Risk of HSIL (CIN 2–3) on Colposcopic Biopsy is Minimal in Postmenopausal Women With LSIL on Cytology and a Negative HRHPV Test

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Background: *Current cervical cancer screening guidelines recommend a 1-year follow-up period for patients with a postmenopausal low-grade squamous intraepithelial lesion (LSIL) who are test negative for high-risk human papillomavirus (HrHPV). The aim of this study was to assess whether such patients had an increased immediate risk of high-grade squamous intraepithelial lesion.*
Methods: *We assessed 54 HrHPV-negative women with postmenopausal LSIL in the Department of Obstetrics and Gynecology of our hospital between 2012 and 2013. All patients underwent liquid-based cytology and reflex HrHPV testing (for human papillomavirus [HPV] types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). Colposcopic examination and guided biopsy were performed by the same gynecologist (MO).*
Results: *The average age of the patients was 53.1 ± 3.2 years. There were 33 patients (61%) with cervical intraepithelial neoplasia (CIN) grade 1 and 21 who were non-dysplastic. None of the patients was positive for CIN 2 or any other lesions.*
Conclusions: *If the HPV test is negative, repeat cytology after 12 months is recommended by the American Society for Colposcopy and Cervical Pathology for cases of HrHPV-negative postmenopausal LSIL. We recommend reflex HPV testing as the best choice for patients who test positive for postmenopausal LSIL by Pap smear, in line with the literature.* Diagn. Cytopathol. 2016;44:969–974. © 2016 Wiley Periodicals, Inc.

Key Words: cervical intraepithelial neoplasia; human papillomavirus DNA tests; low-grade squamous intraepithelial lesions; postmenopausal period

Every year, low-grade squamous intraepithelial lesions (LSIL) are detected in ~2–3% of all patients who undergo cytologic screening in the US.[1] LSIL is the second-most common diagnosis on cervicovaginal smear in Turkey, with a diagnostic frequency of 0.3–0.39%.[2] The frequency of diagnosis of LSIL in Turkish women aged 50–69 years is 0.7%.[2]

Although nearly 50% of LSIL cases resolve spontaneously, particularly in young women, the prevalence of histologic cervical intraepithelial neoplasia grade 2 (CIN 2) or worse in LSIL patients, detected on first colposcopy, has been reported to be 12–16%.[3] In a postmenopausal LSIL group, the rate was 16.3%.[4]

Colposcopy is recommended for the management of LSIL in females, excluding postmenopausal and adolescent cases. The American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines recommend postmenopausal LSIL patients to undergo a repeat cytology after 12 months (in high-risk human papillomavirus (HrHPV)-negative cases with no CIN identified on colposcopy). Colposcopic examination is advised for HrHPV-positive cases.[6]

HrHPV prevalence is high among LSIL patients (< 30 years, 80–85%; aged 30 years [30–49 years], ≈50%; 50–59 years, 19%).[5] Although the proportion of LSIL cases positive for human papillomavirus (HPV) is high among young women, in elderly women, cytologic

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This article was presented as a poster 15. Biannual Meeting of Gynecological Oncology at Melbourne 2014 and International journal of gynecological cancer.
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Received 28 January 2016; Revised 27 July 2016; Accepted 28 July 2016
DOI: 10.1002/dc.23556
Published online 16 August 2016 in Wiley Online Library (wileyonlinelibrary.com).

