

## Clinicopathological importance of Ki-67, p27, and p53 expression in gastric cancer

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**Background/aim:** To assess the prognostic value of Ki-67, p27, and p53 immunoreactivity in human gastric cancer.

**Materials and methods:** A total of 84 patients with gastric cancer participated in our study. We categorized tumors as intestinal and diffuse types, with reference to Lauren's classification. Ki-67, p27, and p53 immunoreactivity were correlated with patient's age, tumor type, grade, lymph node status, extent of invasion, tumor-node-metastasis (TNM) stage, and survival.

**Results:** Decreased expression of p27 (<20% positivity of cells) and increased p53 staining (>50% positivity of cells) were determined in 41 (48.8%) and 29 (36.9%) tumor specimens, respectively, and were connected with both the TNM stage (P = 0.007 and P = 0.039, respectively), and the extent of tumor invasion (P = 0.025 and P = 0.004, respectively). Kaplan–Meier methods showed a remarkable effect of reduced p27 expression on survival time (P = 0.003). In contrast, we observed no notable relationship between survival time and p53 or Ki-67 immunoreactivity (P = 0.372 and P = 0.401, respectively).

**Conclusion:** A decrease in p27 expression and overexpression of p53 or Ki-67 may cause advancing and metastatic illness in patients with gastric carcinoma. In addition, immunopathological identification of p27 may be helpful to define patients with gastric cancer who are at an increased risk of death.

**Key words:** Gastric cancer, p27, p53, Ki-67, prognosis

### 1. Introduction

As a common malignancy, gastric cancer still draws attention as a major public health issue (1). It is the fourth most commonly diagnosed cancer and the second most common cause of cancer-related death worldwide (2–4). The incidence of gastric cancer varies in different parts of the world and among various ethnic groups (1). While it is commonly found in Japan, China, Chile, and East Europe, it has low prevalence in some countries, such as the United States and United Kingdom (5–7). Adenocarcinoma is accepted as the most common type of gastric cancer (6,8). In addition to the classification of the World Health Organization (WHO), 2 histological types of gastric cancer, which are clinically, morphologically, and epidemiologically distinct entities, have been described by Lauren: intestinal and diffuse (1,6,8). In addition, their pathogenesis also varies (9). Intestinal gastric cancer is related to corpus-dominant gastritis, which is usually accompanied by gastric atrophy and intestinal metaplasia, while diffuse gastric cancer is usually seen as pangastritis

without atrophy. Moreover, the intestinal type of gastric cancer is more frequently observed in elderly men, whereas the diffuse type occurs in younger patients, under the age of 50, and is more often found in females (3,10).

The complex natural history of gastric cancer means that its disease mechanisms have not been fully elucidated. Its prevalence varies according to genetic and environmental factors, so it can be claimed as a multifocal disease (3). During the course of the transformation, which is a multistep process involving multiple genetic and epigenetic events, transformation of normal epithelium to stomach cancer can be observed. The study of cell-cycle proteins may help in the understanding of these events.

In cell-cycle regulation, the p53 and p27 tumor suppressor genes play the key role at the G1/S check point (3,11). The p27 gene, located on chromosome 12p13, is a tumor suppressor protein of the CIP/KIP family (12,13) and affects the activity of kinase complexes, which controls the transition of G1/S. Levels of p57 are reduced in many carcinomas, including those of the breast, colon, bladder,

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prostate, esophagus, and lung (14), and this reduction has been attracting attention. Most, although not all, studies have also described an association between p27 loss and poor prognosis in gastric cancer (15,16).

The p53 gene, which is present at very low levels in normal cells, plays a role in cell-cycle regulation as a tumor suppressor. Normal p53 is involved in many cellular functions, which include the regulation of apoptosis and cell proliferation (17). A mutation of the p53 gene is frequently observed during the development of numerous human malignancies (18,19). More stable gene products are translated from those mutated genes. As a result, with the help of immunohistochemical methods, these gene products can easily be detected, thanks to their long half-life. Overexpression of p53 has been shown in numerous human tumors, and high levels of p53 protein have been correlated with malignant progression in colonic tumors and more advanced stages of lung carcinoma. In addition, expression of p53 has been shown to be independently related to poor prognosis in breast carcinomas. However, few studies have been conducted to assess p53 expression in gastric cancer (18).

Ki-67 is a monoclonal antibody that attaches to nuclear antigens expressed in proliferating cells (G1, S, and G2 phases and mitosis), but not in G0. Therefore, it can easily be claimed that Ki-67 antibodies can be used in the detection of cycling cells; that is, its expression can be used as a direct measurement of the growth fraction of the tissue (20–23). This antigen provides information regarding the stage of the cell cycle in which it is presented. While Ki-67 expression diversifies greatly during the cell cycle, it increases in many tumors (24); significantly higher expression has been shown in interstitial gastric neoplasms. However, other studies have not shown a correlation between the replication of neoplastic cells and the histopathological type of tumors according to the Lauren classification (25,26).

