JOURNAL OF GYNECOLOGIC SURGERY
Mary Ann Liebert, Inc., Publishers

Diagnosing Human Papillomavirus of the Female Lower Genital Tract: Failure of the Pap Smear as a Sole Screening Test

NEAL M. LONKY, M.D.,[1] ANTOINETTE MAHONEY, M.D.,[2] and MARK V. SAUER, M.D.[3]

ABSTRACT

Of 197 patients referred for colposcopy who underwent repeat Pap smears and colposcopic biopsies (when indicated), histologic evidence of human papillomavirus (HPV) infection involving the endocervix, cervix, or vagina or all three sites was documented in 109 biopsies. Sixty-six (61%) had normal Pap smears at the time of colposcopy. Despite a specificity of 92% for detecting HPV, the Pap smear demonstrated a low sensitivity (39%), with a positive and negative predictive test value of 88% and 50%, respectively. In patients with biopsies revealing HPV infection without associated dysplasia, false negative Pap smears were found most often in women with strictly vaginal HPV (74%) ($P < 0.05$), followed by those with coexistent cervical and vaginal HPV (65%), and then by those with solely cervical HPV (51%). We question the use of the Pap smear for the detection of lower genital tract HPV, particularly in patients with only vaginal involvement, especially when the smear is repeated at the time of colposcopy. Benefits and disadvantages of other screening tests for HPV are discussed. (J GYNECOL SURG 7:183, 1991)

INTRODUCTION

SCREENING FOR CERVICAL CANCER is based on identifying and eradicating dysplasia prior to its progression to frank carcinoma. Cytologic screening remains the mainstay in the detection of women with pre-malignant (cervical intra-epithelial neoplasia) (CIN) and malignant changes of the cervix. Timely diagnosis of pre-malignant and malignant changes of the cervix has significantly reduced the morbidity and mortality associated with squamous cell carcinoma of the cervix.[1–3]

HPV is believed to be significantly associated with the neoplastic transformation of squamous cells in the lower genital tract.[4,5] HPV infection may involve viral replication and insinuation of viral genome within the infected host's nuclear genetic material.[6] The site of infection and subsequent neoplastic transformation is often multifocal. Evidence of HPV has been found in malignancies involving the cervix, vagina, and vulva, as well as premalignant lesions of these areas.[7–10]

Accuracy in diagnosing HPV infection depends on various skills. The clinical examiner must be able to visualize anatomic features suggestive of warty change. A carefully performed cytologic screen should reveal

[1]Departments of Obstetrics and Gynecology, Kaiser Permanente Anaheim, Anaheim, California.
[2]Department of Pathology, Kaiser Permanente Anaheim, California.
[3]Department of Obstetrics and Gynecology, University of Southern California, Los Angeles, California.

characteristic features, such as koilocytosis and dyskeratosis.[11] Results of molecular virology and DNA hybridization analysis may further identify potentially oncogenic subtypes.[12,13] Finally, histologic diagnosis of biopsy specimens of lesions should be confirmatory.[14] Although some investigators suggest that the Pap smear is adequate for the primary screening of HPV, others doubt its sensitivity and accuracy. Likewise, controversy surrounds the sensitivity of the Pap smear as the sole screening technique in detecting CIN, cervical carcinoma, and other premalignant lesions of the lower genital tract.[15,16]

Koilocytotic changes associated with HPV may decrease the sensitivity of the screening Pap smear for detecting CIN.[17] However, CIN and HPV often coexist. Studies have proven that cytologic evidence of HPV followed by colposcopic evaluation and directed biopsies improves the accuracy of detecting CIN when conditions occur concomitantly.[18,19] Thus, it may be advisable to perform colposcopy on all patients with cytologic evidence of HPV.[20]

The purpose of this investigation is threefold. First, we wish to evaluate the adequacy of the Pap smear as a screening method for the detection of HPV infection of the lower genital tract. Second, we wish to assess whether the Pap smear was a more sensitive screen for the population of patients with HPV and histologic evidence of CIN as opposed to HPV alone. Finally, we hope to determine what effect the site of HPV infection, be it vaginal, cervical, or both, has on the sensitivity and accuracy of the Pap smear.

