Morphometric Analysis of Colonic Adenomatous Polyps

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Morphometric evaluation of adenomas of the colon to determine their degree of dysplasia is still controversial, since subjective factors influence the decision concerning benignancy or malignancy. Some indices have been proposed to assist in categorizing intermediate lesions into benign or malignant. However, these indices show a wide range of distribution. To evaluate the reliability of one such index, the index of structural atypism (ISA, area of glands/area of stroma), we studied polyps as a whole, and found great variability of index values within a single polyp.

Key words: Morphometry — Adenoma — Polyp — Dysplasia — Colon

Adenoma of the colon is a focus of abnormal epithelial tissue that has progressed along the neoplastic pathway but falls short of frank malignancy.¹⁾ This lesion is considered neoplastic and premalignant, according to the adenoma-carcinoma sequence, and its identification is of great importance because it can be removed endoscopically, preventing its malignant transformation.

All adenomas are, by definition, dysplastic, and the dysplasia is classified from mild to severe according to its degree of morphological abnormality. However, standardizing the grade of dysplasia is rather difficult because the experience of the individual observer influences the histologic grading of adenomas.

For objective analysis of structural atypia, some indices were proposed by Nakamura *et al.*, i.e., the index of structural atypism (ISA), and the index of double doughnuts (IDD).²⁻⁴⁾ The ISA is the ratio of the area of glands per area of the stroma in a determined field of the polyp, and the IDD is the number of intraglandular bridgings, glandular ramifications, anastomoses, and loops found by tridimensional analysis.²⁾ According to the values of the indices obtained, they classified lesions as normal mucosa (hyperplastic), benign adenoma, borderline lesion, or carcinoma. Using these indices for neoplastic polyp analysis, they concluded that the majority of colon carcinomas originate *de novo*, as the majority of the index values obtained for adenoma surrounding focal carcinoma were in the range of normal mucosa (hyperplastic) or carcinoma.

Our objective was to evaluate the reliability of one of these indices, i.e., the ISA, and for this purpose we analyzed the whole fields of polyps, to examine the distribution of index values in a single polyp.

MATERIALS AND METHODS

The polyps Three polyps from patients submitted to endoscopic polypectomy were analyzed. The specimens were fixed in formaldehyde, embedded in paraffin, and stained with hematoxylin and eosin. Two of these speci-

mens were adenomas with mild to moderate dysplasia (Figs. 1A and B), and one was a case of *in situ* carcinoma (severe dysplasia) surrounded by adenoma (Fig. 1C), according to the WHO classification.

Photographs Photographs of the specimens were taken, enlarged, and divided in 164, 163, and 157 fields, respectively (average of 161 fields). Each field filled the monitor of an SP 500 Image Processor TVIP-5100 (Olympus, Tokyo) at 200-fold magnification.

Index of structural atypism For morphometric analysis, the ISA was calculated for each field, as reported by Nakamura *et al.*^{2,3)} Using the Auto Image Analyser, all the glands of one field were delimited, and the area was calculated. Then, the area of the stroma was measured. The ISA was obtained by dividing the area of the glands by that of the stroma, as shown below.

$$\text{ISA} = \text{area of glands/area of stroma}$$

The values obtained for each polyp were expressed as means $\pm$ SD, and compared with each other by means of the Wilcoxon test. A *P* value less than 0.05 was considered significant.

False colors were used to express the grade of dysplasia according to the range of ISA values in each field, as shown in Fig. 2. The number of fields of each group was calculated, and expressed as a percentage of the whole polyp. Higher index values reflect more severe dysplasia, and low values correspond to normal mucosa. The percentages of fields with different grades of dysplasia were analyzed for each polyp when the range values were changed.

RESULTS

Analysis of the data The index values obtained from the three polyps ranged from 0.3 to 10.5. The mean values obtained for polyps 1, 2, and 3 were: 2.96 ± 1.62 , 2.59 ± 1.39 , and 3.21 ± 1.73 , respectively. Although polyp 3 contained focal carcinoma, there was no statistically

Fig. 1. Polyps 1 (A) and 2 (B); adenomas with mild to moderate dysplasia. Polyp 3 (C); adenoma with focal carcinoma (carcinoma *in situ*).

significant difference from polyp 1 (Fig. 3). Next, we analyzed the carcinomatous and adenomatous focus separately (Fig. 4), and obtained mean values of 4.09 ± 1.95 for cancer and 2.83 ± 1.48 for adenoma. Wilcoxon analysis showed statistically significant differences between the carcinomatous focus and polyps 1 and 2, and also a significant difference between the carcinomatous and adenomatous focus. The adenomatous focus showed no significant difference from polyps 1 and 2.

Fig. 2. Distribution pattern of ISA in comparison with the histology in polyp 2 (upper panel). Modification in the distribution pattern of ISA in polyp 2 when the range values were changed (lower panel).

