ORIGINAL PAPER

The immunohistochemical expression of the p53-protein in gastric carcinomas. Correlation with clinicopathological factors and survival of patients

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Abstract

Background: P53-tumor suppressor gene has an essential role in controlling cell cycle and initiating carcinogenesis. In the case of gastric cancer, the role of p53-protein accumulation as prognostic factor is controversy. Various results are due to the different methods of study regarding patients' selection, immunohistochemical techniques used and the quantifying systems for immunoreactions. *Aim*: Assessment of p53-immunohistochemical expression in 61 patients with gastric carcinomas and the correlation with clinicopathological factors (gender, age, location, macroscopic, and histological type, degree of tumor differentiation and TNM-stage) and patients' survival. *Material and Methods*: From the total number of 265 patients (186 males and 79 females) diagnosed with gastric cancer in the period 1998–2002, 61 operated patients were selected. On this group, we performed a prospective study regarding the evolution and aggressiveness of gastric cancer, on a duration of five years. Survival time was calculated from the month of the surgical intervention until the month of death or confirmation of survival, and survival rate was represented by the percentage of survivals at the end of the observed interval (in years and months). We used the monoclonal antibody DO7 that detects the wild and mutant form of p53-protein, by EnVision technique and DAB-visualization. We considered positive reaction only in the presence of brown staining of the nuclei. *Results*: P53-immunoreactions were positive in 25 gastric cancers (41%). We obtained positive stainings in 41.9% cases in men and 38.9% women. We found positive p53-immunoreactions in all the carcinomas developed in the upper third of the stomach (100%), in 53.3% of the corporeal tumors, 50% of the pangastric tumors; according to Lauren's classification, we noticed a significantly increased immunoreaction of p53 in the intestinal-type carcinomas. Among histological types, papillary, mucinous, anaplastic and tubular adenocarcinomas presented a relatively increased percentage of p53-positive immunoreactions. P53-positive stainings are more frequently encountered in moderate/poor differentiated carcinomas and those associated with lymphovascular invasion; according to pT- and pN-stage, we remarked a significantly increase of the number of p53-positive cases (p=0.02291 and p=0.038264). Five-year survival rate for patients with p53-positive carcinomas was significantly lower in comparison to the patients p53-negative (8% *vs.* 22.2%, p=0.0326). *Conclusions*: Immunohistochemical evaluation of p53-protein represents in our study an important prognostic factor, allowing the selection of a group of patients with an aggressive therapeutic indication, such as extensive lymphadenectomy and adjuvant chemotherapy.

***Keywords*:** p53, gastric cancer, clinicopathological factors, survival.

Introduction

The tumor-suppressor gene p53, located on the short arm of the 17 chromosome, holds an essential role in controlling the cellular cycle and initiation of tumorigenesis. Unlike normal p53 protein, rapidly removed from the nucleus, mutant forms have a prolonged half-life, which favors intranuclear accumulation, becoming detectable immunohistochemically. Numerous studies reported the correlation between the overexpression of p53 and the worst prognosis of patients with colonic, esophageal, mammary or pulmonary tumors. For gastric cancer, the role of accumulation of p53-protein as prognosis factor is controversial. Various results are due to different study methods concerning selection of patients, immunohistochemical techniques used, and systems of quantifying the immunoreactions.

Material and Methods

From the total number of 265 patients (186 males and 79 females) diagnosed clinically and histopathologically with gastric cancer in the period between 1998 and 2002, 67 patients were selected, who underwent surgery for this pathological condition in the Departments of Surgery of the Emergency County Hospital in Timisoara. On this group, we performed a prospective study regarding the evolution and aggressiveness of gastric cancer, over a period of five years. Surgical interventions performed, with curative or palliative intentions, were not preceded by chemotherapy or radiotherapy. The patients or their families were contacted periodically, on the phone, or through medical letters, at intervals of six months, the survival being monitored over a variable period between one and 68 months.

Patients who died in the period after the surgery, through various complications, or due to other conditions, were excluded from the study. Clinical and morphological (macroscopic and microscopic) data were gathered for each case. Gastric carcinomas were classified and interpreted according to the evaluation protocol recommended by the *American Joint Committee on Cancer* (*AJCC*) and *International Union against Cancer* (*IUCC*).

Survival time was calculated from the month of surgery until the time of death or confirmation of survival, and survival rate was represented by the percentage of survivals at the end of the observed interval (in years and months). From the total of cases included in the prospective study, six patients died at intervals variable between 7 and 26 months, due to other medical causes, being excluded from the study.

