for presence of HPV. It was positive in 8 women, in six of them for HPV 16 (31,58%) and in two for HPV 18 (10,53%). Presence of HPV 31 and 35 wasn't revealed in any patient.

After biopsy was performed results showed one case of invasive stage of cervical carcinoma in patient that was positive for presence of HPV 16, 27 years old and had no children. One HPV 16 positive woman had CIN II, and two women (HPV 16 and HPV 18 positive) had chronic cervicitis. Three women didn't undergo biopsy because of normal clinical examination result.

Three patients with PaP IIIb were tested, and in all of them presence of HPV 16 was proved. Results of biopsy in two of them was CIN III and in one CIN II. (Table No 4)

Results of PaP testing / Results of HPV testing	PaP II	PaP IIIA	PaP IIIB	Total
Positive HPV16	3	6	3	12
Positive HPV18	1	2	0	3
Negative	0	11	0	11
Total	4	19	3	26

Table No 4. Results of HPV testing and its relation to PaP testing results

Total 26 participants out of 100 underwent HPV testing. Test was positive for presence of high carcinogenic types in 15 (57,69%), while in 11 (42,31%) was negative. Biopsy was performed in patients with clinical and colpscopic suspected lesions, and in HPV positive ones. Results showed CIN I in one, CIN II in two, CIN III in five patients, while invasive stage of cervical carcinoma was discovered in one patient. Table No 5.

Results of HPV testing / Results of biopsies	HPV16 positive	HPV18 positive
CIN I	0	1
CIN II	2	0
CIN III	5	0
CA invasivum	1	0
Cervicitis chronica active	1	1
Patients that didn't undergo biopsy because of normal clinical examination result	2	1
Total	12	3

Table No 5. Results of HPV testing in relation to biopsy results

Discussion

PaP testing has low sensitivity ranges 50-70% for detection of HSIL; also 10% of PaP smears classified as LSIL or ASCUS were actually LSIL.(31-33) By use of colposcopy almost all HSIL are detectable, but it has limited specificity in patient with low grade lesions. (34-36) In study performed by Adamopolou et al., it was shown that the best combination for screening is use of colposcopy and HPV testing, which had sensitivity of 97,2% and specificity of 80,8%, while cytology showed to be less efficient in detection of patients under risk in those with ASCUS or LSIL. (9)

Despite the high cervical cancer mortality rate drop since the introduction of PaP testing, considering the above mentioned results of recently conducted studies, as well as the fact that interpretation of PaP smears is highly subjective, all around the world new researches are conducted with an aim of finding the new screening test or combination of test, which would have better combination of sensitivity and specificity, as well as reasonable price.

HPV testing reveals high carcinogenic potential HPV which are related to development of cervical cancer, which is significant in false negative PaP tests, as well as in cases of low grade lesions that are accompanied by presence of HPV infection.

In comparison to cytology, HPV testing has proven to be more sensitive but less specific in identifying CIN III and worse. (16,19,37,38) According to review of several meta-analyses, sensitivity of HPV testing for CIN III and worse was 37% higher while specificity was 7% lower than cytology. (39)

In a review done by Cuzick et al., it was published that HPV testing has higher sensitivity for lesions CIN II I CIN III, in average of 96,1%, regardless of patients age. Specificity was a bit lower, especially for women younger than 35 years, which can be explained by the fact that HPV infection in younger women are more often and in the same time mostly transient, and therefore will not lead to malignant lesions. Average specificity in certain studies was 90,7%, and in older than 35 it was slightly higher 93,3%. On the other hand, average sensitivity of cytology for detection of CIN II and worse was 53% with major variations between reviewed studies, and it was a bit higher for women aged over 50 – 79,3%. Average specificity was 96,3%. In combination, these tests had higher specificity, up to 95,9% for younger and 97,1% for older than 35 years. (15)

Cox et al. have been investigating 10 different screening strategies. As the most sensitive one turned out to be HPV testing with colposcopy of positive patients, without cytology testing, but it also had the most false positive CIN III and worse. As for the efficiency, according to relation of number of colposcopy and biopsy results of CIN II and worse, this strategy has shown to be as efficient as combination of cytology and colposcopy, in which colposcopy was performed on those with ASCUS result or worse.