SUBJECTS AND METHODS

Between July 1986 and April 1989, 197 consecutive women were referred to the Kaiser Permanente-Anaheim colposcopy clinic for evaluation of suspected lower genital tract pathology after noncolposcopic directed biopsy, suspicious lesion(s) seen on routine examination, or abnormal cervical cytology. Abnormal cytology was defined as any Pap smear that did not meet criteria for Class I. The distribution of referral diagnoses of the patients included in this study can be found in Table 1. At colposcopy, all patients first underwent repeat Pap smear tests. A wooden spatula was then used to scrape the cervical transformation zone, followed by endocervical sampling with an endocervical brush. Patients who had undergone prior total hysterectomy ($n = 7$) had upper vaginal cytologic samples obtained using only the spatula. The time interval between the initial abnormal Pap smear and subsequent colposcopic evaluation and repeat Pap testing was 21–90 days.

Following repeat Pap smear, colposcopy was performed after the application of dilute acetic acid to the cervix and vagina. Directed biopsies were taken when suspicious areas were visualized, and endocervical curettage was performed if colposcopic imaging was inadequate or when no exocervical lesions were visible.

Cytopathologists and histopathologists interpreting specimens were blinded as to the patients' identity so not to bias results. All histologic specimens were reviewed by a second pathologist at the termination of the study to confirm pathologic diagnoses.

RESULTS

Of the original 197 patients entered, 109 women (56%) demonstrated histologic evidence of HPV on biopsies of the endocervix, cervix, or vagina. All exhibited characteristic changes of HPV, such as

TABLE 1. REASONS FOR REFERRAL TO COLPOSCOPY CLINIC IN STUDY POPULATION

Method	*Diagnosis*	*n (197)*
Pap smear	CIN	64
	HPV	45
	CIN and HPV	5
	Inconclusive for pathology	69
Examination	Visible lesion on lower genital tract	8
	Positive noncolposcopic lower genital tract biopsy for CIN or HPV	6