In our study, we examined the expression and clinicopathological significance of Ki-67, p27, and p53 in human gastric cancer.

## 2. Materials and methods

### 2.1. Patients and materials

Our sample comprised a series of 84 patients who were diagnosed and underwent potentially curative surgery for gastric cancer at the Hospital of the Faculty of Medicine, Atatürk University, Turkey, between August 2003 and June 2013. The sample consisted of 59 (70.2%) males and 25 (29.8%) females, with a mean age of 58.9 years (range: 32–86).

### 2.2. Pathological review

Surgical resection of specimens was performed using routine procedures, which included fixing the tissue

in 10% formalin, embedding it in paraffin wax, and staining it with hematoxylin and eosin. Two experienced pathologists examined the slides and classified the histological type of the samples on the basis of the criteria of Lauren and the histopathological classification of the WHO (6). The extent of tumor invasion (pT category), lymph node involvement (pN category), and pathological staging of all surgically resected tumors was determined according to the American Joint Committee on Cancer's tumor-node-metastasis (TNM) classification (27).

### 2.3. Immunohistochemical methodology

We performed immunohistochemistry using a Leica BOND-MAX automated immunostainer (Leica Microsystems, UK), following the manufacturer's protocol. We carried out formalin-fixation and paraffin-embedding procedures and then deparaffinized 4- $\mu$ m sections in a dry oven, dewaxed them in xylene, and rehydrated them through graded alcohol. With the help of citrate buffer (pH 6.0), we performed heat pretreatment at 100 °C for 20 min. We then placed the sections in an endogenous peroxide block for 5 min and subsequently applied p27 protein (NCL-p27, mouse monoclonal antibody, 1:35; Leica Microsystems), p53 protein (NCL-L-p53-DO7, mouse monoclonal antibody, 1:100; Leica Microsystems), and Ki-67 protein (NCL-L-Ki67-MM1, mouse monoclonal antibody, 1:60; Leica Microsystems) for 30 min. Antibody binding was detected with the help of a bond polymer refine kit (Leica Microsystems) and a diaminobenzidine tetrahydrochloride solution (Kit HK-153-5K; BioGenex, USA) that was used as a chromogen.

### 2.4. Immunohistochemical evaluation

Two pathologists evaluated the p27-, p53-, and Ki-67 stained sections. We graded the positivity of Ki-67 and P-53 semiquantitatively, based on the Remmele score (28). We divided the cells into 5 categories according to their percentages of positivity: 0–4 (0%, <10%, 10%–50%, 51%–80%, and >80%). The p27 immunostainings were too focal and weak to be measured, so we had to use a 2-point grading system to evaluate their positivity, where a low score was 0%–20% and a high score was 21%–100% (14).

### 2.5. Statistical analysis

We assessed the correlations between Ki-67, p53, and p27 expression and the clinicopathological characteristics of the patients using the chi-square test, Mann–Whitney U test, and analysis of variance. We examined correlations between Ki-67, p53, and p27 expressions using Spearman coefficients. We plotted survival curves using the Kaplan–Meier method and assayed statistical differences using the log-rank test. We used SPSS 15.0 (SPSS Inc., USA) for all statistical analyses and defined significance as a P-value of less than 0.05.

### 3. Results

#### 3.1. Clinicopathological findings

Of the 84 gastric cancer patients, 70.2% (n = 59) were male and 29.8% (n = 25) were female. Their mean age was 58.9 years (range: 32–86). Three of the patients (3.6%) were under the age of 40, 14 (16.7%) were aged 40–49, 25 (29.8%) were aged 50–59, 24 (28.6%) were aged 60–69, 16 (19%) were aged 70–79, and 2 (2.4%) were over the age of 80. In histopathologic examination, 37 (44%) of cases were of the intestinal type, while 47 (23%) were of the diffuse type of gastric cancer. With regard to the classic grading system, 7 (8.3%) were well differentiated (grade 1), 33 (39.3%) were moderately differentiated (grade 2), and 44 (52.4%) were poorly differentiated (grade 3). Lymph nodes were positive for metastasis in 71 (84.5%) cases (1–2 metastases, 11%; 3–6 metastases, 35.7%; 7 or more metastases, 35.7%) and 13 (15.5%) cases were lymph node-negative. Stage evaluation revealed that 3 (3.6%) were in stage IA, 7 (8.3%) were in stage IB, 6 (7.1%) were in stage IIA, 12 (14.3%) were in stage IIB, 29 (34.5%) were in stage IIIA, and 26 (32.1%) were in stage IIIB. Table 1 shows the clinicopathological features of the gastric cancers of the 84 patients included in this study.

