New insights into cervical cancer screening

Jonathan D. Boone[1], Britt K. Erickson[2], Warner K. Huh[2]

[1]Department of Obstetrics and Gynecology, [2]Division of Gynecologic Oncology, University of Alabama at Birmingham, Birmingham, AL, USA

Worldwide, cervical cancer is a leading cause of cancer related morbidity and mortality. For over 50 years, cervical cytology has been the gold standard for cervical cancer screening. Because of its profound effect on cervical cancer mortality in nations that have adopted screening programs, the Pap smear is widely accepted as the model screening test. Since its introduction, many studies have analyzed the Pap smear and found that it is not without its shortcomings including low sensitivity for detection of cervical intraepithelial neoplasia 2/3. Additionally, the discovery of infection with the human papillomavirus (HPV) as a necessary step in the development of cervical cancer has led to the development of HPV testing as an adjunct to cytology screening. More recently, researchers have compared HPV testing and cytology in the primary screening of cervical cancer. In this review, we will discuss cytologic testing limitations, the role of HPV DNA testing as an alternative screening tool, the impact of the HPV vaccine on screening, and future directions in cervical cancer screening.

Keywords: Co-testing, Cytology, HPV vaccine, Pap smear limitations, Screening

INTRODUCTION

Cervical cancer screening is hailed as one of the major public health advances in the 20th century. In the process of evaluating vaginal smears as an indicator of hormone status, Dr. George Papanicolaou [1] incidentally noticed that malignancy could be detected during cytologic evaluation. He and his colleague, Dr. Herbert Traut, eventually published "The diagnostic value of vaginal smears in carcinoma of the uterus" which was the beginning of an era of the Papanicolaou, or "Pap smear," screening [1].

Despite a drastic decrease in cervical cancer morbidity and mortality in communities that have adopted cytology screening programs, cervical cancer is still the third most commonly diagnosed cancer in women with 529,800 new cases estimated worldwide annually [2]. More recent data have suggested

limitations of the Pap smear including low sensitivity, high false negative rates, and interobserver variability. These limitations have forced many to revisit the utility of cytology as a primary screening test particularly when compared to human papillomavirus (HPV) testing.

THE LIMITATION OF PAP SMEARS

As the first cancer screening test of the modern era, the Pap smear was never initially scrutinized through a standard evidence based approach as many of our modern screening tests are today. However, the epidemiologic data are convincing. In nations that have adopted cytologic screening programs, the incidence and mortality from cervical cancer has declined dramatically [3-5]. Because of its success in cervical cancer prevention, the Pap smear has come to be known as the archetype of screening tests [6]. Although it had a profound effect on cervical cancer morbidity and mortality in an era of highly prevalent cervical disease, cytologic screening has inherent limitations, particularly as the patterns of incidence have changed and the morbidity from overtreatment is now

fully appreciated.

Despite the acceptance of cytologic testing as the primary screening method for cervical cancer, it has shown a high false negative rate (i.e., missed cervical intraepithelial neoplasia [CIN] 2+). Studies have shown that 20% to 40% of new cervical cancer cases are diagnosed in women who have had "proper" screening [7-9]. As data from population based trials have emerged, the Pap smear has shown low sensitivity for the detection of CIN 2+ and even more variable sensitivity depending on a woman's age, highest in the 50 and older age group [10]. A systematic review performed by Nanda et al. [11] to evaluate the diagnostic accuracy of the Pap smear found its sensitivity to be 51% (range, 30% to 87%), and specificity to be 98%, (range, 86% to 100%). In a meta-analysis by Spence et al. [12], the false negative rate of cytologic testing was as high as 35.5% on average. Cytologic testing has also been shown to have a lower specificity for high-grade CIN than low-grade lesions, which can lead to overtreatment [11,13]. Additionally, the sensitivity of cervical smears for adenocarcinoma is lower than for squamous cell carcinoma [14]. In conjunction with this and the rising incidence of adenocarcinoma which accounts for nearly a quarter of all newly diagnosed cervical cancers, cytologic tests will continue to become less useful [15,16].

