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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 $39.2 \pm 5.7\%$ 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 in-

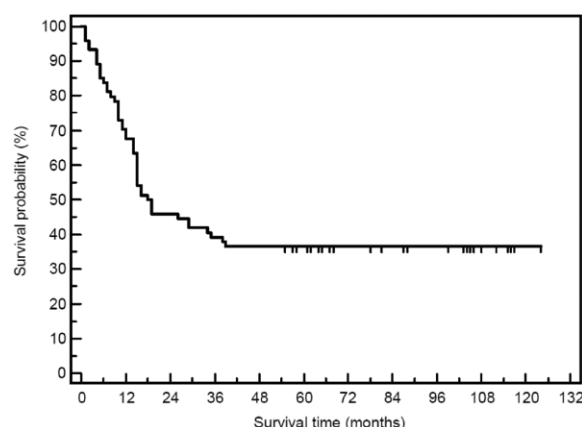


Figure 1. Overall survival of 74 patients in the study, with censored data denoted with signs on the Kaplan-Meier curve. Seventy-four patients who had undergone gastric resections for adenocarcinoma of the stomach in the University Hospital Center of Rijeka, Croatia, were analyzed. None had received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

cluded 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).

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 al.* (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 al.* 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.

variable	univariate		multivariate		
	$\beta \pm \text{S.E.}(\beta)$	P	$\beta \pm \text{S.E.}(\beta)$	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 Setälä *et al.* examined only patients with I-II stage while in our study the patients had I-IV stage of gastric cancer. Ikeguchi *et al.* 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 al.* 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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REFERENCES

