

Histopathological Statistical Discrimination Between Well Differentiated Type of Endometrial Adenocarcinoma and Endometrial Adenomatous Hyperplasia

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Summary. The histopathological distinction was statistically examined between an endometrial adenomatous hyperplasia (aH) and a well differentiated type of invasive endometrial adenocarcinoma (wC). The significant morphometric indices were disclosed as follows: (1) indices related to a structural atypism, based on the index of glandular area per unit area (IGA), the index of cellular area per unit area (ICA), and the index of gland-in-gland configuration per unit area (IGG), which were higher in the wC with the significant levels of 0.001, 0.001, and 0.02, respectively (t-test); (2) indices related to a cellular atypism, being made up of the mean nuclear long axial diameter (NLAD), the mean nuclear short axial diameter (NSAD), the mean nuclear area (NA), and the mean nucleolar maximal diameter (nMD), which were larger in the wC with the statistical significances of 0.1, 0.02, 0.05, and 0.05, respectively (t-test); and (3) the mean age of the wC was older than that of the aH with the significance of 0.001 (t-test). Furthermore, the multivariate analysis with the use of these reliable indices led five perfect linear discriminants. It is concluded that the multivariate analysis is valuable for the histopathological distinction between the wC and the aH.

INTRODUCTION

The endometrial hyperplasia is classified into cystic glandular hyperplasia (cH), adenomatous hyperplasia (aH), and atypical hyperplasia (AH).¹⁻⁶ In the morphological continuum from hyperplasia to carcinoma, the AH is judged as a borderline malignancy between the AH and a well differentiated type of endometrial adenocarcinoma (wC). For the histopathological diagnosis of malignancy, stromal invasion is thought to

be the most reliable histological criterion. However, the stromal invasion is often lacking in a well differentiated type adenocarcinoma, and furthermore, a pseudo-invasion has been difficult to distinguish from a true invasion in a small biopsied specimen. Hence, no evidence of stromal invasion could not always indicate non-carcinomatous nature and vice versa; the AH, diagnosed histologically because of a lack of invasion, always had the possibility of being confused with the wC. As for accuracy and reproducibility, the examined specimens in this study were restricted to histologically authentic cases (aH and wC) excluding the AH as a borderline malignancy.^{5,7,8} The cases of aH were confirmed by the examination of either the repeated full currettaged specimens after hormonal curettage or extirpated uteri, and those of wC were diagnosed by the findings of obvious invasion in extirpated uteri.

However, the histochemical examination of wC may be useful for diagnosis, the judgement of stainabilities still may remain equivocal according to each pathologist's judgement.³⁻⁵ The histopathological studies with the digital quantifications on several indices for atypism have been reported.^{4,5,7,8} In this study, the indices of structural atypism were numerically analyzed and the functions discriminating between the aH and the wC were investigated.

MATERIALS AND METHODS

The examined cases consisted of five normal proliferative endometria (pN), eleven cH, seven aH, and eight

wC. The difference in the age of cases was statistically significant (Table 1). Sections from the formalin-fixed and paraffin-embedded blocks were stained with hematoxylin and eosin. The specimens of pN were obtained from extirpated uteri which were resected because of myoma uteri. In the cH and the aH, each examined area was the field of the *severest* atypism in each series of repeated biopsied specimens or the extirpated uterus. The compared area used as each wC was the region of the *least* atypism in frankly invasive lesions. Hence, in this histopathological study of aH and wC, the definitive wC of the *least* atypism

was compared with the reliable aH of the *severest* atypism.