In addition to its limitations regarding low sensitivity, the Pap smear limitations also include failure to acquire adequate specimens, interobserver bias, and misinterpretations. Inflammation, scant cellularity, and blood contaminating samples have all been cited as reasons for inadequate or unsatisfactory samples. Approximately 1% to 8% of Pap smears have been reported as unsatisfactory [17,18]. In one trial, when inadequate samples were reevaluated, cytologic atypia including high grade lesions and carcinoma was seen in 7% of the samples [18]. Even with satisfactory samples, cytologic interpretation is subject to interobserver variability despite international standards. Woodhouse et al. [19] showed the discrepancy between low and high grade lesions to range from 9% to 15% among different laboratories and their personnel. Furthermore, the diagnosis of atypical squamous cells of undetermined significance (ASC-US) is well-documented as a poorly reproducible category. Even with experienced cytologists and adequate samples, varied interpretations continue to reduce cytologic testing's diagnostic accuracy [20].

The most effective screening tests must achieve a balance between high sensitivity and acceptable specificity. Equally important is identifying a screening interval that is frequent enough to detect lesions before they become invasive while still minimizing cost and morbidity associated with overtreatment. This ensures the low likelihood that an abnormal result,

in this case invasive cervical cancer, will be present before the next screening event. Because of its low sensitivity, cytologic testing alone requires regular exams with diligent follow-up. In a meta-analysis by Spence et al. [12], even when optional screening is available, 65% of women had deficient screening histories and 14% of women had poor follow-up after an abnormal Pap smear. Therefore, a screening test with a high negative predictive value, which safely allows for extension of screening intervals, is of greatest benefit.

THE INFLUENCE OF THE HUMAN PAPILLOMAVIRUS

Infection with the oncogenic HPV is necessary for the development of cervical cancer [21,22]. Dr. Harald zur Hausen [23], a German virologist and physician, first proposed the role of HPV in the development of cervical cancer in the 1970s. By 1984, he had specifically isolated HPV 16 and HPV 18, the two HPV types that today are known to cause approximately 70% of cervical cancer [24]. HPV is now the most commonly diagnosed sexually transmitted infection with prevalence estimated at 43% for females aged 14-59 in the US [25]. Currently, 14 high-risk HPV DNA types have been associated with the development of cervical cancer [26,27].

Young women clear the HPV at a high rate of 40% to 70% in the first year of infection and as high as 70% to 100% two to five years after infection [28-32]. The prevalence of HPV infection along with its subsequent clearance in adolescents is extremely high but the incidence of cervical cancer is negligible at 0.1 per 100,000 [31,33-37]. Additionally, CIN 2 in this same age group will regress 60% of the time within the first three years [38]. Screening adolescents may cause more harm because it increases unnecessary evaluation and treatment [38]. Therefore, regardless of risk factors, screening should begin at age 21.

Low risk HPV clears more quickly than high risk HPV with HPV types 16, 31, 54, and 53 resulting in the longest course of infectivity [29,39]. HPV 16 has been proven to be responsible for most of the persistent infections [13]. These persistent infections have shifted the focus towards high risk HPV DNA testing as the new paradigm for cervical cancer screening.

HPV SCREENING: A NEW HORIZON

In theory, invasive cervical cancer is a preventable disease. Due to the limitations of the Pap smear and an improved understanding of the role of HPV in cervical carcinogenesis, primary prevention has shifted to high-risk HPV (HR-HPV) testing

and HPV vaccination.

The American Society for Colposcopy and Cervical Pathology (ASCCP) has recommended the use of HR-HPV testing in a variety of situations. These include triage for ASC-US Pap smears, initial workup of atypical glandular cell (AGC) Pap smears, "co-testing" with cytology in women over thirty years old, follow-up CIN 1 testing when the preceding Pap was ASC-US, ASC-H, or LSIL, follow-up testing after an excision or ablative procedure is performed for CIN 2/3, and "reflex" testing in postmenopausal women with LSIL cytology. Testing specifically for HPV 16 and 18 is also emerging as an important test for further triage of women greater than 30 years of age who are HR-HPV positive but cytology negative.