Statistical analysis was performed using the EpiInfo 6.04, Epi 3.2.2 and OpenEpi and consisted in computing the frequency counts and percentages for the qualitative variables, the means and standard deviations for the quantitative variables. The comparison of the percentages and the means was performed using the chi-square test and the unpaired *t*-test.

For statistical analysis, *p*-values of less than 0.05 were considered significant, and *p*-values of less than 0.01 were considered very significant.

In our study, we analyzed the immunohistochemical expression of the p53-protein in gastric carcinomas, following the correlations with various clinico-pathological factors (gender and age of patients, location, macroscopic type, histological type, degree of tumor differentiation and the TNM-staging) and survival of patients.

Immunohistochemical reactions were applied to the 61 gastric carcinomas from the respective study group. For these cases, we followed the positivation of p53 in the tumor and the adjacent peritumoral mucosa.

We also used the DO7 monoclonal antibody (Dako, Carpinteria, USA), which detects the wild and mutant form of the p53-protein, through the EnVision technique, with pre-treating in citrate solution (pH 6), for 20 minutes and incubation with the primary prediluted antibody for 30 minutes, visualization with DAB and counterstained with Lillie's modified Hematoxylin. Positive control was represented by a breast carcinoma, with strong nuclear expression for p53 in carcinomatous cells. For negative control, buffer replaced the primary antibody.

We considered a positive reaction only in the presence of immunostained nuclei in brown shades. Quantification of the reaction was performed following the next pattern:

- p53-negative (-): absence of immunostaining or positivation of the reaction in less than 10% of the tumoral nuclei;
- p53-positive (+); positivation of the reaction in over 10% of the nuclei of tumoral cells, regardless of the intensity of the reaction (Figure 1).

On the sections examined, we observed three distribution patterns of the p53-positive tumoral cells:

- a focal model, in which under 5% of neoplastic cells were stained;
- a regional and heterogeneous model, with islets of positive cells separated by negative areas, in which 5 to 50% of the tumoral nuclei became positive;
- a diffuse model, in which over 50% of tumoral cells became positive, these being present on the entire section (Figure 2).

According to their age, the patients were grouped into two categories:

- ≤60-year-old (29 cases);
- ≥61-year-old (32 cases).

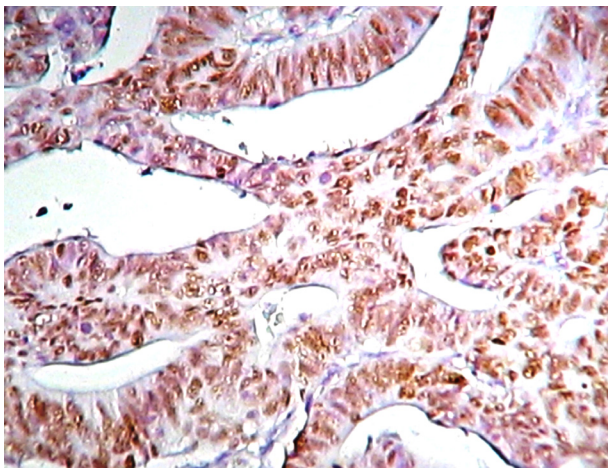


Figure 1 – ***Gastric adenocarcinoma with intensely positive reaction for p53. DAB, ×200.***

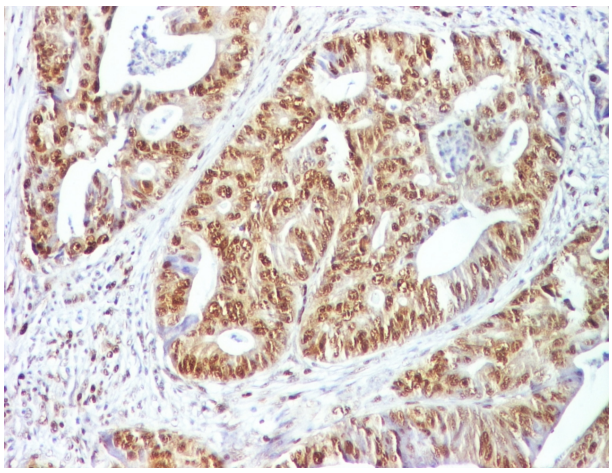


Figure 2 – ***Diffuse model of immunostaining for the p53-protein. DAB, ×100.***

Results

The final group consisted of 61 patients (43 males and 18 females) with ages between 30 and 80 years (average age 59.34 years).

The main clinicopathological features of cases of gastric cancer investigated are presented in Table 1.

