

## Assessment of Colorectal Cancer Prognosis Through Nuclear Morphometry

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**Background.** Due to the fact that different biological parameters play a key role in colonic malignant behavior, with nuclear morphometry being a prognostic marker in many malignancies, then predictive approaches in colorectal cancer (CRC) carried out on histologically well-defined groups may prevent interpretative errors. Subsequently, in the present study, CRC patients were screened according to the morphometric features of tumor cell nuclei, using an accurate histotechnical approach, to analyze their clinical evolution according to Dukes' stratification.

**Materials and methods.** A total of 66 cases were grouped according to Dukes' classification (5 y of follow-up). The perimeter, nuclear area, and shape factor of 50 interphase carcinoma nuclei were recorded through microphotographs obtained from each subject. Nuclei boundaries were drawn by an electronic pencil and examined by a computerized system. Data were submitted to a variance analysis, and a multiple regression model compared results.

**Results.** The sample was made up of 44 males (66.67%) and 22 females (33.33%) aged  $59.7 \pm 6$  y old. Forty-nine patients (74.24%) were classified as stage B, and 17 (25.76%) as stage C. Nuclear homogeneity was confirmed by analysis of variance. The nuclear parameters were (mean  $\pm$  SD): area ( $3.17 \pm 1.74$ ), perimeter ( $6.72 \pm 1.83$ ), and shape factor ( $0.82 \pm 0.03$ ). A multiple logistic regression model showed that stage C subjects had a higher risk of developing a worse clinical evolution than those at stage B ( $P < 0.02$ ), independent of sex and age.

**Conclusions.** Dukes' classification remains the best predictor of evolution. Although nucleomorphometric suitability is still controversial, this technique has become an additional tool to establish CRC prognosis. © 2008 Elsevier Inc. All rights reserved.

**Key Words:** clinical evolution; colorectal cancer; histopathology; image analysis; nuclear morphometry.

### INTRODUCTION

It is highly desirable for surgeons to have at their disposal useful tools for prognosis, to choose the best strategy for patients suffering from colorectal cancer (CRC). Despite many pathological and clinical parameters being related to survival rates in CRC patients, their evolution after apparently successfully operations is still difficult to predict. The most suitable and most frequently used prognostic indicator is Dukes' classification [1]. However, in recent years, morphometric image analysis has also been used in an attempt to better define the postsurgical evolution of several carcinomas. Morphometry consists of the measurement of the length, area, or volume of nuclei, cell, and tissue components. For several varieties of noncolorectal cancer, certain morphometric characteristics of tumor cells correlate fairly well with patient survival and provide valuable new information not readily available during routine examinations of tissue sections. The nuclear morphometry of tumors from different sites have been published [2, 3], and some of these studies on the prognostic value of nuclear morphometry in CRC have shown a strong relationship to survival in operated patients [4]. Moreover, it could even be useful in familial colonic polyposis [5].

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Previously, we showed that various different biological parameters and environmental factors, such as dietary habits, seem to play a key role in the colonic malignant behavior [6]. By considering nuclear morphometry as a simple prognostic marker in CRC [7], future studies carried out on histologically well-defined groups may help to prevent interpretative errors in predictive approaches. Therefore, in the present work, patients were screened according to the morphometric features of tumor cell nuclei, using a reliable histotechnical approach to analyze their clinical evolution.

## MATERIALS AND METHODS

### Subject Data

The charts of 66 selected patients, who had previously had curative resection for colorectal carcinoma, were recorded over a period of 10 y. Information with regard to Dukes' staging, age, sex, location of the lesion, and surgical procedures performed were tabulated. Patients in stage C had received postoperative chemotherapy and/or radiotherapy. Subjects with associated diseases, such as diabetes, immunosuppressive diseases, and other colonic diseases such as ulcerative colitis, Crohn's disease, colonic familial polyposis, or antecedents of malignancies other than colorectal were not included in the present study. All patients were monitored over a period of at least 5 y or until death occurred due to oncological progression. Then, they were divided in 2 groups according to their evolution at the end of the period of study as follows: good evolution (free of any evidence of disease) and poor evolution (death or evidence of recurrence obtained by images or pathologic analysis). Tumor tissues were examined to establish the Dukes' staging.

### Histopathology

The histological samples were obtained from the Department of Pathology of Hospital Privado (Cordoba, Argentina). All specimens were fixed in 10% buffered formalin before embedding them in paraffin blocks. Sections were cut of 6- $\mu$ m thickness, stained with hematoxylin and eosin, and both the central and peripheral areas of the cancer were studied. For the morphometric analysis, the most representative areas upon which the histological grading of the lesion was based (free of necrosis, hemorrhage, technical artifacts, or acute inflammatory infiltrates) were selected.

