JBUON 2021; 26(4): 1466-1478
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

Clinical and pathological significance of proliferation index and p53 expression in gastric adenocarcinoma

Miljan Zindovic[1], Milena Vuletic[2], Sanja Milenkovic[3], Snezana Jancic[2], Miljan Krstic[4], Dunja Zindovic[5], Velimir Milosevic[6]

[1]Clinical Center of Montenegro, Center for Abdominal Surgery, Podgorica, Montenegro. [2]University of Kragujevac, Faculty of Medical Sciences, Department of Pathology, Kragujevac, Serbia. [3]Clinical and Hospital Center Zemun, Clinical Pathology Department, Belgrade, Serbia. [4]University of Nis, Faculty of Medicine, Institute of Pathology, Nis, Serbia. [5]Clinical Center of Montenegro, Clinic for Anestesia, intensive care and pain therapy. [6]Codra Hospital, Podgorica, Montenegro.

Summary

Purpose: *The purpose of our work was to investigate the association between proliferative index [proIDX] and expression index p53 (p53IDX) with the clinical and pathological characteristics of gastric adenocarcinoma.*

Methods: *The biopsy material of 90 patients operated on for gastric cancer was routinely processed in paraffin and archived. After the histopathological report was made, two study groups were formed, the first group (n=45) comprised biopsies with intestinal carcinoma and the second (n=45) biopsies of diffuse gastric cancer. In both cases, the control group consisted of biopsies of surrounding non-tumor tissue The routine Hematoxylin-Eosin and immunohistochemical ABC method with anti-Ki67 and anti-p53 antibodies was applied at sections 3-5 μm thick. The expression of Ki67 and p53 was quantified stereometrically. For statistical analysis SPSS (19.0) was used.*

Results: *Significantly higher Ki67 expression was found in both types of adenocarcinoma compared to the control group, as well as significant association of proIDX with most of testing parameters. Expression of p53 was significantly higher in the intestinal type compared to the diffuse type and the control group and was significantly associated with age and histological grade. Diffuse type particulary showed, significant association of p53IDX with most of the histological parameters tested.*

Conclusion: *Our results point a highly significant correlation of the Ki67 and p53 expression with indicators of gastric adenocarcinoma progression, which may help to identify patients with an aggressive gastric adenocarcinoma phenotype.*

Key words: *stomach, adenocarcinoma, immunohistochemistry, expression of Ki67 and p53*

Introduction

Gastric cancer, behind lung, colorectum, breast, and prostate cancers, is the fifth most common malignant tumor in humans [1] and the second most common cause of death [2]. There were 723,100 deaths reported worldwide just in 2012. The highest rates of cancer patients and deaths are in East Asia (Korea, Mongolia, Japan and China), Central and Eastern Europe and South and Latin America [1, 3]. There are reports that more than 40% of gastric cancer cases in the world are reported in China [1].

Gastric cancer rarely occurs in people under 30 and its incidence in older age groups increases markedly with every decade of life after 55-65 years old, reaching its highest incidence around 75-85 years old [2].

In relation to histogenesis, gastric cancers are the most common adenocarcinomas accounting for more than 95% of malignant gastric tumors [2]. There are numerous histological classification systems, but the most commonly used is the his-

Corresponding author: Snezana Jancic, MD, PhD. University of Kragujevac, Faculty of Medical Sciences, Department of Pathology, Svetozara Markovica 69, Kragujevac, 34000, Serbia.
Tel: +381605776077, Fax +38134306800, Email: sjancic@medf.kg.ac.rs
Received: 21/02/2021; Accepted: 26/03/2021

tological classification of Pekka Lauren from 1965 and the classification of the World Health Organization (WHO) from 2010. According to Lauren, gastric cancers are divided into an intestinal type that occurs in about 50% and a diffuse type that occurs in about 33% of cases, and there is a mixed, unclassified type that accounts for about 17% of cases of gastric cancer [4].

Gastric carcinogenesis is a multi-stage process in which various molecular disorders occur that lead to neoplastic transformation. Numerous epidemiological and genetic studies indicate that gastric cancer is a multifactorial disease resulting from the interaction of endogenous and environmental factors [4-9]. Key developments in this process include alterations of the oncogene and inactivation of the tumor suppressor gene, conditioning cell migration and proliferation, which determine the biological behavior of the tumor. Numerous studies have highlighted the association of cell kinetics and proliferation with aggressive behavioral and tumor prognosis of different histogenesis [10 -13].

One of the most sensitive markers of the cell's proliferative potential is the Ki67 protein encoded by the Ki67 gene, which is located on the 10q25 chromosome [13,14]. Antigen expressed exclusively in the nucleus and only detected in the dividing cell detects monoclonal Ki67 antibodies. The association of proliferative Ki67 antigen with p53 expression has been established in several tumors [14].

The p53 protein is a multifunctional, nucleoprotein encoded by the tumor suppressor TP53 gene located on the short arm of chromosome 17(17p13.1). Unlike normal p53 protein, which rapidly disappears from the nucleus, mutant forms have a prolonged half-life, accumulate in the nucleus and can be detected by immunohistochemical methods [15]. Expression of p53 has been described in many malignant and benign tumors [11,16]. Known as the "guardian of the genome," p53 plays a role in cell cycle control, DNA repair, apoptosis, and prevention of tumor mutations [16].

In this study, we examined the association of proliferation index and p53 expression index with the clinical-pathological characteristics of intestinal and diffuse gastric cancer.

Methods

Patients and tissue samples

The tissue material of patients operated on for gastric cancer at the Surgical Clinic of Clinical and Hospital Center Zemun in the period from the beginning of year 2005 to June 1, 2014. was used for the research. In the Department of Clinical Pathology, according to the established protocol, 10-15 biopsies, including 3-4 biopsies of surrounding non-tumor tissue of the stomach, are taken from each surgical preparation, depending on the size of the tumor. After fixation in 4% neutral buffered formaldehyde solution, biopsy material was routinely processed, paraffin molded, and archived.

Based on the archived standard pathohistological report, from the stated period, two study groups were formed: the first group (n=45) consisted of operative biopsies with intestinal type of cancer, and the second study group (n=45) consisted of operative biopsies of diffuse gastric cancer. The control group of both, the first and the second study group, in this study, are biopsies of proximal non-tumor tissue, which are taken according to the protocol from an operative preparation delivered to the Department of Pathology. Differentiation of the histological type of the tumor was done according to Lauren's criteria. The study protocol was approved by the local Ethics Committee, which gave permission for the use of paraffin embedded tissues.