#### 3.2. Expression of Ki-67, p53, and p27 in gastric cancer

Over the course of our study, we ignored cytoplasmic positivity and only considered nuclear positivities, counting the number of positively stained nuclei in the normal and tumor cells. We used immunohistochemistry to examine 84 gastric cancer tissue specimens for the presence of Ki-67, p53, and p27. Eight (9.5%) of the cases did not show immunoreactivity with Ki-67. Nuclear staining for Ki-67 was detectable in 76 (90.5%) cases: 22 cases (26.2%) with score 1, 18 (21.4%) cases with score 2, 19 (22.6%) cases with score 3, and 17 (20.2%) cases with score 4 (Figures 1A–1D). Six (7.1%) of the samples were not positively stained with p53. We determined nuclear positivity with p53 in 78 (92.9%) cases: 18 cases (21.4%) with score 1, 29 (34.5%) cases with score 2, 24 (28.6%) cases with score 3, and 7 (8.3%) cases with score 4 (Figures 2A–2D). While 41 (48.8%) cases showed low p27 immunoreactivity, 43 (51.5%) cases showed high immunoreactivity (Figures 3A and 3B). Table 2 shows the Ki-67, p53, and p27 immunoreactivity findings.

#### 3.3. Correlations between Ki-67, p53, and p27 expression and clinicopathological parameters

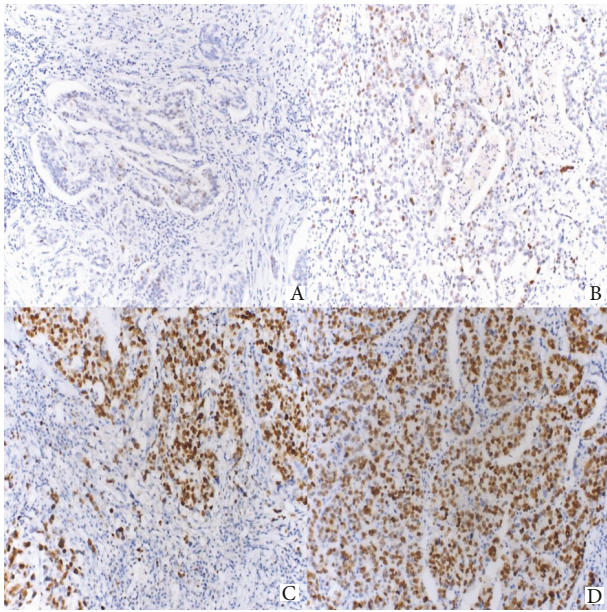
The correlation between p27 expression and clinicopathological diversity is shown in Table 3. In all types of lesions, including advanced gastric cancers, p27 immunostaining scores were usually lower than 20%, which can be evaluated as low positivity. There was no statistically significant difference in age and sex with regard to p27 expression. No significant associations

**Table 1.** Clinicopathological features of 84 patients with gastric cancer.

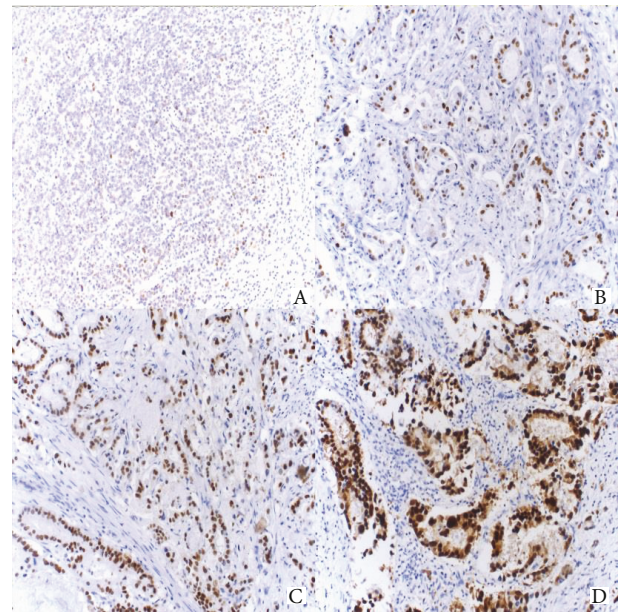
Parameter		n	%
Sex	Male	59	70.2
	Female	25	29.8
Age	<40	3	3.6
	40–49	14	16.7
	50–59	25	29.8
	60–69	24	28.6
	70–79	16	19.0
	80	2	2.4
Tumor size	<5	32	38.1
	≥5	52	61.9
Histological type	Tubular	62	73.8
	Mucinous	11	13.1
	Signet cell	11	13.1
Lauren's	Intestinal	37	44.0
	Diffuse	47	56.0
Histologic grade	Well	7	8.3
	Moderate	33	39.3
	Poor	44	53.4
TNM stage	IA	3	3.6
	IB	7	8.3
	IIA	6	7.1
	IIB	12	14.3
	IIIA	29	34.5
	IIIB	27	32.1