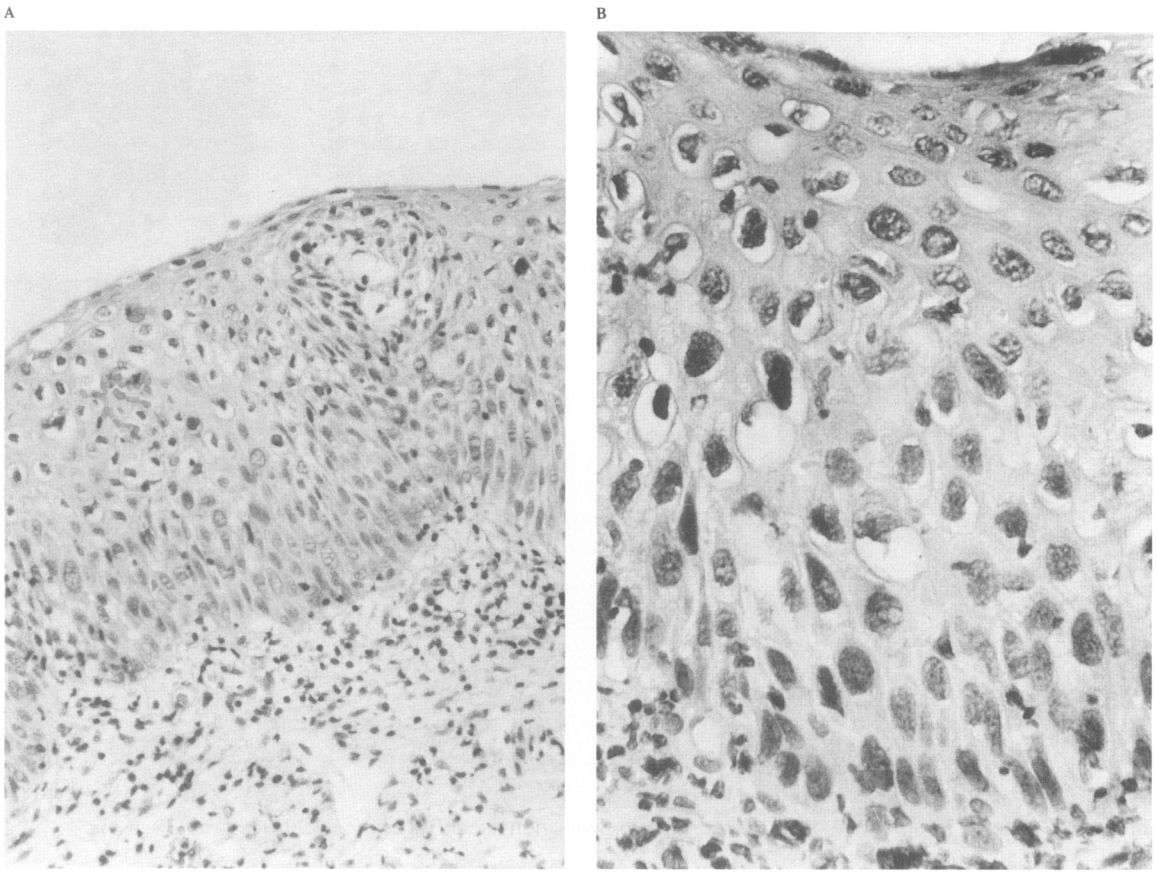

FIG. 1. Cervical biopsy in a patient with a negative Pap smear, showing features of HPV with CIN I at ×20 **(A)** and ×40 **(B)**.

koilocytosis, nuclear atypia, dyskeratosis, and acanthosis. Vaginal biopsies deemed abnormal similarly contained histologic correlates of HPV, as previously described.[21] Sixty-six of 109 patients with HPV proven on biopsy (61%) had normal cytology on their repeated (study) Pap smears (Fig. 1). Table 2 lists study Pap smear results for patients with concomitant (histologic) HPV and CIN and compares these findings to women with HPV without CIN. The study Pap smear was unable to detect the existing abnormality in 54/87 patients (62%) with only HPV on biopsy. This was not significantly different from the 55% (12/22) false negative Pap smear finding in patients with coexisting disease. However, of 22 patients with CIN without histologic features of HPV, only 6 (27%) had negative Pap smears.

The study Pap smear showed a low sensitivity (39%) yet high specificity (92%) for detecting HPV infection of the lower genital tract. The positive and negative predictive value of the Pap smear in this population was 88% and 50%, respectively (Table 3).

TABLE 2. PAP SMEAR SENSITIVITY AND DETECTION OF HPV OF LOWER GENITAL TRACT: INFLUENCE OF CONCOMITANT DYSPLASIA

	Positive HPV on biopsy		
	With CIN	*Without CIN*	*All patients*
Pap smear positive	10	33	43
Pap smear negative	12	54	66
Total	22	87	109
False negative rate	55%	62%	

NS ($p = 0.52$) by Chi square.

TABLE 3. SENSITIVITY, SPECIFICITY, POSITIVE AND NEGATIVE PREDICTIVE VALUE OF THE PAP SMEAR IN DETECTION OF HPV OF FEMALE LOWER GENITAL TRACT

	Colposcopy-directed biopsy		
Pap smear	*+ HPV*	*Negative*	*Total*
Abnormal	43	6	49
Negative	66	66	132
Total	109	72	181

Sensitivity = 39%.
Specificity = 92%.
Positive predictive value = 88%.
Negative predictive value = 50%.

Reviewing results of patients whose biopsies showed histologic evidence of HPV without associated CIN, the Pap smear was significantly least helpful in identifying those with only vaginal HPV involvement, with 74% false negatives. Patients with cervical and vaginal HPV demonstrated a 65% false negative rate, whereas women with only cervical HPV had 51% false readings (Fig. 2).

