Journal of Cellular Biochemistry, Supplement 23:147-150 (1995)

Quantitative Cytopathology of Endometrial Lesions

Fumiaki Tezuka, MD, Ryoji Chiba, MD, and Tohru Takahashi, MD

Department of Pathology, Institute of Development, Aging and Cancer, Tohoku University, Sendai 980, Japan

Abstract Morphometric and multivariate statistical methods were used to discriminate endometrial carcinoma from benign cells in cytologic studies. Clumps of epithelial cells that appeared most diagnostically relevant were selected from aspirated samples of 70 endometrial cancer patients. The cells' cytologic character was reduced to a combination of five quantitative parameters—nuclear size, degree of anisokaryosis, nuclear form index, homogeneity of nuclear chromatin texture, and regularity of nuclear arrangement. The 5-variate cluster analysis demonstrated that the 70 cases could be classified into three definite groups: Group A (17 cases) was characterized by cells of small nuclear size, slight anisokaryosis, homogeneous chromatin texture, and regular nuclear arrangement; Group C (12 cases) by cells of large nuclear size, marked anisokaryosis, heterogeneous chromatin texture, and irregular nuclear arrangement; and Group B (41 cases) by cells of intermediate parameter values. Group C was derived from 10 cases of adenocarcinoma and 2 of atypical hyperplasia, while Groups A and B were not derived from any cases of malignancy. The computer-assisted morphometric statistical method can objectively classify the endometrial cells into malignant and benign, with improved validity and reproducibility. The cytopathologic finding, if detected by this method, may serve as a surrogate endpoint biomarker.

Key words: Aspiration cytology, cluster analysis, computer-assisted image analysis, endometrial carcinoma, morphometry, multivariate analysis

A number of possible candidates could be surrogate biomarkers of endometrial carcinoma, including cytopathologic findings. Indeed, cytopathology provides us with practical markers of carcinogenesis. However, considerable interobserver variation exists, especially in early detection of endometrial carcinoma [1]. This inconsistency is partly because cytologic slides are interpreted subjectively through pattern recognition, and partly because there are currently no generally accepted quantitative criteria for classifying endometrial cells into benign and malignant categories [2]. Some authors believe cellular or nuclear size could be the most important indicator of malignancy because the mean and standard deviation are significantly larger in carcinoma cells [3–5]. However, values in different conditions may overlap to such a degree as to render the cellular or nuclear size an unreliable diagnostic criterion [3,5,6]. Classifying endometrial epithelial cells into benign and malignant categories based on cellular or nuclear size alone has serious limitations.

These observations suggest that the cytologic diagnosis of malignancy should not depend on single parameters, but on a comprehensive recognition of multiple variables [6]. We tried to discriminate carcinoma cells from benign cells in endometrial cytologic studies using both morphometry and multivariate statistical analysis.

Address correspondence to Fumiaki Tezuka, MD, Department of Pathology, Institute of Development, Aging and Cancer, Tohoku University, 4-1 Seiryomachi, Aoba-ku, Sendai 980, Japan.

CYTOLOGIC SAMPLES AND MORPHOMETRY

Our trial examined data from 70 cases in which cytopathologic and histopathologic diagnoses were made almost simultaneously. Histopathologically, they included 10 cases of well-differentiated adenocarcinoma (grade 1), 4 cases of adenomatous and atypical hyperplasia, and 56 "normal" controls. These were chosen in a mass survey from cases that were free from endometrial hyperplasia and carcinoma. In each case, endometrial samples were aspirated by the endocyte technique, fixed in 95% ethanol, and stained by the Papanicolaou procedure. Clumps of more than 100 epithelial cells that seemed the most diagnostically relevant in each case were selected microscopically for morphometric analysis.

The cytologic character of epithelial clumps was characterized quantitatively by a combination of five parameters: nuclear size (mean area, meanarea); degree of anisokaryosis (covariance of areas, cvarea); nuclear form (mean form index, FI); homogeneity of nuclear chromatin texture (homog); and regularity of nuclear arrangement (distribution pattern index, DPI). The definition and measurements of these parameters are detailed in our previous publications [7–9].

MULTIVARIATE STATISTICAL ANALYSIS

Cluster Analysis

After the cytologic character was reduced to a combination of five morphometric parameters, the data were submitted to 5-variate cluster analysis to examine the classifiability of endometrial cells [9–14]. The standard 5-D Euclidean distance was used as a between-individual deviance and Ward's method was used for grouping [9,12].

