Morphological characteristics and distribution patterns of epithelial cells in the cytological diagnosis of gastric cancer

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SUMMARY The morphological features of epithelial cells in gastric biopsy touch smears from 296 cases (248 benign, 48 malignant) were retrospectively reviewed. By subjective criteria no single purely morphological characteristic could be identified that was consistently capable of separating benign from malignant specimens. The value of cell distribution patterns in diagnosis was therefore objectively analysed in the 231 adequate specimens (190 benign, 41 malignant). A score of over 20% for single epithelial cells was found to have a sensitivity of 95.1% and specificity of 99.5% in the diagnosis of carcinoma. Quantitative analysis of cell distribution pattern is a simple test, which does not require special equipment, yet can be helpful in the interpretation of gastric cytology.

The examination of endoscopically collected cytology specimens is now an established diagnostic procedure during the investigation of patients with gastric disease. Brushing is the most widely used sampling technique,¹⁻⁴ direct vision lavage^{5,6} and biopsy touch smear cytology^{4,7-9} are additional methods. However, accurate cytological diagnosis can sometimes be difficult, irrespective of the sampling technique. Although in very occasional cases, equivocal cytological findings may be the reflection of severe epithelial dysplasia,^{10,11} the great majority of difficulties do not arise for this reason. Most problems in the interpretation of gastric cytology are due to two far more common causes. Firstly, marked cellular changes are seen in cytological specimens from benign conditions, such as gastritis and peptic ulcer,^{12,13} which on histology show only inflammatory or degenerative changes not amounting to severe dysplasia. Secondly, cells from gastric adenocarcinomas display a wide variety of morphological appearances.¹⁴ A cytological specimen can appear puzzling for either of these reasons, yet correct interpretation is essential for patient management.

In order to try to identify a simple criterion, which could be used as an objective aid in the interpretation of gastric cytology specimens, a retrospective study of the characteristics of benign atypical and malignant epithelial cells was carried out. From this

subjective study, the single most valuable feature appeared to be an alteration in intercellular relationships. The diagnostic accuracy of cell distribution patterns was therefore examined, as this was the component of intercellular relationship which could be analysed objectively.

Material and methods

The specimens studied were those collected during a previous trial of gastroduodenal cytology.⁴ From a total of 329 patients, 303 had specimens collected from the stomach. The original subjective cytology reports had classified the specimens as normal; showing benign atypia of mild, moderate or severe degree; or malignant, with small or large numbers of malignant cells. "Suspicious" or inconclusive reports were not issued, as a definitive diagnosis was one of the requirements of the trial protocol. The final diagnosis was benign in 248 cases and malignant in 55. The benign diagnoses were substantiated by a period of follow-up ranging from two to four years and the malignant cases were all verified by histology. Six of the tumours were non-epithelial or of uncertain primary origin and these were excluded from the present study. The remaining malignant tumours were all primary adenocarcinomas. There was one case with false-negative cytology, due to sampling error, among these and this was also deleted. A total of 296 cases (248 benign and 48 malignant) was there-

fore available for retrospective study.

In the original trial⁴ both brush and biopsy touch smear cytology specimens were examined. Cellular detail was better in the touch smears than the brushings, due to the smaller quantity of contaminating debris. The "best" biopsy touch smear specimen was therefore selected from each case. The incidence and severity of the cellular characteristics listed in Table 1 were subjectively assessed for each of the four categories: mild, moderate and severe benign atypia and malignancy. Specimens containing small and large numbers of malignant cells were considered together. From this subjective review, it appeared that alteration in cell distribution pattern was a feature, which could possibly be used as an objective discriminating factor between benign and malignant specimens.

To test the validity of this hypothesis, the cell distribution pattern on the 296 slides was examined "blind," without knowledge of the previous cytological classification. In each of the 200 microscopic fields (at $\times 400$ magnification), it was recorded (i) whether the epithelial cell at the top right hand border of the field occurred as a discrete single cell with cytoplasm; (ii) in a small group of two to five cells; (iii) in a large group of six or more cells or (iv) was a bare nucleus without cytoplasm. It was necessary to look at bare nuclei as a separate category, as these may represent either undifferentiated malignant cells, or may occur as artefacts due to cytolysis in normal specimens.

STATISTICAL METHODS

The frequency distribution for each type of cell distribution pattern was constructed for all benign

cases, severe benign atypia cases and malignant cases. These statistical distributions could not be adequately represented by a "normal" curve and, consequently, all statistical tests performed were non-parametric. Comparison was made of the median percentage value between all benign and malignant cases, and between severe benign atypia and malignant cases. These calculations were made for each cell distribution pattern separately. The statistical procedure used was the Mann-Whitney test and the probability levels quoted were based on a two-sided test of significance.