Concerning the measurement of structural atypism, each specimen was first photographed under the $\times 10$ objective lens with a microscope. The photographs then were enlarged 25 times and were traced on sheets of graph-paper, and this procedure magnified the photos five times. Finally indices were calculated on each sheet of graph-paper with three thousand units to count (5 mm-square/unit). These indices consisted of (1) the index of glandular area per unit area (IGA), (2) the index of cellular area per unit area (ICA), (3) the index of

Table 1. Morphometric values and their statistical significance among various endometria of normal proliferative phase, cystic hyperplasia, adenomatous hyperplasia, and well differentiated type endometrial adenocarcinoma

lesions	cases	age	IGA	ICA	INS	IGG	NLAD	NSAD	NA	nMD
pN	5	40±2.9	16.5±7.01	12.7±3.40	1.52±0.110	0.970±0.080	9.00±1.58	4.56±0.768	32.3±8.17	0.800±0.410
cH	11	52±7.3	37.5±14.2	21.4±7.57	1.91±0.360	1.15±0.170	8.12±1.44	5.23±0.663	34.3±9.66	0.640±0.327
aH	7	45±5.8	45.7±7.27	28.9±4.11	2.20±0.750	1.60±1.04	8.64±1.50	5.24±0.555	36.4±8.29	0.561±0.209
wC	8	62±9.6	60.9±12.2	50.9±16.2	2.51±0.350	2.19±1.09	10.3±1.91	6.59±1.23	55.7±21.2	1.20±0.645
p		0.001	0.001	0.001		0.02	0.1	0.02	0.05	0.05

lesions: pN; normal proliferative phase endometrium, cH; cystic endometrial hyperplasia, aH; adenomatous endometrial hyperplasia, wC; well differentiated type endometrial adenocarcinoma, cases: number of cases, morphometric indices: IGA; index of glandular area per unit area, ICA; index of cellular area per unit area, INS; index of nuclear stratification per unit area, IGG; index of gland-in-gland configuration per unit area, NLAD; mean nuclear long axial diameter, NSAD; mean nuclear short axial diameter, NA; mean nuclear area, nMD; mean nucleolar maximal diameter, statistical analyses: p; values of statistical significance between aH and wC in t-test analyses.

Table 2. Discriminant analysis between adenomatous hyperplasia and well differentiated type endometrial adenocarcinoma

indices	X ₁ age	X ₂ IGA	X ₃ ICA	X ₄ INS	X ₅ IGG	X ₆ NLAD	X ₇ NSAD	X ₈ NA	X ₉ nMD	Constant	correct discrimi- nation ratio (aH/wC)
p(t-test)	0.001	0.001	0.001		0.02	0.1	0.02	0.05	0.05		
Q ₁ (X)		0.04999	-0.42731	4.24527			-1.60017	-0.03348	-6.12846	22.15320	1.000/1.000
Q ₂ (X)		-0.02583	-0.34042		3.06000		-4.28872	0.14162	-5.61535	34.46190	1.000/1.000
Q ₃ (X)		-0.07446	-0.28840				-0.26856	-0.04769	-6.42634	24.90360	1.000/1.000
Q ₄ (X)		-0.00446	-0.25497		2.43397		-3.19494			25.89620	1.000/0.875
Q ₅ (X)		0.08570	-0.34808	4.44041			-3.59283			21.54920	1.000/0.875
Q ₆ (X)							-1.64334	0.04137	-1.34555	9.00269	1.000/0.875
Q ₇ (X)		-0.72687	-0.12679	-0.13055	8.42251		-6.55062			72.59260	1.000/1.000
Q ₈ (X)		-0.63286	-0.25638	0.00200	4.55247		-5.52132			73.81120	1.000/1.000

Q_n (X): linear discriminant functions (LDF), X-factors (X_n): corresponded to each index, p(t-test): values of statistical significance between aH and wC in t-test analyses, each value: coefficient of Q_n (X); ex. Q₁ (X)=0.04999X₂-0.42731X₃+4.24527X₄-1.60017X₇-0.03348X₈-6.12846X₉+22.15320, correct discrimination ratio (aH/wC): numerator representing the correctly discriminated numbers of aH cases in total numbers of aH cases by each Q (X) and denominator showing the correctly discriminated numbers of wC cases in total numbers of wC cases by each Q (X), each abbreviation: same as Table 1.