All strategies that included cytology, either as only test, either as base line test with HPV as additional test in ASCUS or worse cases, either as co-testing, had the lowest sensitivity for detection of CIN II or worse. Of all, the most specific strategy was the one with cytology as base line testing and HPV testing as additional. All strategies that were based on co-testing demanded twice more colposcopies than others. To determine which one was the most acceptable, their guidelines were sensitivity for CIN III and worse, and number of colposcopy as a very uncomfortable procedure for women. Their conclusion were strategies that included co-testing cytology and HPV with genotyping, and HPV testing with genotyping followed by cytology as a triage test for eventual colposcopy. (40)

Similar results were published in Finland (41), and by Naucler et al (20). Never the less, they didn't consider economic aspect of HPV testing that much. On the opposite, number of cost/benefit studies as well as meta-analyses suggest cytology as base line test and HPV as additional. (39,42-44)

Four major studies that analyzed and compared results obtained by cytology as only base line test and co-testing, came to conclusion that co-testing didn't lead to discovery of higher number of CIN III and worse after the second round of testing in relation to HPV testing as the only test. On the other side, co-testing identified less CIN III and worse after the second round in relation to cytology alone, which would indicate the reduction of cases with more severe lesions in the ones tested in round one. (21,22, 45, 46)

Also, few European studies revealed that co-testing in comparison with cytology as an only test discovers more CIN III and worse.(16, 20, 21)

Based on the results of many published studies, many authors believe that HPV testing should take the place of cytology as base

line test for screening, with which we disagree. Our research has shown that HPV testing would increase the number of false positive results as well as number of invasive diagnostic procedures, anxiety and discomfort of patients, as well as costs for society, concidering the price of HPV testing. We still believe that for developing countries, such as our, PaP test in combination with clinical examination and colposcopy is more cost effective.

Some studies have shown that combination of HPV and PaP testing can determine only 5% more advanced lesions, and 35% more false positive results. (10)

In study performed by Ferris et al., results showed da sensitivity of PaP testing doesn't increase by adding HPV as co-testing in a second round, if the base line result for colposcopy referral is ASCUS.

Several studies indicated that women with normal PaP test results and were positive for presence of HPV 16 and 18, were in higher risk for developing pre-malignant lesion. They consider that those women are in 10% more of a risk to develop more advanced lesions, and in such cases colposcopy referral is justified. Unless genotyping is available, colposcopy is indicated only in persistence of positive HPV test result during 12 months. (5,10)

HPV testing, considering cost/benefit ratio and many transient infections in more than 70% younger women for 1 to 3 years period, as a co-testing with cytology, is for now justified around the world for women older than 30 years, or for those with abnormal clinical examination, PaP smear or colposcopy. It still doesn't have the use as the only base line test for screening. (10)

Our research has demonstrated that when proven the presence of HPV 16 or18, situation is actually more delicate as it may seem as when only cytology is used. Also, we think that PaP test showed remain as the only base line for women all age, and that in case of abnormal results of PaP smear or clinical examination, colposcopy should be performed, and only after that, if indicated, HPV testing. It is also justified to use HPV results as prognostic marker in disease development n women of all age. Differentiation of high carcinogenic potential HPV is better diagnostic criteria for monitoring the development of pre-malignant lesion and post-operative treatments, which also affects the individualization of treatment protocols and improvement of prevention and treatment of cervical cancer.

Conclusions

HPV testing with clinical and colposcopic examination with false negative PaP test results, as diagnostic criteria is very significant

Detection of high carcinogenic potential HPV in cases of normal PaP smears is prognostic criteria of lesion progression from low to high grade

With abnormal PaP test results, HPV testing could be diagnostic criteria in planning further diagnostic and therapeutic procedures

HPV without clinical examination or PaP testing makes no sense, since it increases the number of false positives results

