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A morphometrical analysis of dysplasia in small adenomas of the large instestine

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Summary. In a morphometrical study of 40 colorectal adenomas less than 10 mm in diameter and of 10 specimens of normal mucosa, it was found that nuclear size, perimeter and shape-factor of the epithelial cells were significantly different in neoplastic when compared with normal tissues. The same was found for the volume fraction of stroma, gland spaces, goblet and non-goblet epithelium, for the gland diameter, nuclear stratification height and stratification index, but not for the epithelial height nor the total volume fraction of the epithelium.

In a multivariate analysis, stratification index and nuclear size of the epithelial cells contributed most significantly to determination of the histological grade of dysplasia in adenomas. By the combination of these two morphometric variables 75% of all adenomas could be correctly allocated to the grade of dysplasia.

Key words: Morphometry – Adenomas – Large intestine – Dysplasia – Normal mucosa

Introduction

There is substantial evidence that a large proportion of colo-rectal carcinomas develop from pre-existing adenomas (Muto et al. 1975; Enterline 1976; Eide 1983). Large size and severe dysplasia increase the risk of the individual adenoma becoming malignant (Muto et al. 1975; Shinya and Wolff 1979). Whereas size is an objective measurement, the grading of dysplasia is based on microscopical criteria which have a considerable subjective element.

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To obtain quantitative information about normal and benign neoplastic tissue of the large intestine and more objective criteria in the microscopic evaluation of dysplasia, a morphometric analysis of structural and cellular features in adenomas and in the normal mucosa was performed.

Material and methods

Forty adenomas, 10 mm or less measured at their largest diameters and removed from surgical specimens of 28 patients with colorectal carcinoma were selected for the study. Before the polyps were removed the resected material was fixed in 10% neutral buffered formalin.

One slide from paraffin embedded material of each adenoma stained with H & E was used in the study. Two to four 5 μm sections were cut from the central part of the polyp head and through the base of the lesion.

The dysplasia of the adenomas was graded in mild, moderate, and severe dysplasia according to Kozuka (1975):

Mild dysplasia was characterized by glandular epithelium with cell nuclei maintaining their basal position with round and elongated nuclear shapes. Pseudostratification of the glandular epithelium is not conspicious (Kozuka grade I+II).

Moderate dysplasia was characterized by glandular epithelium with apparent nuclear stratification and frequent mitotic figures but the level of the nuclear position is limited to the basal half of the hight of the glandular epithelium except for mitotic nuclei (Kozuka grade III).

Severe dysplasia was characterized by glandular epithelium with severe nuclear stratification with the nuclei scattered throughout the whole hight of the glandular epithelium. (Kozuka grade IV+V).

The morphometrical analysis was performed with a Leitz Dialux 20 microscope with a sidearm for visualization of an electronic cursor which could be moved over a morphometric plate ("Bit pad one", Summagraphics) and connected to a Pet Commodore microcomputer (CBM, 4040) where all morphometric data were calculated and stored. The morphometric measurements were performed weeks after the adenomas were histologically graded and all slides were renumbered before being morphometrically evaluated, so that no prior knowledge of the origin of the sections was possible.