### Image Analysis

The basic image analysis system consisted of:

1. An image source, obtained by an Olympus BH 25-RFCA microscope with a vertical tube on which was mounted an Olympus C-35 camera;
2. Microphotographies, obtained using Agfa Pan 100 films and printed on Ilford gloss paper;
3. A PC Pentium 4 with a SnapScan e50 scanner (Agfa-Gevaert AG, Leverkusen, Germany);
4. The SigmaScanPro V. 4.01 Science program image analysis software (SPSS Inc., Chicago, IL).

For well-differentiated and moderately differentiated histologically graded tumors, nuclear measurements were obtained on cells maintaining the colonic tubular architecture and showing the whole malignant epithelial cell layer from the base to the luminal surface. When neoplastic gland architecture and nuclear polarity were not well preserved, nuclear morphometry was evaluated for the most representative cellular areas. The nuclear perimeter, area, and shape factor (defined as  $4\pi \times \text{area/perimeter}^2$ , where the shape

factor of a circle is equal to unity, and the more an object lengthens, the more the shape factor tends toward 0) of 50 interphase nuclei were recorded in per case. The microphotograph was then digitalized, and the nuclear perimeter traced. In this way, the area, perimeter, and shape factor of each nucleus were obtained [8].

### Statistical Analysis

A variance analysis was applied to compare repeated means for the same subjects in 3 or more groups. A predictive model was obtained by multiple logistic regression for clinical evolution, by setting age, sex, Dukes' stage, and the 3 nuclear morphometric parameters as independent variables. A *P* value of less than 0.05 was considered significant.

## RESULTS

The population (44 males, 22 females) was  $59.7 \pm 6$  y old, with 60.6 and 58.2 y being the means for males and females, respectively. Forty-nine patients (74.24%) were classified as stage B, and 17 (25.76%) as stage C, according to Dukes' stratification. Twenty-four patients (36.36%) died within the monitored period as a consequence of cancer progression. On analyzing 50 nuclei per case, patients with a poor evolution tended to have increased nuclear area means (1.5 times). Thoroughly, the nuclear parameters studied were (mean  $\pm$  SD): area ( $3.17 \pm 1.74$ , range: 1.24–8.56), perimeter ( $6.72 \pm 1.83$ , range: 4.24–11.68), and shape factor ( $0.82 \pm 0.03$ , range: 0.75–0.97), with the last one being the least disperse (Table 1).

First, nuclear homogeneity was confirmed by analysis of variance, with representative microphotographies being shown in Fig. 1. Then, when clinical evolution was assessed in patients within a similar age range and with similar nuclear morphometry getting out the sex effect, it was found by a multiple logistic regression model that stage C subjects had a higher risk of developing a worse clinical evolution than those at stage B (*P* < 0.02).

## DISCUSSION

In patients with colorectal cancer, Dukes' classification is by far the most broadly accepted prognostic indicator of survival. Although other variables, such as the degree of differentiation and extension [9], and the set of variables proposed by Jass *et al.*, have also proved to be independent prognostic factors [10], they are much less powerful predictors than Dukes' stratification. Moreover, many other new prognostic features have recently been proposed, such as dosage of carcinoembryonic antigen and the use of other molecular parameters [11, 12]. However, all of these studies also concluded that Dukes' classification remains the best predictor of evolution. Indeed, in the present study, when clinical evolution was assessed in patients within a similar age range and with similar nuclear morphometry, a higher risk of impairing clinical evolution was

TABLE 1  
Clinicopathological and Nucleomorphometric Data (Analysis of Variance)

