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Ki-67 expression in gastric cancer. Results from a prospective study with long-term follow-up

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Abstract

Background: The characteristics of the cellular kinetic reflect the aggressiveness of the tumors and even their prognosis, many studies proving the correlation between the increased proliferation activity and a poor prognosis in a variety of neoplasms. *Aim*: The analysis of immunohistochemical expression of the Ki-67 antigen using the monoclonal antibody MIB1 in 61 patients with gastric cancer, the correlation with clinicopathological factors and the prognosis of the patients. *Material and Methods*: We used the primary MIB1 antibody pre-diluted, using the LSAB technique, DAB visualization. The quantification of the reaction was performed by appreciating the marking index Ki-67 (MI Ki-67). Although all the lesions were positive, we noticed a marked intratumoral heterogeneity regarding the distribution of the Ki-67 score. The tumor cells were considered Ki-67 positive in the presence of brown nuclear staining of granular or diffuse type. The tumor invasion front has shown the most numerous Ki-67 positive cells. *Results*: In the gastric carcinomas, we remarked various Ki-67 scores. For a proper grouping of the results, we classified gastric carcinomas into two categories: carcinomas with high MI Ki-67 (≥45%) and carcinomas with low MI Ki-67 (≤45%). We noticed an increased frequency of high MI Ki-67 carcinomas in elderly patients (p=0.03) and also in the tumors developed at cardia level and those extended in the entire stomach in the moment of diagnosis (p<0.001). The histological forms associated to high Ki-67 values are represented by the anaplastic carcinoma (100% of cases) and papillary adenocarcinoma (60% of cases).We observed a close correlation between the degree of tumor differentiation and the Ki-67 score (p<0.001). The results of our study do not reveal any correlation between the Lauren's Classification of gastric carcinomas, the lymphovascular invasion, the depth of tumor invasion, the TNM stage and the Ki-67 score (p>0.05). *Conclusions*: In our study, immunohistochemical assessment of the tumor proliferation does not represent a prognostic factor, but seems to be useful in identifying of a group of patients with aggressive tumors, needing adjuvant postoperatory chemotherapy.

***Keywords*:** Ki-67, gastric cancer, clinicopathological factors, survival.

Introduction

Ki-67 is a nuclear proliferation-associated antigen expressed in the growth and synthesis phases of the cell cycle but not in the resting phase. This antigen provides information about the proportion of active cells in the cell cycle. The expression of Ki-67 varies greatly during the cell cycle and is increased in many tumors.

The prognosis of patients with gastric cancer can be influenced by the alteration of oncogenes or tumoral suppressor genes, determining alterations of the kinetics of cell proliferation. The characteristics of cellular kinetics reflect the aggressiveness of tumors and even their prognosis, a series of studies demonstrating the correlation between the marked proliferative activity and an unfavorable prognosis in a variety of neoplasias.

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 Timişoara. On this group, we performed a prospective study regarding the evolution and aggressiveness of gastric cancer, over a period of 5 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 6 months, the survival being monitored over a variable period between 1 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 order to assess the aggressiveness and prognosis of gastric carcinomas studied (61 cases), we analyzed the immunohistochemical expression of the Ki-67 antigen using the MIB1 monoclonal antibody, following the correlations with various clinicopathological factors (gender and age of patients, location, macroscopic type, histological type, degree of tumor differentiation and the TNM staging) and survival of patients.

The study material was prepared through fixating in 10% formaldehyde, paraffin inclusion, and sectioning at 3–4 μm. We worked with the MIB1 pre-diluted primary antibody, through the LSAB technique and pre-treating through boiling in Retrieval solution for 60 minutes at 90^0C. The final reaction product is brown in color (visualizing agent – DAB) and has nuclear and sometimes cytoplasmic location. For positive control of the reaction, we included a tonsil fragment in the study, and for negative control, phosphate buffer solution (PBS) replaced the primary antibody.

