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Short communication

Prognostic value of nuclear area in intestinal type of gastric cancer

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Abstract

Background and purpose: *The incidence of gastric cancer decreases but despite declining, it still remains the second cause of death of all malignancies worldwide. Morphometric methods are independent prognostic variables in various cancers and there have been encouraging results in using it for estimating the prognosis of gastric cancer. Aim of this study was to evaluate the influence of nuclear area (morphometric method), on the survival of patients with intestinal type of gastric cancer.*

Materials and methods: *Seventy-four patients who had undergone gastric resections for adenocarcinoma of the stomach in the University Hospital Center, Rijeka, Croatia, were analyzed in this study. None had received pre-operative radiotherapy or chemotherapy. Age, gender, tumor size, tumor depth into the stomach wall (T-category) presence or absence of metastases to regional lymph nodes (N-category) or distant organs (M-category) and nuclear area of tumor cells were estimated. All patients were followed up for at least 36 months or to death.*

Results: *Univariate analysis showed that gender (P = 0.009), nuclear area (P = 0.017), TNM (P = 0.001) and size of the tumor (P = 0.024) have influence on the survival. Patients age at the time of diagnosis showed no influence on the survival in univariate analysis (P = 0.089). In multivariate analysis only TNM (P = 0.048) and size of the tumor (P = 0.020) are independent prognostic factors for planning further therapy.*

Conclusions: *According to our results, reliable prognostic factors in patients with intestinal type of gastric cancer are still TNM and size of the tumor; nuclear area showed no influence on the outcome of the disease.*

INTRODUCTION

The incidence of gastric cancer decreases worldwide. Despite declining, it still remains the second cause of death of all malignancies worldwide (approximately 800,000 every year) *(1)*. There have been major geographical differences in incidence and prevalence of gastric cancer. Almost 66% of cases occur in developing countries and 42% in China alone. The highest rates of gastric cancer (>20 per 100,000) are found in East Asia (China, Japan), Eastern Europe and parts of Central and South America. Low incidence (<10 per 100,000) is found in Southern Asia, North and East Africa, North America, New Zealand and Australia. In Central Africa the incidence is 12.6 per 100,000. Generally, gastric cancer rates are about twice as high in men than in women but recent epidemiological studies reported the same prevalence in both sexes *(2–6)*.

TNM-classification is considered to be the most reliable determinant of the prognosis of gastric cancer. It has been identified as independent predictor of survival in multiple reports with multivariate analysis. The tumor depth into the stomach wall (T-category) and the presence or absence of metastases to regional lymph nodes (N-category) or distant organs (M-category) are important predictors of disease-free and overall survival *(7, 8)*. Nevertheless, it is necessary to consider other parameters that could influence the outcome of patients with gastric cancer such as for example age, sex, smoking or alcoholism, Lauren histotype, localization of the tumor, lymphonodal or distant metastases *(9, 10)*. Recent studies showed that various other factors could be used as a prognostic indicators in gastric cancer, for example the expression of vascular endothelial growth factor receptor 1, 2 and 3 *(11)*, E – cadherin *(12)*, human epidermal growth factor receptor (HER) 3 and 4 *(13)*, CXCR4 chemokine receptor *(14)*, preoperative serum tumor markers CEA, CA 19–9, CA 72–4, AFP *(15)*, Arp2/3 overexpression *(16)*, tensin4 expression *(17),* etc. Morphometric methods are independent prognostic variables in various cancers; papillary thyroid tumor *(18)*, colorectal cancer *(19)*, nasopharyngeal tumor *(20)*, ovarian mucinous tumor *(21)*, basal cell carcinoma *(22)* and ductal breast tumor *(23)*. There have been encouraging results in using morphometric methods for estimating the prognosis of gastric cancer *(24–27)*. According to original Lauren's classification *(28)* gastric cancers are subdivided in two types: intestinal and diffuse. Modified Lauren's classification, currently in use, recognizes diffuse, intestinal and mixed type *(29)*. Intestinal type of tumor follows precancerous state as for example chronic gastritis with intestinal metaplasia, infection with *Helicobacter pylori*, alcohol and smoking. This type depends on environmental factors and is more frequently present in men. Diffuse type is hereditary illness with same incidence in younger women and men, without evident connection with eating habits or life style. Since there are significant differences in etiology, pathogenesis and size of the cell nuclei between these types we decided to examine the nuclear areas only for intestinal type of gastric cancer. Immunohistochemical methods are often expensive, genetic analyses also, so we decided to evaluate the nuclear area as an independent prognostic factor in gastric cancer because the method is simple, cheap (haematoxylin and eosin staining), objective, quickly performed using a light microscope and easily reproducible.