Comparison with histology All the fields of the polyps were stained according to the range values, and compared with the histological findings (Fig. 2). We observed a remarkable heterogeneity in the three specimens. Even in areas with similar grades of dysplasia there was a wide heterogeneity. The area with focal carcinoma showed higher values.

Grading and distribution pattern The number of fields presenting the same grade of dysplasia was calculated and expressed as a percentage of the whole polyp. The percentage of "carcinoma" foci in a polyp changed remarkably with the change of range values (Figs. 5A, B, and C), when the areas with the highest index values are assumed to be carcinoma.

Fig. 3. Statistical analysis of the 3 polyps (Wilcoxon test).

Fig. 4. Statistical analysis of the 3 polyps (Wilcoxon test) when the focal carcinoma region was studied separately from the adenoma region in polyp 3.

DISCUSSION

Colonic adenomas are well known to be neoplastic, with high potential for malignant transformation,^{1,5-8)} although some authors^{4,9)} consider that the majority of colon cancers originate from normal mucosa ("de novo"). However, more recently, flat elevations of the colon have been demonstrated to be adenomatous, with higher potential for malignant transformation^{6,7,10)} and they are assumed to play an important role in the adenoma-carcinoma sequence as a unique precursor of colorectal carcinoma.

Fig. 5. A, B, C: The percentage of fields in each range, and the modification of the distribution pattern by changing the range values for the 3 polyps. The highest range values are considered as "carcinoma."

On the basis of structural morphology, adenomas are classified as mild, moderate, or severe dysplasia. In dysplasia one sees irregularity of the glands with infolding and buckling. The dysplastic epithelial cells tend to have hyperchromatic nuclei, and mucin production is often reduced. Severe dysplasia, or *in situ* carcinoma,

is diagnosed when there is bridging or cribriform growth of epithelial cells with accompanying cytological atypia. Mitoses are increased, and occasionally necrosis is present.⁷⁾ Very often, severe dysplasia is a focal change within an adenoma that is otherwise mildly or moderately dysplastic.¹⁾

However, the grading of dysplasia varies among observers.^{11, 12)} There are lesions that are undoubtedly benign, and others undoubtedly malignant, but there are borderline lesions that are considered benign by some observers and malignant by others. Subjective factors, especially the experience of the pathologist, play a great role in the evaluation.

Morphometric indices were established by Nakamura *et al.*, in an attempt to allow objective classification of these lesions as benign or malignant; they were the ISA, the IDD, and the index of nucleus-gland ratio (ING).²⁻⁴⁾ Nakamura *et al.* used these indices to study the histogenesis of colon carcinoma, and they concluded that the majority of colon carcinoma originates *de novo*. Although there are wide differences of criteria for making the diagnosis of carcinoma among pathologists, they determined the ISA and ING values exclusively based on their own histological criteria for carcinoma diagnosis. The diagnosis may be different when the ISA and/or ING values for carcinoma are shifted to match the carcinoma criteria of other pathologists. Therefore, we tried to determine the ISA values in the whole polyp and to examine the changes of distribution pattern when the range values were altered.

We observed a marked variability in one of these indices (ISA) when whole polyps were analyzed. A carcinomatous focus in general showed higher index values than an adenomatous focus. When these indices were divided in four groups according to the grade of dysplasia, we observed heterogeneity in the distribution pattern. When the ranges of the values were changed, a great variation in the distribution pattern was observed.

The mean values obtained by analysis of a small number of fields showed great variability. This variability decreased in parallel with increasing number of fields

analyzed (data not shown). However, when we analyzed whole polyps and the mean values obtained for each polyp were statistically compared, the results were conflicting. The adenoma containing focal carcinoma showed a statistically significant difference from one, but not from the other adenoma, although both adenomas exhibited mild to moderate dysplasia. When we analyzed the carcinomatous focus and the adenomatous focus separately, however, higher index values were obtained from the carcinomatous region.

Morphometric analysis is accurate and objective, and therefore, the diagnosis of carcinoma based upon this modern technique is expected to be reliable. However, our observations presented here show that this is not the case. As each adenoma has ISA heterogeneity and shows a mosaic pattern in dysplasia grading, the mean value from several fields in an adenoma does not represent the ISA value of the whole adenoma. Moreover, as the diagnosis of carcinoma can change depending on the assigned ISA value range for carcinoma, morphometric diagnosis of cancer is not necessarily objective, since the value range for carcinoma is determined according to the cancer criteria of the individual pathologist. It is also inappropriate to determine the origin of colonic carcinoma based on morphometric analysis. However, as we analyzed only one of the indices proposed by Nakamura *et al.*, i.e., the ISA, we can not be sure whether or not the combination of their three indices would give more reliable results.

The analysis of cellular atypia by means of techniques such as DNA flow cytometry, direct karyotyping with high-resolution banding, or highly sensitive molecular probes, may yield more objective parameters.

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