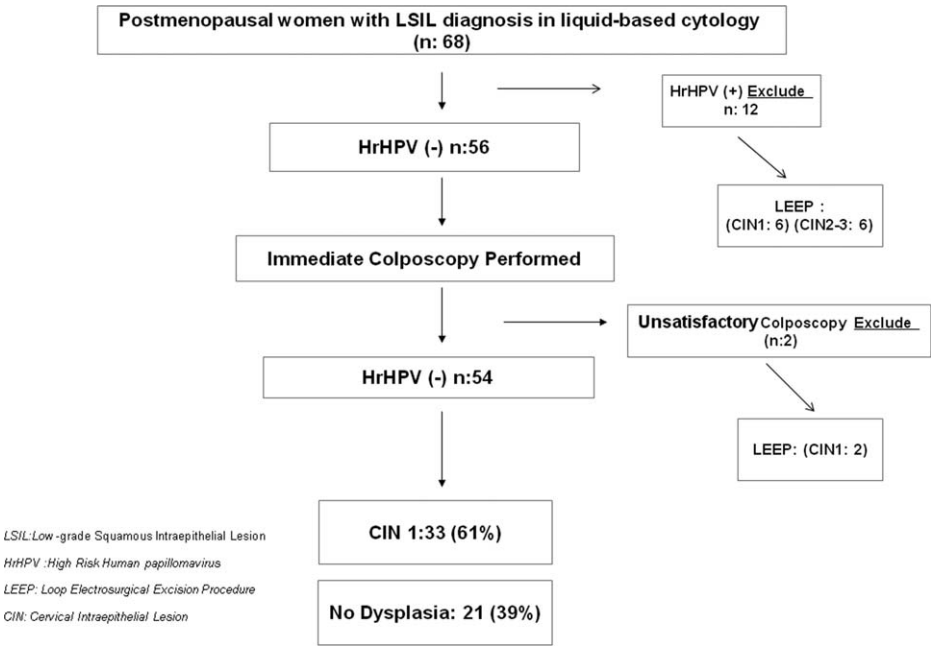

Fig. 1. Study design and categories of patients in current study. Postmenopausal patients with LSIL diagnosis in smear and their HrHPV negative.

findings indicating HPV infection are more common, and the incidence of noncarcinogenic HPV is higher.[7]

In two large studies, the prevalence of high-risk HPV (HrHPV) was lower in postmenopausal women.[8] Datta et al.[9] reported a HrHPV prevalence rate of 6% in women aged 50–65 years, while Zhoa et al.[10] reported a detection rate of 6.3% in women aged 50 years and older, who also tested positive for HrHPV DNA.

The ATHENA (Addressing THE Need for Advanced HPV Diagnostics) screening trial showed that the prevalence of HrHPV in LSIL cases was age-dependent; furthermore, while the rate of HrHPV-positive LSIL cases was 71.2% among the whole population, it dropped to 56.1% in women over 40 years old.[11] Setting an age limit for HPV reflex testing in female LSIL cases may be an acceptable option for triaging postmenopausal women.

To assess the risks and benefits associated with recent HPV testing guidelines, the current study investigated the baseline frequency of high-grade squamous intraepithelial lesion (HSIL; CIN 2–3) in HrHPV-negative postmenopausal LSIL cases.

Materials and Methods

Study Design and Patient Selection

The study received approval from the local ethics committee. Informed consent was obtained from each subject. Between 2011 and 2013, the results of 54 consecutive cases of HrHPV-negative LSIL were evaluated (Fig. 1). There was no prescreening history of an abnormality in any of the cases. All patients were postmenopausal women in whom menstruation ceased at least 12 months before screening. We performed a baseline colposcopic examination of all patients negative for postmenopausal LSIL and concomitant Hr-HPV according to liquid-based cytology (reflex HPV testing) results. Patients who opted for cervical smear screening were enrolled in a follow-up program. Cervical cytological screening was performed using the liquid-based cytological method (ThinPrep Pap test; Hologic UK Ltd., Crawley, UK). Cytological screening results were classified using The Bethesda System (2001).[12] Cases whose smear results indicated postmenopausal LSIL (Figs. 2 and 3) underwent HrHPV detection and genotyping tests.

HrHPV DNA Test

As a routine measure, we utilized a qualitative multiplex assay that provides specific genotyping information for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 (COBAS 4800; Roche Diagnostics, Indianapolis, IN).

Colposcopic Evaluation

All patients were asked to provide informed consent prior to any procedure. The same gynecologist (MO) performed all colposcopic evaluations. Patients who were followed-up previously due to diagnosis of an abnormal cervical smear, showed vaginal bleeding during the procedure, had used vaginal creams within the last month, had sexual intercourse during the 2 days prior to the colposcopy