MATERIAL AND METHODS

Patients

Seventy-four patients who had undergone gastric resections for adenocarcinoma of the stomach at the University Hospital Center Rijeka, Croatia, in the period between January 1, 1993 and December 31, 1999 fulfilled the criteria designed for this study and were analyzed. None had received pre-operative radiotherapy or chemotherapy; follow-up was at least 36 months or to time of death. Bioptic materials were obtained from the files of the Department of Pathology, Faculty of Medicine, University of Rijeka, Croatia. Morphologic examination and classification of the tumors were performed according to the Lauren's classification *(28)* and to the criteria of the Histopathology reporting *(30)*. Multiple macroscopic description and paraffin-embedded samples were available for each tumor.

Histopathological examination

The tissue samples from resected stomachs were cut into 5 mm slices after fixation in 10% buffered formalin. The slices were embedded in paraffin blocks and sections (5 μm thick) were stained with haematoxylin and eosin (H&E) for histopathological examinations.

Computerized nuclear morphometry

The morphometric analysis was performed on H&E stained sections by two observers who have no knowledge of the patients (M.J, D.K.). Sections were viewed under high power microscope (x200, Olympus BX-40, Tokyo, Japan). The images were visualized on a computer display (IBM compatible PC) using a color video camera module (Sony, CCDIRIS, Tokyo, Japan). For each specimen, 10 images of cell fields were captured by each operator, who moved the microscopic field randomly across the specimen. For each slide, a total of 100 cancer cell nuclei with complete and clearly identifiable nuclear outlines were measured. The outline of the cancer cell nucleus on the display was traced using a computer mouse. The mean nuclear areas of 100 cancer cell nuclei per case were calculated using an IBM compatible PC computer program (ISSA – an integrated system for archiving patient/examination data, including images, Ver. 2.95, Copyright(C) VAMS d.o.o. Zagreb, Croatia). For the control the material was taken from 24 patients who underwent gastric surgery for benign gastric ulcers, at the same time period as for these 74 cancer patients. We measured the nuclear areas of epithelial cells in normal gastric mucosa distanced minimally two centimeter from ulcer margin.

Statistical analysis

Data were studied using MedCalc software (MedCalc, Mariakerke, Belgium). Survival was calculated from the date of diagnosis to the last follow-up date or death. Survival curves were calculated using Kaplan-Meier method, presented with survival probability with standard error and compared using nonparametric log-rank test. Cox's proportional hazard regression was performed as a forward stepwise method with original numerical data and nominal variables coded binary. Cox statistics is presented with regression coefficients (β) and their standard errors (S.E.(β)), and with odds ratio and 95% confidence intervals for odds ratio. All *P* values reported refer to two sided tests and only those lower than 0.05 were considered significant.

RESULTS

Basic data of 74 patients in the study are presented in Table 1. Fifty-six patients were male and eighteen were females (76% vs 24%), aging from 42 to 81 years (median 65.5 years). The smallest tumor had a diameter of 2 cm, and the biggest diameter was 8.0 cm (median 4.9 cm). The size of nuclear areas ranged from 17.583 to 95.823 μm^2 (median 44.289 μm^2). According to the TNM classification the tumors were mostly advanced gastric cancers, big in size, infiltrating the adjacent tissues including the regional lymph nodes.

Overall survival of patients in this study is presented in Figure 1. After a three-year period 39.2±5.7% of patients was alive. The median follow-up was 18.5 months (range 1–124 months); for patients that were alive it was 81 months (range 55–124 months), and for those who died it was 12 months (range 1–39 months). Forty-seven patients (63%) died as a result of metastatic dissemination of the disease while the remaining twenty-seven (37%) were alive and without any evidence of residual tumor.

Univariate analysis showed that only age had no influence on survival ($P = 0.089$), and that gender ($P = 0.009$), areas ($P = 0.017$), TNM ($P = 0.001$) and size of the tumor ($P = 0.024$) are the variables that should be included in multivariate analysis. Mean nuclear area is not a prognostic factor according to the results of multivariate analysis ($P = 0.404$). Only TNM ($P = 0.048$) and size of the tumor ($P = 0.020$) can be considered as independent prognostic factors for predicting the course of the disease (Table 2).