nuclear stratification per unit area (INS), and (4) the index of gland-in-gland configuration per unit area (IGG). Each index was calculated as follows (Fig. 1, 2):

$$(1) \text{ IGA} = \frac{\text{Sum of square-units of whole glandular area}}{3000 \text{ (total square-units of a graph paper)}} \times 100$$

$$(2) \text{ ICA} = \frac{\text{Sum of square-units of whole glandular cellular area}}{3000} \times 100$$

$$(3) \text{ INS} = \frac{\text{Total number of nuclei of glandular cells}}{\text{Total number of nuclei lining up on the basement membrane}}$$

$$(4) \text{ IGG} = \frac{\text{Total number of glandular lumens}}{\text{Total number of glands}}$$

Regarding the measurement of cellular atypism, each specimen was microscopically photographed under the $\times 40$ objective lens. Secondly, this photo was printed 25 times enlarged, which was calculated lastly for indices as follows (Fig. 2): (1) the mean nuclear long axial diameter (NLAD), (2) the mean nuclear short axial diameter (NSAD), (3) the mean nuclear area (NA), and (4) the nucleolar maximal diameter (nMD). The nuclear short axis located at a right to the nuclear long axis and the NLAD was the longest diameter in each nucleus. Each nuclear area was briefly calculated by $3.14 \times (\text{NLAD}/2) \times (\text{NSAD}/2)$.

The multivariate analysis was performed by a digital computer (personal computer: PC-9801 VX, NEC, TOKYO, Soft disk: PC-9801/E/F/M/VF/VM/U/UV/VX/XL, 5''2HD, Kyouritsu Press Co., Tokyo).⁷⁾

RESULTS

The values of morphometric indices corresponded with the development of lesions (Table 1). Concerning the indices of structural atypism, the significant differences between the aH and the wC were confirmed in the IGA, the ICA, and the IGG ($p=0.001$, 0.001 , and 0.02 , respectively in t-test). As for the INS, there was no statistical difference between the aH and the wC. Regarding the cellular atypism, the wC was statistically differentiated from the aH by the NLAD, the NSAD, the NA, and the nMD with the critical rates of 0.1 , 0.02 , 0.05 , and 0.05 , respectively (t-test). Furthermore, the age of cases was one of the most important discriminating factors ($p=0.001$). Hence the above significant indices were valuable for

the differentiation of the wC from the aH.

The multivariate statistical analysis was performed with the following factors: age, (X_1); IGA, (X_2); ICA, (X_3); INS, (X_4); IGG, (X_5); NLAD, (X_6); NSAD, (X_7); NA, (X_8); nMD, (X_9). Linear discriminants were then drawn as the following $Q_n(X)$ (Table 2):

- (1) $Q_1(X) = 0.04999X_2 - 0.42731X_3 + 4.24527X_4 - 1.60017X_7 - 0.03348X_8 - 6.12846X_9 + 22.15320$,
- (2) $Q_2(X) = -0.02583X_2 - 0.34042X_3 + 3.06000X_5 - 4.28872X_7 + 0.14162X_8 - 5.61535X_9 + 34.46190$,
- (3) $Q_3(X) = -0.07446X_2 - 0.28840X_3 - 0.26856X_7 - 0.04769X_8 - 6.42634X_9 + 24.90360$,
- (4) $Q_4(X) = -0.00446X_2 - 0.25497X_3 + 2.43397X_5 - 3.19494X_7 + 25.89620$,
- (5) $Q_5(X) = 0.08570X_2 - 0.34808X_3 + 4.44041X_4 - 3.59283X_7 + 21.54920$,
- (6) $Q_6(X) = -1.64334X_7 + 0.04137X_8 - 1.34555X_9 + 9.00269$,
- (7) $Q_7(X) = -0.72687X_1 - 0.12679X_2 - 0.13055X_3 + 8.42251X_4 - 6.55062X_7 + 72.59260$,
- (8) $Q_8(X) = -0.63286X_1 - 0.25638X_2 + 0.00200X_3 + 4.55247X_5 - 5.52132X_7 + 73.81120$.

As to the discriminants of the $Q_{4-6}(X)$, the reliabilities of both the aH, showing a positive value in the $Q(x)$, and the wC, showing a negative value in the $Q(x)$, were 1.000 and 0.875 , respectively. Hence, 12.5% of the wC cases showed a false positive value in the $Q(x)$ and were diagnosed as the aH under these discriminants. Concerning the discriminants $Q_{1-3,7,8}(x)$, the wC was completely differentiated from the aH (both corrective discriminating rate: 1.000).