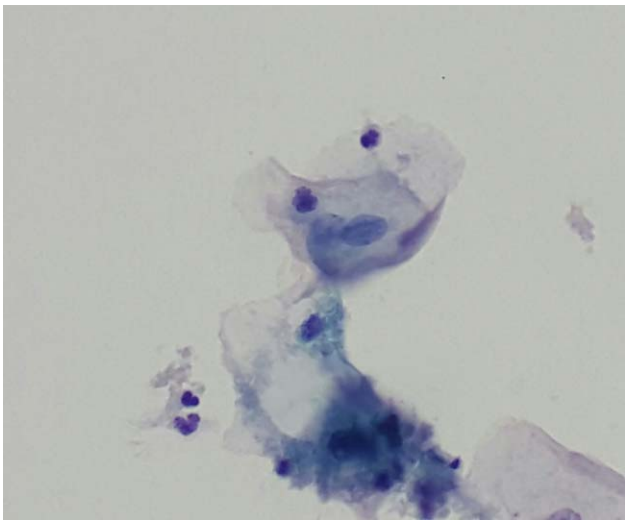

Fig. 2. Binucleation is seen in this LBS. Case was interpreted as low grade squamous intraepithelial lesion. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

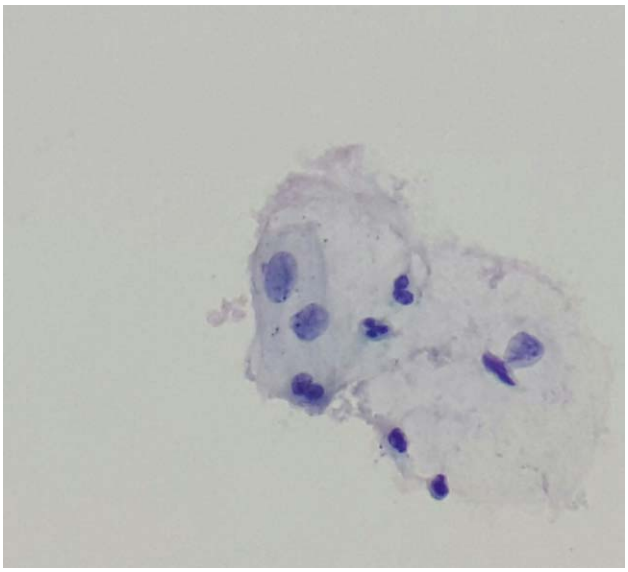

Fig. 3. Liquid based cervicovaginal smear. Cytological changes are subtle. Case was categorized as ASC (TBS 2014). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

examination, or who were currently receiving hormone replacement therapy (HRT) were excluded. The colposcopic examination was deemed sufficient when the squamocolumnar junction (SCJ) could be seen clearly with the colposcope. Data were not included in the analysis when the SCJ was not detected during the colposcopy examination; such cases were classified as unsatisfactory colposcopy (type 3 transformation zone [TZ]). A West Type Fbw colposcope (Leisegang, Berlin, Germany) was used for the colposcopic evaluations. After inserting the speculum, the cervix was flushed with saline, and the SCJ and TZ were observed under ×40 magnification. Then, 3% acetic acid solution was applied to the cervix. A normal TZ with original squamous and columnar epithelium was considered a normal colposcopic finding.[13] Acetowhite spaces, punctuation, mosaicism, iodine-negative epithelial tissue, leukoplakia, and atypical vascularizations were considered abnormal findings; colposcopic-directed biopsy (punch biopsy) was performed on these regions in all such cases.

The colposcopic biopsies were evaluated together with the results of previous liquid-based smears.

Terminology

For classification of smear test results, The Bethesda System (2001) was utilized, whereas for colposcopy, the Barcelona (2002) colposcopic terminology was preferred. For the HrHPV tests, positive results were denoted as "screening HrHPV(+)"; by-genotype positive results were denoted as "16+," "18+," or "other HrHPV(+)."

For histopathologic assessment of the colposcopic biopsies, the novel Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions (LAST) classification system was used.[14] In this system, biopsy material is classified in a similar manner to that of its cytological counterparts. In LAST 2012, under "biopsy material," CIN 1 and equivalent lesions are considered "low-grade SIL," while CIN 2 and 3 lesions are "high-grade SIL,"

The age, gravidity, parity, and smoking habits of the enrolled patients were also recorded.

Data Presentation and Statistical Evaluation

All percentile data were rounded for clarity. Statistical evaluations were performed using the SPSS for Windows software package (ver. 15.0; SPSS Inc., Chicago, IL).

Results

During a 2-year period, 54 patients who met the study criteria were included in the analysis. The median age of the participants was 53.1 ± 3.2 years (range: 48–57 years). In total, 10% (5/54) of the patients were nulliparous, while 90% (49/54) were multiparous. Gravidity status and parity information are provided in Table I. There were no statistically significant differences between the CIN 1 and "no dysplasia" groups in terms of gravidity, parity, or age. The majority of the patients were non-smokers (82.5% [44/54]). There were four smokers in the CIN 1 group and six in the no dysplasia group.