Reflex testing (that is, HPV testing when cytology is abnormal) was first studied and found to be a viable option for screening in the ASC-US/LSIL Triage Study (ALTS) trial. The ALTS trial found that testing for HPV after an ASC-US Pap smear was a sensitive and cost effective strategy. HPV testing detected CIN 3 with a sensitivity of 96% and it decreased the number of colposcopies by 50% [40].

In women over thirty, HPV DNA testing combined with cytology, known as "co-testing", was approved for screening in the US in 2006. Combining these tests results improved detection of pre-invasive and invasive lesions. The natural history of HPV infection has shown decreased incidence in women over the age of 30. Therefore, combining cytology with HR-HPV testing in this age group also allows for extended screening intervals if both tests are negative, given its high negative predictive value [41-43].

Numerous studies have shown a benefit of using HPV testing for the primary detection of cervical dysplasia. Much of the benefit of HPV DNA testing is drawn from increased sensitivity with acceptable specificity and high negative predictive values for detecting CIN 2/3 relative to cervical cytology [41,44-48]. Several large international trials have shown that primary HPV testing has better sensitivity alone than cytology, and when combined with cytology triage, the specificity is similar to cytology alone [20,44-46,48,49]. Ronco et al. [41] suggested that HPV DNA testing might be better than cytology in preventing invasive cancer because it detects high-grade lesions earlier. The Population-Based Screening Study Amsterdam (POBASCAM) trial also confirmed this by showing CIN 2+ was detected earlier with HR-HPV testing than with cytology [46].

In addition to its improved sensitivity, HPV testing has other advantages. HPV testing is more objective and reproducible than the other cervical cancer screening tests while also being less demanding in terms of training and quality assurance [45]. It can be automated, centralized, and be quality-checked for large specimen input while avoiding the subjective interpretation associated with cytology [20]. It may also be more cost-effective than cytology if deployed for high volume testing such as in primary screening.

An additional advantage to primary HPV testing is seen in developing countries where the burden of cervical cancer is highest. Large prospective trials have compared once per lifetimes screening methods of HPV testing, cytology and visual inspection with acetic acid. Compared with a control group, only HPV testing reduced the rate of cervical cancer. Therefore, in low resource settings, HPV testing in women over 30 may be an effective large scale method of cervical cancer screening [45].

Despite the positive results seen with HPV testing, the ASCCP has not yet adopted primary screening with HPV testing because of concerns for an evidence based approach to subsequent follow-up. A new approach which is currently utilized many European nations and is being evaluated in the US is primary HPV testing with cytology triage. In this screening method, a positive HR-HPV test is then followed by cytology. Patients with abnormal cytology then proceed with colposcopic evaluation. With this method, the test with the higher sensitivity (HPV testing) is followed by the test with the higher specificity (cytology), thus improving detection rates while eliminating false positive results. In support of this method, a prospective Finnish trial demonstrated that primary HPV DNA screening with cytology triage had improved sensitivity and equivalent specificity for detection of CIN 2/3. Moreover, in women 35 years or older, HPV testing with cytology triage was more specific than cytology alone and decreased colposcopy referrals and follow-up tests [49].

HPV VACCINATIONS AND ITS EFFECT OF THE VACCINE ON SCREENING

With a better understanding of the biology of HPV infections, vaccinations have been developed to help prevent primary infection. There are currently two commercially available vaccines in the US that protect against cervical cancer and pre-invasive disease by targeting specific HPV types: GARDASIL (Merck & Co. Inc., Whitehouse Station, NJ, USA), which is a quadrivalent vaccine and protects against HPV type 6, 11, 16, and 18, and Cervarix (GlaxoSmithKline, Rixensart, Belgium) which is a bivalent vaccine and protects against HPV types 16 and 18.