DISCUSSION

Identification of the patient with evidence of HPV allows the clinician to counsel the patient and her partner as to the potential for viral transmission. Also, the increased risk of developing neoplasia, followed by a discussion of the various treatment modalities available to the patient, may be addressed. Although a range of therapeutic modalities, such as laser vaporization,[22–24] cryosurgery,[25,26] intralesional injection of interferon,[27,28] and the application of 5-fluorouracil,[29,30] are available, success with regard to complete eradication of HPV and accurate diagnosis of recurrence posttherapy remains controversial.[31–36]

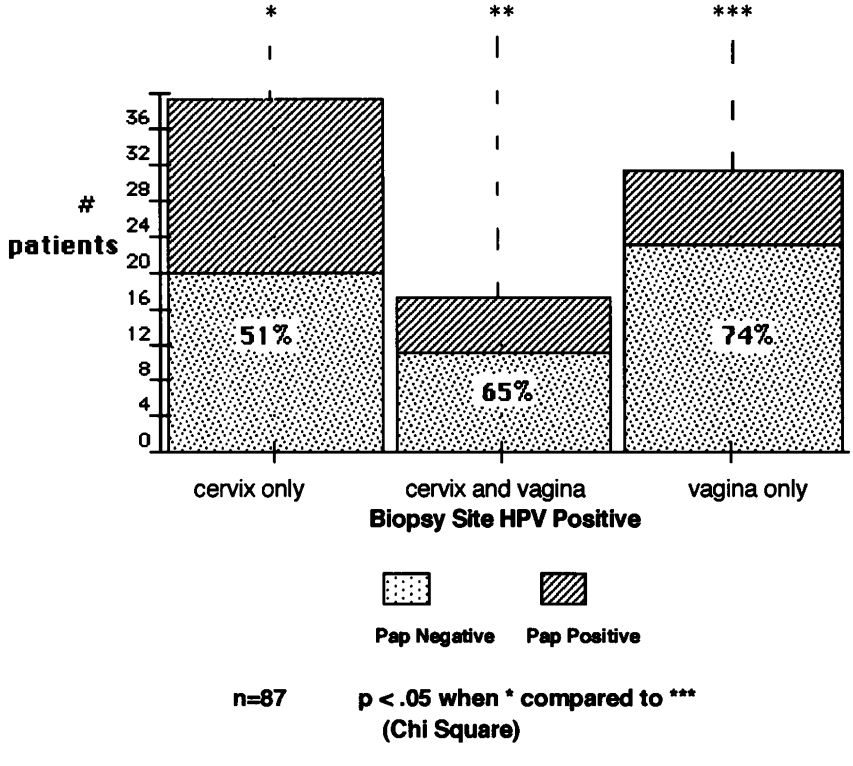

FIG. 2. Accuracy of the Pap smear. Detection of HPV of the lower genital tract with respect to lesion site.

Our study questions the sensitivity of the Pap smear when dysplasia and HPV coexist. Unless patients were diagnosed at primary screening, they are unlikely to ever undergo colposcopy, where the presence of HPV or dysplasia can be visualized and affected areas biopsied. We, therefore, did not include the referral (first) Pap smear in our analysis of the data, since it would reflect the bias of our selected colposcopy clinic population.

This study further suggests that the Pap smear is more sensitive as a screen for cervical HPV as opposed to vaginal HPV. Most likely, this results from the traditional sampling of the endocervix and cervical portio, where any vaginal cells obtained are from those sloughed onto the cervix or posterior fornix.

In patients with dysplasia, the value of repeat cytologic study during colposcopy has been considered misleading.[37] Up to a third of patients with normal cervical cytology on repeat examinations may have dysplasia when biopsied. Our study agrees with these findings. We limited our population to patients at high risk for abnormality. Thus, the likelihood of discovering pathology was increased.

The false negative rate of the Pap smear in our study actually may be underestimated because we consistently performed Pap smear examinations using a wooden spatula and endocervical brush, whereas these tools were not used consistently in the clinics that referred patients to the colposcopy clinic. Use of an endocervical brush has been shown to improve the quality and sensitivity of the cytologic sample obtained.[38] Previous reported reasons for failure of a Pap smear to detect existing abnormalities include improper specimen collection, douching before the examination, poor instrumentation, delayed fixation, improper specimen fixation, and poor laboratory performance.[39] Since repeat examinations were performed in identical fashion and processed by the same clinicians and staff, it is unlikely that these confounding variables had much effect on our results.