References

1. Antonishyn NA, Horsman GB, Kelln RA, Severini A.Human papillomavirus typing and viral gene expression analysis for the triage of women with abnormal results from Papanicolaou test smears to colposcopy. Arch Pathol Lab Med. 2009; 133(10): 1577-86
2. Subramanya D, Grivas PD.HPV and cervical cancer: updates on an established relationship. Postgrad Med. 2008; 120(4): 7-13
3. Silverloo I, Andrae B, Wilander E.Value of high-risk HPV-DNA testing in the triage of ASCUS. Acta Obstet Gynecol Scand. 2009; 88(9): 1006-10
4. Bhatla N, Moda N. The clinical utility of HPV DNA testing in cervical cancer screening strategies. Indian J Med Res. 2009; 130(3):261-5.
5. Chelmow D, Waxman A, Cain JM, Lawrence HC III. The evolution of cervical screening and the specialty of obstetrics and gynecology. Obstet Gynecol 2012; 119: 695-9.
6. Berrington de Gonzalez A, Green J, International Collaboration of Epidemiological Studies of Cervical Cancer. Comparison of risk factors for invasive squamous cell carcinoma and adenocarcinoma of the cervix: collaborative reanalysis of individual data on 8,097 women with squamous cell carcinoma and 1,374 women with adenocarcinoma from 12 epidemiological studies. Int J Cancer 2007; 120: 885–91
7. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJ, Meijer CJ, International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003; 348: 518–27.
8. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Munoz N. Human Papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999; 189: 12–9.
9. Adamopolou M, Kalkani E, Charvalos E, Avgoustidis D, Haidopoulos D, Yapijakis C. Comparison of citology, colposcopy, HPV typing and biomarker analysis in cervical neoplasia. Anticancer Res. 2009; 29: 3401-3410.
10. Saslow D, Solomon D, Lawson H, Killackey M, Kulasingam S, et al. American Cancer Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology Screening Guidelines for the prevention and early detection of cervical cancer. CA Cancer J Clin 2012; 62: 147-172.
11. Walboomers JM, Jacobs MV, Manos MM et al. human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999; 189: 12-19.
12. Munoz N, Bosch FX, de Sanjose S et al. international agency for research on cancer multicenter cervical cancer study group. Epidemiological classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 2003; 348: 518-527.
13. De Sanjose S, quint WG, Alemany 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: 1048-1056.
14. Bosch FX, Burchell AN, Schiffman M, Giuliano AR, de Sanjose S, Bruni L, Tortolero-Luna G, Kjaer SK, Munoz N. Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. Vaccine. 2008; 26 (Suppl): K1–16.

15. Cuzick J, Clavel C. Petry KU et al. Overview of the Europian and North American studies on HPV testing in primary cervical cancer screening. Int J Cancer 2006; 119: 1095-1101.

16. Ronco G, Giorgi Rossi P, Carozzi F 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 randomized controlled trial. Lancet Oncol 2010; 11: 249-257.

17. Mayrand MH, Duarte-Franco E, Rodrigues I et al. Canadian cervical cancer screening trial study group. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. N ENGL J Med 2007; 357: 1579-1588.

18. Anttila A, Kotaniemi-Talonen L, Leinonen M et al. Rate of cervical cancer, severe intraepithelial neoplasia and adenocarcinoma in situ in primary HPV DNA screening with cytology triage: randomized study within organized screening programme. BMJ. 2010; 340: c1804

19. Castle PE, Stoler MH, Wright TC Jr, Sharma A et al. Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. Lancet Oncol. 2011; 12: 880-890.

20. Naucler P, Ryd W, Tornberg S et al. Human papillomavirus and Papanicolaou test to screen for cervical cancer. N Engl J Med 2007; 357: 1589-1597.

21. Bulkmans NW, Berkof J, Rozendal L, et al. Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomized controlled implementation trial. Lancet 2007; 370: 1764-1772.

22. Plummer M, Schiffman M, Castle PE, Maucort-Boulch D, Wesler CM. ALTS group. A 2-year prospective study of human papillomavirus persistence among women with a cytological diagnosis of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. J infect Dis. 2007; 195: 1582-1589

23. Rodriguez AC, Schiffman M, Herrero R et al. Rapid slearence of human papillomavirus and implications for clinical focus on persistent infections. J Natl Cancer Inst. 2008; 100: 513-517.

24. Castle PE, Schiffman M, Wheeler CM, Solomon D. evidence for frequent regression of cervical intraepithelial neoplasia – grade 2. Obstet Gynecol. 2009; 113: 18-25;

25. Castle PE, Stoler MH, Solomon D, Schiffman M. the relationship of community biopsy-diagnosed cervical intraepithelial neoplasia grade – 2 to the quality control pathology – reviewed diagnoses: an ALTS report. Am J Clin Pathol. 2007; 127: 805-815.

26. Trimble CL, Piantadosi S, Gravitt P et al. Spontaneous regression of high-grade cervical dysplasia: effects of human papillomavirus type and HLA phenotype. Clin Cancer Res. 2005; 11: 4717-4723.