Results

MORPHOLOGICAL CHARACTERISTICS OF EPITHELIAL CELLS

The subjective assessment of the incidence and severity of cellular characteristics in the four categories of mild, moderate and severe benign atypia and malignancy are given in Table 1. This is an expanded version of the description of cellular characteristics included in the original report of the gastric cytology trial.⁴

The characteristics fall into four groups. In group 1 are features which occurred on a progressive scale through mild, moderate and severe benign atypia. The degree to which they were found in malignant cells was generally greater than in atypia, but in occasional malignant cases was less marked than in some examples of benign atypia. The features in group 2 were never seen in mild atypia, but occurred to a variable extent in both moderate and severe atypia and malignancy. The characteristics in group 3 were never seen in benign specimens. If present they

Table 1 Incidence and severity of cellular characteristics in benign atypical and malignant gastric cytology specimens

Characteristic	Classification			
	Benign atypia			Malignant
	Mild	Moderate	Severe	
Group 1				
Variation in nuclear/cytoplasmic ratio	+	++	+++	- → + + + +
Anisocytosis	+	++	+++	+ + → + + + +
Anisonucleosis	+	++	+++	+ + → + + + +
Abnormal nucleoli	+	++	+++	+ + → + + + +
Variation in nuclear chromasia, granularity or clumping	+	+ +	- + +	+ + → + + + +
Group 2				
Nuclear pleomorphism	-	+	+ → + +	- → + + + +
Abnormal coarse cytoplasmic vacuolation	-	- → +	- → + +	- → + + + +
Mitotic figures, normal	-	-	- → +	- → +
Group 3				
Mitotic figures, abnormal	-	-	-	- → +
Irregular multinucleation	-	-	-	- → +
Fine "foamy" cytoplasmic vacuolation	-	-	-	- → +
"Opaque glass" nuclei	-	-	-	- → +
Group 4				
Abnormal intercellular relationships (polarity and distribution pattern)	-	+	++	+ + + → + + + +

were indicative of malignancy, but only occurred in cells from a small proportion of carcinomas and therefore could not be used as a consistent basis for diagnosis. Altered intercellular relationships were placed in group 4 as the only characteristic, which was always present in malignant specimens and always present to a more marked degree than in severe benign atypia. Intercellular relationships, however, consist of two closely associated features, polarity and cell distribution pattern. Polarity is the way cells relate to one another within a given group and assessment is essentially a subjective judgement, which is dependent on previous experience and, therefore, not suitable for objective examination. Cell distribution pattern is the way cells relate to one another on the slide as a whole and this appeared to be a factor which could be objectively analysed by simple means.

CELL DISTRIBUTION PATTERN

The material was adequate for the assessment of cell distribution in 231 cases (190 benign, 41 malignant) and inadequate in 65. The definition of an adequate specimen was the presence of well preserved epithelial cells in 200 microscopic ($\times 400$) fields. The overall range of values found for single cells, small and large groups and bare nuclei, in the 231 specimens is shown in Table 2. The original cytological classification of these 231 specimens was then checked. Sixteen had been classified as normal, 76 displayed mild benign atypia, 68 moderate atypia, 30 severe atypia and 41 had been diagnosed as malignant. In the 30 cases with severe benign atypia the final definitive diagnosis was gastric ulcer in 14 patients, chronic gastritis in 12 and gastric erosions in two. One patient was found to have a hyperplastic "polyp" associated with chronic gastritis and one an abnormal gastric fold, also associated with chronic gastritis. Table 3 compares the median values for the percentage of each type of cell distribution in all 190 benign cases, with the values in the 41 malignant cases. Table 4 gives the median percentages for each of the four benign classes (normal, mild, moderate and severe

Table 2 Overall cell distribution pattern in 231 biopsy touch smears

	%
Single cells	0-64.5
2-5 cells	3.0-44.5
≥ 6 cells	2.0-79.0
Bare nuclei	1.5-76.5

Table 3 Median percentage of each type of cell distribution in biopsy smears by original cytological diagnosis

	No of cases	Median values (%)			
		Single cells	2-5 cells	≥ 6 cells	Bare nuclei
Benign	190	6.0	15.5	43.5	28.5
Malignant	41	38.0	13.0	19.5	27.5

benign atypia) compared with malignancy.

