

Papanicolaou Smear Adequacy: The Effect Of The Sampling Sequence

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Abstract: Background: The purpose of this study was to determine the effect of ectocervical sampling methods and sequence on Papanicolaou smear adequacy.

Methods: A randomized clinical trial was performed using either a curved cytologic sampling brush or Ayre spatula for ectocervical sampling before or after a straight sampling brush for endocervical sampling.

Results: The adequacy of ectocervical smears, as measured by the presence of columnar cells or squamous metaplasia, differed significantly among the four techniques studied. When the Ayre spatula was used second, 115 (69 percent) of 167 ectocervical smears were adequate compared with 75 (48 percent) of 156 smears when it was used first. Likewise, when the curved brush was used second, 116 (67 percent) of 172 ectocervical smears were adequate compared with 80 (50 percent) of 160 smears when it was used first. One hundred ninety-six (59 percent) of 332 ectocervical smears obtained with a curved brush were adequate compared with 190 (59 percent) of 323 ectocervical smears obtained with a spatula.

Conclusions: The differences among the four ectocervical sampling techniques studied were due to the sequence of ectocervical sampling rather than the instrument utilized. We recommend that an ectocervical sample be obtained only after a straight brush is used to obtain an endocervical sample. (J Am Board Fam Pract 1993; 2:103-107.)

The adequacy of Papanicolaou smears obtained in various primary care settings increased from 35 to 84 percent to 79 to 98 percent with the introduction of brush instruments for endocervical canal sampling.¹⁻⁷ The endocervical brush improves the adequacy of Papanicolaou smears by obtaining more columnar and metaplastic squamous cells, indicating probable sampling of the transition zone, where cervical cancer and its precursors most frequently develop.⁸ The increased presence of columnar and metaplastic squamous cells on Papanicolaou smears has been associated with an increased detection of cervical cancer and its precursors.^{1,5,9-11} The greater number of abnormal smears recovered using the cytologic sampling brush has not been associated with an increase in the rate of false-positive smears.^{5,12} Thus, the endocervical brush improves the detection of cervical cancer and its precursors.

The transition zone between the columnar epithelium and the squamous epithelium can be on the ectocervix or in the endocervix.^{13,14} The transition zone migrates into the endocervix as a result of aging and pregnancy.¹⁵ Some false-negative Papanicolaou smears are a result of the transition zone being out of reach of the sampling instruments, either within the endocervical canal or on the periphery of the ectocervix.¹⁶ While the majority of cervical intraepithelial neoplasias involve the transition zone, approximately 10 percent are higher in the endocervical canal and 2 to 3 percent are located only on the ectocervix.^{8,17} Therefore, the ectocervix as well as the endocervix must be sampled in all patients.¹⁸

The curved brush for ectocervical sampling was introduced in an attempt to improve the adequacy of Papanicolaou smears.^{19,20} Both the curved brush and the Ayre spatula provide adequate ectocervical samples for Papanicolaou smears, as measured by the quantity of squamous cells.²⁰ A comparison is needed to determine which instrument provides a better yield of ectocervical columnar and metaplastic squamous cells, indicating that an ectocervical transition zone has been sampled. In addition, no studies have determined the effect of the sequence of sampling the endocervix

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and ectocervix on Papanicolaou smear adequacy. The purposes of this study, therefore, were to determine the effects of ectocervical sampling method and sequence on Papanicolaou smear adequacy, as measured by the presence of columnar or metaplastic squamous cells.

Methods

Subjects

Between July 1989 and June 1990, women coming to the Baylor Family Practice Center for routine or repeat Papanicolaou smears were asked to participate in the study. Patients who had had a hysterectomy or were currently pregnant were ineligible for the study. There were 663 eligible patients, and all agreed to participate; 323 patients were randomly assigned to the Ayre spatula and 332 patients to the curved brush for ectocervical sampling. Eight patients whose results could not be analyzed because of insufficient data were excluded. The participants' data were analyzed according to their assigned method as a member of either the Ayre spatula or curved brush group and further by whether the ectocervix or endocervix was sampled first. This design produced four study groups.

Procedure

Eligible patients who gave written informed consent were randomized (using a blocking factor of four) to receive a Papanicolaou smear using either the curved sampling brush or the Ayre spatula for ectocervical sampling either before or after a straight brush was used for endocervical sampling. Assignment was confirmed by placing the study instruments in individual packets. Patients completed a questionnaire requesting information about factors known to affect cell yields, including age, gravidity, parity, current hormonal therapy, menopausal status, and previous cervical surgery.^{15,21-23} A memorandum was sent to each physician regarding the purpose and procedure for the study, and a research assistant monitored compliance.