Immunohistochemical examination

From paraffin blocks, in which samples of tumor tissue and regional lymph glands were molded, 3-5 μm thick cuts were made and heated at 55°C to melt the paraffin, deparaffinized in xylene (3 times per 5 min) and then rehydrated trough graded ethanols. Antigen retrieval was enhanced by autoclaving slides in sodium citrate buffer (pH 6.0) for 30min. Endogenous peroxidase activity was blocked by 0.3% hydrogen peroxide-methanol buffer for 25 min. Mouse monoclonal p53 antibody (1:100, DAKO, Denmark) and rabbit monoclonal Ki67 antibody (1:100, Abcam, Burlingame, CA, USA) were incubated at +4°Covernight. Immunostaining was performed by the avidin-biotin peroxidase complex (ABC) method (Vectastain ABC-*Elite* kit, Vector Laboratories, Burlingame, CA, USA). Staining was visualized with 3.3 diaminobenzidine tetrachloride (DAB). The slides were counterstained with Mayer hematoxylin and mounted in Canada balsam. In negative controls the primary antibody was replaced with phosphate buffered saline (PBS).

Quantification of immunohistochemical staining

For the determination of Ki67 and p53 values, only the discoloration of the nuclei was taken into account, and for the determination of the density of Ki67 and p53 positive cells in mm^2 of surface, we used the multifunctional test system M42 by Weibel. An objective micrometer (Reichert Wien 2mm / 200) calibrated the test system on a Nikon Eclipse Ni microscope (MBP 99 400) at a magnification of 400 (10 eyepiece x 40 lens), with a measurement field of 0.016mm^2. For the assay of Ki67 and p53 positive cells / mm^2 density, 10 "hot spots" were counted successively. The absolute value of the density of positive cells in the hot spot was determined stereometrically [17]. The arithmetic mean of the obtained hotspot values represents the finite number of positive cells in mm^2 per case. Then, the median was determined and the subjects were divided into two groups: those with low levels of expression (values less than or equal to the values of the median) and those with high

Table 1. Clinical and pathological features of gastric carcinoma studied

Clinical and pathological factors	*Number of cases (n=90) n (%)*
Gender	
Males	53 (58.9)
Females	37 (41.1)
Intestinal carcinoma	45 (50)
Males	33 (73.3)
Females	12 (26.7)
Diffuse carcinoma	45 (50)
Males	20 (44.4)
Females	25 (55.6)
Average age (min-max) [years]	64,11 (37-84)
Intestinal carcinoma	67,11 (33-81)
Diffuse carcinoma	61,11 (37-84)
Location	
Intestinal carcinoma	
The upper half of the stomach	15 (33)
The lower half of the stomach	30 (66,7)
Diffuse carcinoma,	
The upper half of the stomach	17 (37,8)
The lower half of the stomach	28 (62,2)
Growth pattern	
Intestinal carcinoma/Diffuse carcinoma	
Ulcerative type	16 (35,6)/8 (17,8)
Ulceroinfiltrative type	9 (20)/20 (44,4)
Vegetative type	4 (8,9)/2 (4,4)
Vegetative infiltrative type	11 (24,4)/5 (11,1)
Infiltrative type	0 (0)/6 (13,3)
Ulcerovegetative type	5 (11,1)/4 (8,9)
Grade of tumor	
Intestinal carcinoma	G1 0 (0); G2 31 (68,9); G3 14 (31,1);
Diffuse carcinoma	G1 0 (0); G2 8 (17,8); G3 37 (82,2);
Lymphatic invasion	
Intestinal carcinoma	L0 11 (24,4); L1 34 (75,6);
Diffuse carcinoma	L0 14 (31,1); L1 31 (68,9)
Vascular invasion	
Intestinal carcinoma	V0 25 (55,6); V1 20 (44,4)
Diffuse carcinoma	V0 27 (60); V1 18 (40)
Grade of tumor	
Intestinal carcinoma pT1a/T1b/T2/T3/T4	1 (12,2); 0 (0); 7 (15,6); 34 (75,6); 3 (8,7)
Diffuse carcinoma pT1a/T1b/T2/T3/T4	4 (8,9); 5 (11,1); 3 (6,7); 26 (57,8); 7 (15,6)
Lymph node metastases	
Intestinal carcinoma pN0/N1/N2/N3a/N3b	13 (28,9); 10 (22,2); 9 (20,0);11 (24,4); 2 (4,4)
Diffuse carcinoma pN0/N1/N2/N3a/N3b	17 (37,8); 5 (11,1); 6 (13,3); 8 (17,8); 9 (20,0)
Distant metastases	
Intestinal carcinoma pM0/M1	42 (93,3); 3 (6,7)
Diffuse carcinoma pM0/M1	40 (88,9); 5 (11,1)
Stage of tumor	
Intestinal carcinoma IA/IB/IIA/IIB/IIIA/IIIB/IV	2 (4,4); 4 (8,9); 8 (17,8); 11 (24,4); 8 (17,8); 10 (22,2); 2 (4,4)
Diffuse carcinoma IA/IB/IIA/IIB/IIIA/IIIB/IV	8 (17,8); 3 (6,7); 7 (15,6); 4 (8,9); 6 (13,3); 12 (26,7); 5 (11,1)

L0, V0 - negative lymph and blood vessels; L1, V1 - positive lymph and blood vessels

levels of expression (values greater than the values of the median). From the absolute values of p53 and the absolute values of the proliferative antigen Ki67 relative to deviations from the median, we obtained the expression index p53 (p53IDX) and the proliferation index (proIDX). Expression of the investigated markers was evaluated by two pathologists.

Statistics

The statistical software package SPSS for Windows (19.0 IBM Corp) was used for conducting statistical analysis. For the purpose of conducting the analysis of the significance of differences of parametric and nonparametric features, between and within the groups, were used x^2-test (chi-square test), the Mann-Whitney U-test, Fisher exact test and Student's t-test. Afterwards, the Kolmogorov-Smirnov normality test, univariate statistical analysis and correlation analysis (Spearman's rank correlation coefficient) were used. P values less than 0.05 were considered statistically significant.

Results

Observed groups of subjects with gastric cancer

This study included 90 subjects with intestinal and diffuse gastric cancer, of which 45 were with intestinal and as many subjects with diffuse gastric cancer. Of the total number 53 (58.9%) were male and 37 (41.1%) were female. The main clinical and pathological characteristics of intestinal and diffuse gastric carcinoma are shown in Table 1.

Ki67 and p53 expression in gastric adenocarcinoma and in adjacent non-tumor tissue

High or moderate intranuclear expression of Ki67 was observed in all cases of both intestinal and diffuse gastric cancer. Also, intranuclear, high or moderate expression of p53 was found in 31 (68.9%) cases of intestinal and in 24 (53.3%) diffuse adenocarcinomas.

Statistical expression of Ki67 and p53 expression obtained absolute distribution of immunohistochemically positive cells in mm^2 of tissue. The basic characteristics of these sizes (median, minimum and maximum values, significance of differences) for expression of Ki67 and p53 in intestinal, in diffuse cancer and in the control group are shown in Table 2.

The applied median test for expression of Ki67 immunopositive cells shows that Ki67 expression is similar in both histopathological types of gastric cancer, i.e. that the difference in median is not significant (approximately both types of tumors have about 3000 Ki67 immunopositive cells in mm^2 of tissue) but relative to the control group the median test showed significantly higher Ki67 expression values in both intestinal and diffuse gastric cancer.

