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Risk-Adapted Multimodal Laboratory Cervical Screening – Application of New Technologies to Cervical Cancer Prevention

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Abstract The objective of screening for cervical cancer is to reduce the mortality and incidence of the disease. To date, there is extensive and strong evidence that this can be achieved by cytology-based screening programs, which continue to be the mainstay of cervical prevention worldwide despite their inherent methodological limitations. This article presents a review on the utility of conventional, ancillary, and experimental methods for cervical screening both as single tests and test-combinations, and describes possible future directions for enhanced screening accuracy using risk-adapted protocols.

Current Epidemiological Status of Cervical Cancer

Carcinoma of the uterine cervix is the second most common cancer in women worldwide, with approximately 500,000 new cases diagnosed and 230,000 deaths each year. Almost 80% of new cases occur in the developing world, where it is the leading cause of cancer-related death among women (1). In the European Union (EU) before its recent enlargement, cervical cancer was estimated to comprise about 3% of cancers in women; ranking eighth in importance and being the tenth most common cause of cancer-related deaths in women in 1998 (2). The recent expansion of the EU in May 2004 will certainly cause significant changes in cervical cancer rates, because there is substantial excess in female mortality for the disease in most central and eastern European countries (3).

After the implementation of regular, population-based cervical screening programs in most developed countries in the 1960s, the incidence of and mortality from cervical cancer has decreased substantially. This has been mainly attributed to early detection and treatment of precancerous lesions. Where screening quality and coverage has been high, these efforts have reduced invasive cervical cancer by up to 90 percent (4,5). Also in Germany, the age-standardized rates for cervical cancer incidence and mortality declined by 73% and 74% from 1960 to 1997, after the introduction of the statutory opportunistic cancer-screening program in the Western part of the country in 1971 (6). Despite significant efforts in population-based screening that makes a free annual Papanicolau (Pap) test available to all women of 20 years of age and older covered by statutory health insurance (slightly above 90% of the adult female population) (7), the age-standardized annual incidence of 13.8 and mortality rate of 4.5 per 100,000 women were among the highest in Europe for the 1998-2000 period (8). This can be partly explained by the generally poorer effectivity of an opportunistic screening program compared to an active invitation system (9).

In the meantime, accumulating data from organized screening programs indicate that

the marked declines seen until the mid-1980s have been slowing and may even be increasing in certain countries (10). This could reflect increased cancer detection by using new diagnostic techniques, such as human papillomavirus (HPV) testing and cervicography, or it might be the result of a cohort effect. Another factor with a potential effect on incidence trends is the increase in rates of adenocarcinomas and adenosquamous carcinomas, which account for about 10% of all cervical cancers in Western populations (11). These tumor types and especially their precursors are frequently missed by conventional Pap smears. These data suggest that the maximum effect of Pap smear-based screening could have been reached and further reduction in cervical cancer rates will require the introduction of new technologies and/or more efficient population screening strategies.

Cytology-Based Cervical Screening

Screening for cervical cancer and its precursors have been performed by the conventional Pap smear method over the last half-century (12), with well-published public health success and inherent methodological limitations. High-quality cytology is a highly specific screening test with estimates of an average of 97% (range 86-100%). In contrast, sensitivity of a single smear may be between 30-87% (average of 51%), although the sensitivity for high-grade disease alone is between 70% and 80% (13).

Newer technologies have been developed, with the intention of improving the detection of cytological abnormalities, including liquid-based, thin layer cytology (ThinPrep, Autocyte), computerized re-screening (PAPNET), and algorithm-based computer re-screening (AutoPap). Several sub-optimal studies (split-sample or historical) have been performed to determine the sensitivity, specificity, and predictive values of these new methods; however lack of an adequate reference standard in most of the studies hampers proper assessment and comparison of test characteristics (14). Taken as a whole, the available evidence indicates that using liquid-based cytology, sensitivity is modestly higher for detecting any degree of cervical intraepithelial neoplasia (CIN), whereas specificity is modestly lower than with conventional Pap smears (13). This supports the conclusion that liquid based cytology is an acceptable alternative to conventional cervical cytology smears, which is reflected by the Food and Drug Administration (FDA) of the US approval of two liquid-based Pap systems for routine use.