There was found to be a significant difference in the median values of the distribution of both single cells ($p < 0.001$) and large groups ($p < 0.01$), not only between benign and malignant specimens overall, but also between severe benign atypia and malignancy, the distinction which causes most problems during routine reporting. In both instances the difference was greater for the distribution of single cells than for large groups. The frequency distribution of single cells in all the 190 benign cases in comparison with the 41 malignant cases is shown in Fig. 1 and the frequency distribution in the 30 cases of severe benign atypia in comparison with malignancy is shown in Fig. 2. The data for the region where the values for benign and malignant overlap is shown in greater numerical detail in Table 5. It can now be seen that all benign cases except one had 20% or less single cells and that all except two malignant cases had more than 20% single cells. If a score of more than 20% single cells is therefore considered as an indication of malignancy, the diagnostic sensitivity of this observation is 95.1%, the specificity 99.5% and the efficiency 98.7%.

Twenty-nine of the 30 cases displaying severe

Table 4 Median percentages of each cell distribution in biopsy touch smears by cytological classification

Classification	No of cases	Median values (%)			
		Single cells	2-5 cells	≥ 6 cells	Bare nuclei
Normal	16	3.5	15.0	43.5	33.5
Mild benign atypia	76	5.5	11.5	38.0	28.5
Moderate benign atypia	68	7.0	16.6	43.5	27.5
Severe benign atypia	30	10.0	23.0	46.0	27.5
Malignant	41	38.0	13.0	19.5	27.5
Differences between all benign and malignant specimens		$p < 0.001$	NS	$p < 0.01$	NS
Difference between severe benign atypical and malignant specimens		$p < 0.001$	NS	$p < 0.01$	NS

Fig. 1 Frequency distribution of single cells in benign and malignant cases.

Fig. 2 Frequency distribution of single cells in benign cases with severe atypia and in malignant cases.

Table 5 Percentage of single cells in benign and malignant cases

Percentage of single cells	All benign cases (n = 190)	Severe benign atypia (n = 30)	All malignant cases (n = 41)
<15.0	180	25	1
15.1-17.5	3	1	0
17.6-20.0	6	3	1
20.1-22.5	1	1	2
22.6-25.0	0	0	5
>25.0	0	0	32

atypia were correctly diagnosed as benign by assessment of single cell distribution pattern. The one so-called false-positive case with a slightly raised single cell score of 21.5% was from this group. The patient had a gastric ulcer, which in the original endoscopic biopsies had shown dysplasia, although the final definitive diagnosis, after follow-up, was benign. Two malignant cases had false-negative scores, one had 15% single cells and the other 18%. Both these specimens were from very poorly differentiated tumours and the slides contained large numbers of undifferentiated malignant cells, which appeared as bare nuclei in the cell distribution counts. The bare nuclei scores for these two cases were 56.5% and 31.5%. In practical terms neither would have presented a diagnostic problem as both looked obviously malignant on microscopic examination due to extreme chromatin abnormalities.

Discussion

Gastric epithelial cells exhibit great morphological variety in their response to disease. The cells from two carcinomas are never quite alike¹⁴ and benign specimens also show a great range of appearances, particularly in the cells arising from areas of atrophic gastritis or the regenerating edges of gastric ulcers.

Cells from one carcinoma may display a particular morphological abnormality to such a degree that the diagnosis of malignancy is obvious, yet the same abnormality may be almost completely absent from the cells of another tumour, which in turn may exhibit a quite different characteristic of malignancy. The majority of carcinomas display gross stigmata of malignancy and the "overall disturbance" of morphology occurs on a progressive scale through mild, moderate and severe benign atypia to malignancy. However, the degree to which each abnormal characteristic develops shows such variation that no single purely morphological feature was identified which could be used as a constant discriminating factor between benign and malignant specimens. This is in keeping with the findings of Kalnins *et al*¹⁵ based on discriminant function and multiple regression analysis by computer of variable morphological factors in cells arising from gastric carcinomas.

A group 1 characteristic may occasionally be less pronounced in malignancy than in severe benign atypia. For example, anisocytosis and anisonucleosis are less pronounced in the small cell anaplastic type of cellular picture associated with Lauren's diffuse carcinoma,^{16 17} than in groups of cells arising from the regenerating edges of occasional benign ulcers. The cellular changes in group 2, though not seen in mild atypia, occur to such a variable degree both in benign and malignant lesions that they are un-

reliable. Nuclear pleomorphism is a serious abnormality and if present to more than a moderate degree is indicative of malignancy, but occasional carcinomas occur that are surprisingly monomorphic.¹⁸ Coarse cytoplasmic vacuolation may be seen in intestinal metaplasia and conversely some poorly differentiated carcinomas show no evidence of mucin secretion, even with special staining techniques, in cytological preparations. Mitotic figures are seen in both benign and malignant specimens and with routine light microscopy it is sometimes difficult to distinguish normal from abnormal forms. They are a known cause of false-positive reports.¹⁹