The expression of p53 shown by the number of positive cells in mm^2 of tissue (median=2462.522) in intestinal gastric cancer was significantly higher compared to the diffuse gastric cancer and control group.

Association of Ki67 expression and clinicopathological characteristics of gastric adenocarcinoma (Table 3)

Expression of immunoreactive Ki67 cells in mm^2 or proliferation index (proIDX) was not significantly associated with the sex of subjects, either in intestinal or diffuse gastric adenocarcinoma. However, a slightly higher incidence of high proIDX in males was observed in both histopathological types of adenocarcinoma.

No significant difference in Ki67 / proIDX expression was observed with respect to age of subjects by type of gastric adenocarcinoma. In both types of cancer, the prevalence of low proIDX was observed in subjects younger than 60 years, while in diffuse cancer, a higher incidence of high proIDX was observed in subjects older than 60 years, but without statistical significance.

Ki67 / proIDX expression was not significantly associated with macroscopic tumor type in either intestinal or diffuse adenocarcinoma, but it was observed that high proIDX was significantly more common in ulcerative vegetative macroscopic tumor types in both histological types (in intestinal in 60% and in diffuse adenocarcinoma in 75% of cases).

Table 2. Expression of Ki67 and p53 positive cells per mm^2 in examened and control group

Groups	*Intestinal type*		*Diffuse type*		*Control group*	
Marker	*Ki67*	*p53*	*Ki67*	*p53*	*Ki67*	*p53*
Median	2795.617	2462.522	3200.089	1070.662	547.227	142.755
Minimum	1617.889	0.000	1439.445	0.000	452.057	0.000
Maximum	3759.213	10159.390	4235.063	7934.793	761.360	606.708
Significance (p)	0.522	0.010	<0.001	<0.001	<0.001	<0.001

*significant at p<0.05, Median test

Table 3. Association of Ki67 and p53 expression with clinical and pathological parameters

Type of tumor	*Intestinal type*					*Diffuse type*				
Markers	*Ki67*			*p53*		*Ki67*			*p53*	
Tested parameters, n (%)	*low*	*high*	*0*	*low*	*high*	*low*	*high*	*0*	*low*	*high*
Localization										
Upper parts of stomach	5 (33,3)	10 (66,7)	2 (13,3)	4 (26,7)	9 (60,0)	8 (47,1)	9 (52,9)	9 (52,9)	0 (0)	8 (47,1)
Lower parts of stomach	18 (60,0)	12 (40,0)	12 (40,0)	4 (13,3)	14 (46,7)	14 (50,0)	14 (50,0)	12 (42,9)	2 (7,1)	14 (50,0)
Significance, p value	0.92			0,163		0,546			0,481	
Histological grade										
moderately differentiated	20 (64,5)	11 (35,5)	13 (41,9)	6 (19,4)	12 (38,7)	8 (100)	0 (0)	8 (100)	0 (0)	0 (0)
poorly differentiated	3 (21,4)	11 (78,6)	1 (7,1)	2 (14,3)	11 (78,6)	14 (37,8)	23 (62,2)	13 (35,1)	2 (5,4)	22 (59,5)
Significance, p value	0.07*			0,032*		0,001*			0,004*	
Lymphatic invasion										
not identified	11 (100)	0 (0)	5 (45,5)	2 (18,2)	4 (36,4)	14 (100)	0 (0)	12 (85,7)	1 (7,1)	1 (7,1)
lymphatic invasion present	12 (35,3)	22 (64,7)	9 (26,5)	6 (17,6)	19 (55,9)	8 (25,8)	23 (74,2)	9 (29,0)	1 (3,2)	21 (67,7)
Significance, p value	<0,001*			0,453		<0,001*			0,001*	
Lymph nodes metastases										
no	13 (100)	0 (0)	5 (38,5)	3 (23,1)	5 (38,5)	17 (100)	0 (0)	13 (76,5)	1 (5,9)	3 (17,6)
1 to 6 lymph nodes	9 (47,4)	10 (52,6)	6 (31,6)	4 (21,1)	9 (47,4)	5 (45,5)	6 (54,5)	5 (45.5)	0 (0)	6 (54,5)
7 and more	1 (7,7)	12 (92,3)	3 (23,1)	1 (7,7)	9 (69,2)	0 (0)	17 (100)	3 (17,6)	1 (5,9)	13 (76,6)
Significance, p value	<0,001*			0,582		<0,001*			0,011*	
Distant metastases										
not identified	23 (54,8)	19 (45,2)	14 (33,3)	7 (16,7)	21 (50,0)	22 (55,0)	0 (45,0)	21 (52,2)	1 (2,5)	18 (45,0)
present metastases	0 (0,0)	3 (100)	0 (0)	1 (33,3)	2 (66,7)	18 (0)	5 (100)	0 (0)	1 (20)	4 (80,0)
Significance, p value	0,067			0,452		0,020*			0,030*	
Pathological stage of the tumor										
pT_1-pT_2	8 ((100)	0 (0,0)	3 (37,5)	2 (25,0)	3 (37,5)	12 ((100)	0 (0,0)	11 (91,7)	0,0	1 (8,3)
pT_3-pT_4	15 (40,5)	22 (59,5)	11 (29,7)	6 (16,2)	20 (54,1)	10 (30,3)	23 (69,7)	10 (30,3)	2 (6,1)	21 (63,6)
Significance, p value	0,002*			0,682		<0,001*			0,001*	
Stage of tumor disease										
I	5 (83,3)	1 (16,7)	3 (50,0)	1 (16,7)	2 (33,3)	11 ((100))	0 (0,0)	11 ((100)	0 (0,0)	0 (0,0)
II	17 (89,5)	2 (10,5)	6 (31,6)	4 (21,1)	9 (47,4)	11 ((100))	0 (0,0)	6 (54,5)	1 (9,1)	4 (36,4)
III-IV	1 (5,0)	19 (95,0)	5 (25,0)	3 (15,0)	12 (60,0)	0 (0,0)	23 ((100))	4 (17,4)	1 (4,3)	18 (78,3)
Significance, p value	<0,001*			0,760		<0,001*			<0,001*	

*significant difference $p<0.05$, x^2 test, Fisher's exact test

It was observed that in intestinal adenocarcinoma located in the upper half of the stomach, proIDX is high in about two thirds of cases (66.7%), which is significantly higher than low proIDX (x^2 test, p=0.025). However, there is no significant difference in Ki67 expression, i.e. the incidence of high and low proIDX in intestinal cancers located in the lower half of the stomach. No significant difference in Ki67 / proIDX expression was observed in diffuse adenocarcinoma in relation to the location of gastric cancer.

Significant association of Ki67/proIDX expression with histological grade of gastric adenocarcinoma was observed. Specifically, high proIDX was associated with poorly differentiated intestinal cancer in 78.6% of cases (p=0.033). In diffuse adenocarcinoma, low proIDX in significant 100% of cases was associated with moderately differentiated tumor (G2), while high proIDX in most (62%) cases, but with no significant difference with low proIDX, was associated with poorly differentiated tumor (G3).