There are three major advantages of liquid based cytology over conventional Pap smears: 1) many investigators agree that liquid based cytology markedly improves specimen adequacy (15-17); 2) the residual material can be used for ancillary testing (e.g., for HPV DNA); and 3) recent studies have shown an improvement in sensitivity and specificity for biopsy proven adenocarcinoma *in situ* (AIS) and adenocarcinoma (18,19).

HPV DNA as Marker of Precursor Lesions

It is now well established that the vast majority of cervical carcinomas and its precursors worldwide are caused by persistent infections with certain high-risk types of human papillomaviruses (HR-HPV) (20-22). Under optimal testing conditions, HR-HPV DNA can be identified in nearly all specimens of invasive cervical cancer (99.7%), in at least 70% of CIN1, 80% of CIN2, and 96% of CIN3 precursor lesions. Using the Bethesda system nomenclature, HR-HPV DNA can be identified in some 50% of borderline cytology lesions (ASCUS), 80% of low-grade squamous intraepithelial lesions (LSIL), and 90-95% of high-grade intraepithelial lesions (HSIL) and invasive cancer cases (23,24). In terms of public health, these data indicate that the existence of HPV-negative cervical cancer cases is negligible and does not require any interventional targeting by screening.

However, epidemiological studies have shown not only that women without HPV do not get cervical cancer but also that most women with HPV do not get cervical cancer. This is due to the fact that most HPV infections are transient in nature, especially in younger age groups, resulting in no symptoms or minimal cellular changes, or low-grade intraepithelial lesions (25,26).

Research is ongoing to determine acceptable protocols for HPV testing for three main screening- or management-related purposes: 1) as a primary screening in asymptomatic women with negative cytology results for intraepithelial neoplasia or malignancy, for estimating prevalence and distribution of HPV in the normal screening population, and to define baseline HPV status in these women for diagnostic follow-up (27); 2) re-

flex HPV DNA testing for triage of women with initial equivocal and abnormal Pap smear (≥ASCUS) (28); and 3) follow-up for treated cases for improved surveillance of residual disease or recurrence (29).

Primary screening studies have demonstrated HPV testing to be more sensitive than cytology alone, whereas the specificity of HPV-tests is age-dependent. In the younger age groups, specificity is lower than for cytology and in age groups of 35 years and older (also country-dependent) the specificity of the tests is similar (28,30,31). One of the strongest gains of the combination of HPV tests and cytology lies in the very high negative predictive value of >99% for detecting CIN3 or cancer (27). Such a testing combination could potentially allow screening intervals to be increased; e.g. from the minimum of 3 years up to 5 years or longer, depending on the population and risk profile (32). Furthermore, HPV DNA detection can successfully be performed on self-collected samples as well, which may be advantageous in specific patient groups (33). Additionally, unlike the Pap smear, which can determine only whether abnormal cells are presently detected, molecular HPV testing has predictive value for lesions that may develop in the future (23,34). HPV DNA testing also appears to represent a significant enhancement for detection of endocervical adenocarcinomas, which are otherwise difficult to detect and prevent (35). One of the main drawbacks of this approach is the loss in specificity with respect to either test in isolation, due to the excessive number of patients who would need to be referred for colposcopy (36). Nevertheless, results from large investigational trials, including the ASC-US/Low-grade Squamous Intraepithelial Lesion Triage Study (ALTS), provide an abundance of data to justify the use of HPV testing for triaging ASC-US cases and as a follow-up test (37).

At present, there are two HPV testing systems in widespread clinical use. Current hybrid capture technology (HC2 test) detects the presence of 13 types of oncogenic HPVs and 5 low-risk types using respective probe cocktails. Results are group-specific and do not allow distinction between different HPV genotypes. For clinical purposes, only the high-risk probe cocktail is used, with a reported sensitivity for detecting high-grade cervical intraepithelial neoplasias (CIN 2-3) between 84% and 100% (24,38,39).