Using techniques previously described,^{4,19} the Papanicolaou smear was obtained by the faculty or resident physician with whom the patient's appointment was made. A water-lubricated speculum was used for the examination. If the cervix was covered with excessive mucus, it was gently cleansed with a large cotton swab.²² In the Ayre

spatula group, the larger portion of the irregular end of the Ayre spatula was inserted into the cervical os and rotated 360-degrees to obtain an ectocervical sample. In the curved brush group, the angle of curvature of the brush was adjusted to accommodate the shape of the patient's cervix. The curved brush then was placed on the ectocervix with the handle aligned with the external os and rotated 360-degrees to obtain an ectocervical sample. Nurses prepared the smear by rocking the sampling brush in a 180-degree arc while spreading it along a slide. In both groups, a straight brush was used for endocervical sampling, and that sample was submitted on a separate slide.

All slides were immediately sprayed with fixative and sent to one of two laboratories, depending on the patient's insurance. Cytotechnologists registered by the American Society of Clinical Pathologists analyzed all smears for the outcome variable—the presence or absence of columnar or metaplastic squamous cells. The cytotechnologists were blinded to the patient's ectocervical sampling technique assignment.

Results

Statistical Analysis

Frequencies, means, and standard deviations were calculated for all variables for the entire study group and within each diagnostic method using the SPSS/PC+ software package.²⁴ Student's t-test was used to compare the two groups for differences in age, gravidity, and parity. Chi-square analysis was used to compare the two groups for differences in menopausal status, oral contraception, previous cervical surgery, Papanicolaou smear classification, and presence or absence of endocervical cells.

Demographic Data

Table 1 provides descriptive statistics for the 655 patients studied. The mean age was 33.2 years for all participants. The mean gravidity was 1.3, and the mean parity was 0.9. Five hundred ninety-eight (91 percent) of the patients were premenopausal, and 57 (9 percent) were postmenopausal. Thirty patients (5 percent) had previous cervical surgery, and 224 (34 percent) were taking oral contraceptives. Although the difference in the age of patients was statistically significant (32.5 versus 34.5, $P < 0.05$), this difference was not clinically significant. There were no significant differences

Table 1. Demographic Characteristics of the Study Sample.

Variables	Entire Sample (n = 655)	Laboratory 1 (n = 409)	Laboratory 2 (n = 246)	Significance
	Mean \pm SD	Mean \pm SD	Mean \pm SD	
Age	33.2 \pm 10.8	32.5 \pm 11	34.5 \pm 10.4	$P < 0.05$
Gravidity	1.3 \pm 1.7	1.2 \pm 1.7	1.4 \pm 1.7	NS
Parity	0.9 \pm 1.4	0.9 \pm 1.5	1 \pm 1.4	NS
	No. (%)	No. (%)	No. (%)	
Postmenopausal	57 (9)	33 (8)	24 (10)	NS
Previous surgery on cervix	30 (5)	16 (4)	14 (6)	NS
Oral contraception	224 (34)	146 (36)	78 (32)	NS

NS = not significant.

between the experimental groups in any of the other demographic variables studied.

Primary Study Findings

Sampling the endocervix and then the ectocervix resulted in more ectocervical smears containing columnar or squamous metaplastic cells. In the curved brush group, 116 (67 percent) of 172 ectocervical samples obtained second contained columnar or metaplastic squamous cells compared with 80 (50 percent) of 160 ectocervical samples obtained first. In the Ayre spatula group, likewise, 115 (69 percent) of 167 ectocervical samples obtained second contained columnar or squamous metaplastic cells, compared with 75 (48 percent) of 156 ectocervical samples obtained first. The sequence of sampling, therefore, affected ectocervical smear adequacy.

Table 2. Effect of Sampling Sequence and Method on Ectocervical Smear Adequacy by Pathology Laboratory.

Sampling Sequence and Method	Adequacy			
	Laboratory 1*		Laboratory 2†	
	No.	Percent	No.	Percent
Curved brush first	54/92	59	26/68	38
Curved brush second	79/104	76	37/68	54
Ayre spatula first	55/103	53	20/53	38
Ayre spatula second	82/110	75	33/57	58

* $\chi^2 = 17.65$, $df = 3$, $P < 0.05$, power = 0.68 (curved brush) and 0.86 (Ayre spatula).