Following the colposcopy-guided biopsies, 33 cases received a histopathological diagnosis of LSIL (61%) (Figs. 4 and 5); the remaining 21 cases were classified as having no dysplasia. Adenocarcinoma in situ and invasive cervical cancer were not detected in any case.

Table I. The Demographic Characteristics of the Participants

Postmenopausal LSIL	*No Dysplasia (n:21) Mean (Min–Max)*	*CIN 1 (n: 33) Mean (Min–Max)*	P
Gravida	2.5 ± 0. 8 (1–4)	2.6 ± 0.7 (1–4)	*NS*
Parite	2.3 ± 0.7 (1–3)	2.4 ± 0.9 (1–4)	*NS*
Age	51.1 ± 1.9 (48–54)	52.6 ± 2.4 (49–57)	*NS*

LSIL: low grade squamous intraepithelial lesion; CIN: cervical intraepithelial lesion.

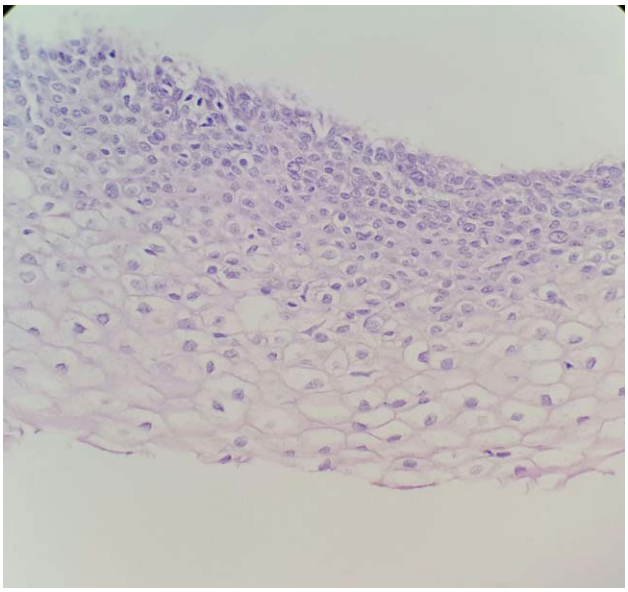

Fig. 4. Medium power magnification of cervical biopsy. Disorganization is confined to basal one third of epithelial thickness. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Patients who were found to have LSIL on colposcopic biopsy were scheduled for a 1-year follow-up.

Discussion

For postmenopausal LSIL cases, ASCCP guidelines recommend a repeat cytology after 12 months if they test negative on the HrHPV test.[6] An ideal management strategy for postmenopausal LSIL remains to be identified.

Cervical screening in older women presents many special challenges[15] associated with postmenopausal hormonal changes (hypoestrogenism), which in turn cause atrophic changes. This may increase the likelihood of insufficient or atypical findings on cervical cytology, in which degenerative changes in immature squamous cells can mimic squamous intraepithelial lesions and even invasive cancer. Colposcopic evaluations can be difficult to perform, and the SCJ is not usually seen; this renders such evaluations unsatisfactory.[16]

Cervical cancer screening researchers have shown great interest in the potential of HPV testing for cervical cancer

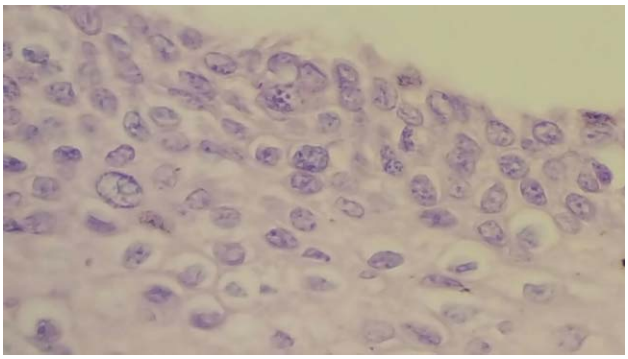

Fig. 5. High-power magnification of cervical biopsy. Epithelial cells with pleomorphic nuclei and nuclear enlargement are mainly at lower levels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

screening. It is likely that the rationale for establishing clinical guidelines based on HPV results has resulted from growing confidence in HPV testing.