However, because only women with long-lasting latent HPV infections, even with low levels of oncogenic types, are at high risk for developing HSIL (40), discrimination between transient and persistent infection with high-risk HPV types is essential for risk-adapted screening protocols. Furthermore, the persistence of at least one oncogenic HPV type is necessary for the emergency of pre-cancer (41), which can only be defined by genotyping of two consecutive probes because of the possibility of a new infection with another high-risk type during the follow up period. Since the median duration of transient infection is 6-11 months (25,31), a second type (variant)-specific HPV test about 12 months after the first positive genotype test should identify persistent type specific infections.

Genotyping can be performed by using different established polymerase chain reaction (PCR) techniques (PGMY09/11-Amplicor LBA, GP5+/6+-EIA, SPF10-LIPA, PPF1/CP5-Sequencing) (42,43). Amplified HPV DNA is identified by either microplate hybridization for the detection of PCR amplicons (GP5+/6+-EIA) or a reverse hybridization line blot assay (PGMY09/11-LBA, SPF10-LIPA) which provides information on the specific type(s) detected (44,45).

Although simultaneous reporting of cytology and HPV results seems to be ideal, it is not always feasible, e.g. due to reimbursement issues in several countries. However, stringent cytomorphological criteria for minor HPV-associated cellular changes could help in pre-selecting high-risk patients with borderline smear abnormalities for subsequent molecular HPV testing (17,46,47).

In our own experience, sensitivity and specificity of the combination of classical and non-classical HPV signs have nearly achieved 100% for PCR-based detection of HPV infection in women with squamous intraepithelial lesions (SIL) and high-grade intraepithelial lesions, respectively (48,49). Since >90% of HSIL is HPV infected, identification of even minor cytological changes suggestive of HPV infection could raise awareness of the screening cytologist more carefully to search for atypical cells. With a complete lack of such minor abnormalities, HPV infection and consequently the presence of HSIL is practically excluded and no further molecular HPV tests are needed (17).

Surrogate Biomarkers of Neoplastic Transformation

Theoretically, certain DNA, RNA, or protein markers associated with neoplastic transformation of cervical epithelium subsequent to HPV infection could be applied in screening, diagnosis, and prognosis. Because oncogenic HPVs are causative agents in cervical carcinogenesis and act via altering the cell cycle in infected epithelial cells, host genes interacting directly or indirectly with HPV oncoproteins have been extensively investigated *in vitro* (50,51). The effect of the high-risk HPV early protein E7 on the function of the tumor suppressor pRB, which leads to over-expression of p16INK4A, a cyclin-dependent kinase inhibitor involved in cell cycle control has been investigated by several groups and a simple immunohistochemical assay has been developed for detecting p16 expression in both cell smears and tissue sections (52). Diffuse, full thickness P16INK4A expression was found to discriminate low-grade from high-grade CIN and claimed to be a marker of high-risk HPV integration into DNA of infected squamous epithelial cells (53). However, P16INK4A positivity in cervical glandular lesions was equivocal in different studies indicating limited utility of this biomarker in diagnosing suspicious glandular lesions, particularly in cytopathology (54).

Among the markers of proliferative activity and differentiation, including Ki67, cell cycle regulators (Rb, p53, Cyclin A, E, and D, p16, p21, p27, and telomerase), and cellular differentiation products (involucrin, CK13, CK14) combined quantitation of Ki67, Rb, CK13, and CK14 was found to predict progression risk of early CIN lesions (55).

Other recent studies, including ours, have reported that DNA ploidy measurement by image cytometry on cervical smears positive for HR-HPV help to detect women at high risk for developing high-grade cervical lesions (56,57). This is supported by experimental results suggesting that increasingly deregulated expression of the E6-E7 oncogenes of HR-HPVs in epithelial stem cells first results in chromosomal instability and induces DNA aneuploidy followed either by subsequent integration of the HR-HPV genome into the affected cell clone (58) or alternative oncogene activation mechanisms (59). Clinical studies support the concept that cervical lesions with an aneuploid DNA profile are more likely to persist or progress than those with diploid or polyploid DNA content (60).