The reaction was considered positive for any nuclear staining, regardless of the intensity of the reaction. Quantifying of the reaction was achieved through the assessment of a marking index Ki-67 (MI Ki-67), expressed as percentage result of the number of Ki-67+ cells reported to 500 cells (Ki-67+ and Ki-67-). In the gastric carcinomas studied, MI Ki-67 was evaluated in the area with the highest density of Ki-67 nuclei.

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.

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

Even though all lesions were positive, we noted great intratumoral heterogeneity in the distribution of the Ki-67 score. Tumoral cells were considered positive in the presence of nuclear coloration in brown of a granular or diffuse type. Cells in mitosis associated the nuclear and cytoplasmic stain.

The gastric mucosa adjacent to the neoplastic proliferation presented intense and relatively homogenous nuclear markings at the level of neck of glands (Figure 1) and in the germinative centers of lymphoid follicles. In general, the front of tumoral invasion presented most Ki-67 positive cells.

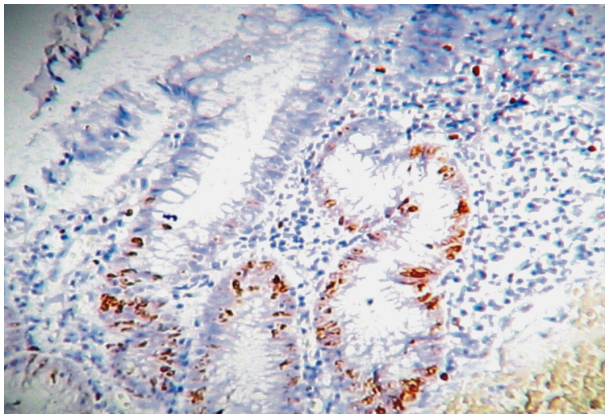


Figure 1 – *Immunostained nuclei in the epithelial cells at the neck of gastric glands. Ki-67 immunoreaction, DAB, ×200.*

The Ki-67 score was reported at the clinicopathological factors: sex and age of patients, location and macroscopic type of tumor, histological type, degree of tumor differentiation, lymphovascular invasion, and the TNM stage.

In the gastric tumors studied, we noted varied Ki-67 scores (10.2–92.5%), indicating different proliferative activities. MI Ki-67 presented an average value of 46.4%. For a better systematizing of the results, we classified gastric carcinomas into two categories:

- carcinomas with high MI Ki-67 ≥45% – 33 cases (54.1% – Figure 2);
- carcinomas with low MI Ki-67 <45% – 28 cases (45.9% – Figure 3).

We noted a much higher frequency of carcinomas with high Ki-67 scores in male patients, however without any statistical significance (51.2%) (Table 2).

In females, carcinomas with low MI Ki-67 were predominant (66.7%) (p=0.007195) (Figure 4). Elderly patients develop more frequently tumors with high proliferative index, in a percentage of 59.4% of cases (Figure 5).