DISCUSSION

In the stratum of an endometrial hyperplasia to carcinoma, the endometrial hyperplasia was regarded as a foregoing lesion of carcinoma. In this study the ages of patients with aH were statistically younger than those with wC ($p=0.001$, t-test). For eliminating the overdiagnosis of carcinoma, therefore, repeated biopsies or the re-biopsies after hormonal curettage were significant for an early premenopausal patient in her thirties or forties.^{2,5)}

In the histological distinction, the wC shows a stromal invasion, i.e., an irregular glandular budding with surrounding stromal reactions, cribriform patterns, and prominent papillary growths.^{1-3,6)} However, the wC could not always accompany these typical configurations, especially in a small preoperative biopsy specimen; the AH, therefore, could often be confused

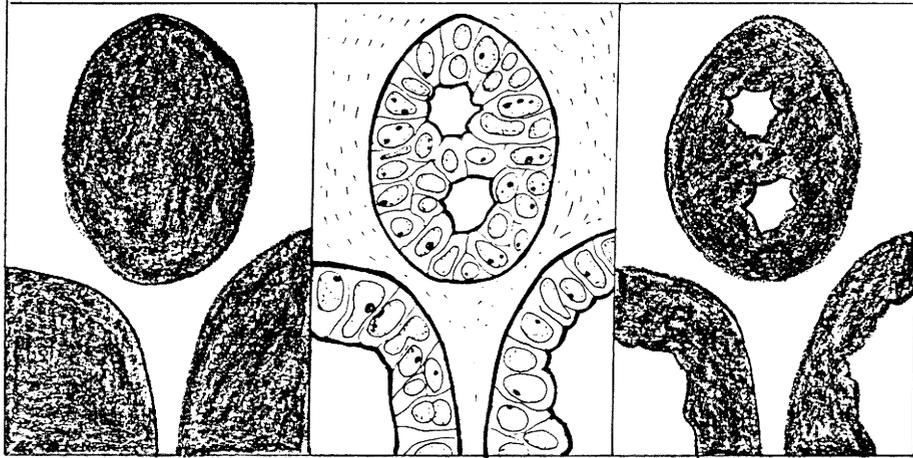


Fig. 1. Left: The index of glandular area per unit area (IGA) was calculated as the black area, showing the whole glandular area in the central schema, divided by total area (percentage). Right: The index of cellular area per unit area (ICA) was led by the black area, showing the whole cellular area of the glands in the central schema, divided by total area (percentage). The index of gland-in-gland configuration per unit area (IGG) was counted as the total number of glandular lumens divided by the total number of glands, e.g., IGG in this schema = $4/3 = 1.33$.

