Karyometric Analysis of Precancerous Lesions of Human Colorectal Mucosa

Dragan Mihailovic, Institute of Pathology, University of Nis, Serbia, Yugoslavia

INTRODUCTION
Colorectal carcinoma is one of the commonest neoplasms affecting individuals living in industrialized nations. Colorectal cancer is the third ranking cancer worldwide, accounting for approximately 9% of all cancers. Complex interactions between inherited and acquired genomic and other biological changes associate with both benign and malignant large bowel neoplasms. Colon cancer is highly curable if it is diagnosed in its early stages [1].

Most carcinomas develop from adenomas, their precursor lesions. These adenomas occur sporadically or as a part of a polyposis syndrome. Adenomas are benign by definition, although they are neoplastic and they may harbour an invasive carcinoma [1]. Large size and severe dysplasia increase the risk of the individual adenoma becoming malignant. Whereas size is an objective measurement, the grading of dysplasia is based on micrscopic criteria, which have a considerable subjective element [2]. Despite the findings of inter-observer studies, dysplasia has been a successful marker in clinical practice [3]. Carcinomas also arise in areas of dysplasia in patients with idiopathic inflammatory bowel disease [1].

Morphometry has the advantage, compared with conventional histological grading, of being a method suitable for numerical classification [4]. Meijer and Baak [5] showed that a clear three-group distinction (mild, moderate, severe) could not be obtained in colorectal dysplasia. Similarly to gastric dysplasia, the morphometric features might be used to design two-grade (low, high) rather than a three-grade system to assess the degree of dysplasia.

The aim of this study was to estimate sensitivity, specificity and efficiency of a karyometric method for the early detection of colorectal carcinoma.

MATERIALS AND METHODS
This study included 48 men and 38 women aged from 28 to 79 years. Endoscopic biopsies of colonic carcinoma (n=42), mucosa surrounding carcinoma (n=42), colonic adenoma with low-grade dysplasia (n=18), and chronic colitis (n=25) were analyzed. After standard fixation and paraffin embedding, 4-micrometer thick sections were stained with haematoxylin and eosin, and alcian-blue periodic acid-Schiff reagents (AB-PAS), pH 2.5 (Fig 1).

The mean volume-weighted nuclear volume of epithelial cells was estimated by the point-sampled nuclear intercept method, by an original test system, using a 100x objective and a total magnification of 1200x [6]. Nuclear area, perimeter, circularity and integrated optical density (IOD) were estimated by an image analyzer (Lucia M 3.52 ab, Nikon, Tokyo, Japan), using a 40x (NA 0.65) objective, after manual editing of binary images (Fig 2). In each case a hundred nuclei were measured. Nuclear volume values over 100 µm³ or nuclear area values over 20 µm² were considered as positive findings. The significance of differences...
between groups was estimated by the Mann-Whitney test.

The statistical significance of the diagnostic value of the mean volume weighted nuclear volume or the nuclear area was calculated using the following criteria and formulae [7]:

- True positive (TP) = both colonic carcinoma and the mean nuclear volume or the nuclear area higher than 100 µm³ or 20 µm², respectively.
- True negative (TN) = both colonic carcinoma and the mean nuclear volume or the nuclear area not higher than 100 µm³ or 20 µm², respectively.
- False positive (FP) = the mean nuclear volume or the nuclear area higher than 100 µm³ or 20 µm², respectively, but colonic carcinoma was not present.
- False negative (FN) = colonic carcinoma was present but the mean nuclear volume or the nuclear area were not higher than 100 µm³ or 20 µm², respectively.

Diagnostic sensitivity = \( \frac{TP}{TP + FN} \)

Specificity = \( \frac{TN}{FP + TN} \)

Predictive value of positive test = \( \frac{TP}{TP + FP} \)

Predictive value of negative test = \( \frac{TN}{TP + FN} \)

Efficiency of the test of patients correctly classified = \( \frac{TP + TN}{TP + FP + FN + TN} \)

**RESULTS**

The mean volume-weighted nuclear volume of epithelial cells in colonic mucosa surrounding carcinoma was significantly higher than in adenoma with low-grade dysplasia and chronic colitis (Figs 3b and c). No significant differences in circularity were found (Fig 3d). The lowest IOD was found in adenomas with low-grade dysplasia (Fig 3e). Other differences between groups were not statistically significant.

