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The Application of Morphometry in Gastric Cytological Diagnosis

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Summary. The applicability of morphometry in the cytological diagnosis of adenocarcinoma of the stomach was tested. Useful morphometric variables were extracted from 41 cases with known histology, and applied to 33 other cases: in all these cases the histological diagnosis were successfully predicted. Morphometry was applied an additional 39 cases selected for difficulties in cytodiagnosis. In the group of cases with suspicious cytology in particular, refinement of cytodiagnosis was achieved by the application of morphometry.

Key words: Morphometry – Gastric carcinoma – Gastric cytology

Introduction

Brush cytology of the gastric mucosa is of considerable value in the diagnosis of carcinoma of the stomach (Prolla et al. 1977, Kasugai et al. 1978; Young and Hughes 1980). This technique has progressed rapidly in countries where gastrofiberoscopy has been widely used (Shida 1965). The success of the method however, is greatly dependent on the expertise of the cytologist interpreting the smears. In this paper, we attempt to evaluate the role of morphometry in the application of gastric cytology. Our purposes were the following: to determine which morphometric variables are essential in distinguishing benign from malignant lesions, and whether morphometry can be of value in aiding cytological diagnosis in difficult cases. Successful application of morphometry to gastric cytology has been reported by Danno, (1975), who has shown that the nuclear cytoplasmic ratio (N/C ratio) of malignant cells and benign cells differed significantly. From his data it can be anticipated that other morphometric data, including nuclear area, may be of additional value in separating benign from malignant lesions.

The cytological evaluation of a smear can be separated into two steps: first the cytologist identifies the abnormal cell population, and second these

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cells are interpreted as either atypical or malignant. In the application of morphometry in cytodiagnosis as presented in this paper the first step is identical to routine cytodiagnosis: i.e., the abnormal cell population is identified by the cytologist. However, the second step differs: the analysis of the abnormal cell population as regard to its morphometric variables is performed by a computer instead of by a cytologist (Boon et al. 1980).

For the extraction of useful morphometric variables in gastric cytology material with known histology was used (reported by Keighley et al. 1979). To test the application of morphometry in difficult or doubtful cases, an additional series of cases was tested using the extracted morphometric variables.

Material and Methods

Brush cytology smears obtained during endoscopy from patients with gastric lesions were treated by wet fixation in 75% ethyl alcohol. The smears were stained by the Papanicolaou technique and reported by the cytologist (H.T.) in the Department of Histology, General Hospital, Birmingham. Gastric biopsies were also taken from each patient and reported independently without knowledge of the cytology.

A total of 74 smears (Keighley et al. 1979 original series) were submitted to the Department of Cytology, Delft, for morphometric analysis. There were 34 benign lesions (negative cytology and tumour negative follow-up) and 40 positive smears (confirmed by histology). This material was randomly divided into two groups. In the first group of 41 cases (the learning group) the knowledge of the histological reports was used to develop the morphometric criteria which differentiated the benign from malignant cases. Using these criteria the second group of 33 cases (the testing group) were divided into presumed benign and malignant, and than compared with the known histological reports.

It was possible to differentiate successfully the benign and malignant cases and thus the same morphometric criteria were applied to a third group of cases. This third group of cases was selected by an independent arbiter, Prof. D.B. Brewer (D.B.B.) to include cases with difficulty in cytologic diagnosis. This material was reviewed independently by the cytologists (H.T. and D.B.B.). Morphometric analysis was made without knowledge of the cytological diagnoses. The cytological interpretation, the morphometric analysis and the histological diagnosis was submitted to the arbiter (D.B.B.), for determination of the accuracy of the cytological and morphometric interpretations. This audit material consisted of: a) 13 benign lesions with negative cytology and tumour negative follow-up, b) 9 positive smears confirmed by histology, c) 4 cases with positive cytology, negative histology and clinical cancer, d) 3 cases with suspicious cytology and negative follow-up, e) 10 cases with suspicious cytology and positive histology, f) 1 case with suspicious cytology and a histologic report of dysplasia.

Quantitative Studies

For the planimetric studies a graphic tablet was used, equipped with a camera lucida system. The digital cursor of the system was seen in the microscopical field under study, and thus the chosen object could be outlined and its features calculated bya minicomputer attached to the graphic tablet. In each smear, 25 abnormal cells were chosen: from each cell the contour of the nucleus and the cytoplasm was delineated on the graphic tablet with the cursor, and from these contours the following five features were calculated by the minicomputer: perimeter of the nucleus, area of the nucleus, perimeter of the cell, area of the cell and nuclear cytoplasmic area ratio. From each measured slide, 10 variables could be calculated, being mean and standard deviation of the five enumerated features. The measurements were performed by one cytopathologist and one well-instructed cytotechnologist: there were no statistical differences between the measurements of these two observers.