† $\chi^2 = 8.13$, $df = 3$, $P < 0.05$, power = 0.40 (curved brush) and 0.47 (Ayre spatula).

There was a significant difference between the two laboratories in the reporting of columnar or metaplastic squamous cells on ectocervical smears ($\chi^2 = 22.58$, $df = 1$, $P < 0.05$). Regardless of the laboratory, however, the sampling sequence continued to have a significant effect (laboratory 1: $\chi^2 = 17.65$, $df = 3$, $P < 0.05$; laboratory 2: $\chi^2 = 8.13$, $df = 3$, $P < 0.05$) (Table 2).

The curved brush was equivalent to the Ayre spatula for ectocervical sampling. Of the 332 ectocervical smears obtained with the curved brush, 196 (59 percent) contained columnar cells compared with 190 (59 percent) of the 323 smears obtained with the Ayre spatula. When the observed results were reanalyzed according to pathology laboratory, the method of ectocervical sampling continued to have no significant effect on ectocervical smear adequacy (Table 3).

Eighty-one of the ectocervical smears (19.8 percent) showed some abnormality. More abnormalities were detected when the ectocervical sample was obtained after the endocervical sample.

Table 3. Effect of Sampling Method on Ectocervical Smear Adequacy by Pathology Laboratory.

Sampling Method	Adequacy			
	Laboratory 1*		Laboratory 2†	
	No.	Percent	No.	Percent
Curved brush	133/196	68	63/136	46
Ayre spatula	137/213	64	53/110	48

* $\chi^2 = 0.57$, $df = 1$, $P > 0.05$.

† $\chi^2 = 0.08$, $df = 1$, $P > 0.05$.

Discussion

The improved yield of columnar and metaplastic squamous cells on the ectocervical smears that was achieved by sampling the endocervix and then the ectocervix could have been a result of the endocervical brush dragging columnar cells from the endocervical canal to the cervical os, where they were picked up by the instrument sampling the ectocervix. The increase in the number of ectocervical smears containing columnar or metaplastic squamous cells that was observed should improve the detection of cervical cancer and its precursors, however, because more potentially abnormal cells were available for pathology laboratory screening. If the transition zone were on the ectocervix, however, the increased number of columnar cells from the endocervix could dilute the number of potentially abnormal cells obtained from the ectocervix. Because the transition zone is located either in the endocervical canal or at the cervical os in the majority of women,¹⁴ however, this potential risk would be minimal compared with the benefit obtained by sampling the endocervix and then the ectocervix.

This study has several potential threats to internal validity. The Bethesda system²⁵ for reporting Papanicolaou smear results was adopted by one of the laboratories during the course of the study. This change, however, improved the reliability of the Papanicolaou smear reports from this laboratory by providing uniform criteria for reporting adequacy of the smears and any abnormalities. The results were not affected by repeated measurements because one sample was from the endocervix whereas the other was from the ectocervix. Observer bias was controlled by blinding the cytotechnologist to the method used to obtain the smear. Initially, two physicians were not compliant with the method assigned to their patients by randomization, choosing instead their own preferred method of sampling. This form of observer bias was extinguished by reeducation. The two subjects were not removed from the analysis because study instruments were utilized to obtain the smears. Bias caused by attrition of subjects was minimal, as the results from only 8 of the 663 patients randomized were not analyzed. In spite of these potential limitations, the findings of this study are consistent with those of related studies in other primary care settings¹⁻⁷ and thus should be generalizable.

Sampling of the transition zone could have been verified by performing colposcopy on every patient. Given the current practice in the United States of primarily using colposcopy to examine patients with abnormal Papanicolaou smears, it was believed that this method would have been unrealistic and less generalizable. Thus, presence of columnar or squamous metaplastic cells was used as the indicator that the transition zone had been sampled.

Several physicians participating in the study reported that the curved brush was difficult to use because it was difficult to maintain contact with the cervix while rotating the instrument in a 360-degree arc. Both the Ayre spatula and curved brush, however, provided equally adequate ectocervical smears. Because the Ayre spatula is less expensive, seems easier to use, and appears to cause no more bleeding than the curved brush, it could be the preferable choice for ectocervical sampling.

Conclusion

To increase the yield of columnar or squamous metaplastic cells on Papanicolaou smears, and thereby the detection of cervical cancer and its precursors, we recommend that an ectocervical sample be obtained only after a straight brush is used to sample the endocervix.