were observed between p27 expression and tumor size, histological grade, and histological type, but p27 protein expression was markedly correlated with extent of tumor invasion, lymph node metastasis, and TNM stage. Immunohistochemically, p27 scores decreased with tumor progression. The mean scores in pT1 cases were much higher compared with those in pT2 or pT3 cases. According to the Mann–Whitney U test, a reduction in p27 expression correlated significantly with extent of tumor invasion ( $P = 0.025$ ). Similarly, increased lymph node involvement and TNM stage correlated significantly with loss of p27 expression (chi-square,  $P = 0.001$  and  $P = 0.007$ , respectively). Table 3 includes the correlation between p27 expression and clinicopathological variables.

In the cases of progressive disease, Ki-67 and p53 staining showed scores of over 3, which can be evaluated as high positivity. Immunohistochemically, we observed overexpression of the p53 oncoprotein in all cases of pT2



**Figure 1.** Immunohistochemical staining for Ki-67 in gastric cancer. **A)** Score 1 (<10%), **B)** score 2 (10%–50%), **C)** score 3 (51%–80%), and **D)** case showing high expression (>80%) of Ki-67 (score 4).



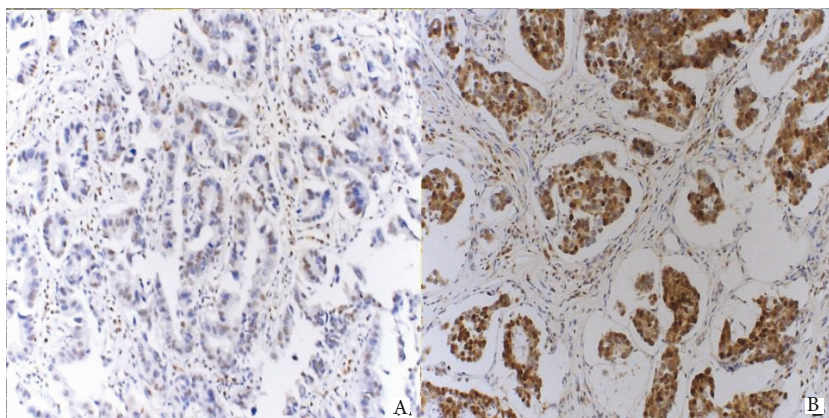
**Figure 2.** Immunohistochemical detection of p53 in gastric adenocarcinoma. **A)** Score 1 (<10%), **B)** score 2 (10%–50%), **C)** score 3 (51%–80%), and **D)** score 4 (>80%) of p53.

and pT3, but pT1 tumors had low positivity. We detected score 3 and 4 positivity with p53 in 67.7% of pT3 tumors. Moreover, we found score 5 (>80%) staining only in pT3 tumors. Similarly, we determined high Ki-67 expression in 79.4% of pT3 tumors. Expression of Ki-67 and p53 was significantly correlated with extent of tumor invasion ( $P = 0.021$  and  $P = 0.004$ , respectively; Tables 4 and 5). In addition, there was a statistically significant correlation between TNM stage and p53 expression ( $P = 0.039$ ). Nevertheless, there was no association between Ki-67 or p53 expression and sex, age, tumor site, histological grade, or histological type. Furthermore, we could not find an

association between Ki-67 or p53 expression and p27 expression according to the Mann–Whitney U test ( $P = 0.63$  and  $P = 0.143$ , respectively).

### 3.4. Survival analysis

We retrospectively evaluated all 84 patients in terms of survival, reviewing their 5-year survival rates after resection. We used the Kaplan–Meier method to correlate Ki-67, p53, and p27 expression with patient survival. We also investigated the correlation between survival rates and classical prognostic parameters. The median and mean of entire patient survival were 40.63 and 35.0 months, respectively (range: 5–72 months, SD = 25.20 months).



**Figure 3.** Representative illustration of p27 expression. Immunohistochemical staining in formalin-fixed paraffin-embedded gastric adenocarcinomas: **A)** low expression (0%–20%), **B)** high expression (21%–100%).