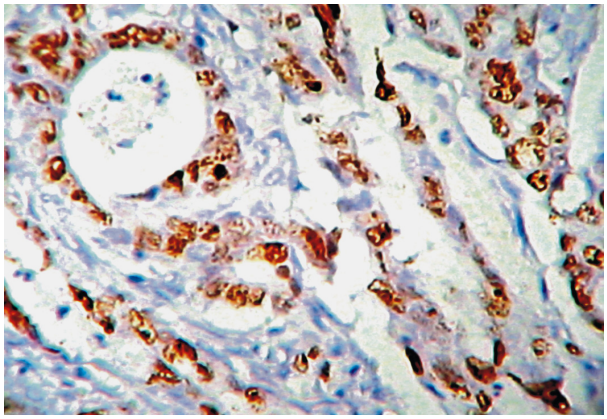


Figure 2 – ***Gastric carcinoma with high Ki-67 score. DAB, ×400.***

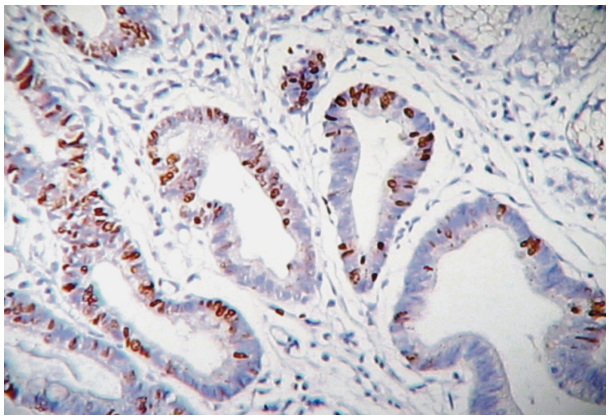


Figure 3 – ***Gastric carcinoma with low Ki-67 score. DAB, ×200.***

Table 2 – ***Correlation between the Ki-67 score and the gender, age of patients, and location of tumors***

Clinicopathological factors		Carcinomas with MI Ki-67 ↓ (n=33)	Carcinomas with MI Ki-67 ↑ (n=28)	*p*
Gender	*Males*	21 (48.8%)	22 (51.2%)	0.174421
	Females	12 (66.7%)	6 (33.3%)	
Age	*≤60 years*	20 (69%)	9 (31%)	0.032068
	≥61 years	13 (40.6%)	19 (59.4%)	
Location	*Antrum*	18 (58.1%)	13 (41.9%)	<0.001
	Gastric body	9 (60%)	6 (40%)	
	Pangastric	4 (40%)	6 (60%)	
	Cardia	0 (0%)	2 (100%)	
	Gastric stump	2 (66.7%)	1 (33.3%)	

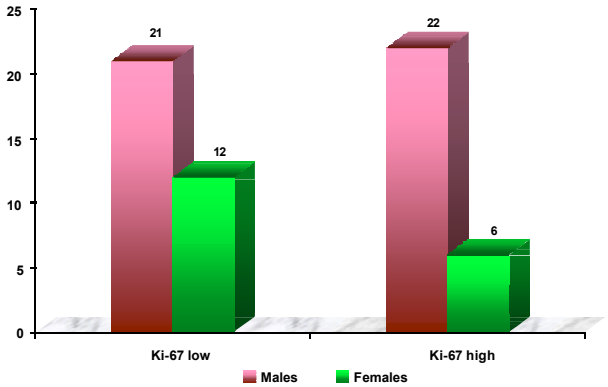


Figure 4 – ***Relation between Ki-67 and patients' gender.***

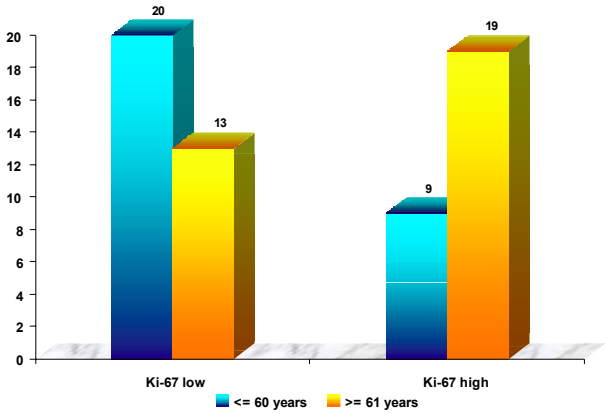


Figure 5 – ***Relation between Ki-67 and patients' age.***

Cancers developed at the level of the cardia and those extended in the entire stomach at the time of detection proved to be particularly aggressive neoplastic proliferations, with high MI Ki-67 in a proportion of 100% and 60% of cases. We noted 41.9% antral carcinomas, 40% carcinomas of the gastric body, and 33.3% carcinomas developed on the gastric stump with high values of MI Ki-67. The high proliferation index was significantly more frequently observed in carcinomas of the cardia *vs.* those located at the antrum.