For the entire study group, p53-immunoreactions became positive in 25 gastric cancers, representing 41% of the total of cases. Nuclear staining was in general intense and moderate, and was limited only to neoplastic cells, without interesting the stromal nuclei.

We have obtained p53-positive immunohistochemical reactions in 41.9% of males and 38.9% of females, noting a slight predominance of cases for the males (Table 2). P53-positive tumors are found more

frequently in elderly patients (≥61 years: 43.7%; ≤60 years: 38%) (Figure 3).

Table 1 – *Clinicopathological features of gastric cancers studied*

Clinicopathological factors		No. of cases
Males		43
Females		18
Average age (min.–max.) [years]		59.34 (30–80)
Location	Antrum	31
	Body	15
	Pangastric	10
	Eso-cardia	2
	Gastric stump	3
Early carcinoma		5
Advanced carcinoma		56
Borrmann	I	5
	II	20
	III	22
	IV	9
pTis/T1/T2/T3/T4		4/6/7/21/23
pN0/N1/N2/N3		18/16/23/4
pM0/M1		47/14

Table 2 – *Correlations between gender and age of patients, location of tumor and p53*

Clinicopathological factors		p53+ cases (%)	p53- cases (%)	*p*
No. of cases		25 (41%)	36 (59%)	
Gender	Males	18 (41.9%)	25 (58.1%)	0.8295
	Females	7 (38.9%)	11 (61.1%)	
Age	≤60 years	11 (38%)	18 (62%)	0.6444
	≥61 years	14 (43.7%)	18 (56.3%)	
Location	Antrum	9 (29%)	22 (71%)	0.0789
	Body	8 (53.3%)	7 (46.7%)	
	Pangastric	5 (50%)	5 (50%)	
	Cardia	2 (100%)	–	
	Gastric blunt	1 (33.3%)	2 (66.7%)	

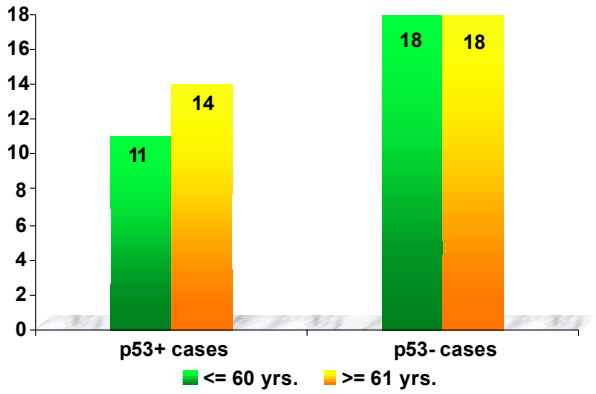


Figure 3 – *Expression of p53 and age of patients.*

In our study, p53-positive immunoreactions were observed in all cases of carcinomas developed in the proximal 1/3rd of the stomach (100%), in 53.3% of tumors of the gastric body, 50% of pangastric tumors, and much rarer in tumors occurring on the gastric stump (33.3%) or in the antral region (29%) (Figure 4).

According with the Lauren's classification, we noted the significantly greater frequency of p53-positivation in intestinal type carcinomas (47.4% – Figure 5) in comparison with diffuse type carcinomas (29.4%) (p=0.0212) (Table 3, Figure 6).

Among histological forms, papillary adenocarcinoma (60%), mucinous adenocarcinoma (50%), anaplastic carcinoma (33.3% – Figure 7) and tubular adenocarcinoma (42.9%) have relatively high proportions of p53-positive immunohistochemical reactions. The only exception is represented by the signet ring cell carcinoma. Of the total of 17 cases, we noted only five p53-positive cases (29.4%).