Combination of Different Modalities

There is an increasingly large research literature on possible applications of new visual, microscopical, and virological screening methods for the prevention of cervical cancer (61). Adding a second sensitive test to cytology, such as HR-HPV detection, yields a substantial increase in sensitivity and negative predictive value for high-grade CIN and cancer at the cost of concomitant decrease in specificity (31), which is of particular concern for large populations. The second test can be used sequentially as a triage method, with the aim to restrict the number of screen-positives requiring referral. Among women with equivocal cytology results, HPV DNA testing is more accurate for detecting underlying CIN3 or cancer than repeat cytology. HPV DNA testing is not useful for triage of low-grade squamous intraepithelial lesions (LSIL in the Bethesda system) because of the very high HPV positivity (39). Nevertheless, screening performance is largely influenced by population characteristics, especially the prevalence of underlying HPV infection. Therefore, all fundamental cervical screening statistics will vary greatly by region (61).

However, even the highly sensitive test combination of cytology (preferably liquid based) and HR-HPV genotyping cannot assess the biological potential of prevalent cervical intraepithelial neoplasia towards progression or regression. An ideal test combination would indicate that an oncogenic HPV virus has already enhanced genetic instability and rendered cells susceptible to malignant transformation and consequent progression if left untreated. This can only be assessed by using an adequate biomarker in combination with morphological and/or HPV tests.

Sporadic published results of such preliminary approaches are available only from experimental or study settings up to date (55-60). Our institution is the first in Germany which introduced a multimodal, risk-adapted cervical screening protocol using the combination of liquid based cytology, HPV genotyping, and DNA image cytometry in a pure clinical setting, including a routine screening population of about 30,000 women from the Bonn region in Western Germany. Since January 1999, all cervical samples sent to our institute by referral gynecologists from

the region have been processed according to this screening profile (Fig. 1). Our preliminary results from the year 2002 showed that the combination of all three test modalities resulted in an up to 6.9% increase in positive predictive value (PPV) for moderate to high-grade cervical dysplasias and carcinomas (≥CIN2) compared to single tests or double combinations (62). This combined approach had the additional benefit of being able to predict the possible outcome of histologically proven CIN1 lesions detected as false positives by single tests. The positivity for HR-HPV and DNA aneuploidy in a CIN1 lesion signalize a high risk for progression, whereas HR-HPV positivity with diploid DNA content indicate a probable benign course. Accordingly, our multimodal cervical screening protocol may permit identification of those women with low-grade squamous intraepithelial lesions (LSIL/CIN1) likely to progress at earlier and curable stage of disease and distinguish them from transient minor lesions caused by productive HPV infection. Using a risk-adapted combination of methods, although more expensive per screening, might be cost effective if the increased sensitivity permits lengthening of the screening interval and prevent many women from unnecessary colposcopy and conization (63-64).