Until improvements in the primary gynecologic screening examination for HPV occurs, we cannot expect to reduce the true incidence of HPV in the community at large. Cytology combined with colposcopic screening remains the most comprehensive and accurate method for detecting and localizing lower genital tract HPV infection with or without associated dysplasia.[40,41] Colposcopic screening of patients referred for abnormal cervical cytology is of proven benefit in identifying lesions associated with HPV infection of the cervix and vagina and is a superior screen compared to cytology alone.[41,42] If performed concomitantly with primary cervical cytology, sensitivity of the primary cancer screening examination is improved.[43] The patient with a vaginal or cervical lesion can be counseled immediately. Those with false negative colposcopic findings benefit from further information afforded by the Pap smear. It has been suggested that follow-up examinations after therapy for CIN or condyloma are most accurately performed when patients are screened using cytology and colposcopy.[44]

Critics of colposcopy argue that the technique is expensive and time consuming, requires specialized training to perform, and is not universally available to all gynecologists.[45] Although simultaneous colposcopy and cervical cytology has been described for over 30 years as a screening method for detecting cervical dysplasia, it has not gained favor as a primary gynecologic screening procedure.[46] Furthermore, it is unlikely that this approach will gain popularity as a screening method in discovering viral infection, even though the presence of HPV is linked to the subsequent development of neoplasia.

Because of the difficulties inherent in the use of colposcopy, other methods, such as cervicography, DNA hybridization probe analysis of cervical cytologic samples, and use of the polymerase chain reaction technology,[47–50] have been suggested as primary screens. They may be technically difficult and not always readily available. Furthermore, although cervicography has been shown to improve the sensitivity of the primary cervical cancer screening examination,[45] it offers little information about the rest of the genital tract. Similar to a Pap test, results are not immediately available. This delay fosters noncompliance. In situ DNA hybridization studies for detecting HPV are sensitive but nonspecific with respect to the site of infection.[49] However, DNA typing may be of benefit when isolating HPV subtypes associated with oncogenic transformation.[51]

Our study suggests that gynecologic examinations miss a significant number of patients with HPV infection despite screening with cervical cytology. This is especially true of women with vaginal HPV involvement. Perhaps improvement can be achieved if a more comprehensive approach to screening is used. This might include consideration of the results of careful visual inspection of the entire lower genital tract (visual evidence of a suspicious papillomatous lesion of the lower genital tract), the inclusion of vaginal sampling into the cytologic screening technique, and scrutiny of patients with persistent clinical symptoms of HPV infection (such as recurrent, unexplained vaginal infections or the presence of chronic pruritus). This may lead to the performance of more confirmatory tests and will improve our ability to identify more accurately and counsel these patients with a sexually transmissible viral infection.