As more women are successfully vaccinated there will be a reduction in prevalence of cytological abnormalities and this may further limit the effectiveness of cytology as a primary screening tool. It is estimated that there will be a reduction

from the current 50% to 70% positive predictive value of cytology to 10% to 20% if there is a decrease in prevalence of pre-invasive cervical lesions. HPV testing may therefore be more effective in regions and countries with a lower HPV prevalence due to effective vaccination programs [20].

The second major impact of the vaccine on Pap testing is a change in cytologic interpretation. With fewer abnormal lesions, there may be greater interobserver variability in cytologic interpretation. This could raise false-negative diagnoses and further reduce the sensitivity of cytology [20].

FUTURE DIRECTIONS

Improved screening algorithms, which may in the future include primary HPV testing, followed by cytology triage will likely continue to change as data from large prospective trials emerge. This method has shown some promise by maintaining high sensitivity, prolonged screening intervals, and may ultimately prove to be more efficacious in the post-vaccinations era. Other areas of current research include identification of other novel molecular markers associated with protein expression and cell cycle regulators that are present in high-grade lesions. E6 and E7 viral oncogenes are necessary for HPV carcinogenesis and tests for E6/E7 mRNA, already commercially available, could help identify women at higher risk for developing cancer [50]. Staining for p16 overexpression has already shown promise in the triage of abnormal cytology, specifically in those with ASC-US, ASC-H, and LSIL cytology [50,51]. Additionally, high-grade lesions have genetic expression profiles that resemble invasive disease [50]. Therefore, DNA microarray analysis may be able to better stratify a woman's risk in the setting of a positive HR-HPV test [50].

The utilization of the Pap smear in preventive care and cervical cancer screening has been a cornerstone in women's health for over 70 years. Decline of cervical cancer rates after implementation of cytology programs is considered one of the greatest successes in cancer prevention of all time. Through a better understanding of the role of HPV in cervical cancer carcinogenesis and the development of HR-HPV tests, cervical cancer screening strategies have already shown a drastic shift from conventional annual cytology to a more complex interplay of HPV triage, extended screening intervals, and varying methods of follow-up. These changes likely represent just the beginning of a paradigm shift in cervical cancer prevention. As we move forward with cervical cancer screening programs, HPV testing will likely emerge as a primary screening method followed by triage with either cytology, HPV genotyping, or other genetic profiling, which will more efficiently guide clinicians in the prevention of invasive disease.

CONFLICT OF INTEREST

The author, Warner K. Huh has worked as a consultant for Roche Diagnostics and Merck & Co. Inc.

REFERENCES

1. Papanicolaou GN, Traut HF. The diagnostic value of vaginal smears in carcinoma of the uterus. 1941. Arch Pathol Lab Med 1997;121:211-24.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
3. Quinn M, Babb P, Jones J, Allen E. Effect of screening on incidence of and mortality from cancer of cervix in England: evaluation based on routinely collected statistics. BMJ 1999;318:904-8.
4. Sasieni P, Adams J. Effect of screening on cervical cancer mortality in England and Wales: analysis of trends with an age period cohort model. BMJ 1999;318:1244-5.
5. van der Aa MA, Pukkala E, Coebergh JW, Anttila A, Siesling S. Mass screening programmes and trends in cervical cancer in Finland and the Netherlands. Int J Cancer 2008; 122:1854-8.
6. Sawaya GF, Brown AD, Washington AE, Garber AM. Clinical practice: current approaches to cervical-cancer screening. N Engl J Med 2001;344:1603-7.
7. Subramaniam A, Fauci JM, Schneider KE, Whitworth JM, Erickson BK, Kim K, et al. Invasive cervical cancer and screening: what are the rates of unscreened and under-screened women in the modern era? J Low Genit Tract Dis 2011;15:110-3.
8. Leyden WA, Manos MM, Geiger AM, Weinmann S, Mouchawar J, Bischoff K, et al. Cervical cancer in women with comprehensive health care access: attributable factors in the screening process. J Natl Cancer Inst 2005; 97:675-83.
9. National Institutes of Health. NIH Consensus Statement Online 1996 April 1-13 [Internet]. Bethesda, MD: National Institutes of Health; [cited 2012 Jun 15]. Available from: http://consensus.nih.gov/1996/1996cervicalcancer102html.htm.
10. Cuzick J, Clavel C, Petry KU, Meijer CJ, Hoyer H, Ratnam S, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer

screening. Int J Cancer 2006;119:1095-101.