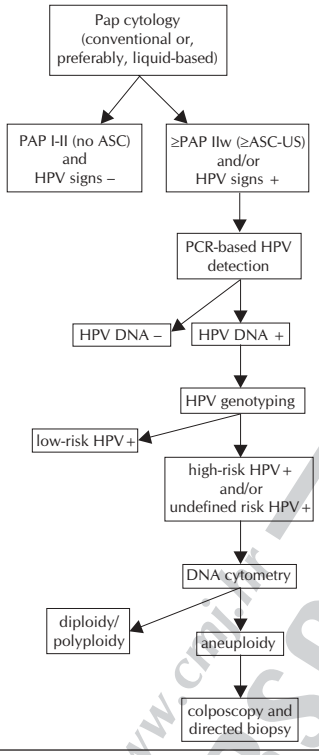

Figure 1. Risk-adapted, multimodal cervical screening program (the Bonn-scheme) using a combination of cytology and PCR-based HPV detection with adjuvant DNA cytometry in the routine clinical setting. Papanicolau (Pap) cytology interpretations are given according to the revised Munich II Classification, which is the standard in Germany, and the Bethesda System (in brackets). For the ideal case, if all screening examinations can be done consecutively, the major steps include: 1) conventional Pap-test, or preferably by the ThinPrep technique with special focus on assessing HPV-related cytomorphological changes (classic and non-classic HPV signs) (17); 2) cytology results of ≥PapIIw are managed by using reflex PCR-based HPV DNA testing; 3) in HPV DNA+ cases, genotyping by sequencing; 4) in high-risk HPV+ cases, DNA cytometry for assessing gross chromosomal instability as progression marker (14); 5) only DNA aneuploid cases should be referred to immediate colposcopy and directed biopsy; 6) treatment is reserved for biopsy-confirmed ≥CIN3 cases; and 7) after ablation, Pap-smears are to be scheduled at 6 months with a repeated HPV DNA test. ASC-US – atypical squamous cells of undetermined significance; HPV – human papillomavirus, PCR – polymerase chain reaction; PAP IIw – an unofficial class in the Munich II classification, indicating inadequate specimens or minimal cytological abnormalities roughly equating to ASC-US.

We conclude that, to date, there are several good methods available for cervical screening. With appropriate screening programs and early diagnosis and treatment, cervical cancer may become a preventable public health issue in the foreseeable future. Based on good evidence, highly accurate screening for cervical cancer and high-grade intraepithelial neoplasias is now technically feasible. Given the abundant options for detecting its precursors, it is not a conceptual or technical challenge, but a matter of health care and financing which delay the elimination of this malignancy.

References

1 Parkin DM, Bray FI, Devesa SS. Cancer burden in the year 2000. The global picture. Eur J Cancer. 2001;37 Suppl 8:S4-66.

2 Ferlay J, Bray F, Sankila R, Parkin DM. EUCAN: Cancer incidence, mortality and prevalence in the European Union 1998. version 5.0. IARC CancerBase No. 5. Lyon: IARCPress; 1999.

3 Levi F, Lucchini F, Negri E, La Vecchia C. Trends in mortality from major cancers in the European Union, including acceding countries, in 2004. Cancer. 2004; 101:2843-50.

4 Gustafsson L, Ponten J, Zack M, Adami HO. International incidence rates of invasive cervical cancer after introduction of cytological screening. Cancer Causes Control. 1997;8:755-63.

5 Nieminen P, Kallio M, Hakama M. The effect of mass screening on incidence and mortality of squamous and adenocarcinoma of cervix uteri. Obstet Gynecol. 1995;85:1017-21.

6 Becker N. Epidemiological aspects of cancer screening in Germany. J Cancer Res Clin Oncol. 2003;129:691-702.

7 Schenck U, von Karsa L. Cervical cancer screening in Germany. Eur J Cancer. 2000;36:2221-6.

8 Working group of population-based cancer registries in Germany: cancer in Germany: prevalence and trends (a comprehensive brochure) [In German]. Available at: www.rki.de/KREBS. Accessed: November 6, 2004.

9 Nieminen P, Kallio M, Anttila A, Hakama M. Organised vs. spontaneous Pap-smear screening for cervical cancer: A case-control study. Int J Cancer. 1999;83:55-8.

10 Vizcaino AP, Moreno V, Bosch FX, Munoz N, Barros-Dios XM, Borras J, et al. International trends in incidence of cervical cancer: II. Squamous-cell carcinoma. Int J Cancer. 2000;86:429-35.

11 Vizcaino AP, Moreno V, Bosch FX, Munoz N, Barros-Dios XM, Parkin DM. International trends in the incidence of cervical cancer: I. Adenocarcinoma and adenosquamous cell carcinomas. Int J Cancer. 1998;75:536-45.