REFERENCES

1. Miller A, Lindsay J, Hill G. Mortality from cancer of the uterus in Canada and its relationship to screening for cancer of the cervix. Int J Cancer 1976;17:602.
2. Stafl A. Colposcopy. Cancer 1976;38(suppl):432.
3. Talebian F, Shayan A, Krumholz B, et al. Colposcopic evaluation of patients with abnormal cervical cytology. Obstet Gynecol 1976;49:670.
4. Reid R, Greenberg M, Jenson B, et al. Sexually transmitted papillomaviral infections. I. The anatomic distribution and pathologic grade of neoplastic lesions associated with different viral types. Am J Obstet Gynecol 1987;156:212–222.
5. Sutton G, Stehman F, Ehrlich C, Roman A. Human papillomavirus deoxyribonucleic acid in lesions of the female genital tract, evidence for type 6 and 11 in squamous carcinoma of the vulva. Obstet Gynecol 1987;70:564–568.
6. Howett M, Rapp F. Basic biology of papillomaviruses. Contemp Obstet Gynecol 1986;28:110–123.
7. Crum C, Kenberg H, Richart R, et al. Human papillomavirus type 16 and early cervical dysplasia. N Engl J Med 1981;310:880.
8. Schneider A, de Villers E, Schneider V. Multifocal squamous neoplasia of the female genital tract, significance of human papillomavirus infection of the vagina after hysterectomy. Obstet Gynecol 1987;70:294–298.
9. Spitzer M, Krumholz B, Seltzer V. The multicentric nature of disease related to human papillomavirus infection of the female lower genital tract. Obstet Gynecol 1989;73:303–311.
10. Wilbur D, Bonfiglio T, Stoler M. Continuity of human papillomavirus (HPV) type between neoplastic precursors and invasive cervical carcinoma: an in-situ hybridization study. Am J Surg Pathol 1988;12:182–186.
11. Koss L. Cytologic and histologic manifestations of human papillomavirus infection of the female genital tract and their clinical significance. Cancer 1987;60:1942–1950.
12. Burk R, Kasdish A, Calderia S, Romney S. Human papillomavirus infection of the cervix detected by cervico-vaginal lavage and molecular hybridization: Correlation with biopsy results and Papanicolaou smear. Am J Obstet Gynecol 1986;154:982.
13. Wagner D, Ikenberg H, Boehm N, Gissman L. Identification of human papillomavirus in cervical swabs by deoxyribonucleic acid in-situ hybridization. Obstet Gynecol 1984;64:767–772.
14. Saigo P. Cytology of condyloma of the uterine cervix. Semin Diagn Pathol 1986;3:204–210.
15. Coppelson L, Brown B. Estimation of the screening error rate from observed detection rates in repeated cervical cytology. Am J Obstet Gynecol 1974;119:953.
16. Koss L. The Papanicoloau test for cervical cancer detection: A triumph and a tragedy. JAMA 1989;261:737.
17. Woodman C, Yates M, Ward K, et al. The confounding effect of koilocytosis on the predictive accuracy of a cervical smear. J Obstet Gynecol 1988;8:345–348.
18. Carmichael J, Maskens P. Cervical dysplasia and human papillomavirus. Am J Obstet Gynecol 1989;160:916–918.
19. Gruenbaum A, Sedlis A, Sillman F, Fruchter R, Stanek A, Boyce J. Association of human papillomavirus infection with cervical intra-epithelial neoplasia. Obstet Gynecol 1983;62:448–455.
20. Richart R. Causes and management of cervical intra-epithelial neoplasia. Cancer 1987;60:1951–1959.
21. Nuovo G, Blanco J, Silverstein S, Crim C. Histologic correlates of papillomavirus infection of the vagina. Obstet Gynecol 1988;72:770.
22. Calkins JW, Masterson B, Magrina J, Cape C. Management of condylomata acuminata with the carbon dioxide laser. Obstet Gynecol 1981;59:105–112.
23. Ferenczy A. Laser therapy of genital condylomata accuminata. Obstet Gynecol 1984;63:703–706.
24. Krebs H, Wheelock J. The CO_2 laser for recurrent and therapy-resistant condylomata acuminata. J Reprod Med 1985;30:489–492.
25. Matsunaga J, Bergman A, Bhatia N. Genital condylomata acuminata in pregnancy, effectiveness, safety and pregnancy outcome following cryotherapy. Br J Obstet Gynaecol 1987;94:168–172.
26. Silva P, Micha J, Silva D. Management of condylma acuminatum. J Am Acad Dermatol 1985;13:457–463.
27. Eron L, Judson F, Tucker S, et al. Interferon therapy for condyloma acuminata. N Engl J Med 1986;315:1059–1064.
28. Gall S, Hughes CE, Trofatter K. Interferon for therapy of condyloma acuminatum. Am J Obstet Gynecol 1985;153:157–163.

29. Krebs H. Treatment of vaginal condylomata acuminata by weekly topical application of 5-fluorouracil. Obstet Gynecol 1987;70:68–80.

30. Krebs H. Treatment of vaginal intra-epithelial neoplasia with laser and topical 5-fluorouracil. Obstet Gynecol 1989;73:657–660.

31. Baggish M. Management of cervical intra-epithelial neoplasia by carbon dioxide laser. Obstet Gynecol 1981;60:377–384.