11. Nanda K, McCrory DC, Myers ER, Bastian LA, Hasselblad V, Hickey JD, et al. Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. Ann Intern Med 2000;132: 810-9.

12. Spence AR, Goggin P, Franco EL. Process of care failures in invasive cervical cancer: systematic review and meta-analysis. Prev Med 2007;45:93-106.

13. Elfgren K, Rylander E, Radberg T, Strander B, Strand A, Paajanen K, et al. Colposcopic and histopathologic evaluation of women participating in population-based screening for human papillomavirus deoxyribonucleic acid persistence. Am J Obstet Gynecol 2005;193:650-7.

14. Scheiden R, Wagener C, Knolle U, Dippel W, Capesius C. Atypical glandular cells in conventional cervical smears: incidence and follow-up. BMC Cancer 2004;4:37.

15. Silverberg SG, Ioffe OB. Pathology of cervical cancer. Cancer J 2003;9:335-47.

16. Howlader N, Noone AM, Krapcho M, Neyman N, Aminou R, Altekruse SF, et al. SEER cancer statistics review, 1975-2009 (Vintage 2009 oopulations) [Internet]. Bethesda, MD: National Cancer Institute; c2012 [cited 2012 Jun 15]. Available from: http://seer.cancer.gov/csr/1975_2009_pops09/.

17. Bentz JS, Rowe LR, Gopez EV, Marshall CJ. The unsatisfactory ThinPrep Pap test: missed opportunity for disease detection? Am J Clin Pathol 2002;117:457-63.

18. Islam S, West AM, Saboorian MH, Ashfaq R. Reprocessing unsatisfactory ThinPrep Papanicolaou test specimens increases sample adequacy and detection of significant cervicovaginal lesions. Cancer 2004;102:67-73.

19. Woodhouse SL, Stastny JF, Styer PE, Kennedy M, Praestgaard AH, Davey DD. Interobserver variability in subclassification of squamous intraepithelial lesions: results of the College of American Pathologists Interlaboratory Comparison Program in cervicovaginal cytology. Arch Pathol Lab Med 1999;123:1079-84.

20. Franco EL, Cuzick J, Hildesheim A, de Sanjose S. Chapter 20: issues in planning cervical cancer screening in the era of HPV vaccination. Vaccine 2006;24 Suppl 3:S3/171-7.

21. 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;189:12-9.

22. Franco EL, Rohan TE, Villa LL. Epidemiologic evidence and human papillomavirus infection as a necessary cause of cervical cancer. J Natl Cancer Inst 1999;91:506-11.

23. zur Hausen H. Condylomata acuminata and human genital

cancer. Cancer Res 1976;36:794.

24. Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlen W, zur Hausen H. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. EMBO J 1984;3:1151-7.

25. Hariri S, Unger ER, Sternberg M, Dunne EF, Swan D, Patel S, et al. Prevalence of genital human papillomavirus among females in the United States, the National Health And Nutrition Examination Survey, 2003-2006. J Infect Dis 2011;204:566-73.

26. Parkin DM, Bray F. Chapter 2: the burden of HPV-related cancers. Vaccine 2006;24 Suppl 3:S3/11-25.

27. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens- Part B: biological agents. Lancet Oncol 2009;10:321-2.

28. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 1998;338:423-8.

29. Richardson H, Kelsall G, Tellier P, Voyer H, Abrahamowicz M, Ferenczy A, et al. The natural history of type-specific human papillomavirus infections in female university students. Cancer Epidemiol Biomarkers Prev 2003;12:485-90.

30. Dalstein V, Riethmuller D, Pretet JL, Le Bail Carval K, Sautiere JL, Carbillet JP, et al. Persistence and load of high-risk HPV are predictors for development of high-grade cervical lesions: a longitudinal French cohort study. Int J Cancer 2003;106:396-403.