- WHO 2009 *Cancer (Fact sheet No297)*
- DE VRIES A C, MEIJER G A, LOOMAN C W, CASPARIE M K, HANSEN B E, VAN GRIEKEN N C, KUIPERS E J 2007 Epidemiological trends of pre-malignant gastric lesions: a long-term nationwide study in the Netherlands. *Gut* 56(12):1665–70
- PARKIN D M, BRAY F, FERLAY J, PISANI P 2005 Global cancer statistics, 2002. *CA Cancer J Clin* 55(2): 74–108
- D. M. PARKIN F B 2006 International patterns of cancer incidence and mortality. In: Schottenfeld D, Fraumeni J F (eds) *Cancer epidemiology and prevention*. Oxford University Press, New York, 2006.
- WHO 2003 *The World Health Report 2003* (Geneva, 2003).
- LEE K J, INOUE M, OTANI T, IWASAKI M, SASAZUKI S, TSUGANE S 2006 Gastric cancer screening and subsequent risk of gastric cancer: a large-scale population-based cohort study, with a 13-year follow-up in Japan. *Int J Cancer* 118(9): 2315–21
- H ALEXANDER D K, TEPPER J 1993 Cancer of the Stomach. In: Vita V, Hellman S, Rosenberg S, In: *Cancer, Principles and Practice of Oncology*, Lippincott J B (ed) Philadelphia.
- AMERICAN JOINT COMMITTEE ON CANCER 1993 Manual for Staging of Cancer In: Lippincott J. B (ed), Philadelphia.
- GUIDA F, FORMISANO G, ESPOSITO D, ANTONINO A, CONTE P, BENCIVENGA M, PERSICO M, AVALLONE U 2008 [Gastric cancer: surgical treatment and prognostic score]. *Minerva Chir* 63(2): 93–9
- KIM J H, BOO Y J, PARK J M, PARK S S, KIM S J, KIM C S, MOK Y J 2008 Incidence and long-term outcome of young patients with gastric carcinoma according to sex: does hormonal status affect prognosis? *Arch Surg* 143 (11): 1062–7; discussion 1067
- HIRASHIMA Y, YAMADA Y, MATSUBARA J, TAKAHARI D, OKITA N, TAKASHIMA A, KATO K, HAMAGUCHI T, SHIRAO K, SHIMADA Y, TANIGUCHI H, SHIMODA T 2008 Impact of vascular endothelial growth factor receptor 1, 2, and 3 expression on the outcome of patients with gastric cancer. *Cancer Sci*
- LAZAR D, TABAN S, ARDELEANU C, DEMA A, SPOREA I, CORNIANU M, LAZAR E, VERNIC C 2008 The immunohistochemical expression of E-cadherin in gastric cancer; correlations with clinicopathological factors and patients' survival. *Rom J Morphol Embryol* 49(4): 459–67
- HAYASHI M, INOKUCHI M, TAKAGI Y, YAMADA H, KOJIMA K, KUMAGAI J, KAWANO T, SUGIHARA K 2008 High expression of HER3 is associated with a decreased survival in gastric cancer. *Clin Cancer Res* 14(23): 7843–9
- TSUBOI K, KODERA Y, NAKANISHI H, ITO S, MOCHIZUKI Y, NAKAYAMA G, KOIKE M, FUJIWARA M, YAMAMURA Y, NAKAO A 2008 Expression of CXCL12 and CXCR4 in pT3-stage gastric cancer does not correlate with peritoneal metastasis. *Oncol Rep* 20(5): 1117–23
- UCAR E, SEMERCI E, USTUN H, YETIM T, HUZMELI C, GULLU M 2008 Prognostic value of preoperative CEA, CA 19–9, CA 72–4, and AFP levels in gastric cancer. *Adv Ther* 25(10): 1075–84
- ZHENG H C, ZHENG Y S, LI X H, TAKAHASHI H, HARA T, MASUDA S, YANG X H, GUAN Y F, TAKANO Y 2008 Arp2/3 overexpression contributed to pathogenesis, growth and invasion of gastric carcinoma. *Anticancer Res* 28(4B): 2225–32
- SAKASHITA K, MIMORI K, TANAKA F, KAMOHARA Y, INOUE H, SAWADA T, HIRAKAWA K, MORI M 2008 Prognostic relevance of Tensin4 expression in human gastric cancer. *Ann Surg Oncol* 15(9): 2606–13
- NAGASHIMA T, SAKAKIBARA M, NAKATANI Y, MIYAZAKI M 2008 Preoperative cytologic morphometry may assist in predicting patient outcome after surgery in papillary thyroid cancer. *Anal Quant Cytol Histol* 30(4): 231–6
- EYNARD H G, SORIA E A, CUESTAS E, ROVASIO R A, EYNARD A R 2009 Assessment of colorectal cancer prognosis through nuclear morphometry. *J Surg Res* 154(2): 345–8
- HUANG T, CHEN M, WU M, WU X 2008 Image analysis of DNA content and nuclear morphometry for predicting radiosensitivity of nasopharyngeal carcinoma. *Anal Quant Cytol Histol* 30(3): 169–74
- VERSA-OSTOJIC D, STANKOVIC T, STEMBERGER-PAPIC S, VRDOLJAK-MOZETIC D, MANESTAR M, KRASEVIC M 2008 Nuclear morphometry and AgNOR quantification: computerized image analysis on ovarian mucinous tumor imprints. *Anal Quant Cytol Histol* 30(3): 160–8
- CHERETIS C, ANGELIDOU E, DIETRICH F, POLITI E, KIARIS H, KOUTSELINI H 2008 Prognostic value of computer-assisted morphological and morphometrical analysis for detecting the recurrence tendency of basal cell carcinoma. *Med Sci Monit* 14(5): MT13–19
- EL SHARKAWY S L, FARRAG A R 2008 Mean nuclear area and metallothionein expression in ductal breast tumors: correlation with estrogen receptor status. *Appl Immunohistochem Mol Morphol* 16(2): 108–12
- FICSOR L, VARGA V, BERCZI L, MIHELLER P, TAGSCHEERER A, WU M L, TULASSAY Z, MOLNAR B 2006 Automated virtual microscopy of gastric biopsies. *Cytometry B Clin Cytom* 70(6): 423–31
- MEGALOPOULOU T M, KOUTROUMBAS K, POULIAKIS A, SIVOLAPENKO G, KARAKITSOS P 2006 The potential of feature selection by statistical techniques and the use of statistical classifiers in the discrimination of benign from malignant gastric lesions. *Oncol Rep* 15 (Spec no.): 1033–6
- KARAKITSOS P, MEGALOPOULOU T M, POULIAKIS A, TZIVRAS M, ARCHIMANDRITIS A, KYROUDIS A 2004 Application of discriminant analysis and quantitative cytologic examination to gastric lesions. *Anal Quant Cytol Histol* 26(6): 314–22
- IKEGUCHI M, CAI J, OKA S, GOMYU Y, TSUJITANI S, MAETA M, KAIBARA N 2000 Nuclear profiles of cancer cells reveal the metastatic potential of gastric cancer. *J Pathol* 192(1): 19–25

28. LAUREN P 1965 The Two Histological Main Types of Gastric Carcinoma: Diffuse and So-Called Intestinal-Type Carcinoma. An Attempt at a Histo-Clinical Classification. *Acta Pathol Microbiol Scand* 64: 31–49
29. TEGLBJAERG P S, VETNER M 1977 A case of massive, unilateral oedema of the ovary simulating tumour. *Acta Obstet Gynecol Scand* 56(2): 157–9
30. ALLEN D 2000 *Histopathology reporting – Guidelines for Surgical Cancer*. Springer, New York.
31. SHAO L 1992 [Morphometric analysis of gastric dysplasia and malignancy]. *Zhonghua Zhong Liu Za Zhi* 14(4): 264–6
32. HAMILTON P W, WYATT J I, QUIRKE P, WATT P C, ARTHUR K, WARD D C, JOHNSTON D 1992 Morphometry of gastric carcinoma: its association with patient survival, tumour stage, and DNA ploidy. *J Pathol* 168(2): 201–8
33. SETÄLÄ L, LIPPONEN P, KOSMA V M, MARIN S, ESKELINEN M, SYRJÄNEN K, ALHAVA E 1997 Nuclear morphometry as a predictor of disease outcome in gastric cancer. *J Pathol* 181(1): 46–50
34. IKEGUCHI M, OKA S, SAITO H, KONDO A, TSUJITANI S, MAETA M, KAIBARA N 1999 Computerized nuclear morphometry: a new morphologic assessment for advanced gastric adenocarcinoma. *Ann Surg* 229(1): 55–61