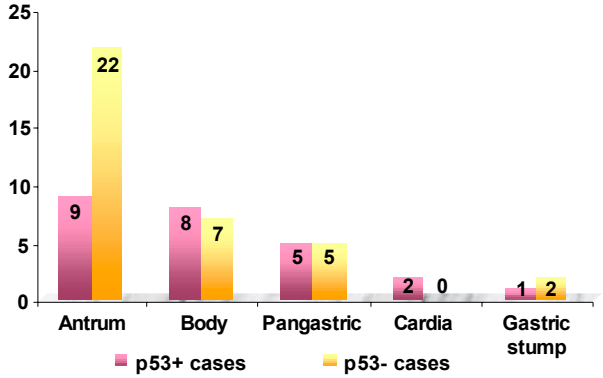


Figure 4 – *Expression of p53 and location of tumors.*

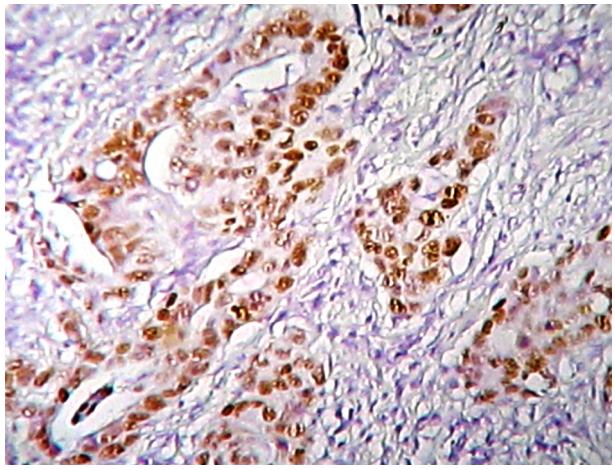


Figure 5 – *Intestinal type gastric carcinoma. P53-positive immune reaction. DAB, ×200.*

Table 3 – *Correlations between morphological factors and p53*

Clinicopathological factors		p53+ cases (%)	p53- cases (%)	*p*
Lauren's classification	Intestinal	18 (47.4%)	20 (52.6%)	0.0212
	Diffuse	5 (29.4%)	12 (70.6%)	
	Mixed	2 (33.3%)	4 (66.7%)	
Histological type	TA	12 (42.9%)	16 (57.1%)	0.1519
	PA	3 (60%)	2 (40%)	
	MA	4 (50%)	4 (50%)	
	SRCC	5 (29.4%)	12 (70.6%)	
	AC	1 (33.3%)	2 (66.7%)	
Tumor grade	G1	–	2 (100%)	0.0390
	G2	7 (35%)	13 (65%)	
	G3	18 (46.1%)	21 (53.9%)	
Lymphovascular invasion	Present	18 (47.4%)	20 (52.6%)	0.1924
	Absent	7 (30.4%)	16 (69.6%)	

TA: tubular adenocarcinoma; PA: papillary adenocarcinoma; MA: mucinous adenocarcinoma; SRCC: signet ring cell carcinoma; AC: anaplastic carcinoma.

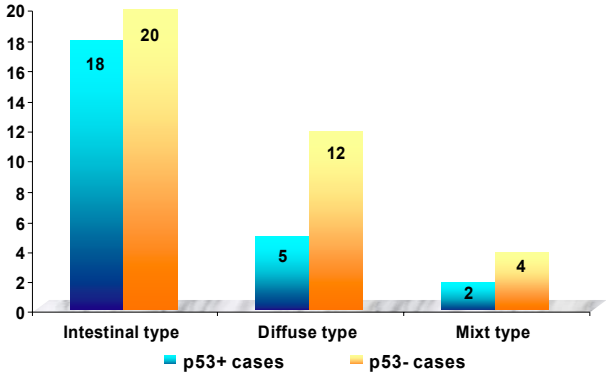


Figure 6 – *p53-expression and histological type of gastric carcinoma.*

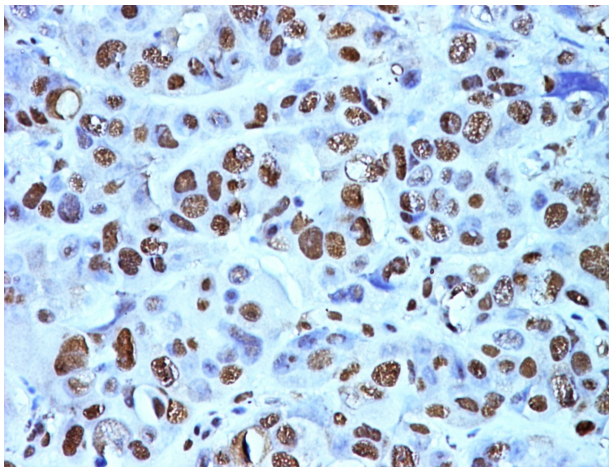


Figure 7 – *Anaplastic carcinoma. P53-immune reaction. DAB, ×400.*

We have observed a significant correlation between the tumor grade and positivation of p53. We did not note well-differentiated tumors (G1) with positive immune reaction. P53-positive tumors are averagely differentiated (35%), and particularly poorly differentiated (46.1%) (Figure 8).