Abnormal cells of any kind were selected for morphometry. The selection of cells for morphometry was done independently of the original cytological interpretation. The abnormal cells selected were cells with enlarged nuclei, abnormal nuclear shapes and abnormal chromatin pattern. Abnormal

cells without visible cytoplasm were also included in the measurements, because they are considered to be poorly differentiated cells which have lost their cytoplasm (Spaander et al. 1981). The N/C ratio of these cells is 1.0. Among the cell groupings, only the cells on the edges with well spread cytoplasm were included, and the centrally localized cells were excluded from measurement.

Statistical Analysis

Statistical analysis of the original series of 74 cases was carried out on a PDP 11 DEC computer. The descriptive statistics were calculated for the group of benign lesions and for the group of malignant lesions respectively. Wilcoxon's test was used to establish the difference between the two groups; as a level of significance, $P<0.05$ was adopted.

The learning group of 41 cases was used to extract the classification rule. A multivariate normality and equality of the within group variance covariance matrices was established, therefore a stepwise discrimination analysis could be used. STATPAC (Digital, Maynard, Mass) was applied. The F-test was used in selecting variables. It was found that the two variables, mean N/C ratio and standard deviation of the nuclear area, were essential for discrimination between the two groups, and therefore they were used for computation of the classification rule according to Cooley and Lohnes (1971).

Results

The significant differences between the group of benign lesions and malignant lesions of the 74 cases is shown in Table 1. It is evident that among the 10 variables, 8 are significant in the discrimination of the two groups. The two most discriminating, variables the mean of the N/C ratio and the standard deviation of the nuclear area, are very successful in separating benign from malignant lesions (Fig. 1).

It was computed that there were no significant differences between the data of the learning group and the testing group, pointing to an absence of bias.

It was therefore justifiable to extract a classification rule from the learning group. When the established classification rule was applied respectively to the learning and the testing group, a high probability for a correct classification was computed, in the learning group ranging from 91–100%, which was surpassed in the testing group (96–100%). Cases with probabilities of malignancy

Table 1. The significant differences and their means in the benign and malignant lesions (74 cases)

Variables	Benign	Diff	Malignant
Mean nuclear perimeter	28.01[a]	*	31.58
S.D. nuclear perimeter	30.05	***	5.79
Mean nuclear area	53.39		60.15
S.D. nuclear area	10.28	***	21.14
Mean cell perimeter	79.32	***	36.59
S.D. cell perimeter	6.33		6.59
Mean cell area	156.99	***	73.88
S.D. area	38.03	**	25.28
Mean N/C area	0.58	***	0.87
S.D. N/C area ratio	0.06	***	0.11

* $P\leqq0.05$; ** $P\leqq0.01$; *** $P\leqq0.001$

[a] In m μ

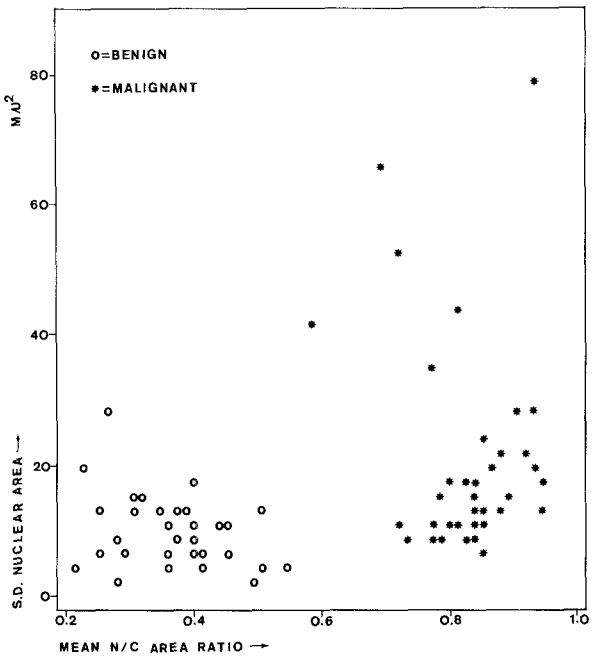

Fig. 1. Scattergram of the morphometric results of the 74 cases of the original series with known histology. Each symbol represents one case

Table 2. Classification table of the 74 lesions

Learning group	Benign[a]	Malignant[a]
1. Benign 19	19	0
2. Malignant 22	0	22
Test group	**Benign[a]**	**Malignant[a]**
1. Benign 15	15	0
2. Malignant 18	0	18

[a] Computer aided classification

ranging from 0–24 were classified as benign, between 25–75 as inconclusive, and over 75 as malignant. Applying these threshold criteria, all cases in both the learning and the testing groups were correctly classified (Table 2).