27. Nishino HT, Tambouret RH, Wilbour DC. Testing for human papillomavirus in cervical cancer screening. Cancer cytopathology

28. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet. 2007; 370: 890-907.

29. Stanley M. Immune responses to human papillomavirus. Vaccine. 2006; 24 (suppl 1): S16-S22.

30. Woodman CB, Collins SI, Young LS. The natural history of cervical HPV infection: unresolved issues. Nat Rev Cancer. 2007; 7: 11-22.

31. Kinney WK, Manos MM, Hurley LB and Ransley JE. Where is the high-grade cervical neoplasia? The importance of minimally abnormal Papanicolaou diagnoses. Obstet Gynecol. 1998; *91*: 973-976.

32. Wright TC, Jr, Cox JT, Massad LS, Carlson J, Twiggs LB and Wilkinson EJ. 2001 consensus guidelines for the management of women with cervical intraepithelial neoplasia. Am J Obstet Gynecol 2003; 189: 295-304.

33. Monsonego J, Bosch FX, Coursaget P, Cox JT, Franco E, FrazerI, Sankaranarayanan R, Schiller J, Singer A, Wright TC Jr, Kinney W, Meijer CJ, Linder J, McGougan E and Meijer C. Cervical cancer control, properties and new directions. Int J Cancer 2004; 108: 329-333.

34. Follen Mitchell M, Schottenfeld D, Tortolero-Luna G, Cantor SB and Richards-Korturn R. Colposcopy for the diagnosis of squamous intraepithelial lesions: a meta-analysis. Obstet Gynecol 1998; 91: 626-631.

35. Huntington J, Oliver LM, St Anna L and Hill J: What is the best approach for patient with ASCUS detected on Pap smear? J Fam Pract 2004; 53: 240-241.

36. Hatch KD, Schneider A and Abdel-Nour MW: An evaluation of human papillomavirus testing for intermediate and high-risk types as triage before colposcopy. Am J Obstet Gynecol 1995; 172: 1150-1157.

37. Rijkaart DC, Berkhof J, Rozendaal L, van Kemenade FJ, Bulkmans NW, Heideman DA, Kenter GG, Cuzick J, Snijders PJ, Meijer CJ. Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial.

38. Pajtler M, Milicić-Juhas V, Milojković M, Topolovec Z, Curzik D, Mihaljević I. Assessment of HPV DNA test value in management women with cytological findings of ASC-US, CIN1 and CIN2. Coll Antropol. 2010; 34(1):81-6.

39. Arbyn M, Sasieni P, Meijer CJ, Clavel C, Kollopoulos G, Dillner J. Chapter 9: Clinical aplications of HPV testing: a summary of meta-analyses. Vaccine. 2006; 24 (suppl 3): S3/78-89

40. Cox JT, Castle PE, Behrens CM, et al. Comparison of cervical cancer screening strategies incorporating different combinations of cytology, HPV testing, and genotyping for HPV 16/18: results from the ATHENA HPV study. Am J Obstet Gynecol 2013; 208: 184.e1-11.

41. Leinonen M, Nieminen P, Kotaniemi-Talonen L, et al. Age-specific evaluation of primary human papillomavirus screening vs conventional cytology in a randomized setting. J Natl Cancer Inst 2009; 101: 1612-23.

42. Kim JJ, Wright TC, Goldie SJ. Cost-effectiveness of alternative triage strategies for atypical squamous cells of undetermined significance. JAMA 2002; 287: 2382-90.

43. Kim JJ, Wright TC, Goldie SJ. Cost-effectiveness of human papillomavirus DNA testing in the United Kingdom, The Netherlands, France, and Italy. J Natl Cancer Inst 2005; 97: 888-95.

44. Kulasingam SL, Kim JJ, Lawrence WF, et al. Cost-effectiveness analysis based on the atypical squamous cells of

undetermined significance/low-grade squamous intraepithelial lesion Triage Study (ALTS). J Natl Cancer Inst 2006; 98: 92-100.

45. Ronco G, Segnan N, Giorgi-Rossi P, Zappa M, Casadei GP, Carozzi F, et al. New Technologies for Cervical Cancer Working Group. Human papillomavirus testing and liquid-based cytology: results at recruitment from the new technologies for cervical cancer randomized controlled trial. J Natl Cancer Inst. 2006; 98: 765-74.

46. 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: 672-82.