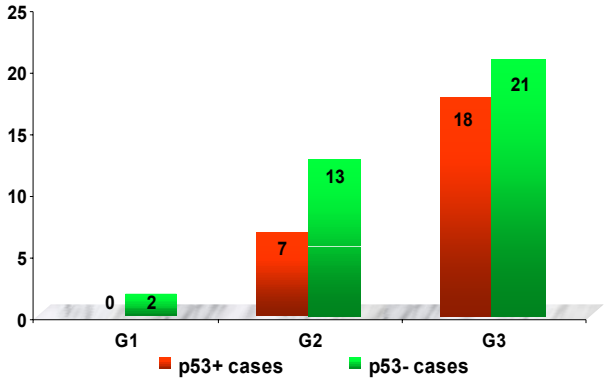


Figure 8 – *p53-expression and the tumor grade.*

In our study, p53-positive immune reactions are more frequent in carcinomas associated with aspect of lymphovascular invasion (47.4% – Figure 9).

According to the pT-stage (Table 4), we noted a progressive increase in the number of the p53-positive cases. The pTis case did not show immunostaining for p53. We identified p53-positive immunoreactions in 25% of pT1-carcinomas, 33.3% of pT2-carcinomas, 47% of pT3-carcinomas and 43.3% of pT4-carcinomas.

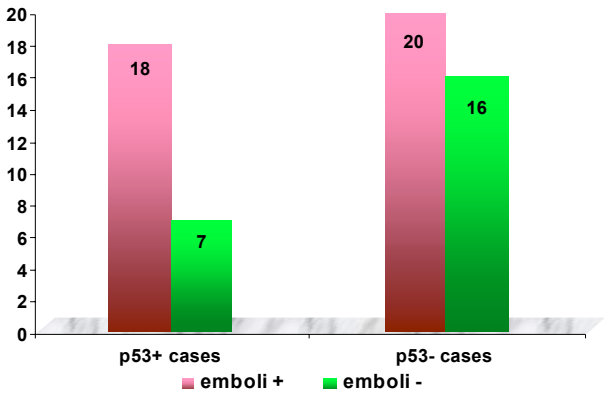


Figure 9 – *p53-expression and lymphovascular invasion.*

Table 4 – *Correlation between the TNM stage and p53*

Clinicopathological factors		p53+ cases (%)	p53- cases (%)	*p*
pT	Tis	–	1 (100%)	0.0229
	T1	1 (25%)	3 (75%)	
	T2	3 (33.3%)	6 (66.7%)	
	T3	8 (47%)	9 (53%)	
	T4	13 (43.3%)	17 (56.7%)	
pN	N0	5 (27.8%)	13 (72.2%)	0.0382
	N1	6 (37.5%)	10 (62.5%)	
	N2	11 (47.8%)	12 (52.2%)	
	N3	3 (75%)	1 (25%)	
pM	M0	19 (40.4%)	28 (59.6%)	0.1425
	M1	6 (42.8%)	8 (57.2%)	
pTNM	0	–	1 (100%)	0.7707
	IA	1 (33.3%)	2 (66.7%)	
	IB	2 (40%)	3 (60%)	
	II	3 (42.8%)	4 (57.2%)	
	IIIA	2 (18.2%)	9 (81.8%)	
	IIIB	3 (37.5%)	5 (62.5%)	
	IV	14 (53.8%)	12 (46.2%)	

Our results show a significant correlation between pN and p53-immunoreaction (Figure 10). We have obtained p53-positive immunoreactions in 27.8% of the pN0-carcinomas, 37.5% of the pN1-carcinomas, 47.8% of the pN2-carcinomas and 75% of the pN3-carcinomas.

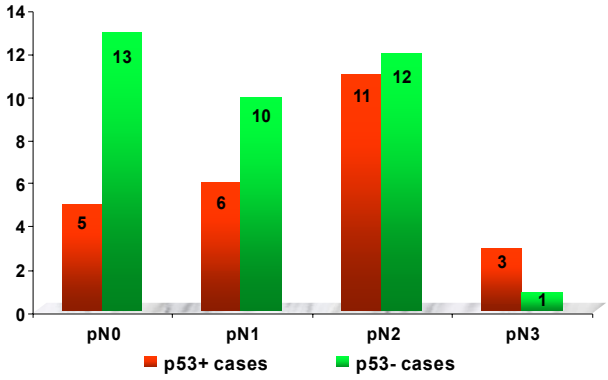
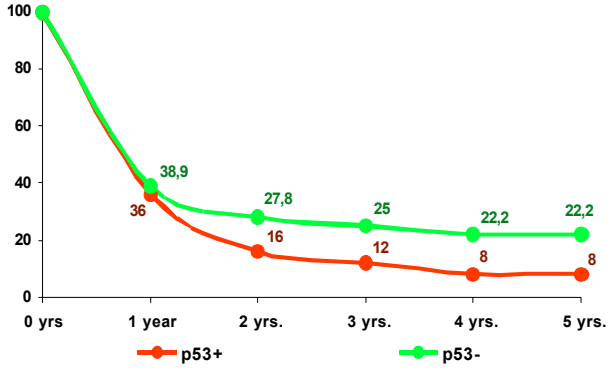