Ki67 / proIDX expression is significantly associated with invasion of lymphatic vessels in both intestinal and diffuse gastric carcinoma. In both histologic types of carcinomas in which no lymphatic invasion was identified, low proIDX was found in significant 100% of cases (p=0.001). In the case of intestinal carcinoma, in the case of lymphatic invasion, a high but not statistically significant percentage (64.7%) was recorded with high proIDX, while in the case of diffuse carcinoma with present lymphatic invasion a significantly higher incidence of high proIDX was observed (74.2%).

No significant association of proIDX with vascular invasion was observed in intestinal gastric cancer. However, in diffuse cancer, a significant association of high proIDX with vascular invasion was shown.

There is a significant association of proIDX with lymph node metastases in both cancer histological types. Low proIDX was found in 100% of cases in tumors without metastasis in the lymph nodes, both in intestinal and diffuse gastric cancer. In both histological types, a significant association of high proIDX with metastases was found in more than 7 lymph nodes (in 92.3% of cases in intestinal and in 100% in diffuse cancer).

High proIDX is associated with distant metastases in diffuse gastric cancer in 100% of the cases.

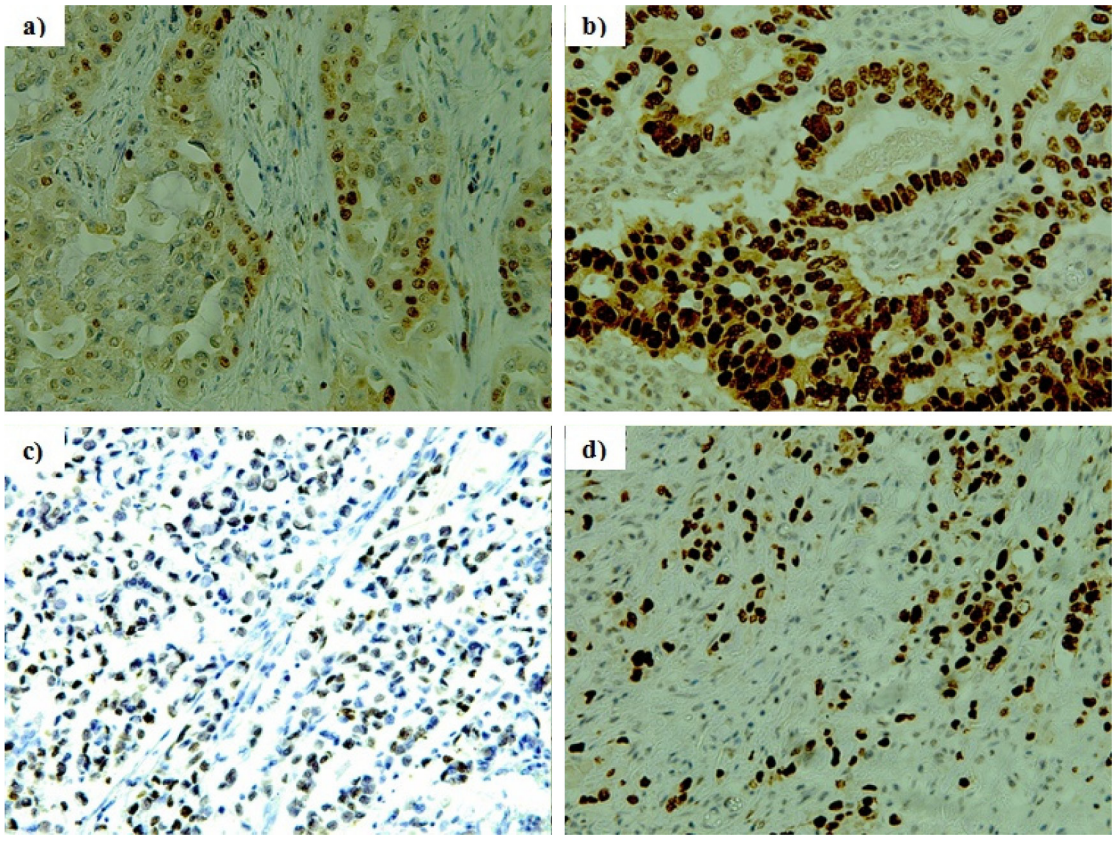

Figure 1. Gastric adenocarcinoma: **a:** Low proliferation index in intestinal type of gastric adenocarcinoma (Ki67×200). **b:** Overexpression of p53 in intestinal type of gastric adenocarcinoma (p53×200). **c:** High proliferative index in diffuse type of gastric adenocarcinoma (Ki67×200). **d:** Moderate to pronounced expression of p53 in diffuse type of gastric adeocarcinoma (p53×200).

However, no statistically significant association of proIDX with distant metastasis was observed in intestinal cancer.

In both types of gastric cancer, a significant association of pT1-pT2 stages with low pro IDX was observed in 100% of cases. No statistically significant difference in the incidence of low and high proIDX was observed in intestinal cancer in pT3-pT4, although high proIDX was present in most cases (60% vs. 40%, p=0.250). However, in diffuse cancer there was a significant association of high proIDX pT3-pT4 (p=0.024).

Significant association of low proIDX with stage I-II tumor (83.3-89.5%, p=0.001) and high proIDX with stage III-IV (95.0%, r <0.001) was observed in intestinal cancer. In diffuse cancer, a significant association of proIDX with the stage of tumor disease was also noted, so that low proIDX in 100% of cases was associated with stage I-II and high in 100% with stage III-IV of tumor.

Association of p53 expression and clinicopathological characteristics of gastric adenocarcinoma (Table 3)

No significant association of p53 expression index (p53IDX) with sex and age of subjects was observed, although there had been a slight dominance of high p53IDX in men with intestinal cancer and women with diffuse cancer. No significant association of p53IDX with macroscopic type and tumor location was observed.

In a significant number of cases (78.6%; p=0.002), high p53IDX was associated with poorly differentiated intestinal cancer. In most cases of moderately differentiated intestinal cancer, p53 expression was not verified (41.9%), with no statistical significance, but in poorly differentiated tumors of this histologic type, high p53IDX was found in significant 78.6% of cases (p=0.002).

In moderately differentiated diffuse gastric cancer, p53 expression was not found in 100% of cases. In the poorly differentiated type of this tumor, no significant difference was observed between absence (35.1%) and high p53IDX (62.2%, p=0.128).

It was observed that p53IDX in intestinal gastric cancer was not significantly associated with lymphatic vessel invasion, that is, no significant difference in p53IDX level between cases with invasion and those without lymphatic invasion was found.

Significant association of p53IDX with lymphatic vessel invasion was identified in diffuse gastric cancer. In a significantly high number (85.7%, p <0.001), no p53 expression was found in the absence of lymphatic invasion, while high p53IDX was significantly higher (67.7%, p <0.001) in diffuse gastric cancer with invasion of lymphatic vessels.