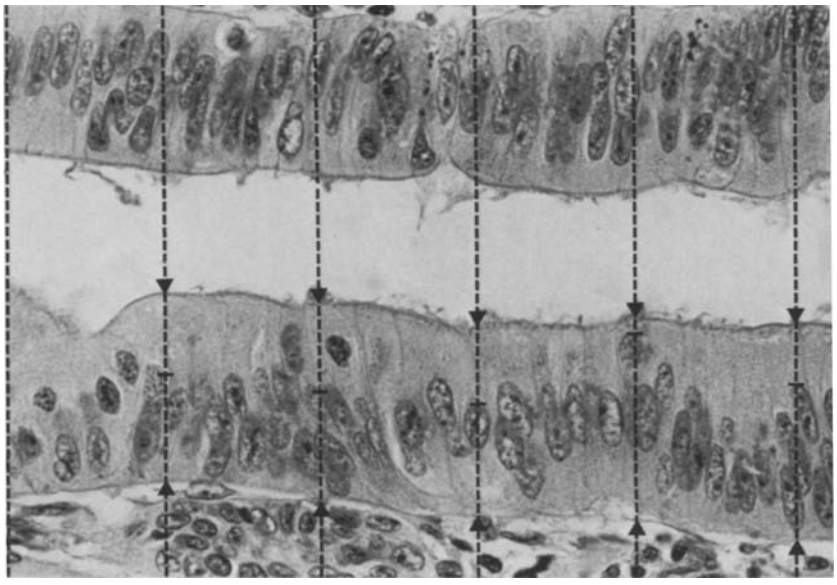

Fig. 1. The mean epithelial height and mean nuclear stratication height in each adenoma were determined by measuring ten times the distance from the basal membrane to the surface of the epithelium (▶----◀) and the distance from the basal membrane to the apical margin of the epithelial nuclei (|---◀). The points for the measurements were defined where the lines in the graticule, placed in one of the oculars with a grid 10 × 10 mm (10 mm = 100 intervals) crossed perpendicular to the surface of the epithelium, the apical margin of the epithelial nuclei and the basal membrane. The measurements were performed in the area with the most advanced dysplasia in the upper third of the cryptal epithelium using the 25 × objective. Crypts tangentionally sectioned were avoided. (HE, 775 ×)

The morphometric variables included in the study were (i) the mean profile area, (ii) the mean perimeter and (iii) the mean shape factor, determined by tracing the outlines of 25 epithelial nuclei with the electronic cursor (objective magnification; 40 ×) in the upper third of one or more crypts within the most advanced dysplastic area in each adenoma. Further; (iv) the mean epithelial height measured by the distance from the basal membrane to the brush border of the epithelial lining; (v) the nuclear stratification height measured by the distance from the basal membrane to the apical margin of the epithelial nuclei; (vi) the stratification index calculated by the ratio of the mean nuclear stratification height (v) and mean epithelial height (iv) were estimated. The means were calculated from ten measurements in every case, (objective magnification; 25 ×) as shown in Fig. 1.

The mean gland (vii) diameter was determined by measuring the distance between the basal membranes on both sides of each crypt with the electronic cursor in 15 consecutive glands. The measurements were performed parallel to the surface of the adenoma at one third of the distance between the surface and muscularis mucosa (objective magnification; 10 ×).

The volume fraction of epithelial cells; both (viii) the goblet and (ix) the non-goblet structures, (x) the stroma fraction and (xi) the fraction of gland spaces were calculated by a direct point-counting technique in the microscope by placing a graticule in one ocular. For the computation of the volume fractions of the various tissue elements a total of hundred points were counted in each adenoma (objective magnification; 10 ×). All measurements were performed in the area showing the most advanced dysplasia.

The crude adenoma volume was determined by the product of the three dimensions. The distribution of the crude adenoma volume was asymmetric. This asymmetry was normalized by utilizing a logarithmical value in the statistical analysis.

For comparison, normal tissue from the mucous membrane was analysed morphometrically in the same way in 10 of the 28 surgical specimens.

Results

The means of all morphometric variables estimated in the adenomas and normal mucosa are demonstrated in Table 1. Except for the epithelial height, and the total volume fraction of the epithelium, all variables showed significant differences at the 1% level between normal and neoplastic tissue by the use of Wilcoxon rank-sum test.

Of the 40 adenomas included, 11 showed mild, 18 moderate and 11 severe dysplasia.

Figure 2 shows the morphometric variables, the plot of the mean for each adenoma and the mean for the respective group of adenomas. From the figure it can be seen that there is a trend of increasing means of all variables, with increasing grade of dysplasia and the differences are statistically significant for nuclear area, nuclear perimeter, nuclear stratification height, stratification index and maximum diameter, using one-way variance analysis.

With increasing grade of dysplasia, a trend of decreasing volume fraction of stroma and goblet structures, but increasing volume fraction of non-goblet structure and gland spaces could be observed (Fig. 3). However, the differences are non-significant.

Due to a simple correlation analysis, variables shown to be significantly correlated to the grade of dysplasia were selected for a multiple regression analysis. Table 2 shows the independent variables selected; the grade of dysplasia being the dependent variable. Both the stratification index and the nuclear size independently and significantly contribute to the variation of dysplasia with a magnitude of 24.3% and 20.1% respectively.