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References

1. Boon ME, Alons-van Kordelaar JJ, Rietveld-Schefers PE. Consequences of the introduction of combined spatula and Cytobrush sampling for cervical cytology. Improvements in smear quality and detection rates. *Acta Cytol* 1986; 30:264-70.
2. Reissman SE. Comparison of two Papanicolaou smear techniques in a family practice setting. *J Fam Pract* 1988; 26:525-9.
3. Deckert JJ, Staten SF, Palermo V. Improved endocervical cell yield with Cytobrush. *J Fam Pract* 1988; 26:639-41.
4. Noel ML. Papanicolaou smear adequacy: the cervical cytobrush and Ayre spatula compared with the extended-tip spatula. *J Am Board Fam Pract* 1989; 2:156-60.
5. Murata PJ, Johnson RA, McNicoll KE. Controlled evaluation of implementing the Cytobrush technique to improve Papanicolaou smear quality. *Obstet Gynecol* 1990; 75:690-5.

6. Ruffin MT 4th, Van Noord GR. Improving the yield of endocervical elements in a Pap smear with the use of the cytology brush. *Fam Med* 1991; 23:365-9.
7. Davey-Sullivan B, Gearhart J, Evers CG, Cason Z, Replogle WH. The Cytobrush effect on Pap smear adequacy. *Fam Pract Res J* 1991; 11:57-64.
8. Marsh M. Original site of cervical carcinoma. Topographical relationship of carcinoma of the cervix to the external os and to the squamocolumnar junction. *Obstet Gynecol* 1956; 7:444-52.
9. Elias A, Linthorst G, Bekker B, Vooijs PG. The significance of endocervical cells in the diagnosis of cervical epithelial changes. *Acta Cytol* 1983; 27:225-9.
10. Vooijs PG, Elias A, van der Graaf Y, Veling S. Relationship between the diagnosis of epithelial abnormalities and the composition of cervical smears. *Acta Cytol* 1985; 29:323-8.
11. Woodman CB, Williams D, Yates M, Tomlinson K, Ward K, Luesley D. Indicators of effective cytological sampling of the uterine cervix. *Lancet* 1989; 2:88-90.
12. Van Erp EJ, Blaschek-Lut CH, Arentz NP, Trimpos JB. Performance of the Cytobrush in patients at risk for cervical pathology: does it add anything to the wooden spatula. *Eur J Gynaecol Oncol* 1988; 9:456-60.
13. Fluhmann CF. The squamocolumnar transitional zone of the cervix uteri. *Obstet Gynecol* 1959; 14:133-48.
14. Frost JK. Diagnostic accuracy of "cervical smears." *Obstet Gynecol Surv* 1969; 24:893-908.
15. Ostergard DR. The effect of age, gravidity, and parity on the cervical squamocolumnar junction as determined by colposcopy. *Am J Obstet Gynecol* 1977; 129:59.
16. Spurrett B, Ayer B, Pacey NF. The inadequacies of instruments used for cervical screening. *Aust NZ J Obstet Gynecol* 1989; 29:44-6.
17. Abdul-Karim FW, Fu YS, Reagan JW, Wentz WB. Morphometric study of intraepithelial neoplasia of the uterine cervix. *Obstet Gynecol* 1982; 60:210-4.
18. Richart RM, Vaillant HW. Influence of cell collection techniques upon cytological diagnosis. *Cancer* 1965; 18:1474-8.
19. Kawaguchi K, Nogi M, Ohya M, Nishikawa Y, Kobayashi TK. The value of the Cytobrush for obtaining cells from the uterine cervix. *Diagn Cytopathol* 1987; 3:262-7.
20. Dotters DJ, Carney CN, Droegemueller W. Nylon brush improves collection of cervical cytologic specimens. *Am J Obstet Gynecol* 1988; 159:814-9.
21. Allingham JD, King A. Patient characteristics and endocervical cell recovery on Papanicolaou smears. *J Fam Pract* 1985; 20:185-90.
22. Hamblin JE, Brock CD, Litchfield L, Dias J. Papanicolaou smear adequacy: effect of different techniques in specific fertility states. *J Fam Pract* 1985; 20:257-60.
23. Vooijs PG, Van der Graaf Y, Elias AG. Cellular composition of cervical smears in relation to the day of the menstrual cycle and the method of contraception. *Acta Cytol* 1987; 31:417-26.
24. SPSS/PC+ software package, version 2.0. Chicago: SPSS, Inc., 1988.
25. The 1988 Bethesda System for reporting cervical vaginal/cytological diagnoses. National Cancer Institute Workshop. *JAMA* 1989; 262:931-4.