31. Moscicki AB, Hills N, Shiboski S, Powell K, Jay N, Hanson E, et al. Risks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females. JAMA 2001;285:2995-3002.

32. Ferreccio C, Van De Wyngard V, Olcay F, Dominguez MA, Puschel K, Corvalan AH, et al. High-risk HPV infection after five years in a population-based cohort of Chilean women. Infect Agent Cancer 2011;6:21.

33. Winer RL, Lee SK, Hughes JP, Adam DE, Kiviat NB, Koutsky LA. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. Am J Epidemiol 2003;157:218-26.

34. Winer RL, Feng Q, Hughes JP, O'Reilly S, Kiviat NB, Koutsky LA. Risk of female human papillomavirus acquisition associated with first male sex partner. J Infect Dis 2008;197:279-82.

35. Burchell AN, Winer RL, de Sanjose S, Franco EL. Chapter 6: epidemiology and transmission dynamics of genital HPV infection. Vaccine 2006;24 Suppl 3:S3/52-61.

36. Munoz N, Mendez F, Posso H, Molano M, van den Brule AJ, Ronderos M, et al. Incidence, duration, and determinants

of cervical human papillomavirus infection in a cohort of Colombian women with normal cytological results. J Infect Dis 2004;190:2077-87.

37. Centers for Disease Control and Prevention (CDC). Human papillomavirus-associated cancers - United States, 2004-2008. MMWR Morb Mortal Wkly Rep 2012;61:258-61.

38. Moscicki AB, Cox JT. Practice improvement in cervical screening and management (PICSM): symposium on management of cervical abnormalities in adolescents and young women. J Low Genit Tract Dis 2010;14:73-80.

39. Woodman CB, Collins S, Winter H, Bailey A, Ellis J, Prior P, et al. Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. Lancet 2001;357:1831-6.

40. Solomon D, Schiffman M, Tarone R; ALTS Study group. Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. J Natl Cancer Inst 2001;93:293-9.

41. Ronco G, Giorgi-Rossi P, Carozzi F, Confortini M, Dalla Palma P, Del Mistro A, 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.

42. Naucler P, Ryd W, Tornberg S, Strand A, Wadell G, Elfgren K, et al. Human papillomavirus and Papanicolaou tests to screen for cervical cancer. N Engl J Med 2007;357:1589-97.

43. Bulkmans NW, Berkhof J, Rozendaal L, van Kemenade FJ, Boeke AJ, Bulk S, 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.

44. Mayrand MH, Duarte-Franco E, Coutlee F, Rodrigues I, Walter SD, Ratnam S, et al. Randomized controlled trial of human papillomavirus testing versus Pap cytology in the primary screening for cervical cancer precursors: design, methods and preliminary accrual results of the Canadian cervical cancer screening trial (CCCaST). Int J Cancer 2006;119:615-23.

45. Sankaranarayanan R, Nene BM, Shastri SS, Jayant K, Muwonge R, Budukh AM, et al. HPV screening for cervical cancer in rural India. N Engl J Med 2009;360:1385-94.

46. 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 intra-epithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial. Lancet Oncol 2012;13:78-88.

47. 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.

48. Kotaniemi-Talonen L, Nieminen P, Anttila A, Hakama M. Routine cervical screening with primary HPV testing and cytology triage protocol in a randomized setting. Br J Cancer 2005;93:862-7.

49. Leinonen M, Nieminen P, Kotaniemi-Talonen L, Malila N, Tarkkanen J, Laurila P, 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.

50. Cuzick J, Mayrand MH, Ronco G, Snijders P, Wardle J. Chapter 10: new dimensions in cervical cancer screening. Vaccine 2006;24 Suppl 3:S3/90-7.

51. Reuschenbach M, Clad A, von Knebel Doeberitz C, Wentzensen N, Rahmsdorf J, Schaffrath F, et al. Performance of p16INK4a-cytology, HPV mRNA, and HPV DNA testing to identify high grade cervical dysplasia in women with abnormal screening results. Gynecol Oncol 2010;119:98-105.