In order to demonstrate discrimination of the different grades of dysplasia on basis of the stratification index and nuclear size a scatter-plot of the

Table 1. The mean of the various morphometric variables in adenomas and normal mucosa

Variables	Adenomas ($n=40$)		Normal mucosa ($n=10$)	
	Mean	s.d	Mean	s.d
Nucleus				
Perimeter (μm)[a]	28.6	2.8	21.4	3.1
Area (μm^2)[a]	47.0	9.7	30.3	6.0
Shape factor[+a]	0.72	0.057	0.83	0.067
Epithel and gland				
Epithelial height (μm)	37.9	7.8	35.1	8.1
Nuclear stratification height (μm)[a]	23.1	7.4	11.7	4.6
Stratification index[a]	0.60	0.11	0.33	0.12
Gland diameter (μm)[a]	111.8	33.8	78.3	11.2
Volume (%)				
Stroma fraction[a]	23.1	7.5	36.3	10.6
Epithel fraction (total)	67.2	8.3	61.1	10.6
Goblet fraction[a]	13.9	8.5	24.2	11.4
Non-goblet fraction[a]	53.4	11.4	36.8	8.2
Gland lumen fraction[a]	9.7	6.8	2.7	2.2

[+]Shape factor $\frac{4\pi \cdot \text{Area}}{\text{Perimeter}^2}$

n = number of specimens
s.d = standard deviation

[a] Significant different at the 1% level between adenomas and normal mucosa by the use of Wilcoxon rank-sum test

two measurements is shown in Fig. 4. This figure shows that the combinations of these two variables, permits a correct grading of 30 of 40 (75%) of the adenomas.

Discussion

The histological diagnosis of pathological processes in tissue sections usually relies upon recognition of abnormal morphological features. While this qualitative assessment serves quite well for much diagnosis it is clearly inadequate for discriminating among certain pathological entities (Paplanus et al. 1985). This is due to the fact that these lesions represent merely different points on a continuum of increasing deviation of the normal. Morphometry has the advantage, compared with conventional histological grading, of being a method suitable for numerical classification (Baak and Oort 1983). Thus, discrete differences of microscopical features can be measured, which cannot be accurately assessed by the naked eye.

In this study, the measurements were restricted to the upper third of the cryptal epithelium since these areas are the most relevant for comparing changes in epithelial differentiation in large intestinal adenomas. This procedure is in accordance with the principals of selective morphometry (Baak and Ooort 1983). Random sampling would have required a much higher number of computations and probably could not be performed without completely automatic image-analysis.

The estimated mean of all morphometric variables included in the study, except the height of the cryptal epithelium and the total volume fraction of epithelium, were found to be significantly different in adenomas compared with normal mucosa. Variables discriminating different grades of dysplasia were found to be statistically significant only for nuclear size and perimeter and nuclear stratification height and index.

The morphometric analysis displayed striking differences of nuclear size and shape in normal and neoplastic tissue. In normal epithelium the nuclei are small and almost spherical. In adenomatous tissue the nuclei are more elongated, but rather small in mild dysplasia. By increasing grade of dysplasia the size of the nuclei increase and they

Table 2. Simple correlation (r) and multivariate analysis (r^2) of variables contributing to the grade of dysplasia in adenomas

Variables	Simple Correlation r[a]	Multivariate analysis	
		Partial r^2	Probability
Stratification index	0.69	0.243	$P=0.001$
Nuclear area	0.67	0.201	$P=0.004$
Crude volume	0.61	0.074	$P=0.098$
Gland diameter	0.40	0.048	$P=0.185$
Nuclear stratification height	0.62	0.007	$P=0.621$
Stroma fraction	−0.32	0.006	$P=0.641$
Nuclear shape factor	0.26	0.004	$P=0.694$
Epithelial goblet fraction	−0.21	0.002	$P=0.770$