12 Papanicolaou GN. A survey of the actualities and potentialities of exfoliative cytology in cancer diagnosis. Ann Intern Med. 1949;31:661-74.

13 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.

14 Hartmann KE, Nanda K, Hall S, Myers E. Technologic advances for evaluation of cervical cytology: is newer better? Obstet Gynecol Surv. 2001;56:765-74.

15 Diaz-Rosario LA, Kabawat SE. Performance of a fluid-based, thin-layer papanicolaou smear method in the clinical setting of an independent laboratory and an outpatient screening population in New England. Arch Pathol Lab Med. 1999;123:817-21.

16 Saslow D, Runowicz CD, Solomon D, Moscicki AB, Smith RA, Eyre HJ, et al. American Cancer Society guideline for the early detection of cervical neoplasia and cancer. CA Cancer J Clin. 2002;52:342-62.

17 Bollmann M, Bankfalvi A, Trosic A, Speich N, Schmitt C, Bollmann R. Can we detect cervical human papillomavirus (HPV) infection by cytomorphology alone? Diagnostic value of non-classic cytological signs of HPV effect in minimally abnormal Pap tests. Cytopathology. 2005;16:13-21.

18 Bai H, Sung CJ, Steinhoff MM. ThinPrep Pap Test promotes detection of glandular lesions of the endocervix. Diagn Cytopathol. 2000;23:19-22.

19 Ashfaq R, Gibbons D, Vela C, Saboorian MH, Iliya F. ThinPrep Pap Test. Accuracy for glandular disease. Acta Cytol. 1999;43:81-5.

20 Schiffman MH, Bauer HM, Hoover RN, Glass AG, Cadell DM, Rush BB, et al. Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. J Natl Cancer Inst. 1993;85:958-64.

21 Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. J Natl Cancer Inst. 1995;87:796-802.

22 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.

23 Nobbenhuis MA, Walboomers JM, Helmerhorst TJ, Rozendaal L, Remmink AJ, Risse EK, et al. Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study. Lancet. 1999;354:20-5.

24 Clavel C, Masure M, Bory JP, Putaud I, Mangeonjean C, Lorenzato M, et al. Human papillomavirus testing in primary screening for the detection of high-grade cervical lesions: a study of 7932 women. Br J Cancer. 2001;84:1616-23.

25 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.

26 Moscicki AB, Shiboski S, Hills NK, Powell KJ, Jay N, Hanson EN, et al. Regression of low-grade squamous intra-epithelial lesions in young women. Lancet. 2004;364:1678-83.

27 Sherman ME, Lorincz AT, Scott DR, Wacholder S, Castle PE, Glass AG, et al. Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. J Natl Cancer Inst. 2003;95:46-52.

28 Schiffman M, Herrero R, Hildesheim A, Sherman ME, Bratti M, Wacholder S, et al. HPV DNA testing in cervical cancer screening: results from women in a high-risk province of Costa Rica. JAMA. 2000;283:87-93.

29 Wright TC Jr, Schiffman M, Solomon D, Cox JT, Garcia F, Goldie S, et al. Interim guidance for the use of human papillomavirus DNA testing as an adjunct to cervical cytology for screening. Obstet Gynecol. 2004;103:304-9.

30 Schneider A, Hoyer H, Lotz B, Leistritza S, Kuhne-Heid R, Nindl I, et al. Screening for high-grade cervical intra-epithelial neoplasia and cancer by testing for high-risk HPV, routine cytology or colposcopy. Int J Cancer. 2000;89:529-34.

31 Cuzick J, Szarewski A, Cubie H, Hulman G, Kitchener H, Luesley D, et al. Management of women who test positive for high-risk types of human papillomavirus: the HART study. Lancet. 2003;362:1871-6.

32 Advisory Committee on Cancer Prevention. Recommendations on cancer screening in the European Union. Eur J Cancer. 2000;36:1473-8.

33 Dannecker C, Siebert U, Thaler CJ, Kiermeir D, Hepp H, Hillemanns P. Primary cervical cancer screening by self-sampling of human papillomavirus DNA in internal medicine outpatient clinics. Ann Oncol. 2004;15:863-9.