The result of 5-variate cluster analysis demonstrated that 70 cases were ultimately classifiable into three groups, A (17 cases), B (41 cases) and C (12 cases). From the mean and standard deviation of the parameters, each group could be characterized quantitatively. The first group (A) included cells of small nuclear size (meanarea = 26.5 ± 12.5 μm^2), slight anisokaryosis (cvarea = 2.47 ± 0.45), homogenous chromatin texture (homog = 0.68 ± 0.08), and regular isodistant nuclear arrangement (DPI = 0.79 ± 0.03), while the third group (C) was comprised of cells with large nuclear size (meanarea = 48.5 ± 9.3 μm^2), marked anisokaryosis (cvarea = 3.31 ± 0.66), heterogeneous chromatin texture (homog = 0.50 ± 0.07), and irregular nuclear arrangement (DPI = 0.70 ± 0.02). The second group (B) included cells

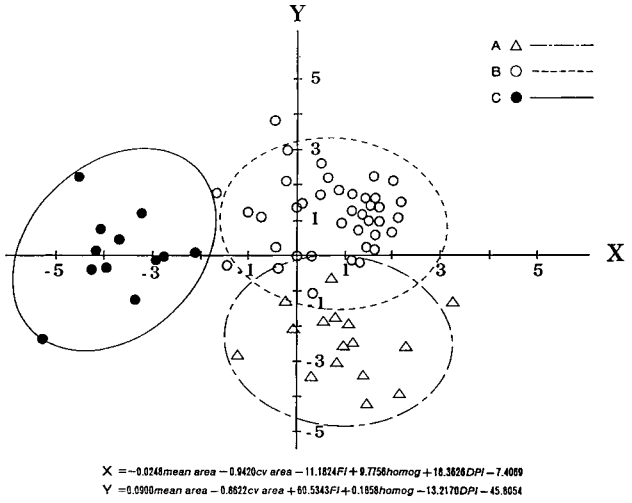

Fig. 1. A scattergram of canonical discriminant analysis [9]. It demonstrates that 70 cases are apparently clustered in three separate territories, each of which is enclosed by a regression ellipse of a 95% confidence limit. They correspond to Groups A, B and C, respectively.

of intermediate values for all parameters. Nuclear shape (FI) did not seem to be an important discriminator among the three groups.

Canonical Discriminant Analysis

The validity of cluster analysis was tested by canonical discriminant analysis. It created linear equations from the original parameters to maximize the between-group variation and visualized how definitely the groups were separated in a 2-D scattergram [9,11]. Figure 1 is a scattergram of 70 cases, where X and Y are the first and second canonical variates, respectively. Seventy cases were clustered into three separate territories corresponding to Groups A, B and C, respectively. The separation among the three groups was not ambiguous, although regression ellipses of a 95% confidence limit showed slight overlapping between A and B.

TABLE I. Histopathologic Diagnosis and Cluster Analysis

Histopathologic Diagnosis	Cluster Analysis		
	A	B	C
Normal	17 (100%)	39 (95.1%)	0
Hyperplasia	0	2 (4.9%)	2 (16.7%)
Adenocarcinoma	0	0	10 (83.3%)
TOTAL	**17**	**41**	**12**

Comparison of Cluster Analysis and Histopathologic Diagnosis

The three-group classification by cluster analysis was compared with histopathologic diagnosis as shown in Table I [9]. Seventeen cases of Group A were all histopathologically "normal". Group B included 39 normal cases (95.1%) and two cases of adenomatous hyperplasia (4.9%). No cases of adenocarcinoma were included in Groups A and B. In contrast, Group C included 10 cases of adenocarcinoma (83.3%) and two of atypical hyperplasia (16.7%).

The present study was comprised of four cases of endometrial hyperplasia. They were not relegated to a single group, but were assigned to two groups (B and C). The cytologic character of the two cases assigned to Group C appeared very similar to that of other cases of histopathologic adenocarcinoma.

Multigroup Linear Discriminant Analysis

The reproducibility of cluster analysis was measured by multigroup linear discriminant analysis, using linear discriminant formulae [11]. Table II shows the result of this analysis. The rates of correct re-discrimination using these formulae were 94.1%, 92.7%, and 100.0% for Groups A, B and C, respectively.