In contrast to adolescents and premenopausal women with LSIL, some authors have suggested that performing the HrHPV test in postmenopausal women with LSIL might be useful for triage to colposcopy, since the prevalence of HPV infection is lower among female LSIL patients.[9,17] Another, larger study showed that the prevalence of HPV-positive diagnoses decreased with age among female LSIL patients (30–34 years, 88%; 60–64 years, 72%; $P = 0.0001$).[18] Therefore, specific triage tests are needed for postmenopausal LSIL to allow evaluation of women at increased risk of cervical cancer before any intervention. A search of the Cochrane database showed that, depending on the local prevalence, repeat cytology overlooks 23–57 female LSIL cases per 1,000, whereas the HPV test overlooks only 4 CIN 2 cases in a low-prevalence situation and 23 CIN 2+ cases in a high-prevalence situation per 1,000 female LSIL cases.[19] HrHPV triage may be useful for postmenopausal LSIL due to the low prevalence of HPV positivity in that population compared with younger women.

How much risk is actually being taken by deferring 1-year repeat cytological testing for HPV-negative postmenopausal LSIL patients?

The significance of management of postmenopausal LSIL remains unclear. The risk of HSIL after 5 years in HPV-negative LSIL cases aged 30–64 years has been reported to be 5.1% for CIN 2+ and 2.0% for CIN 3.[19] In another report, the CIN detection rate in female HPV-positive LSIL cases (aged 50 years and older) was significantly higher than that in HPV-negative LSIL cases (68.3% [56/82] and 45.8% [11/24], respectively). No women in the HPV-negative LSIL group had a follow-up tissue diagnosis of CIN 2 or 3.[10]

The Trial of Management of Borderline and other Low-grade Abnormal smears (TOMBOLA), conducted in the UK, found that a single HPV test in women aged 40

years and older could help in the decision regarding whether to conduct colposcopy or cytologic surveillance for low-grade lesions. However, this test was not useful for triage of younger women.[20] The ATHENA HPV Study concluded that women with LSIL should undergo HrHPV testing to detect high-grade cervical lesions and HrHPV-negative cases, to help avoid unnecessary referrals for immediate colposcopy and potential overtreatment of non-progressive lesions, especially for patients aged 40 years and older.[11]

In the present study, we conducted a colposcopic examination of a specific group of patients who were reflex HrHPV-negative and had a diagnosis of postmenopausal LSIL. At baseline screening (i.e., the date on which the patient applied for screening), we evaluated HSIL status on concomitant or immediate colposcopy: no lesions of CIN 2+ were detected, but a CIN 1 lesion was observed in 61% (33/54) of all postmenopausal LSIL cases.

Co-testing is recommended for the management of women with CIN 1 lesions at 1 year.[7] Whether immediate colposcopy or repeat co-testing at 1 year in postmenopausal LSIL patients may be regarded as having same risk for CIN 1. These strategies are supported by the spontaneous regression rates reported for CIN 1 lesions.[6]

Our findings regarding histopathologic detection of HSIL in postmenopausal LSIL cases with negative HPV test results are consistent with other reports.[21,22] Our results also showed that the negative predictive value of HrHPV-negative cases for detecting HSIL was 100%. These findings support reflex HPV testing as an alternative to routine colposcopic referral in older women.

We believe that triaging of postmenopausal LSIL cases with HrHPV testing could be useful to avoid overtreatment. Based on our observations and pertinent literature, we conclude that, when the HrHPV test is negative, the likelihood of a high-grade lesion is very low, such that follow-up with repeat cytologic evaluation at 12 months is a reasonable option.

Acknowledgment

Authors would like to thank Hakan ÇERMİK MD (Associate Professor Etimesgut Military Hospital Pathology Department) for helping during this study. The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see: http://www.textcheck.com/certificate/zGGHeR