**Table 2.** The immunoreactivity findings of Ki-67, p53, and p27 in gastric cancer.

	Staining incidence	n	%
P27	Low (<20%)	41	48.8
	High (>20%)	43	51.2
Ki-67	0%	8	9.5
	<10%	22	26.2
	10%–50%	18	21.4
	51%–80%	19	22.6
	>80%	17	20.2
P53	0%	6	7.1
	<10%	18	21.4
	10%–50%	29	34.5
	51%–80%	24	28.6
	>80%	7	8.3

We assessed the clinical results in conformity with TNM stage. We found that 2 (28.6%) of the 7 stage IB, 4 (66.7%) of the 6 stage IIA, 5 (41.7%) of the 12 stage IIB, 24 (82.8%) of the 29 stage IIIA, and 23 (75%) of the stage IIIB tumors caused the death of the patients during the follow-up period. All 3 stage IA patients survived (Figure 4). This difference was statistically significant according to the log-rank test ( $P = 0.004$ ; Figure 5). We then interpreted the survival rates of the patients according to the status of lymph node involvement. The survival rate was highest (76.9%) in patients without lymph node metastasis, while we observed the lowest survival rates (13.3%) in N3 patients (Figure 6). We determined a statistically significant correlation between survival rates and lymph node involvement ( $P = 0.001$ ).

When we evaluated the relationship between survival rates of patients and Ki-67, p53, or p27 expression, we found a significant correlation between p27 expression and survival rates ( $P = 0.003$ ). A total of 42 (79.2%) of the 53 patients with low p27 expression and 16 (51.6%) of the 31 patients with high p53 expression died as a result of their disease during the follow-up period.

The Kaplan–Meier curves of survival of patients with high versus low expression of p27 showed a highly significant separation ( $P = 0.003$  by log-rank test; Figure 7). Conversely, we observed no statistically significant associations between survival rates and Ki-67 or p53 expression ( $P = 0.372$  and  $P = 0.401$ , respectively; Figures 8 and 9).

#### 4. Discussion

Although gastric cancer incidence has decreased, it remains an important health problem. In addition, this

type of neoplasm is commonly observed in Japan and other Asian countries (29). TNM criteria are used to measure the extent of neoplastic disease, and this can be claimed as the most important prognostic factor. However, different clinical courses can sometimes be observed in tumors with similar pathological or clinical characteristics. Tumor cell kinetics currently attracts attention because it is thought that it reflects tumor aggressiveness, and a correlation between proliferative activity and poor prognosis has been indicated in numerous human malignancies (30,31). Accumulation of genetic alterations plays a role in cancer progression, so development of human cancers can be claimed as a multistep process. Cellular proliferation follows an organized and timely regulated progression through the cell cycle, which is regulated by cell-cycle regulators, including p27 and p53 (32,33).

In our study, we evaluated p27, p53, and Ki-67 expression in gastric carcinoma using immunocytochemistry. In addition, we assessed the correlations between their expression and other clinicopathological findings and survival.

Belonging to the Cip1/Kip1 family of cyclin-dependent kinase inhibitors, p27 plays an important role in the cell cycle. Although alterations in the integrity of the human p27 gene in human primary tumors and cancer cell lines are quite rare, loss of the p27 protein is a frequent event in numerous human malignancies. However, its independent prognostic potential has been shown in colon, breast, esophagus, bladder, and lung carcinomas (34).

The results of the present study showed that loss of p27 protein expression was significantly correlated with extent of invasion, lymph node metastases, and TNM stage, which is in accordance with a number of previous studies. These studies demonstrated that the lower the expression of p27 protein, the stronger the correlation with highly invasive tumors, lymph node metastases, and/or poor clinical outcomes (35–37). In the current study, reduced p27 expression in cases of gastric carcinoma was higher in patients with positive lymph node metastases ( $P = 0.001$ ). Previous studies by Mori et al. (38), Yasui et al. (39), and Kim et al. (40) also showed a significant correlation between reduced p27 expression and lymph node metastases.

The p27 protein plays a role in cell growth, which is adhesion-dependent. Therefore, in light of the findings previously described, we can claim that the p27 protein is involved in tumor cell growth in the presence of altered extracellular matrix properties and altered adhesion. In addition, with the help of this mechanism, cells will be capable of avoiding apoptosis, and metastasis is assured (14).

The correlation between the decrease of p27 expression and extent of invasion that we showed is in accordance

**Table 3.** The correlation between p27 expression and clinicopathological variables.

Clinicopathologic data		P27 staining				P-value
		Low (<20%)		High (>20%)		
		n	%	n	%	
Sex	Male	38	64.4%	21	35.6%	0.702
	Female	15	60.0%	10	40.0%	
Age	<40	3	100%	0	0%	0.273
	40-49	9	64.3%	5	35.7%	
	50-59	12	48.0%	13	52.0%	
	60-69	15	62.5%	9	37.5%	
	70-79	12	75.0%	4	25.0%	
	>80	2	100%	0	0%	
Histological type	Tubular	29	46.8%	33	53.2%	0.392
	Mucinous	6	54.5%	5	45.5%	
	Signet cell	6	54.5%	5	45.5%	
Lauren's Intestinal		20	54.1%	17	45.9%	0.394
	Diffuse	21	44.7%	26	55.3%	
Histological grade	Well	3	42.9%	4	57.1%	0.492
	Moderate	22	66.7%	11	33.3%	
	Poor	28	63.6%	16	36.4%	
Lymph node status	0	5	38.5%	8	61.5%	0.001*
	1-2	4	36.4%	7	63.6%	
	3-6	17	56.7%	13	43.3%	
	≥7	27	90.0%	3	10.0%	
Depth of invasion (pT)	Mucosa or submucosa	2	66.7%	1	33.3%	0.025*
	Muscularis propria	4	30.8%	9	69.2%	
	Subserosa	47	69.1%	21	30.9%	
TNM stage	IA	2	66.7%	1	33.3%	0.007*
	IB	2	28.6%	5	71.4%	
	IIA	2	33.3%	4	66.7%	
	IIB	5	41.7%	7	58.3%	
	IIIA	18	62.1%	11	37.9%	
	IIIB	24	88.9%	3	11.1%	