In this study, no significant association was found with p53IDX with vascular invasion in either intestinal or diffuse gastric cancer.

In intestinal gastric cancer, p53IDX was not significantly associated with lymph node metastases, but in diffuse type it was significantly associated with the presence of metastases and the number of lymph nodes involved. In a significant number (76.5%) of cases, p53 expression was not present in diffuse cancer when there was no lymph node metastasis. When there were metastases in 7 or more lymph nodes, high p53IDX was present (76.5%, p=0.012). However, in diffuse gastric cancer, no significant difference (p=0.763) was found between absence of p53 expression (45.5%) and high p53IDX (p=0.763) if metastases were present in 1-6 lymph nodes.

No significant association of p53IDX with distant metastasis was found in intestinal cancer. However, in diffuse carcinoma, p53IDX in cases where no metastases were identified was significantly different from those where they were present. In the presence of distant metastases, p53IDX was in most cases high (80% vs. 20% low, p=0.180).

The expression index of p53 was not statistically significant in the pathological (pT) stage of intestinal gastric cancer. However, in diffuse cancer, p53 expression was not found in the pT1-pT2 stage in a significant number of cases (91.7%, p=0.004). Also in pT3-pT4, there was a statistically significant difference in the frequency of high p53IDX and in the absence of its expression (63.6% vs. 30.3%, p=0.001).

No association of p53IDX with the stage of disease was observed in intestinal gastric cancer. In diffuse cancer, p53 expression was absent in 100% of the cases in stage I. In stage II, no difference was found between high p53IDX and the absence of its expression (p=0.527). In this type of tumor, a significant association of high p53IDX with stage III-IV of disease was found (78.3%, p=0.003).

Correlation analysis of proliferation index, p53 expression index and clinical and pathological characteristics of gastric cancer

In the previous analyses of the proliferation index, the p53 expression index and the clinical and pathological characteristics of gastric cancer, it was noted that there were highly significant association in individual relationships. A measure of their correlation is shown by a correlation analysis where the significance of that relationship is proved by the significance of the correlation coefficient and the strength of the relationship by its magnitude.

Table 4. Correlation matrix - association between Ki67 and p53 expression with clinical and pathological parameters

Variable / parameter	*Sex*	*Age*	*MA type*	*Local.*	*H. grade*	*ILS*	*IKS*	*MET Lymph n*	*Dist. metast.*	*pT*	*Stage*	*Gastric cancer*
Ki67												
r	-0.188	-0.070	0.289	-0.251	0.399	0.556	0.288	0.756	0.273	0.503	0.722	intestinal
p	0.217	0.646	0.054	0.096	0.007*	0.001*	0.055	0.001*	0.069	0.001*	0.001*	
r	-0.070	0.034	0.065	-0.029	0.475	0.687	0.436	0.898	0.346	0.598	0.881	diffuse
p	0.650	0.823	0.672	0.852	0.001*	0.001*	0.003*	0.001*	0.020*	0.001*	0.001*	
p53												
r	-0.087	0.348	-0.099	-0.199	0.391	0.186	0.255	0.243	0.132	0.163	0.189	intestinal
p	0.570	0.019*	0.517	0.190	0.008*	0.222	0.091	0.107	0.389	0.285	0.213	
r	0.021	0.230	0.106	0.064	0.486	0.556	0.306	0.554	0.280	0.555	0.656	diffuse
p	0.889	0.129	0.486	0.677	0.001*	0.001*	0.041*	0.001*	0.062	0.001*	0.001*	

r - correlation coefficient (Spearman's); p - significance r (* - r <0.05)

In Table 4, in the correlation matrix, the correlation coefficient (r) with its statistical significance (p) shows all the parameters that were the subject of our previous analyzes in intestinal and diffuse gastric cancer.

In intestinal gastric cancer, the proliferation index / Ki67 expression was significant and highest correlation coefficient associated with lymph node metastases and tumor stage (r=0.756; r=0.722). A slightly lower but still high and significant correlation coefficient was proIDX associated with lymphatic vessel invasion, pT tumor stage, and tumor histological grade (r=0.556; r=0.503; r=0.399).

In diffuse cancer type, proIDX was also with very high positive correlation coefficients associated with lymph node metastases and tumor stage (r=0.898, r=0.881). High, significant, and positive correlation coefficients, higher than those of the intestinal type, made proIDX associated with lymphatic vessel invasion, pT tumor stage, and histological grade (0.687, 0.598, 0.475). In diffuse cancer, two slightly weaker links were identified, which were not found in intestinal cancer. Specifically, proIDX with moderate, significant, and positive correlation coefficients is associated with vascular invasion and distant metastasis (0.436 and 0.346).

Index of p53 expression (p53IDX) in intestinal cancer was positive but with lower correlation coefficients associated with the histological grade and age of the subjects (r=0.391; r=0.348) (Figure 1).

Index of p53 expression in diffuse cancer was with significant, positive, high and moderate correlation coefficients associated with tumor stage (r=0.656), lymphatic vessel invasion (r=0.556), pT stage (r=0.555), lymph node metastases (r=0.554), histological grade (r=0.486), and invasion of blood vessels (r=0.306).

Unlike intestinal, a significant positive association of proIDX with the expression index of p53 was observed in diffuse gastric cancer (r=0.614).

Discussion

During multi-stage carcinogenesis of H. pilory-induced chronic gastritis via multifocal atrophy, intestinal metaplasia, and dysplasia to gastric carcinoma (Correa sequence), disorders of cell proliferation, adhesion, differentiation, signal transduction and telomerase gene activity with alteration occur [18,19].

The biological behavior of gastric cancer is primarily defined on the basis of its locally invasive and destructive growth. In the context of aggressive tumor behavior, numerous studies have focused on the study of cell proliferation, which is one of the most important biological mechanisms

in carcinogenesis [12-15]. Nuclear antigen was isolated from proliferating cells to which a monoclonal Ki67 IgG1 class antibody was made [20]. The antigen detected by this antibody is present in cell nuclei during the late G1, S, G2, and M phase, and cannot be detected in the G0 phase [21]. The prognostic significance of proliferative activity has been established in many tumors [12-14,21], but reports regarding the importance of proliferation in gastric cancer are heterogeneous and inconsistent [22-24].

In this study, when examining the expression of nuclear antigen of proliferating Ki67 cells in the intestinal and diffuse Lauren subtype of gastric carcinoma, we observed that their proliferative activity was similar, i.e. no statistically significant difference was found in the expression of this antigen (about 3000 Ki67 immunopositives were verified in both cell types in mm^2 of tumor tissue). Compared to the control group, both types of adenocarcinoma show significantly higher Ki67 expression values, i.e. significantly higher proliferation index (proIDX), which is consistent with numerous literature reports [24-26].