Figure 10 – *p53-expression and the pN-stage.*

P53-immunoreactions performed did not show a relation with the presence of distance metastases (pM-stage). We noted positive reactions in 40.4% of pM0-carcinomas and in 42.8% of pM1-gastric carcinomas.

Also, there is no significant correlation between p53-immunoreactions and the pTNM-stage. In the IA-stage 33.3% of tumors became positive, in the IB-stage 40% of tumors became positive, in stage II we noted 42.8% of tumors p53-positive, in the stage IIIA only 18.2% of tumors positive and in the stage IIIB 37.5% of tumors p53-positive. The only exception is represented by the IVth stage in which we encountered 53.8% of the carcinomas p53-positive.

Immunohistochemical evaluation of the p53-protein constitutes in our study an important prognosis factor. Survival at five years for patients with p53-positive carcinomas is of 8%, significantly lower than the average rate of survival at five years for patients with p53-negative carcinomas (22.2%) (p=0.0326) (Figure 11).



	1 year	2 years	3 years	4 years	5 years
p53+ carcinomas	36%	16%	12%	8%	8%
p53- carcinomas	38.9%	27.8%	25%	22.2%	22.2%

Figure 11 – ***Survival at five years according to the p53-immunohistochemical expression of gastric carcinomas (%).***

Patients with p53-positive carcinomas have survived on average 13.1 months, significantly lower in comparison with patients whose carcinomas did not express immunohistochemically the p53-protein (20.4 months) (Figure 12).

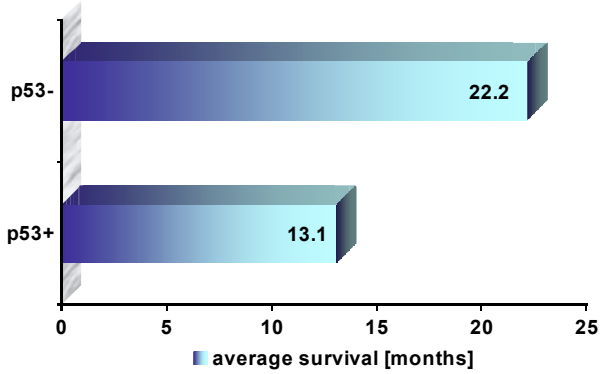


Figure 12 – ***Average survival of patients according to p53.***

Discussion

P53-gene is considered "guardian of the genome" and represents a tumor suppressor gene located on the 17p-chromosome, coding a protein of 53 kD [1]. P53-protein is essential for control of tumor growth, apoptosis and maintaining genome stability. Losing of the p53-function caused by genome alterations or interactions with products from the environment and bacterial products is considered to represent a critical stage in gastric carcinogenesis [2].

Mutations of the p53 have been observed in a wide variety of human carcinomas, such as colorectal carcinoma, breast carcinoma, gallbladder carcinoma, esophageal carcinoma, and gastric carcinoma [3–6].

Numerous data were obtained in regard to the clinical relevance of p53-alterations in gastric cancer, however there are still some controversial aspects, such as the relation between the p53-expression and the Lauren's type of gastric cancer [6, 7], the reserved prognosis of patients with gastric neoplasm expressing p53 [8, 9], and utilizing the expression of p53 as indicator of responsiveness to chemotherapy in gastric cancer [10–12].

For the entire study group, p53-immunoreactions became positive in 25 gastric cancers, representing 41% of the total of cases. Nuclear staining was generally intense and moderate, and was limited only to neoplastic cells, without interesting stromal nuclei. Pinto-de-Sousa J *et al.* [13], analyzing the expression of the p53-protein on a group of 163 patients resected for a gastric neoplasm, have obtained a positivity of the protein in 25.2% of cases. Some studies have demonstrated a p53-immunoreactivity of 19–29% [8, 14–17], while others have obtained higher percentages, of 34–65% [6, 18–25]. The variability of the percentage of cases with CG-positive for p53 in various studies performed resides probably in the differences of the methods used for evaluation of the expression of p53-mutations, as well as due to the characteristics of cases included in the study.