\*: Lymph node metastasis, extent of invasion (pT), and TNM stage in particular were highly significantly related to reduced p27 expression (P = 0.001, P = 0.025, and P = 0.007, respectively).

with the results of Kim et al. (40) and Liu et al. (41). These studies suggested that loss of p27 protein can result both in cellular proliferation and in tumor development and progression.

When we considered the relationship between p27 expression and patient age or histological type of tumor, we did not observe significant correlations with either

parameter; Kim et al. (40) obtained similar results. Conversely, Al-Moundhri et al. (35) showed a significant association between p27 expression and patient age, but, in accordance with our results, they observed a nonsignificant correlation between p27 expression and histological type. In another series, Liu et al. showed that p27 was negative in tumors that were histologically classified as the diffuse

**Table 4.** The correlation between Ki-67 expression and extent of invasion (pT).

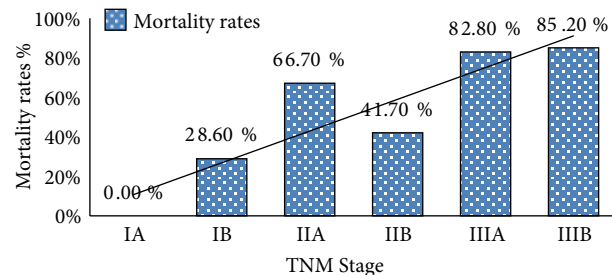
		Ki-67					P-value
		0 (n: 8)	<10% (n: 22)	10%–50% (n: 18)	50%–80% (n: 19)	>80% (n: 17)	
Depth of invasion (pT)	Mucosa or submucosa	25.0%	0	5.6%	0 / 0	0 / 0	0.021
	Muscularis propria	25.0%	22.7%	0	15.8%	17.6%	
	Subserosa	50%	77.3%	94.4%	84.2%	82.4%	

**Table 5.** The correlation between p53 expression and extent of invasion (pT) or TNM stage.

		P53					P-value
		0% (n: 6)	<10% (n: 18)	10%–50% (n: 29)	50%–80% (n: 24)	>80% (n: 7)	
Depth of invasion (pT)	Mucosa or submucosa	33.3%	5.6%	0	0	0	0.004
	Muscularis propria	33.3%	22.2%	13.8%	12.5%	0	
	Subserosa	33.3%	72.2%	86.2%	87.5%	100%	
TNM stage	IA	33.3%	5.6%	0	0	0	0.039
	IB	16.7%	16.7%	6.9%	4.2%	0	
	IIA	16.7%	16.7%	6.9%	0	0	
	IIB	0	5.6%	17.2%	20.8%	14.3%	
	IIIA	33.3%	27.8%	37.9%	37.5%	28.6%	
	IIIB	0	27.8%	31.0%	37.5%	57.1%	

type (41). According to Dominguez et al. (16), although no association was found between p27 reactivity and TNM stage, there was a significant association between histotype and p27 expression (P = 0.004). However, in our study, there was a significant correlation between TNM stage and loss of p27 expression (P = 0.007) and no relationship between histological type and loss of p27 expression.

Ki-67 expression shows fluctuations during the cell cycle and is increased in many tumors (24). Some studies have shown that significantly higher values of Ki-67 are expressed in the intestinal type of gastric carcinoma (42).