CONCLUSION

The results demonstrated that endometrial epithelial cells were objectively classifiable ac-

TABLE II. Reproducibility of the Three-Group Classification Tested by Multigroup Linear Discriminant Analysis

Group	No. of cases	No. rediscriminated*			Rate of correct rediscrimination	
		A	B	C	Number	%
A	17	16	1	0	16	94.1
B	41	38	1	2	38	92.7
C	12	0	0	12	12	100.0

* Discriminant formulae for Group A, B and C:
A = 2.70 meanarea + 38.88 cvarea + 4414.82 FI + 190.41 homog – 50.25 DPI – 2076.55;
B = 3.02 meanarea + 35.95 cvarea + 4626.32 FI + 189.37 homog – 100.26 DPI – 2230.35;
C = 3.02 meanarea + 41.17 cvarea + 4605.49 FI + 145.87 homog – 167.82 DPI – 2149.32.

cording to their morphologic characters, and that morphometric-statistical analysis could classify them into three groups. Comparing this classification with the histopathologic diagnosis revealed, with a small number of exceptions, that Group C corresponded to carcinoma, while Groups A and B corresponded to non-carcinoma.

This classification proved to be valid and reproducible, according to tests of both canonical and multigroup linear discriminant analyses. Endometrial cancer cells can be detected using the discriminant formulae obtained from multigroup linear discriminant analysis. Thus, the morphometric-multivariate statistical method can be of great help in improving the cytodiagnostic validity and reproducibility of endometrial carcinoma. If detected by this method, the cytopathologic finding may serve as a surrogate biomarker of cancer.

REFERENCES

1. Tezuka F, Namiki T, Higashiiwai H: Observer variability in endometrial cytology using kappa statistics. J Clin Pathol 45:292–294, 1992.
2. Reagan JW: Can screening for endometrial cancer be justified? Acta Cytol 24:87–89, 1980.
3. Fu YS, Ferenczy A, Huang I, Gelfand MM: Digital imaging analysis of normal, hyperplastic and malignant endometrial cells in the endometrial brushing samples. Analyt Quant Cytol 10:139–149, 1988.
4. Ng ABP, Reagan JW, Cechner RL: The precursors of endometrial cancer: A study of their cellular manifestations. Acta Cytol 17:439–448, 1973.
5. Skaarland E: Nuclear size and shape of epithelial cells from the endometrium: Lack of value as a criterion for differentiation between normal, hyperplastic, and malignant conditions. J Clin Pathol 38:502–506, 1985.
6. Baak JPA, Nauta JIP, Wisse-Brekelmans ECM, Bezemer PD: Architectural and nuclear morphometrical features together are more important prognosticators in endometrial hyperplasia than nuclear morphometrical features alone. J Pathol 154:335–341, 1988.
7. Tezuka F, Sato I, Higashiiwai H, Endo N, Ito K, Kasai M: Method for quantitative evaluation of the distribution pattern of nuclei in normal and malignant endometrial epithelia. Analyt Quant Cytol 12:237–241, 1990.
8. Tezuka F, Higashiiwai H, Namiki T: Quantitative analysis of nuclear distribution pattern differentiating carcinoma from hyperplasia in endometrial cytology. Am J Clin Pathol 96:648–653, 1991.
9. Tezuka F, Chiba R, Takahashi T: Morphometric and multivariate statistical detection of cancer cells in endometrial cytology. Analyt Quant Cytol Histol 16:332–338, 1994.
10. Kendall MG (ed): "Multivariate Analysis." London: Charles Griffin & Co. Ltd, 1980, pp 31–46.
11. Johnson RA, Wichern DW: "Applied Multivariate Statistical Analysis." Prentice-Hall: 2/E, 1988. (Translated into Japanese by Nishida T, published by Gendai Sugakusha, Kyoto, 1992, pp 637–693.)
12. Ward Jr JH: Hierarchical grouping to optimize an objective function. J Am Stat Assoc 58:236–244, 1963.
13. Anderberg MR: "Cluster Analysis For Applications." New York: Academic Press, 1973. (Translated into Japanese by Nishida H, published by Uchida Rokakuho, 1988, pp 170–278.)
14. Hawkins DM: "Topics In Applied Multivariate Analysis." London: Cambridge University Press, 1981. (Translated into Japanese by Igakutokei Kyokai, published by MPC, Toyko, 1988, pp 325–389.)