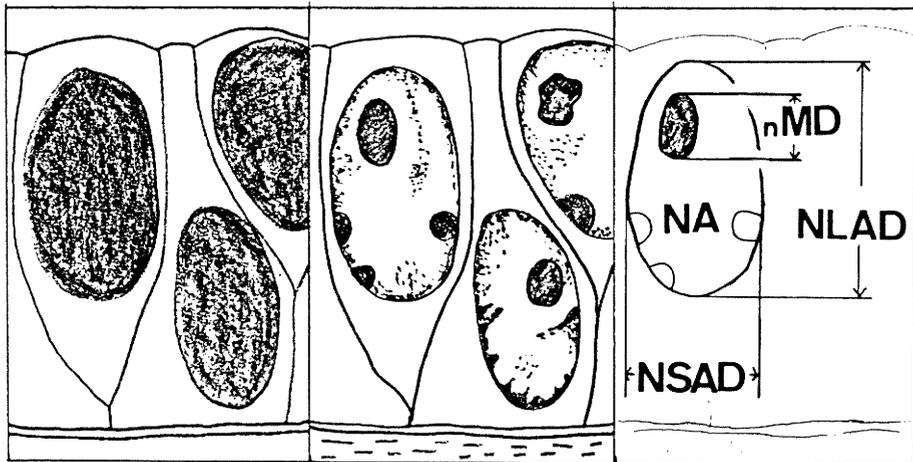


Fig. 2. Left: The index of nuclear stratification per unit area (INS) was calculated as the total number of nuclei of glandular cells divided by the total number of nuclei lining up on the basement membrane, e.g., the INS in this schema = $3/1 = 3$. Right: The nuclear long axial diameter (NLAD) and the nuclear short axial diameter (NSAD) were shown at right angles to each other. The nuclear area was led as $3.14 \times (NLAD/2) \times (NSAD/2)$. The nucleolar maximal diameter (nMD) was the longest nucleolar diameter (shown as paired thick lines) in all nucleoli (four in this schema).

with the wC.^{2,3)} In our study, we excluded the cases of AH and chose authentic cases of both the wC and the aH.⁵⁾ The pathological distinction has looked into objectively by the mathematico-statistical analysis of glandular atypism.^{4,5,7,8)} We chose eight morphometric indices: IGA, ICA, INS, IGG, NLAD, NSAD, NA, and nMD.^{5,7,8)} These indices, except the INS, were statistically significant to differentiate the wC from the aH. The most reliable ones were the IGA and the ICA ($p=0.001$), which means that the wC is more distinguishable by its greater compactness of glands than by its cellular atypism. We believe that the mathematical measurement is essential for the histopathological distinction because structural differences are too delicate to identify by a subjective evaluation under the microscope.^{5,7,8)}

Important as each index was, these two lesions could not completely be separated by a single index. It was necessary to combine various indices into a multivariate discriminant.^{5,7,8)} As emphasized in our previous paper,⁵⁾ the combination of reliant indices of age and structural atypism as IGA and ICA could not make up any complete linear discriminants. In this study, however, the indices of cellular atypism were useful as additional ones in spite of their low statistical significance. Five linear discriminants could perfectly segregate the wC from the aH in this multivariate analysis. The difficulty in the histopathological distinction between the wC and the AH was reconfirmed because many morphometric indices were recommended in the linear discriminants. Further multivariate analysis is necessary to diagnose the wC precisely and to clear the histogenesis of the wC.^{5,7,8)}

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REFERENCES

- 1) Dallenbach-Hellweg G, Poulsen H: Adenomatous hyperplasia. In: Dallenbach-Hellweg G, Poulsen H (eds) Atlas of endometrial histopathology. 1st ed. Munksgaard Co., Copenhagen 1985, p 88-103, 166-169.
- 2) Hendrickson MR, Kempson RL: Endometrial hyperplasia, metaplasia and carcinoma. In: Fox H (ed) Obstetrical and gynecological pathology. 3rd ed. Churchill Livingstone Co., Edinburgh-London-Melbourne-New York 1987, p 354-404.
- 3) Ikarashi T, Takeuchi S: Immunohistochemical localization of placental proteins and tumor-associated antigens in endometrial cancer and endometrial hyperplasia. *Acta Obstet Gynaecol Jap* 39: 1634-1640, 1987.
- 4) Ikarashi T: Histochemical and morphometric studies of endocervical type adenocarcinoma in the cervix uteri. *Acta Med Biol* 36: 75-84, 1988.
- 5) Ikarashi T, Sudo N, Kato M, Hirasawa H, Ishii M, Kaneko H: Histopathological differentiation of endometrial adenomatous hyperplasia from a well differentiated type of endometrial adenocarcinoma by statistical methods. *Acta Med Biol* 37: 51-54, 1989.
- 6) Japan Society of Obsteric and Gynecology, The Japanese Pathological Society, and Japan Radiological Society (eds) The general rules for clinical and pathological management of uterine corpus cancer. 1st ed. Kanehira Press Co., Tokyo 1987, p 35-46.
- 7) Ohkura Y, Nakamura K: Histopathological study on the histogenesis of colorectal carcinoma. *I to Cho* 22: 431-41, 1987. (In Japanese with English abstract)
- 8) Togo S, Kikuchi M, Nakamura K: Histopathological study on adenoma and carcinoma of the large intestine: Morphometrical analysis of structural atypism. In: Proceedings of 13th International Cancer Congress, Seattle September 1982, p 388.