	Nuclear parameters (mean $\pm$ SD)		
	Area ( $\mu\text{m}^2$ )	Perimeter ( $\mu\text{m}$ )	Shape factor
Clinical evolution (n)			
Poor (24)	3.42 $\pm$ 1.92	6.97 $\pm$ 1.96	0.81 $\pm$ 0.02
Good (42)	3.02 $\pm$ 1.96	6.56 $\pm$ 1.74	0.82 $\pm$ 0.02
P value	0.37	0.38	0.46
Dbs (95% CI)	0.40 (-0.6 to 1.4)	0.41 (-0.52 to 1.34)	0.01 (0.0 to 0.02)
Dukes stage (n)			
B (49)	3.04 $\pm$ 1.55	6.6 $\pm$ 1.7	0.81 $\pm$ 0.02
C (17)	3.45 $\pm$ 1.98	7.04 $\pm$ 2.01	0.81 $\pm$ 0.02
P value	0.39	0.39	0.24
Dbs (95% CI)	0.41 (-0.53 to 1.35)	0.52 (-0.56 to 1.44)	0 (0 to 0)
Topography (n)			
Left colon (28)	3.3 $\pm$ 1.62	6.86 $\pm$ 1.82	0.81 $\pm$ 0.02
Rectum (22)	3.28 $\pm$ 1.75	6.87 $\pm$ 1.79	0.81 $\pm$ 0.02
Right colon (16)	2.86 $\pm$ 1.69	6.37 $\pm$ 1.77	0.82 $\pm$ 0.02
P value	0.70	0.65	0.52
Dbs (95% CI)	0.44 (-0.6 to 1.48)	0.50 (-0.69 to 1.69)	0.01 (0.00 to 0.02)

CI = confidence interval; Dbs = difference between samples; SD = standard deviation.

found for stage C subjects with respect to those at stage B, independently of the patient gender. Demographics showed a prevailing male incidence at the third age (~60 y). Regarding the pathological data, the sample included principally stage B subjects (~75%), with the others being at stage C, with 36% of deaths.

Summing up, the computerized image analysis of histopathological samples is an easy tool with a low

cost, which allows accurate tumor characterization. Even though the biological mechanism that influences the nuclear morphology of tumor cells remains unclear, nuclear evaluations in different carcinomas have proved to be useful prognostic factors [13, 14]. Other authors, however, suggest that single morphometry is of little prognostic value [15]. Nonetheless, the nucleus-to-cytoplasm ratio and the variations in the

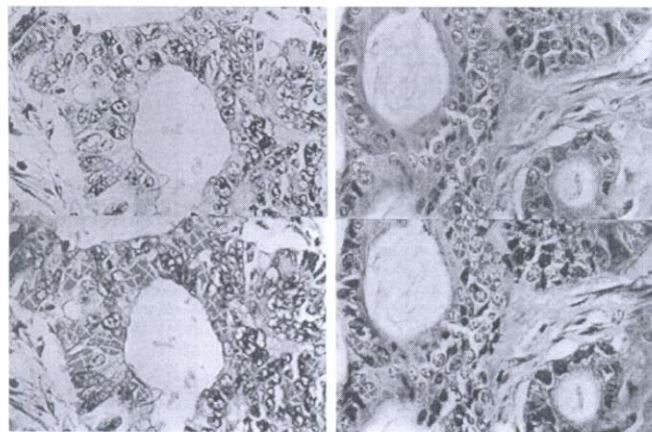


FIG. 1. Nuclear morphometry in colorectal adenocarcinoma. Two representative histopathological areas are shown (hematoxylin and eosin, 600 $\times$ ), obtained from a 49-y-old woman at stage B with good evolution (means for area, perimeter, and shape factor: 5.40  $\mu\text{m}^2$ , 9.36  $\mu\text{m}$ , 0.78; left), and from a 52-y-old man at stage C with poor evolution (means for area, perimeter, and shape factor: 4.57  $\mu\text{m}^2$ , 8.28  $\mu\text{m}$ , 0.82; right). (Below, left and right) Image nucleomorphometric analysis.



cell nucleus have also been suggested as alternative prognostic variables in several diseases [16]. In previous research, shape factor measurements have been applied in the patient evaluation with good results, with increased nuclear area being significantly associated with a poorer outcome [17]. Furthermore, the progressive roundness of the cell nucleus as well as increasing values in the area and perimeter, seem to correspond to an increased neoplastic cell proliferation, enhanced metastatic potential, and poor prognosis [18]. Tumor nuclear grade characterization also has clinicopathological significance, since it allows the grouping of patients to improve outcome prediction. This technology could also be useful in cellular phenotype analysis, such as in chemotherapy-induced apoptosis [19, 20].

Due to their complexity, long-term illnesses with polymorphic clinical expression need to be studied exhaustively, using integrated biological, clinical, and epidemiological data, to improve the prognostic and therapeutic assessments [21]. It is possible that morphometric nuclear evaluations may be done in samples obtained from colorectal biopsies before surgery to permit the analysis of a larger number of nuclei, identify premalignant conditions, and provide predictive data. This could be of great value in preoperative management decisions, thus allowing for a more appropriate selection of patients to be sent for adjuvant therapy.

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