Our results do not show a correlation between the Lauren's Classification of gastric carcinomas and the Ki-67 score (Table 3).

Table 3 – ***Correlation between the histological type, degree of tumor differentiation, and the Ki-67 score***

Clinicopathological factors		Carcinomas with MI Ki-67 ↓ (n=33)	Carcinomas with MI Ki-67 ↑ (n=28)	*p*
Lauren's Classification	*Intestinal type*	20 (52.6%)	18 (47.4%)	0.788077
	Diffuse type	9 (52.9%)	8 (47.1%)	
	Mixed type	4 (66.7%)	2 (33.3%)	
Histological type	*TA*	16 (57.1%)	12 (42.8%)	0.006927
	PA	2 (40%)	3 (60%)	
	MA	6 (75%)	2 (25%)	
	SRCC	9 (52.9%)	8 (47.1%)	
	AC	0 (0%)	3 (100%)	
Degree of tumor differentiation	*G1*	2 (100%)	0 (0%)	0.000216
	G2	13 (65%)	7 (35%)	
	G3	18 (46.1%)	21 (53.2%)	
Lympho-vascular invasion	*Present*	21 (55.3%)	17 (44.7%)	>0.05
	Absent	12 (52.2%)	11 (47.8%)	

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

High values of MI Ki-67 were noted in 47.4% of the intestinal type carcinomas (Figure 6) and 47.1% of the diffuse type carcinomas (Figure 7).

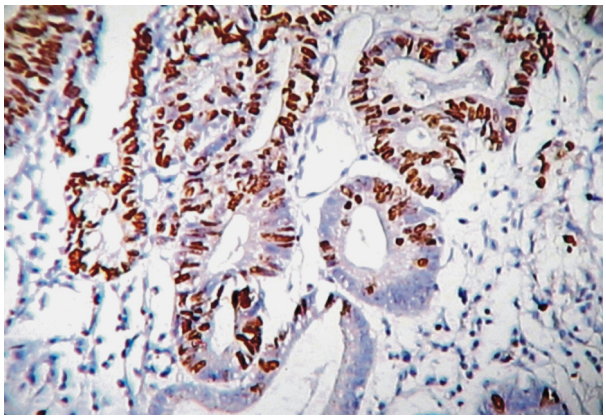


Figure 6 – ***Gastric carcinoma of intestinal type. Ki-67 immunoreaction, DAB, ×200.***

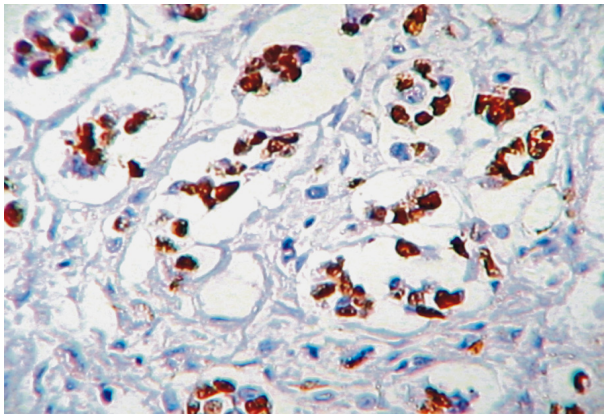


Figure 7 – ***Gastric carcinoma of diffuse type. Ki-67 immunoreaction, DAB, ×400.***

Histological forms associated to certain high Ki-67 values are represented by the anaplastic carcinoma (100% of cases) and papillary adenocarcinoma (60% of cases). Mucinous adenocarcinoma presents an elevated MI Ki-67 only in a percentage of 25%, significantly lower compared with papillary and anaplastic adenocarcinoma. We noted cases with high Ki-67 scores in 42.8% of tubular adenocarcinomas and in 47.15% of signet ring cell carcinomas.