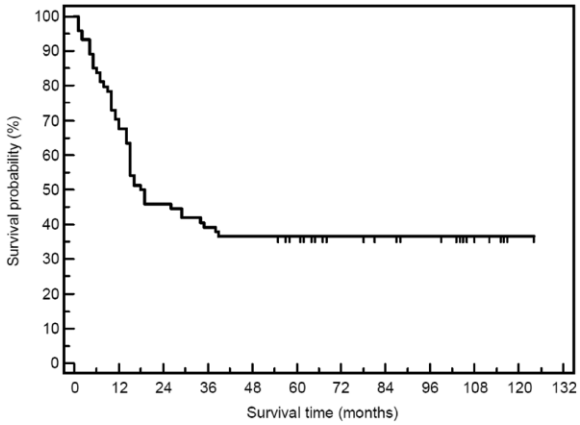

Figure 1. *Overall survival of 74 patients in the study, with censored data denoted with signs on the Kaplan-Meier curve. Seventy-four patients who had undergone gastric resections for adenocarcinoma of the stomach in the University Hospital Center of Rijeka, Croatia, were analyzed. None had received pre-operative radiotherapy or chemotherapy. All patients were followed up for at least 36 months or to death.*

TABLE 1

Basic data on parameters of 74 patients in the study.

Parameter		N (%)	Median	Range
age (yrs.)			65.5	42–81
gender	males	56 (76%)		
	females	18 (24%)		
tumor diameter (cm)			4.9	2.0–8.0
TNM	T2N0M0	29 (39%)		
	T2N1M0	6 (8%)		
	T3N0M0	24 (32%)		
	T3N1M0	3 (4%)		
	T3N1M1	1 (1%)		
	T3N2M0	4 (6%)		
	T3N2M1	7 (10%)		

DISCUSSION AND CONCLUSION

Morphometric methods have been studied for almost fifteen years. Shao *et all.* (1992) morphometrically examined more than hundred specimen of dysplasia and carcinoma of gastric mucosa and showed that computer-assisted morphometry can offer objective criteria in the differential diagnosis of gastric dysplasia and carcinoma *(31)*. The same year Hamilton et all. reported the results of their study performed on patients with gastric cancer; they compared morphometric data with patient survival, clinico-pathological status and DNA ploidy *(32)*. The results showed that the nuclear size variation is significantly associated with the presence of lymphatic invasion and resection margin involvement. Other investigators reported that nuclear area and perimeter and their variation were closely related to survival in univariate, but not

TABLE 2

Results of univariate and multivariate analysis.

	univariate		multivariate		
variable	β±S.E.(β)	P	β±S.E.(β)	P	Odds ratio
gender	1.05±0.40	0.009	0.64±0.37	0.083	–
age	0.03±0.02	0.089	–	–	–
areas	0.02±0.01	0.017	0.01±0.01	0.404	–
TNM	0.80±0.24	0.001	0.15±0.08	0.048	1.17 (1.01–1.36)
size	0.26±0.12	0.024	0.28±0.12	0.020	1.33 (1.05–1.68)

in the multivariate analysis *(33)*. We report similar results in our study; the only difference is that Setala et all. examined only patients with I-II stage while in our study the patients had I-IV stage of gastric cancer. Ikeguchi et all. showed that nuclear area is an independent prognostic factor in multivariate analysis, and that lymph node metastasis, lymphatic invasion and venous invasion were more frequently detected in patients with large nuclear areas. Our results showed that nuclear area could not be used as an independent prognostic factor *(34)*. Possible explanation may be in different number of patients included in two studies; Ikeguchi *et al. (34)* examined 202 patients and in our study only 74 patients were examined. In another study Ikeguchi et all. showed that nuclear area correlate strongly with haematogenous and lymph node recurrence or relapse after gastrectomy and that nuclear area of cancer cells was identified as independent prognostic factor in gastric cancer *(27)*. This study was also performed on a large number of patients (400 patients). Morphometric methods showed encouraging results in gastric cancer *(24–27, 31–34)* and also in other cancers *(18–23)*. The method is simple, quick, reproducible, and the data are objective and can be quickly derived using conventional microscopic analysis. However, there are only few studies that investigated the influence of nuclear area on survival of patients with gastric cancer. The results are also controversial, only a few studies showed that nuclear area is an independent prognostic factor according to the multivariate analysis. We can conclude that the method is simple, but since the results are controversial (our results showed that nuclear area should not be used as an independent prognostic factor, $P = 0.404$) there is need of further studies on a larger number of patients.

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