We further concluded that there is no significant association of proIDX with sex, age, and macroscopic tumor type, in either intestinal or diffuse type of carcinoma. However, in both histologic types, there was a higher incidence of high proIDX in men and a dominance of high proIDX in the index in male subjects, but unlike us, they reported the prevalence of a high proliferation older than 60 years, but without statistical significance, which is in agreement with the observations of other authors [24]. Amrani et al, 2014, also observed the prevalence of a high proliferation index in subjects younger than 60 years but again without statistical significance [27].

In both histological types of adenocarcinomas, proIDX is significantly associated with histological grade, lymphatic vessel invasion, lymph node metastases, pT1 and pT2, and stage of tumor disease, such that low proIDX is significantly associated with absence of lymphatic invasion, absence of lymph node metastases, pT1-pT2 and stage I-II, and high proIDX with lymphatic invasion, with metastases in more than 7 lymph nodes and with stage III-IV of disease (TNM) Amrani et al also found significantly low proIDX in pT1-pT2 adenocarcinomas and significantly high proIDX in tumors with lympho-nodular metastases, with pT3-pT4, and in stage III-IV of disease [27]. Significant association of proIDX with histologic grade and pT1, pT2, and pT3 tumors has been observed by other authors, however, their results do not indicate an association of proIDX with lympho-vascular invasion, lymphatic metastasis, and tumor stage [24,28]. In a large-scale meta-analysis involving 29 studies and 5600 patients with gastric cancer, no significant association of high proIDX with histological grade and lymph node metastasis was observed. However, essentially the proliferation index in this meta-analysis indicates that there is a significant relationship between Ki67 expression and an unfavorable prognosis in patients with stage III-IV tumor disease [29].

In intestinal type of gastric cancer, high proIDX was significantly associated with tumor localization in the upper half of the stomach, but no statistically significant association of proIDX level with gastric tumor localization was observed in diffuse cancer. It has been pointed out in the literature that gastric tumor localization is an important prognostic factor [30,31], and in support of this there are differences in incidence and mortality between proximal and distal localization tumors. The incidence of proximal and carcinomas of cardia has been increasing, especially in the male population [30, 32, 33]. It is further noted that gastric cancers located in cardia are mainly diffuse type and that the cancers located in the distal part of the stomach are predominantly intestinal [34]. Proximal carcinomas have also been observed to have a significantly poorer prognosis, with shorter five-year survival and higher operative mortality [35, 36].

In contrast to the intestinal one, in the diffuse type, pro IDX is significantly associated with vascular invasion, distant metastasis, and pT3-pT4 tumor stage. In diffuse cancer, high proIDX in 77.8% of cases was associated with vascular invasion, in significant 69.7% with pT3-pT4 and in 100% of cases with distant metastases. Consistent with our results, a significant association of high proliferation index with pT3-pT4 tumors has been found by other authors [28,37].

Ki67 expression varies during the cell cycle, whereby it becomes detectable during the mid- to late G1 phase, when levels are low and then its expression increases through the S and G2 phase, and peaks in the early M phase. In the late stages of mitosis (during anaphase and telophase), Ki67 expression decreases rapidly and disappears. Antigen cannot be detected in the G0 phase. Notably, during mitosis, Ki67 is subject to phosphorylation and dephosphorylation, and its amount is regulated by proteolytic pathways by the involvement of protease complexes. All these result in a short Ki67 half-life of 60-90 minutes [14].

An important role in the regulation of the cell cycle and apoptosis is played by the tumor suppressor TP53 gene located on the short arm of chromosome 17 [17p13.1]. It encodes a protein that inhibits tumor formation by affecting genome stability,

cell growth and differentiation, and stimulation of apoptosis. There is *Wild* type (normal form) and mutant type p53, where *Wild* type p53 is tumor suppressor [38].

P53 performs its function of "guardian of the genome" under conditions of DNA damage, by inducing cell cycle arrest at the G1/S regulatory point so that DNA repair proteins have time to repair the damaged DNA before cell division occurs. If DNA repair is unsuccessful then it activates apoptotic mechanisms by which the damaged cells are removed. The pro-apoptotic effect of p53 manifests itself by inhibiting the transcription of the anti-apoptotic Bcl-2 gene on the one hand, while inducing the production of pro-apoptotic Bax protein, FAS and other death receptors from the tumor necrosis factor [TNF] family [39]. The amount of accumulated p53 in cells determines the mode of response to damage. If the amount of p53 accumulated is smaller, the cell cycle will stop and DNA repair will occur, and if the accumulation is greater the apoptosis will occur [40].

In normal cells, the *Wild* p53 type has a short half-life (6-30 minutes) and due to its constant degradation is found in very small amounts and is difficult to identify by standard immunohistochemical methods. Various forms of stress, especially genotoxic damage through post-transcriptional modifications, stabilize p53 and it is not susceptible to degradation, has no tumor-suppressor characteristics, remains stable for a long time and can be immunohistochemically detected [38, 41]. In addition to the role of transcription factor, p53 exerts its tumor-suppressor activity through its ability to modulate cell migration. Loss of p53 function increases cell motility, which contributes to tumor invasiveness [42].

The mutated gene changes the structure and function of the encoded protein, initiates carcinogenesis and, due to impaired degradation, leads to p53 accumulation in tumor cells. Point mutations, deletions, and epigenetic gene inactivation are responsible for the mechanisms of tumor-suppressor gene inactivation, with point mutations being the most common [38]. In about 75% of cases, these are missense mutations. These are mutations in which the replacement of a single nucleotide results in the synthesis of a mutated protein that accumulates in the cell and differs from the wild allele in a single amino acid [41]. Numerous carcinogens act differently on the TP53 gene and the pattern of mutations is specific to each tumor. Unlike cancers, where missense point mutations are most common, sarcomas have deletions and insertions most commonly, and some sarcomas have found amplifications of the MDM2 oncogene whose product inactivates p53.Such cells are genetically unstable and accumulate mutations resulting in tumor progression [43,44].

In agreement with most authors [15,16,45] we also found in our study that p53 expression, expressed by the number of immunoreactive cells in mm^2 in the intestinal type, was significantly higher than the diffuse type and control group, but there are reports in the literature in which no significant difference in p53 expression was found between these two types of adenocarcinoma [46].

In no histologic type did we identify a significant association of p53IDX with sex, macroscopic type, tumor localization in the gastric and vascular invasion, but a slight dominance of high p53I DX in men was observed in intestinal gastric cancer, and in diffuse type dominance of high p53IDX in women in both cases without statistical significance. In our study, Spearman's correlation analysis showed that p53IDX, in intestinal cancer, had a positive but poor correlation coefficient [r=0.348] related to the age of the subjects. Several studies have highlighted higher expression of p53 in men and persons over 60 years of age, but, unlike us, they also observed a significant correlation of p53 expression with the location of tumors in the upper third of the stomach [15,45]. In 2015 Weii et al also showed, in a meta-analysis covering 32 studies, a significant association of high levels of p53 expression with the sex of the patient [47], and Poteca et al, 2014 highlighted a significant correlation of p53 expression with the male gender, advanced age and with the location of tumors in the upper gastric parts [16].