The diagnostic value of nuclear size in colonic lesions is shown in Table 1. The best results were obtained with the mean volume-weighted nuclear volume; sensitivity and specificity were 90.5% and 92.7%, respectively.

**DISCUSSION**

The best way to reduce the incidence of colorectal cancer mortality would be to prevent this cancer. However, none of the biomarkers proposed can accurately identify persons at increased risk of colorectal cancer or those at low risk. The finding of early genetic changes in histologically normal mucosa and non-neoplastic disorders should contain clinically useful information about the development of early neoplasia.

Two main genetic pathways leading to colorectal adenocarcinoma actually can be distinguished. The first and more common pathway is characterized by sequential inactivation of a series of tumour-suppressor genes, such as APC (localized on chromosome 5q), p53 (chromosome 17p), and genes on chromosome 18q (DCC, Smad2, and Smad4). Tumours generated by the classic ‘suppressor’ pathway display marked chromosomal instability with frequent cytogenetic abnormalities and allelic losses [8].

The second genetic pathway is involved in the development of tumours with hereditary nonpolyposis colorectal cancer (HNPCC), an autosomal-dominant condition that accounts for 2-5% of all colorectal carcinomas and for approximately 15% of sporadic large bowel adenocarcinomas. The hallmark of this alternative ‘mutator’ pathway is widespread microsatellite instability (MSI), which is characterized by the accumulation of somatic alterations in the length of simple, repeated sequences called microsatellites. High-frequency MSI (MSI-H) (instability at >30% of microsatellite loci) results from defects in the DNA mismatch repair system (hMLH1 and hMSH2 genes) [9]. Colorectal carcinomas originating by the first (suppressor) and the second (mutator) pathways differ in several pathologic features. Tumours with widespread MSI, both sporadic and HNPCC, are located predominantly in the proximal colon and demonstrate poor differentiation, mucinous or medullary architecture, and prominent peritumoral lymphocytic infiltration more often than microsatellite-stable tumours and tumours with low-frequency MSI (instability at <3% of microsatellite loci) [10]. Furthermore, patients with MSI-H tumours have a more favorable survival than patients with low frequency MSI/microsatellite–stable tumours [11,12]. A single mutation in an oncogene such as K-ras can be found in simple non-neoplastic normal mucosa [13], colonic metaplastic polyps, and aberrant crypt foci [14] as well as in neoplastic adenomas and colorectal adenocarcinomas. Fleischhacker et al. [15] analyzed the DNA aneuploidy of large bowel cancers and their morphologically normal colorectal tissue from the same individuals. Hypodiploid aneuploidy occurred in morphologically normal colonic tissue from a total of 6% of colon cancer cases. In these cases, no K-rasmutations were found in the normal tissue. We show for the first time increased nuclear size in morphologically normal mucosa surrounding colorectal carcinoma.

Melville et al. [3] examined 86 patients with dysplasia, and the overall agreement between the 5 experienced pathologists grading the specimens was poor; each pair agreed on between 42% and 65% of the slides. The best agreement was for slides that were said to show no dysplasia [3]. Comparison with clinical outcome indicated that the pathologists most likely to diagnose dysplasia in patients with...
carcinoma were also most likely to diagnose dysplasia in patients who did not go on to develop carcinoma. Despite the finding of this inter-observer study, dysplasia has been a successful marker in clinical practice. Pathologists and clinicians must work closely together if dysplastic biopsy specimens are to be correctly interpreted. Re-biopsy should usually be performed before a decision is made concerning surgery, particularly if acute inflammation is present. Meijer and Baak [4] noted that a clear three-group distinction (mild, moderate, severe) could not be obtained in colorectal dysplasia [4]. Karyometric findings in this examination also indicated that the two-grade (low, high) rather than a three-grade system might be used to assess the degree of dysplasia.

CONCLUSIONS
Increased nuclear size in apparently normal mucosa should be a useful marker for identifying persons at higher risk of colorectal cancer. The best results were obtained with the mean volume-weighted nuclear volume.

REFERENCES