We obtained p53-positive immunohistochemical reactions in 41.9% of males and in 38.9% of females, remarking a slight predominance of cases for the male gender. P53-positive tumors occur more frequently in the elderly patients (≥61-year-old: 43.7%; ≤60-year-old: 38%). Some studies (Honda T *et al.*) [26] have shown a p53-immunoreactivity rate significantly lower in young patients. A study recently performed in Korea [27] analyzed the polymorphism of the p53-gene (codon 72) in 291 patients with gastric cancer. Although over 75% of gastric neoplasms presented overexpression of the p53-protein, only fewer than 30% presented mutations at the level of this gene. A significant difference was observed compared with the control group, the patients with gastric tumors presenting Arg/Arg genotype in 48.6% of cases and Pro/Pro in 3.6% of cases, in comparison with 41.5% and 10.9% in healthy subjects. A higher frequency of the Arg/Arg was remarked in patients over 75-year-old with gastric cancer, in comparison with younger patients. It was suggested that the prognosis of patients with Pro allele was more unfavorable compared with the Arg/Arg genotype and that the preferential frequency of the codon 72 Arg p53 acts as a survival factor in patients with gastric cancer, conferring an early onset of the conditions, in comparison with patients presenting Pro allele.

In our study, p53-positive immunoreactions occurred in all cases of carcinomas developed in the proximal 1/3rd of the stomach (100%), in approximately half of

the tumors of the gastric body and pangastric tumors, respectively, and much rarer in tumors that occurred on the gastric stump (33.3%) or in the antral region (29%). Some studies failed to prove a significant association between the p53-expression and the location of the gastric carcinoma [13, 28], attributing the greater aggressiveness of proximal gastric neoplasms to the more advanced pTNM-stage and to the high percentage of positivity of the c-erbB-2 protein [29, 30]. However, other studies noted a relatively higher expression of the p53 protein in proximal *vs.* antral tumors [31–33].

According to the Lauren's classification, we noted the significantly greater frequency of p53-positivation in intestinal type carcinomas (47.4%) in comparison with diffuse type carcinomas (29.4%) (p=0.0212).

Most histological types presented in relatively high proportions p53-positive immunohistochemical reactions. In the case of signet ring cell carcinoma, 29.4% of cases were p53-positive.

We noted a significant correlation between the degree of tumor differentiation and positivation of p53, p53-tumors being averagely differentiated (35%), and especially poorly differentiated (46.1%) (p=0.039).

Some studies, including that of Pinto-de-Sousa J *et al.* [13, 29], did not obtain significant differences in regard to the expression of p53 in intestinal type of gastric cancer *vs.* diffuse type, data that does not support the idea of using p53 as marker or indicator of gastric differentiation [34, 35]. It was observed, however, that the expression of p53 is associated with a more reduced survival rate in patients with intestinal type adenocarcinoma, but not also in the diffuse type [6, 13, 36]. These results are in inconsistency with other publications, which have showed that the p53-status influences survival only in diffuse type carcinomas [7, 21]. These inconsistencies can be explained due to the methods used in detecting the p53-expression/mutation, as well as due to the subjectivity of histological classifications of gastric carcinomas.

A significantly greater rate of the Pro p53-genotype was showed in patients with signet ring cell carcinoma. The association with histological grading can suggest that p53 polymorphism is involved in survival as factor of clinical prognosis, as well as for susceptibility for cancer [27, 37].

In our study, p53-positive immunoreactions are more frequent in carcinomas associated with aspects of lymphovascular invasion (47.4%). P53 suppresses angiogenesis through reducing VEGF and reducible synthase of nitrous oxide (iNOS). Mutant p53 intensifies the induction of VEGF-expression by the C-protein kinase (Zhang LL *et al.*, 2000) [38].

Some studies show that local progression of gastric carcinoma is associated with the expression of p53-protein [39]. Since the accumulation of p53-protein was correlated with a poor differentiation and with the TNM-stages T2, T3 and T4 of gastric cancer, one can conclude that this accumulation could determine the loss of control on the cellular proliferation and on the inhibition of iNOS and VEGF, possibly due to the mutations of the p53-gene. Recent studies performed on Japanese patients with gastric cancer revealed that the increased expression of VEGF and the accumulation of p53-protein are associated with intratumoral angiogenesis (Saito H *et al.*, 1999) [40] and with a non-favorable prognosis (Maeda K *et al.*, 1998; Sakatani T *et al.*, 2000; Ikeguchi M *et al.*, 2000) [22, 41, 42]. Other studies do not reveal a significant association between the p53-expression and the depth of tumor invasion [6–8].