In our study, in both histologic types, there was a relationship between p53IDX and tumor histologic grade. In intestinal gastric cancer, this relationship is defined by a significant, positive but low correlation coefficient (r=0.391). In diffuse gastric cancer, the bond is stronger and is defined by a significant, positive, moderate correlation coefficient (r=0.486). Unlike intestinal, in diffuse gastric cancer, p53IDX with significant high and moderate correlation coefficients is associated with tumor stage (r=0.656), lymphatic vessel invasion (r=0.556), pT stage (r=0.555), lymph node metastases (r=0.554), vascular invasion (r=0.306), and distant metastases (r=0.280). In a meta-analysis involving 6599 subjects with gastric cancer, a significant association of high levels of p53 expression with patient gender was found, with Lauren classification, depth of invasion, lymph node metastases, TNM stage of tumor, and lymphovascular invasion [47].

We found in diffuse gastric cancer another connection that was not found in the intestinal type. In diffuse gastric cancer there is a high and sig-

nificant interdependence between the proliferation index and the p53 expression index (r=0.614). The accumulation of p53 resulting from the TP53 mutation most likely leads to a loss of control over cell proliferation, and our results are related to the significant increase in p53IDX in pT3-pT4 and in stage III-IV of diffuse gastric cancer.

Somatic mutations of the TP53 gene are the most frequent mutations in human cancers. They are estimated to occur in 5-90% of malignant tumors, depending on the type and stage of the tumor [48,49]. There are reports in the literature that more than 35,000 TP53 gene mutations have been reported in different types of tumors in 2,700 published papers [49]. There is a suggestion in the literature that a mutation of the TP53 gene is an early event in gastric carcinogenesis, since p53 expression is present in gastric mucosa in chronic gastritis and intestinal metaplasia, with the expression index increasing with lesion progression (dysplasia, early and invasive carcinoma) and the highest index is in metastatic cancer [15]. However, the results of the study by Busuttil et al. 2014 indicate that mutations of the TP53 gene in gastric carcinogenesis occur late and contribute to the final transition to cancer [50]. In our study, the high p53 expression index was most frequent in the III-IV stages of tumor disease in both intestinal and diffuse adenocarcinoma (60%, 79.3% of cases).

Although Ki67 and p53 expression levels have been identified as independent prognostic survival factors in several studies [25,51], there are still no unique criteria for p53 status assessment in the diagnosis and prognosis of tumor disease. The reason for this is probably the large heterogeneity of TP53 mutations, the diversity in patient selection, the choice of immunohistochemical methods and methods for quantifying immunoreactions [15].

There is strong evidence that Ki67 plays a key role in the proliferation and control of tumor cell vitality, since its suppression by antisense oligonucleotides (ASOs) prevents cell proliferation [39,52,53]. Regarding to this, it has been pointed out that the antisense strategy against Ki67 mRNA represents a promising antiproliferative approach in the treatment of tumors [14].

Finally, our study confirms the importance of Ki67 and p53 expression in the pathogenesis of gastric adenocarcinoma.

As our results point to a highly significant correlation of the indexes of expression of Ki67 and p53 with indicators of gastric adenocarcinoma progression, this study supports the view that the indexes of expression of Ki67 and p53 may help to identify patients with an aggressive gastric adenocarcinoma phenotype.

Conflict of interests

The authors declare no conflict of interests.

References

1. Yang L, Zheng R, Wang N et al. Incidence and mortality of stomach cancer in China, 2014. Chin J Cancer Res. 2018; 30:291-8.
2. Piazuelo MB, Correa P.Gastric cancer: Overview. Colomb Med (Cali). 2013;44:192-201.
3. Torre LA, Siegel RL, Ward EM, Jemal A. Global Cancer Incidence and Mortality Rates and Trends-An Update. Cancer Epidemiol Biomarkers Prev. 2016;25:16-27.
4. Lauwers GY, Carneiro F, Graham DY et al. Gastric carcinoma. In: Bosman FT, Carniero F, Hruban RH, Theise ND (eds): WHO Classification of Tumours of the Digestive System. 4th edn IARC press, Lyon 2010.
5. Park JY, von Karsa L, Herrero R.: Prevention strategies for gastric cancer: a global perspective. Clin Endosc 2014;47:478-89.
6. Loh YH, Jakszyn P, Luben RN, Mulligan AA, Mitrou PN, Khaw KT. N-Nitroso compounds and cancer incidence: the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study. Am J Clin Nutr 2011;93:1053-61.
7. Gang Zhou, Jing Yang: Correlations of gastrointestinal hormones with inflammation and intestinal flora in patients with gastric cancer. JBUON. 2019;24:1595-600.
8. Jakszyn P, Bingham S, Pera G et al. Endogenous versus exogenous exposure to N-nitroso compounds and gastric cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST) study. Carcinogenesis 2006;27:1497-1501.
9. Saeki N, Ono H, Sakamoto H, Yoshida T. Genetic factors related to gastric cancer susceptibility identified using a genome-wide association study. Cancer Sci 2013;104:1-8.
10. Budak E, Solakoglu Kahraman D, Budak A et al. The prognostic significance of serum CA125 levels with ER, PR, P53 and Ki-67 expression in endometrial carcinomas. Ginekol Pol 2019;90:675-83.
11. Imaizumi K, Suzuki T, Kojima M et al. Ki67 expression and localization of T cells after neoadjuvant therapies as reliable predictive markers in rectal cancer. Cancer Sci. 2020;111:23-35.