[a] $r \geq \pm 0.31$ (two-tail), $P<0.05$

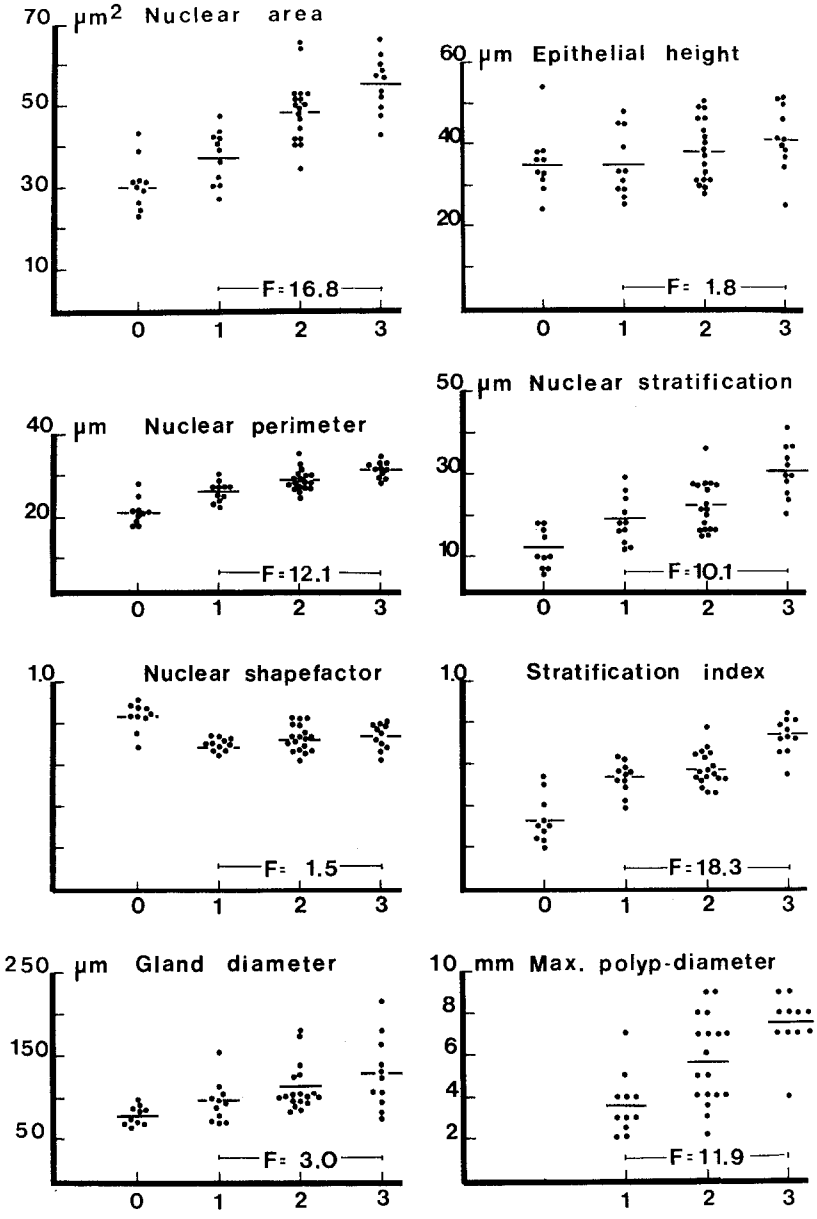

Fig. 2. The plot indicates the mean for each adenoma and the short line the mean for the three groups of adenomas and normal mucosa 0 = normal mucosa, 1 = mild dysplasia, 2 = moderate, 3 = severe. F is the ratio of mean squares between (degree of freedom = 2) and within (degree of freedom = 37) the three groups of adenomas. $F > 3.25$, $P < 0.05$

tend to be more spherical again. Similar observations has been done in a morphometric study of nasal metaplastic and dyplastic epithelium (Boysen and Reith 1983).

Though it is reasonable to suggest that nuclear size has only a modest influence in the visual grading of dysplasia it is more likely that the stratification of epithelial nuclei contribute more to the visual assessment of dysplasia. Kozuka (1975) found that when the histological grading of large intestinal polyps were based on epithelial pseudostratification this was in agreement with the general atypia studied. Determination of nuclear stratification height and stratification index in this study showed a high correlation with the grade of dysplasia, and in the multiple regression analysis of all the morphometric variables the stratification index contributed most significantly deciding the grade of dysplasia.

It is worth noticing that an increase in nuclear stratification height in dysplastic epithelium is not followed by a similar increase in epithelial height and there is no significant difference in the height of the epithelium in normal and adenomatous tissue. This indicates that the length of epithelial cells do not change at any large extent by adenomatous development in small adenomas.