34 Schlecht NF, Platt RW, Duarte-Franco E, Costa MC, Sobrinho JP, Prado JC, et al. Human papillomavirus infection and time to progression and regression of cervical intraepithelial neoplasia. J Natl Cancer Inst. 2003; 95:1336-43.

35 Davey DD, Zarbo RJ. Human papillomavirus testing – are you ready for a new era in cervical cancer screening? Arch Pathol Lab Med. 2003;127:927-9.

36 Franco EL. Chapter 13: Primary screening of cervical cancer with human papillomavirus tests. J Natl Cancer Inst Monogr. 2003;(31):89-96.

37 Schiffman M, Solomon D. Findings to date from the ASCUS-LSIL Triage Study (ALTS). Arch Pathol Lab Med. 2003;127:946-9.

38 Wright TC Jr, Denny L, Kuhn L, Pollack A, Lorincz A. HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer. JAMA. 2000;283:81-6.

39 Arbyn M, Buntinx F, Van Ranst M, Paraskevaidis E, Martin-Hirsch P, Dillner J. Virologic versus cytologic triage

of women with equivocal Pap smears: a meta-analysis of the accuracy to detect high-grade intraepithelial neoplasia. J Natl Cancer Inst. 2004;96:280-93.

40 Ho GY, Burk RD, Klein S, Kadish AS, Chang CJ, Palan P, et al. Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. J Natl Cancer Inst. 1995;87:1365-71.

41 Kjaer SK, van den Brule AJ, Paull G, Svare EI, Sherman ME, Thomsen BL, et al. Type specific persistence of high risk human papillomavirus (HPV) as indicator of high grade cervical squamous intraepithelial lesions in young women: population based prospective follow up study. BMJ. 2002;325:572.

42 Coutlee F, Gravitt P, Kornegay J, Hankins C, Richardson H, Lapointe N, et al. Use of PGMY primers in L1 consensus PCR improves detection of human papillomavirus DNA in genital samples. J Clin Microbiol. 2002;40:902-7.

43 Poljak M, Marin IJ, Seme K, Vince A. Hybrid Capture II HPV Test detects at least 15 human papillomavirus genotypes not included in its current high-risk probe cocktail. J Clin Virol. 2002;25 Suppl 3:S89-97.

44 Kay P, Meehan K, Williamson AL. The use of nested polymerase chain reaction and restriction fragment length polymorphism for the detection and typing of mucosal human papillomaviruses in samples containing low copy numbers of viral DNA. J Virol Methods. 2002;105:159-70.

45 Perrons C, Kleter B, Jelley R, Jalal H, Quint W, Tedder R. Detection and genotyping of human papillomavirus DNA by SPF10 and MY09/11 primers in cervical cells taken from women attending a colposcopy clinic. J Med Virol. 2002;67:246-52.

46 Schneider A, Meinhardt G, De-Villiers EM, Gissmann L. Sensitivity of the cytologic diagnosis of cervical condyloma in comparison with HPV-DNA hybridization studies. Diagn Cytopathol. 1987;3:250-5.

47 Cecchini S, Confortini M, Bonardi L, Cipparrone G, Galante L, Iossa A, et al. "Nonclassic" cytologic signs of cervical condyloma. A case-control study. Acta Cytol. 1990;34:781-4.

48 Bollmann R, Mehes G, Torka R, Speich N, Schmitt C, Bollmann M. Human papillomavirus typing and DNA ploidy determination of squamous intraepithelial lesions in liquid-based cytologic samples. Cancer. 2003;99:57-62.

49 Speich N, Schmitt C, Bollmann R, Bollmann M. Human papillomavirus (HPV) study of 2916 cytological samples by PCR and DNA sequencing: genotype spectrum of patients from the west German area. J Med Microbiol. 2004;53(Pt 2):125-8.