References

1. The Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study Group (ALTS). Human papillomavirus testing for triage of women with cytologic evidence of low grade squamous intraepithelial lesions: Baseline data from a randomized trial. J Natl Cancer Inst 2000;92: 397–402.
2. Sengul D, Altinay S, Oksuz H, Demirturk H, Korkmazer E. Population-based cervical screening outcomes in turkey over a period of approximately nine and a half years with emphasis on results for women aged 30–34. Asian Pac J Cancer Prev 2014;15:2069–2074.
3. Wright TC Jr, Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D. 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. Am J Obstet Gynecol 2007;197:346–355.
4. Khuakoonratt N, Tangjitgamol S, Manusirivithaya S, et al. Prevalence of high grade squamous intraepithelial lesion (HSIL) and invasive cervical cancer in patients with low grade squamous intraepithelial lesion (LSIL) at cervical Pap smear. Asian Pac J Cancer Prev 2008;9:253–258.
5. American Society for Colposcopy and Cervical Pathology (ASCCP). 2012 Updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. J Lower Genital Tract Disease 2013;17:S11–S12.
6. Markowitz LE, Hariri S, Lin C, et al. Reduction in human papillomavirus (HPV) Prevalence among young women following HPV vaccine introduction in the United States, National Health and Nutrition Examination Surveys, 2003–2010. J Infect Dis 2013;208: 385–393.
7. Castle PE, Jeronimo J, Schiffman M, et al. Age-related changes of the cervix influence human papillomavirus type distribution. Cancer Res 2006;66:1218–1224.
8. Sherman ME, Schiffman M, Cox JT; ALTS Group. Effects of age and human papilloma viral load on colposcopy triage: Data from the randomized Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion Triage Study (ALTS). J Natl Cancer Inst 2002;94:102–107.
9. Datta SD, Koutsky LA, Ratelle S, et al. Human papillomavirus infection and cervical cytology in women screened for cervical cancer in the United States, 2003–2005. Ann Intern Med 2008;148: 493.
10. Zhao C, Zhao S, Heider A, Austin RM. Significance of high-risk human papillomavirus DNA detection in women 50 years and older with squamous cell papanicolaou test abnormalities. Arch Pathol Lab Med 2010;134:1130–1135.
11. Cuzick J, Cox T, Zhang G, et al. Human papillomavirus testing for triage of women with low-grade squamous intraepithelial lesions. Int J Cancer 2013;132:959–966.
12. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System: Terminology for reporting results of cervical cytology. J Am Med Assoc 2002;287:2114–2119.
13. Stafl A, Wilbanks GD. An international terminology of colposcopy: Report of the Nomenclature Committee of the International Federation of Cervical Pathology and Colposcopy. Obstet Gynecol 1991; 77:313–314.
14. Darragh TM, Colgan TJ, Cox JT, et al.; Members of LAST Project Work Groups. The lower anogenital squamous terminology standardization project for HPV-associated lesions: Background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. J Lower Genit Tract Dis 2012;16:205–242.
15. Brooks SE. Cervical cancer screening and the older woman: Obstacles and opportunities. Cancer Pract 1996;4:125–129.
16. Dresang LT. Colposcopy: An evidence-based update. J Am Board Fam Med 2005;18:383–392.
17. Katki HA, Schiffman M, Castle PE, et al. Five-year risks of CIN 2+ and CIN 3+ among women with HPV-positive and HPV-negative LSIL Pap results. J Lower Genit Tract Dis 2013;17: 43–49.
18. Arbyn M, Roelens J, Simoens C, et al. Human papillomavirus testing versus repeat cytology for triage of minor cytological cervical lesions. Cochrane Database Syst Rev 2013;3:CD008054. DOI: 10. 1002/14651858.

19. Katki HA, Schiffman M, Castle PE, et al. Benchmarking CIN3+ risk as the basis for incorporating HPV and Pap cotesting into cervical screening and management guidelines. J Lower Genit Tract Dis 2013;17:28–35.
20. Cotton S, Sharp L, Little J, et al. (Trial Of Management of Borderline and Other Low-grade Abnormal Smears Group TOMBOLA) The role of human papillomavirus testing in the management of women with low-grade abnormalities: Multicentre randomised controlled trial. BJOG 2010;117:645–659.
21. Bandyopadhyay S, Austin RM, Dabbs D, Zhao C. Adjunctive human papillomavirus DNA testing is a useful option in some clinical settings for disease risk assessment and triage of females with ASC-H Papanicolaou test results. Arch Pathol Lab Med 2008;132: 1874–1881.
22. Ronco G, Cuzick J, Segnan N, et al.; NTCC Working Group, HPV triage for low grade (LSIL) cytology is appropriate for women over 35 in mass cervical cancer screening using liquid-based cytology. Eur J Cancer 2007;43:476–480.