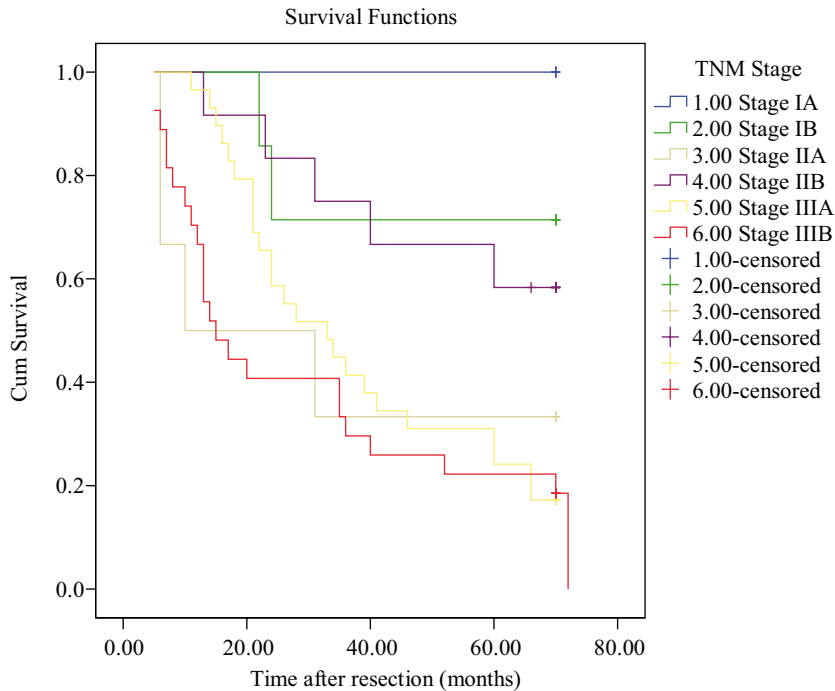


**Figure 4.** Mortality rates in gastric cancer patients, according to TNM stage. Mortality rates increased with increasing TNM stage.

This is not in line with our results, as we did not find any correlation between Ki-67 expression and histological type according to the Lauren classification. In addition, our data showed no association between Ki-67 expression and sex, age, tumor site, and histological grade.

Some studies demonstrated that the more serious the invasion and node metastases are, the higher the Ki-67 activity is (20,42). Similarly, we found a correlation between Ki-67 activity and extent of invasion. However, this conflicts with the results of several previous studies (42–44). Moreover, few authors have shown a relationship between Ki-67 and clinicopathological findings in gastric cancer, including lymph node metastases and venous invasion (24,45).

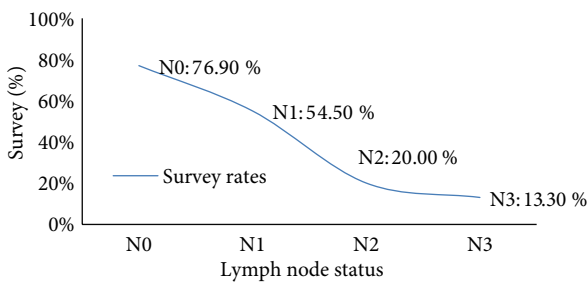
The p53 gene is located on 17p13 and can be claimed to play a role as a tumor-suppressor gene in light of the results of in vitro studies. The evidence accumulated to date suggests that the most frequent genetic abnormality in human cancer may be a mutation of p53, and it is known that such a mutation plays a significant role in the carcinogenesis of colonic carcinoma and gastric carcinoma (46). In previous studies, the incidence of p53 abnormalities varied on the basis of the histological type of cancer. In our series, we also showed a higher percentage



**Figure 5.** The Kaplan–Meier curves of survival of patients with stage IA versus stage IIIB tumors showed a highly significant separation.

of p53 expression for diffuse-type, rather than intestinal-type, lesions, but the difference was not significant (47). Kakeji et al. (48) reported that gastric cancer with p53 overexpression has a high potential for metastasizing to lymph nodes. Similarly, we found a significant association between overexpression of p53 and lymph node metastases. In addition, we observed a significant relationship between overexpression of p53 and depth of tumor invasion.

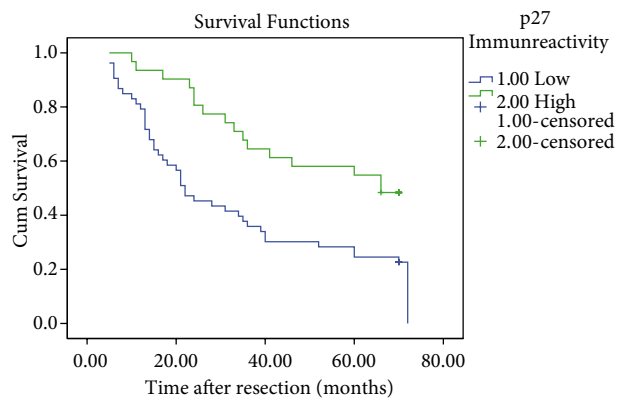
Liu et al. (41) and Al-Moundhri et al. (35) reported that the aggressive characteristics of a tumor are often closely associated with abnormal expression of cell cycle-related proteins. Reduced expression of p27 acting as a negative cell-cycle regulator may be predicted to increase cyclin-dependent kinase activity, resulting in active proliferation