32. MacKnab J, Walkinshaw S, Cordiner J, Clements J. Human papillomavirus in clinically and histologically normal tissue in patients with genital cancer. N Engl J Med 1986;315:1052.

33. Richart R, Townsend D, Crisp W, et al. An analysis of "long-term" follow-up results in patients with cervical intra-epithelial neoplasia treated by cryotherapy. Am J Obstet Gynecol 1980;137:823–826.

34. Riva J, Sedlacek T, Cunnane M, Mangan CE. Extended carbon dioxide laser vaporization in the treatment of subclinical papillomavirus infection of the lower genital tract. Obstet Gynecol 1989;73:25–30.

35. Stuart G, Flagler E, Nation J, Duggan M, Robertson D. Laser vaporization of vaginal intra-epithelial neoplasia. Am J Obstet Gynecol 1988;158:240–243.

36. Townsend D, Richart R. Cryotherapy and carbon dioxide laser management of cervical intra-epithelial neoplasia, A controlled comparison. Obstet Gynecol 1983;61:75–78.

37. Wheelock J, Kaminski P. Value of repeat cytology at the time of colposcopy for the evaluation of cervical intra-epithelial neoplasia on papanicolaou smears. J Reprod Med 1989;34:815–817.

38. Boon ME, Alons-Van Kordelaar J, Rietveld-Scheffers P. Consequences of the introduction of combined spatula and cytobrush sampling for cervical cytology: Improvements in smear quality and detection rates. Acta Cytol 1986;29:264.

39. Rubio C. False negatives in cervical cytology. Can they be avoided? Acta Cytol 1981;25:199.

40. Patsner B, Mann W, Mitchell M, Zuna R, Chumas J. Accuracy of Pap smear and colposcopy in the detection of condyloma of the cervix. Colposc Gynecol Laser Surg 1987;3:81–85.

41. Schneider A, Sterzik K, Buck G, de Villiers E. Colposcopy is superior to cytology for the detection of early genital human papillomavirus infection. Obstet Gynecol 1988;71:236–241.

42. Paavonen J. Colposcopic findings associated with human papillomavirus infection of the vagina and cervix. Obstet Gynecol Surv 1985;40:185–189.

43. Navarre G. Screening with colposcopy in a prepaid health center. J Reprod Med 1982;27:98–100.

44. Falcone T, Ferenczy A. Cervical intraepithelial neoplasia and condyloma: An analysis of diagnostic accuracy of post-treatment follow-up methods. Am J Obstet Gynecol 1986;154:260–264.

45. Tawa K, Forsythe A, Cove J, Saltz A, Peters H, Watring W. A comparison of the Papanicolaou smear and the cervigram, sensitivity, specificity, and cost analysis. Obstet Gynecol 1988;71:229–235.

46. Navratil E, Burghardt E, Bajardi F, et al. Simultaneous colposcopy and cytology used in the screening for carcinoma of the cervix. Am J Obstet Gynecol 1958;75:1292.

47. Stafl A. Cervicography: A new method for cervical cancer detection. Am J Obstet Gynecol 1981;139:815–820.

48. Tidy J, Mason W, Farrell P. A new and sensitive method for screening for human papillomavirus infection. Obstet Gynecol 1989;74:410–413.

49. Ritter D, Kadish A, Sten H, Romney S, Villari D, Burk R. Detection of human papillomavirus deoxyribonucleic acid in exfoliated cervico-vaginal cells as a predictor of cervical neoplasia in a high-risk population. Am J Obstet Gynecol 1988;159:1517–1525.

50. Bauer HM, Yi Ting MS, Greer CE, et al. Genital human papillomavirus infection in female university students as determined by a PCR-based method. JAMA 1991;265:472–477.

51. Walker J, Bloss J, Shu-Yuan L, Berman M. Human papillomavirus genotype as a prognostic indicator in carcinoma of the uterine cervix. Obstet Gynecol 1989;74:781–787.

Address reprint requests to:
Neal M. Lonky, M.D.
Kaiser Permanente Medical Center
Department of Obstetrics and Gynecology
1188 N. Euclid Street
Anaheim, CA 92801