The third group consists of characteristics which only occur in malignant cells and are not found in severe benign atypia. However, unfortunately they can only be seen in the cells from a minority of carcinomas. Abnormal mitotic figures are indicative of malignancy, but are seldom sufficiently bizarre to be identified with certainty. Irregular multinucleation has not been observed in benign columnar cells, but occurs only infrequently in malignant cells. There are also two unusual varieties of malignant cells, which display specific morphological abnormalities not encountered in cells showing benign atypical changes. The first is a large cell with abundant finely vacuolated "foamy" cytoplasm and a prominent cell membrane. The second type of cell has a peculiar "thick" nucleus, which looks like "opaque glass" and appears to rise above the surrounding cytoplasm.

Altered intercellular relationship (polarity and cell distribution pattern) is the only characteristic, which appears to be displayed by all gastric adenocarcinomas, irrespective of the morphological variety of the malignant cells. It is also the only feature which is always more pronounced in malignancy than in severe benign atypia. Alteration of polarity is a constant feature in groups of malignant cells, but judgement of this depends on experience and it is impossible to quantify. Moreover, in occasional specimens malignant cells may occur almost exclusively as discrete, single cells and groups of malignant cells may be of small size or few in number. In these cases loss of polarity may be inconspicuous, even to an experienced observer. The distribution pattern of the cells on the slide as a whole, however, is easy to measure and requires no special equipment.

The objective assessment of the cell distribution pattern in the 231 specimens examined confirmed the subjective impression that malignant specimens contained greater numbers of single cells and fewer groups of cells than benign specimens. Furthermore, this difference remained significant even when severe atypia and malignancy were compared. The sensitivity (95.1%) and specificity (99.5%) of the

observation that more than 20% single cells indicates malignancy are sufficiently high for this measurement to be clinically useful when assessing specimens with equivocal cellular changes. Two specimens, which contained large numbers of undifferentiated (bare nuclei) malignant cells, gave false-negative results. The nuclei of both these tumours, however, showed extreme chromatin abnormalities and neither would have presented any difficulty on routine screening. The nuclear structure must therefore always be carefully scrutinised in specimens with a high count of bare nuclei, so that undifferentiated malignant cells are separated from bare nuclei due to cytolysis.

The specimens from the 30 cases with severe benign atypia were, as expected, from patients with chronic atrophic gastritis or from the regenerating edges of gastric ulcers. In one case there was an associated hyperplastic polyp and in another an abnormal gastric fold. We agree with Husain *et al*²⁰ that this is a group of patients who require special identification and follow-up. The cases in the present series were followed for a period ranging from two to four years and none has developed carcinoma. However Morson *et al*¹¹ stress that it is epithelial dysplasia which is actually the marker in increased risk of malignant change. It is therefore patients with this particular lesion who must be singled out for especially thorough follow-up. In the present study, histology of endoscopic biopsies showed epithelial dysplasia in only one patient with cytological severe benign atypia. It was this patient (with a gastric ulcer) who had the raised single cell score (21.5%) among the benign cases. This probably represented the equivocal state of the epithelium at the time of endoscopy. Morson *et al*¹¹ state that it is important to realise that the degree of risk associated with epithelial dysplasia is not yet established. They consider that even severe dysplasia, on its own, is not an indication for surgical intervention, though they stress close follow-up is necessary. The patient who had dysplasia in the present series remains well and the gastric ulcer has healed without problems. Further studies of cell distribution pattern in patients with histologically proven dysplasia are required to see if this test can "pick out" true epithelial dysplasia from the cytological atypias and thus help to identify the "high risk" patient, who requires vigilant observation.

The altered cell distribution seen in malignant specimens is due to the reduced mutual adhesiveness of malignant cells first noted by Coman in 1944,²¹ in connection with squamous cell carcinomas. Study of the ultrastructure and surface properties of tumour cells has subsequently greatly expanded this observation and shown that a wide variety of factors determine cell to cell adhesion.

Estimation of cell distribution pattern in biopsy touch smears is an easy test, which can be carried out with routine equipment. In this study it proved helpful in the interpretation of gastric cytology, although it was recognised that a theoretical problem could arise with a very poorly differentiated or undifferentiated tumour. Further studies on brush specimens and in cases of severe epithelial dysplasia might reveal extended applications of the method.

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