Consequently, the extracted classification rule was applied to the difficult cases of the third group as audit material. The results are depicted in Table 3. All benign and malignant cases thought to be difficult to interpret were correctly classified. The three cases without histological confirmation of the clinical diagnosis of cancer were classified as malignant. In the 14 cases with suspicious cytology, 12 were correctly classified. In one of these cases the classification was dubious (probability of 65%), which was in accordance with the histological diagnosis of dysplasia. In the remaining two cancer cases the classification

Table 3. Classification of the additional audit series (39 cases)

	Computer-aided assessment			
	Benign	Dubious	Malignant	Total
A. Cytology negative/histology negative	13	0	0	13
B. Cytology positive/histology positive	0	0	9	9
C. Cytology positive/histology negative/ clinical cancer	0	0	3	3
D. Cytology suspicious/follow-up negative	3	0	0	3
E. Cytology suspicious/histology positive	2	0	8	10
F. Cytology suspicious/histology dysplasia	0	1	0	1
	18	1	20	39

was negative which suggests an inadequate sample of the cytological brush material.

Discussion

In routine laboratories, brush cytology is a very successful method (Keighley et al. 1979), however in a small group of cases which are in the middle of the spectrum from benign to malignant the cytological diagnosis is difficult. These cases might be interpreted as either benign or malignant by different cytologists, or a definitive diagnosis is avoided and the report reads simply "suspicious for malignancy". It is this group of cases in which morphometry adds to the accuracy of cytodiagnosis and, in addition, it is useful in false positive and false negative cases.

In 41 cases, in which the absence or presence of gastric cancer was definitely known, it was found that the mean N/C ratio and the standard deviation of the nuclear area were essential in discriminating between benign and malignant lesions (Fig. 1). A classification rule was computed with the morphometric data of these 41 cases (the learning group), and applied on a series of 33 cases with known histology (the testing group). The morphometric variables successfully determined the verified histological diagnosis all cases in this testing group.

In addition, the extracted classification rule was applied on the audit material of 39 cases selected for difficulties in cytodiagnosis. In the morphometric classification of the three cases in which the clinician was certain of gastric malignancy but in which the histological material was inconclusive ("false positive cytological diagnoses"), the clinical diagnosis was endorsed. In addition, the morphometric analysis proved to be very helpful in the group of 14 cases with "dubious" cytology. In two cases it became evident that the cytological underdiagnosis was due to a sample error. One of the cases was also morphometrically classified as dubious: histologically this was a dysplasia and thus was a true representative case for the middle of the spectrum from benign to malignant. In the remaining 11 cytologically dubious cases the correct diagnosis was also determined by morphometry. The two aims of this study are positively answered. First we have found that a high N/C ratio and a large variation in nuclear size identifies

the malignant cell population in gastric smears. Second, when the abnormal cell population, identified by the cytologist, is further quantified morphometrically, cytodiagnosis can be refined in difficult cases when these contain 25 or more cells. The measurement of one case takes only 15 min with available equipment. Thus it can be applied in routine diagnostic cytology.

References

Boon ME, Löwhagen T, Willems JS (1980) Planimetric studies on fine needle aspirates from follicular adenoma and follicular carcinoma of the thyroid. Acta Cytologica 24:145–148

Cooley WW, Lohnes PR (1971) Multivariate data analysis. John Wiley and sons, New York, pp 262–286

Danno M (1975) Statistical criteria for the cytology of gastric cancer. A proposal of distance index. Acta Cytologica 20:466

Kasugai T, Kobayashi S, Kuno N (1978) Endoscopic Cytology of the oesophagus, stomach and pancreas. Acta Cytologica 22:327–330

Keighley MRB, Thompson H, Moore J, Hoare AM, Allan RN, Dykes PW (1979) Comparison of brush cytology before and after biopsy for the diagnosis of gastric carcinoma. Br J Surg 66:246

Prolla JC, Kirsner JB, Cockerham L (1977) Direct vision endoscopic cytology and biopsy in the diagnosis of oesophageal and gastric tumours. Acta Cytologica 21:399–402

Shida S (1965) Diagnosis of early gastric cancer, In: Gastric cytology, Vol 4. Bonic Yodo Co, Ltd., Tokyo

Spaander PJ, Ruiter DJ, Hermans J, de Voogt HJ, Brussee J, Boon ME (1981) The implications of subjective recognition of malignant cells in aspirations for the grading of prostatic cancer using cell image analysis. Analyt Quant Cytol (in press)

Young JA, Hughes HE (1980) Three year trial of endoscopic cytology of the stomach and duodenum. Gut 21:241–246

Young JA, Hughes HE, Lee FD (1980) Evaluation of endoscopic brush and biopsy touch smear cytology and biopsy histology in the diagnosis of carcinoma of the lower oesophagus and cardia. J Clin Pathol 33:811–814

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