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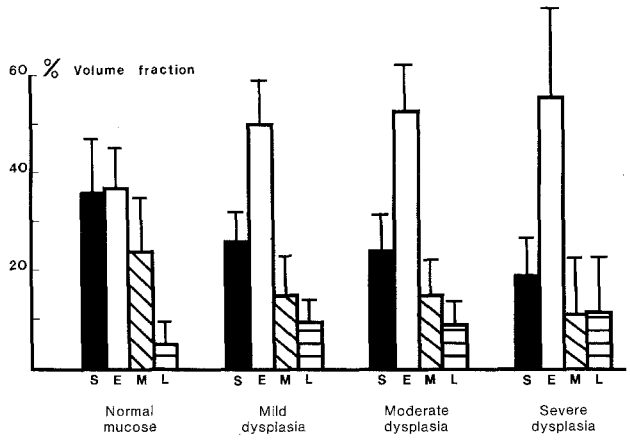

Fig. 3. The mean and standard deviation of volume fraction of stroma (*S*), non-goblet epithelium (*E*), goblet structures (*M*) and gland lumen (*L*) in normal and neoplastic tissue

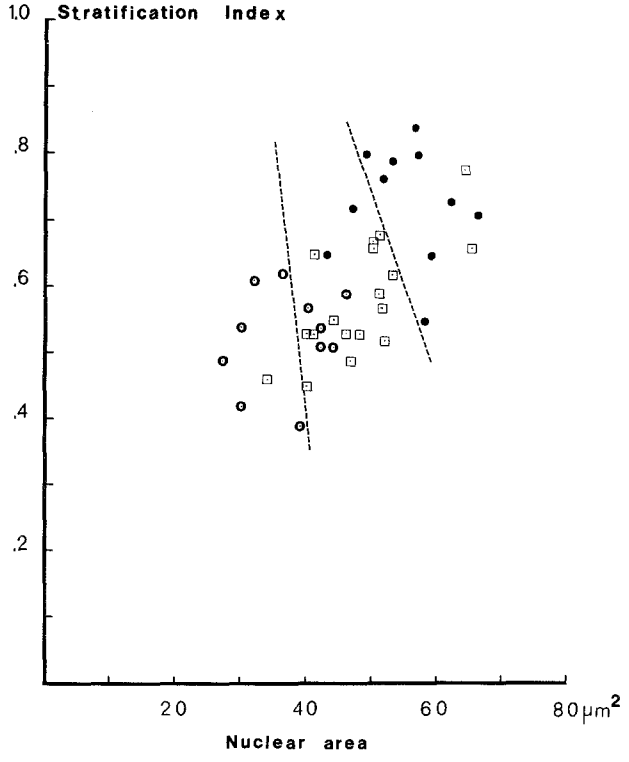

Fig. 4. Mild dysplasia (*open circles*), moderate dysplasia (*open squares*) and severe dysplasia (closed circles) determined by the mean of the stratification index and nuclear area for each adenoma. The discrimination lines indicate that 30 of 40 (75%) adenoma can be allocated to the respective group of dysplasia by determining the mean stratification index and nuclear area for each adenoma

of intestinal tumors" of WHO (Morson and Sobin 1976) that the amount of mucous secretion usually diminish with increasing grade of dysplasia. However, a decrease in the goblet volume fraction was not found to contribute statistically to the grade of dysplasia in this study. Individual differences of adenomas in expressing both dysplastic changes of various features and the number and size of mucous secreting cells, may be the main reason.

Morphometrical evaluation of adenomas has the advantage, compared to histological grading, that different variables can be individually assessed and statistically tested. Though many variables were significantly correlated to the grade of dysplasia it was found that some of these were intercorrelated and did not by themselves contribute to the grade of dysplasia in the multiple variate analysis. Only the nuclear size and the stratification index independently and significantly contribute to the explanation of dysplasia. Though the purpose of morphometry is that continuous lesions (Langley 1978) can be numerically classified it is interesting that as many as 75% of all adenomas could be correctly grouped according to the visual grading procedure by the use of these two morphometric variables.

The present study has shown that by the use of routine sections from paraffin embedded material and relatively few morphometric computations for each variable, significant differences can be obtained not only between normal and adenomatous tissue, but also between different grades of dysplasia in small adenomas of the large intestine. Except for the tracing of epithelial nuclei, the other measurements can be carried out rather quickly and the method assessing the stratification of epithelial nuclei described here is simple and much less time-consuming and circumstantial than the method recently described by Paplanus et al. (1985). Thus, morphometric analysis of large intestinal adenomas may have practical implications not only for research, but also in diagnostic work for the assessment of dysplasia.

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