50 Alazawi W, Pett M, Arch B, Scott L, Freeman T, Stanley MA, et al. Changes in cervical keratinocyte gene expression associated with integration of human papillomavirus 16. Cancer Res. 2002;62:6959-65.

51 Al Moustafa AE, Foulkes WD, Wong A, Jallal H, Batist G, Yu Q, et al. Cyclin D1 is essential for neoplastic transformation induced by both E6/E7 and E6/E7/ErbB-2 cooperation in normal cells. Oncogene. 2004;23:5252-6.

52 Klaes R, Friedrich T, Spitkovsky D, Ridder R, Rudy W, Petry U, et al. Overexpression of p16(INK4A) as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. Int J Cancer. 2001;92:276-84.

53 Hu L, Guo M, He Z, Thornton J, McDaniel LS, Hughson MD. Human papillomavirus genotyping and p16INK4a expressipon in cervical intraepithelial neoplasia of adolescents. Mod Pathol. 2005;18:267-73.

54 Murphy N, Heffron CC, King B, Ganuguapati UG, Ring M, McGuinness E, et al. p16INK4A positivity in benign, premalignant and malignant cervical glandular lesions: a potential diagnostic problem. Virchows Arch. 2004; 445:610-5.

55 Kruse AJ, Skaland I, Janssen EA, Buhr-Wildhagen S, Klos J, Arends MJ, et al. Quantitative molecular parameters to identify low-risk and high-risk early CIN lesions: role of markers of proliferative activity and differentiation and Rb availability. Int J Gynecol Pathol. 2004;23:100-9.

56 Lorenzato M, Bory JP, Cucherousset J, Nou JM, Bouttens D, Thil C, et al. Usefulness of DNA ploidy measurement on liquid-based smears showing conflicting results between cytology and high-risk human papillomavirus typing. Am J Clin Pathol. 2002;118:708-13.

57 Bollmann R, Mehes G, Torka R, Speich N, Schmitt C, Bollmann M. Determination of features indicating progression in atypical squamous cells with undetermined significance: human papillomavirus typing and DNA ploidy analysis from liquid-based cytologic samples. Cancer. 2003;99:113-7.

58 Melsheimer P, Vinokurova S, Wentzensen N, Bastert G, von Knebel Doeberitz M. DNA aneuploidy and integration of human papillomavirus type 16 e6/e7 oncogenes in intraepithelial neoplasia and invasive squamous cell carcinoma of the cervix uteri. Clin Cancer Res. 2004; 10:3059-63.

59 Sathish N, Abraham P, Peedicayil A, Sridharan G, John S, Chandy G. Human papillomavirus 16 E6/E7 transcript and E2 gene status in patients with cervical neoplasia. Mol Diagn. 2004;8:57-64.

60 Bibbo M, Dytch HE, Alenghat E, Bartels PH, Wied GL. DNA ploidy profiles as prognostic indicators in CIN lesions. Am J Clin Pathol. 1989;92:261-5.

61 Ferreccio C, Bratti MC, Sherman ME, Herrero R, Wacholder S, Hildesheim A, et al. A comparison of single and combined visual, cytologic, and virologic tests as screening strategies in a region at high risk of cervical cancer. Cancer Epidemiol Biomarkers Prev. 2003;12:815-23.

62 Bollmann R, Bankfalvi A, Griefingholt H, Trosic A, Speich N, Schmitt C, et al. Validity of combined cytology and human papillomavirus (HPV) genotyping with adjuvant DNA-cytometry in routine cervical screening: results from 31031 women from the Bonn-region in West Germany. Oncol Rep. 2005;13:915-22.

63 Maxwell GL, Carlson JW, Ochoa M, Krivak T, Rose GS, Myers ER. Costs and effectiveness of alternative strategies for cervical cancer screening in military beneficiaries. Obstet Gynecol. 2002;100:740-8.

64 Goldie SJ, Kim JJ, Wright TC. Cost-effectiveness of human papillomavirus DNA testing for cervical cancer screening in women aged 30 years or more. Obstet Gynecol. 2004;103:619-31.

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