**Figure 6.** Survival rates of gastric cancer patients according to lymph node status.

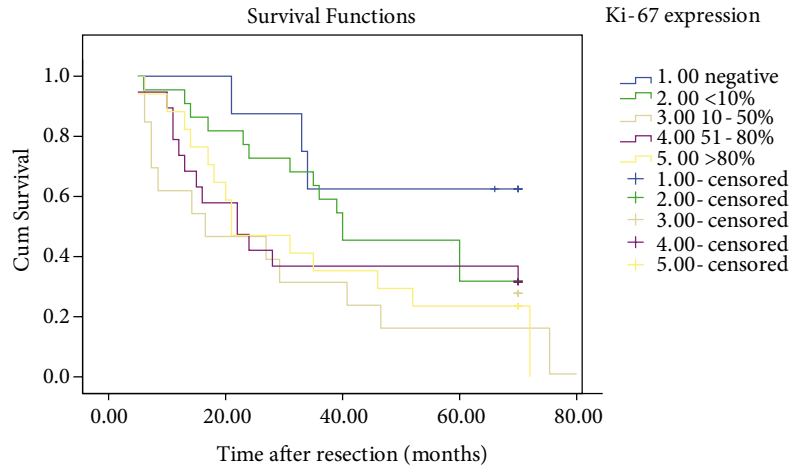
of tumor cells, while a mutated p53 gene results in the loss of an inhibitory effect on cell cycle progression (40). In accordance with the previous reports, Sgambato et al. (17) noticed that reduced expressions of both p27 and p53 were significantly associated with poor prognosis in univariate analyses. In the present study, reduced p27 expression was highly correlated with survival in gastric cancer. However, there was no significant relationship between p53 expression and survival rates.

Lazar et al. (24) showed that patients with gastric carcinomas with low Ki-67 scores had a 5-year survival

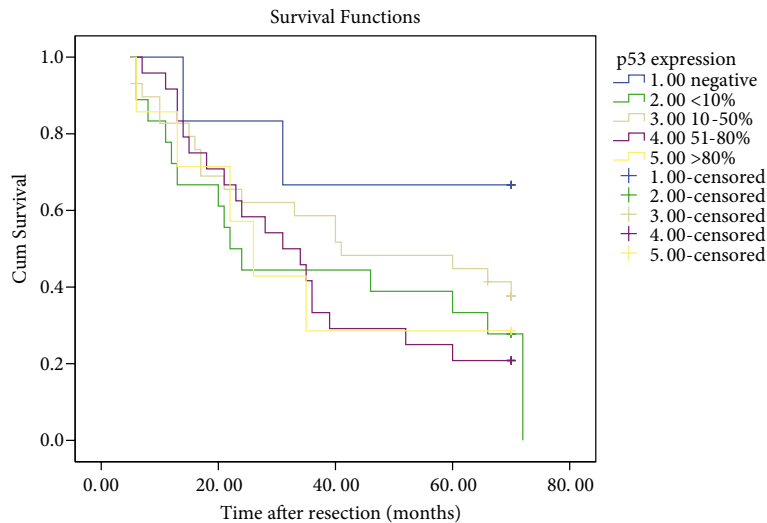


**Figure 7.** The Kaplan–Meier curves of survival of patients with high versus low expression of p27 showed a highly significant separation.





**Figure 8.** Kaplan–Meier survival curve correlating survival with expression of Ki-67. There was no significant correlation between survival and Ki-67 expression.



**Figure 9.** Kaplan–Meier survival curve correlating survival with expression of p53. There was no significant correlation between survival and p53 expression.

rate of 17.9%, while those patients with high Ki-67 scores had a survival rate of 15.2%. Therefore, we could say that there is no significant difference between them. Similarly, when the survival rate was calculated in months, relatively close values were again obtained for carcinomas with low and high Ki-67 scores: 18.8 and 16.2 months, respectively. Therefore, based on this study, the Ki-67 activity in gastric cancers cannot be accepted as a prognostic factor, and it does not appear to affect patient survival. Similarly, in our study, there was no significant correlation between Ki-67 activity and survival rates.

Mattioli et al. (1) showed a very strong positive correlation ( $P < 0.01$ ) between Ki-67 and p53 expression levels. However, in our study, the relationship between the status of p53 expression and Ki-67 was not statistically

significant. This may indicate that p53 mutation is predominantly associated with apoptosis, but not cell proliferation. Furthermore, there was no correlation between reduced p27 expression and the abnormal accumulation of p53.

Therefore, in conclusion, reduced expression of p27 and overexpression of p53 or Ki-67 may influence the progression and metastases of gastric tumors to lymph nodes. In addition, p27 expression may play an important role in the biological behavior of human gastric carcinoma. It is recommended that further studies on gene expression in human cancer be conducted to provide essential information for improved understanding of the mechanisms regulating gastric carcinogenesis, metastatic potential, and survival.

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