12. Pergolini I, Crippa S, Pagnanelli M et al. Prognostic impact of Ki-67 proliferative index in resectable pancreatic ductal adenocarcinoma. BJS Open 2019;3:646-55.
13. Sun G, Wang S, Wang Y. Expressions of Topo IIα and Ki67 in breast cancer and its clinicopathologic features and prognosis. Pak J Med Sci 2019;35:715-20.
14. Li L, Jiang G, Chen Q, Zheng JN. Ki67 is a promising molecular target in the diagnosis of cancer (review). Mol Med Rep 2015;11:1566-72.
15. Lazăr D, Tăban S, Sporea I et al. The immunohistochemical expression of the p53-protein in gastric carcinomas. Correlation with clinicopathological factors and survival of patients. Rom J Morphol Embryol. 2010;51: 249-57.
16. Potecă T, Potecă A, Sajin M, Comănescu M. Biological prognostic parameters in gastric carcinomas. Chirurgia (Bucur). 2014;109:347-54.
17. Mouton PR. Unbiased Stereology: A Concise Guide. Baltimore John Hopkins University Press, 2011, first edition, 1-171.
18. Shin CM, Kim N, Jung Y et al. Role of Helicobacter pylori infection in aberrant DNA methylation along multistep gastric carcinogenesis. Cancer Sci 2010;101:1337-46.
19. Correa P, Piazuelo MB. The gastric precancerous cascade. J Dig Dis 2012;13:2-9.
20. Preusser M, Heinzl H, Gelpi E et al. Ki67 index in intracranial ependymoma: a promising histopathological candidate biomarker. Histopathology. 2008;53(1):39-47.
21. Foltyn W, Zajęcki W, Marek B et al. The value of the Ki-67 proliferation marker as a prognostic factor in gastroenteropancreatic neuroendocrine tumours. Endokrynol Pol 2012;63:362-6.
22. Tsamandas AC, Kardamakis D, Tsiamalos P et al. The potential role of Bcl-2 expression, apoptosis and cell proliferation (Ki-67 expression) in cases of gastric carcinoma and correlation with classic prognostic factors and patient outcome. Anticancer Res 2009;29:703-9.
23. Xiao LJ, Zhao S, Zhao EH et al. Clinicopathological and prognostic significance of Ki-67, caspase-3 and p53 expression in gastric carcinomas. Oncol Lett 2013;6:1277-84.
24. Lazar D, Taban S, Sporea I et al. Ki-67 expression in gastric cancer. Results from a prospective study with long-term follow-up. Rom J Morphol Embryol 2010;51:655-61.
25. Tzanakis NE, Peros G, Karakitsos P et al. Prognostic significance of p53 and Ki67 proteins expression in Greek gastric cancer patients. Acta Chir Belg 2009;109:606-11.
26. Min KW, Kim DH, Son BK et al. A High Ki67/BCL2 Index Could Predict Lower Disease-Free and Overall Survival in Intestinal-Type Gastric Cancer. EurSurg Res. 2017;58:158-68.
27. Amrani HJ, Marchoudi N, Sadaoui I et al. Ki-67 Expression in Gastric Cancer and Correlation with Clinico-Pathological Characteristics. Int J Sci Res Publ 2014;4:2250-3153.
28. Abdel-Aziz A, Ahmed RA, Ibrahiem AT. Expression of pRb, Ki67 and HER 2/neu in gastric carcinomas: Relation to different histopathological grades and stages. Ann Diagn Pathol 2017;30:1-7.
29. Luo G, Hu Y, Zhang Z et al. Clinicopathologic significance and prognostic value of Ki-67 expression in patients with gastric cancer: a meta-analysis. Oncotarget 2017;8:50273-83.
30. Abdi-Rad A, Ghaderi-Sohi S, Nadimi-Barfroosh H, Emami S. Trend in incidence of gastric adenocarcinoma by tumor location from 1969-2004: a study in one referral center in Iran. Diagn Pathol 2006;1:5.
31. Fenoglio-Preiser C, Carniero F, Correa P et al. Gastric carcinoma In: Hamilton SR, Aaltonen LA(eds): World Health Organisation Classification of Tumours: Tumours of the Digestive System, IARC Press, Lyon, 2000;37-53.
32. Terry MB, Gaudet MM, Gammon MD. The epidemiology of gastric cancer. Semin Radiat Oncol 2002;12:111-27.
33. Brown LM, Devesa SS. Epidemiologic trends in esophageal and gastric cancer in the United States. Surg Oncol Clin N Am 2002;11:235-56.
34. Oliveira C, Seruca R, Carneiro F. Genetics, pathology, and clinics of familial gastric cancer. Int J Surg Pathol 2006;14:21-33.
35. Crew KD, Neugut AI. Epidemiology of gastric cancer. World J Gastroenterol 2006;12:354-62.
36. Zheng L, Wu C, Xi P et al. The survival and the long-term trends of patients with gastric cancer in Shanghai, China. BMC Cancer 2014;14:300-13.
37. Li Y, Shi F, Wu C, Yang F. Differential expression of midkine and Ki67 in gastric carcinoma and the clinical implications. Nan Fang Yi Ke Da Xue Xue Bao 2014;34:1534-6.
38. Olivier M, Petitjean A, Marcel V et al. Recent advances in p53 research: an interdisciplinary perspective. Cancer Gene Ther 2009;16:1-12.
39. Benjamin CL, Ananthaswamy HN. P53 and the pathogenesis of skin cancer. Toxicol Appl Pharmacol 2007;224:241-8.
40. Benjamin CL, Ullrich SE, Kripke ML, Ananthaswamy HN. p53 tumor suppressor gene: a critical molecular target for UV induction and prevention of skin cancer. Photochem Photobiol 2008;84:55-62.
41. Stojnev S, Golubović N, Babović P. TP53 gene mutations-from guardian of the genome to oncogene. Acta Medica Medianae 2010;49:59-63.
42. Gadea G, de Toledo M, Anguille C, Roux P. Loss of p53 promotes RhoA-ROCK-dependent cell migration and invasion in 3D matrices. J Cell Biol 2007;178:23-30.
43. Fujii T, Nakagawa S, Hanzawa M et al. Immunohistological study of cell cycle-related factors, oncogene expression, and cell proliferation in adenocarcinoma developed in Barrett's esophagus. Oncol Rep 2003;10:427-31.
44. Claudio PP, Zamparelli A, Garcia FU et al. Expression of cell-cycle-regulated proteins pRb2/p130, p107, p27(kip1), p53, mdm-2, and Ki-67 (MIB-1) in prostatic gland adenocarcinoma. Clin Cancer Res 2002;8:1808-15.
45. Fenoglio-Preiser CM, Wang J, Stemmermann GN, Noffsinger A.TP53 and Gastric Carcinoma: A Review. Hum Mutat 2003;21:258-70.

46. Pinto-de-Sousa J, Silva F, David L et al. Clinicopathological significance and survival influence of p53 protein expression in gastric carcinoma. Histopathology 2004;44:323-31.
47. Wei K, Jiang L, Wei Y et al. The prognostic significance of p53 expression in gastric cancer: a meta-analysis. J Cancer Res Clin Oncol 2015;141:735-48.
48. Fresquet V, Rieger M, Carolis C, García-Barchino MJ, Martinez-Climent JA. Acquired mutations in BCL2 family proteins conferring resistance to the BH3 mimetic ABT-199 in lymphoma. Blood 2014;123:4111-9.
49. Edlund K, Larsson O, Ameur A et al. Data-driven unbiased curation of the TP53 tumor suppressor gene mutation database and validation by ultradeep sequencing of human tumors. Proc Natl Acad Sci U S A. 2012;109:9551-6.
50. Busuttil RA, Zapparoli GV, Haupt S et al. Role of p53 in the progression of gastric cancer. Oncotarget 2014;5:12016-26.
51. Ayed DB, Khabir A, Abid M et al. Clinicopathological and Prognostic Significance of p53, Ki-67, and Bcl-2 Expression in Tunisian Gastric Adenocarcinomas. Acta Histochem 2014;116:1244-50.
52. Rubenstein M, Hollowell CM, Guinan P. Suppression of BCL2 by Antisense Oligonucleotides and Compensation by Non-Targeted Genes May Enhance Tumor Proliferation. In Vivo 2015;29: 687-93.
53. Kausch I, Lingnau A, Endl E et al. Antisense treatment against Ki-67 mRNA inhibits proliferation and tumor growth in vitro and in vivo. Int J Cancer 2003;105:710-16.