According to the pT-stage, we noted a progressive increase in the number of p53-positive cases. The pTis case did not show immune coloration for p53. We identified p53-positive immune reactions in 25% of pT1-carcinomas, 33.3% of pT2-carcinomas, 47% of pT3-carcinomas and 43.3% of the pT4-carcinomas (p=0.022).

Numerous studies show a significant association between the expression of p53 and the presence of lymph node metastases [18, 20, 43, 44]. In the study elaborated by Pinto-de-Sousa J *et al.* [13], an association between the vascular invasion and the expression of p53 was described. The relation between p53, vascular invasion and the presence of lymph node metastases contributes to the understanding of the manner in which the expression of p53 is associated with an aggressive tumor behavior.

Our results show a significant correlation between pN and the p53-immunoreactions. We obtained p53-positive immunoreactions in 27.8% of pN0-carcinomas, 37.5% of pN1-carcinomas, 47.8% of pN2-carcinomas and 75% of pN3-carcinomas (p=0.038).

Although some studies suggest an association between the expression of p53 and the metastatic status [45–47], p53-immunoreactions performed in our study did not show a relation with the presence of distant metastases (pM-stage). We noted positive reactions in 40.4% of pM0-carcinomas, respectively 42.8% of pM1-gastric carcinomas.

Also, there is no significant correlation between p53-immunoreactions and the pTNM-stage. In the IA-stage 33.3% of tumors became positive, in the IB-stage 40%, in stage II we noted 42.8% of tumors p53-positive, in stage IIIA only 18.2% of tumors positive and in stage IIIB 37.5% of tumors p53-positive. The only exception is represented by stage IV where we noted 53.8% p53-positive carcinomas.

Immunohistochemical evaluation of the p53-protein constitutes in our study an important prognosis factor, survival at five years for patients with p53-positive carcinomas being significantly lower than the average rate of survival at five years for patients with p53-negative carcinomas (8% *vs.* 22.2%) (p=0.0326). Patients with p53-positive carcinomas survived on average 13.1 months, significantly less in comparison with patients whose carcinomas did not express immunohistochemically the p53-protein (20.4 months).

Following the univariate analysis, other studies also showed that the expression of p53 influences survival, patients with p53-positive tumors having a more decreased survival rate in comparison with those p53-negatives [5, 8, 19, 22, 32, 48]. However, applying the Cox regression model, p53 did not demonstrate prognostic value in combination with well-known prognosis factors in gastric cancer, such as tumor stage, venous invasion, and the c-erbB-2 expression [20, 29, 30, 43, 49–51].

In conclusion, detecting the expression of p53 can assist in identifying a subgroup of patients with aggressive gastric neoplasm, which might benefit from neoadjuvant chemotherapy [52–54].

Conclusions

The immunohistochemical study for p53-protein has proved the high level of expression in gastric carcinomas, the reactions being positive in 41% of cases, with various distribution models.

According to the Lauren's classification, we noted the greater frequency of p53-positivation in intestinal type carcinomas (47.4%) in comparison with diffuse type carcinomas (29.4%), without however reaching a statistic significance (p>0.05).

We observed a significant correlation between the tumor grade and positivation of p53 (p=0.039), p53-positive tumors being averagely differentiated (35%) and especially poorly differentiated (46.1%).

According to the pT-stage, we noted a progressive increase in the number of p53-positive cases, parallel with the depth of invasion (p=0.022). The pTis case did not show immunostaining for p53. We identified p53-positive immunoreactions in 25% of pT1-carcinomas, 33.3% of the pT2-carcinomas, 47% of pT3-carcinomas, and 43.3% of pT4-carcinomas.

Our results show a significant correlation between pN and p53-immunoreaction (p=0.038). We obtained p53-positive immunoreactions in 27.8% of pN0-carcinomas, 37.5% of pN1-carcinomas, 47.8% of pN2-carcinomas, and 75% of pN3-carcinomas.

Although some studies suggest an association between the expression of p53 and metastatic status, p53-immunoreactions performed in our study did not show a relation with the presence of distant metastases (pM-stage); there is no significant correlation between the p53-immunoreactions and the pTNM-stage.

Immunohistochemical evaluation of the p53-protein constitutes in our study an important prognosis factor, survival at five years for patients with p53-positive carcinomas being significantly lower than the average rate of survival at five years for patients with p53-negative carcinomas (8% *vs.* 22.2%) (p=0.0326). Overexpression of p53 selects a group of patients with aggressive therapeutic indication, extensive lymphadenectomy and adjuvant chemotherapeutic treatment.

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