We observed however a tight correlation between the degree of tumor differentiation and the Ki-67 score. The well-differentiated carcinomas (G1) presented high Ki-67 values. In a significantly greater percentage (53.2%), poorly differentiated carcinomas were characterized through intense proliferative activities, with high Ki-67 scores (Figure 8).

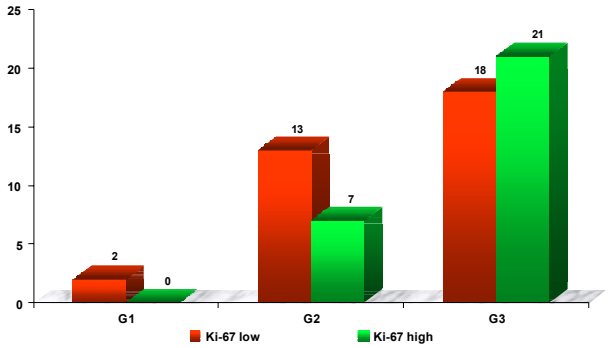


Figure 8 – ***Relation between Ki-67 and the degree of tumor differentiation.***

The values obtained do not indicate the existence of a correlation between the lymphovascular invasion and the Ki-67 score. Among the carcinomas with high MI Ki-67, 44.7% presented lymphovascular invasion, and 47.8% did not associate intravascular tumoral emboli.

We did not note a correlation between the level of tumor invasion and the value of Ki-67 (Table 4).

Table 4 – ***MI Ki-67 according to the pTNM stage***

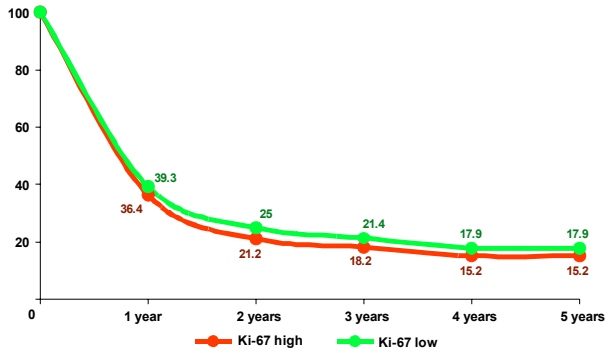
Clinicopathological factors		Carcinomas with ↓ MI Ki-67 (n=33)	Carcinomas with ↑ MI Ki-67 (n=28)	*p*
pT	*Tis*	1 (100%)	0 (0%)	0.584218
	T1	2 (50%)	2 (50%)	
	T2	4 (44.4%)	5 (55.6%)	
	T3	10 (58.8%)	7 (41.2%)	
	T4	16 (53.3%)	14 (46.7%)	
pN	*N0*	8 (44.4%)	10 (55.6%)	0.181171
	N1	8 (50%)	8 (50%)	
	N2	14 (60.9%)	9 (39.1%)	
	N3	3 (75%)	1 (25%)	
pM	*M0*	25 (53.2%)	22 (46.8%)	0.788077
	M1	8 (57.1%)	6 (42.9%)	
pTNM	*0*	1 (100%)	0 (0%)	0.785909
	IA	2 (66.7%)	1 (33.3%)	
	IB	2 (40%)	3 (60%)	
	II	3 (42.9%)	4 (57.1%)	
	IIIA	6 (54.5%)	5 (45.5%)	
	IIIB	5 (62.5%)	3 (37.5%)	
	IV	14 (53.8%)	12 (46.2%)	

We noted high scores for Ki-67 in 50% of pT1 carcinomas, 55.6% of pT2 carcinomas, 41.2% of pT3 carcinomas and 46.7% of pT3 carcinomas.

Also, the level of lymphonodular invasion is not correlated with the Ki-67 value. For pN2 and pN3 carcinomas, we noted high Ki-67 scores in only 39.1% and 25% of cases. High values for Ki-67 were obtained in very close percentages in carcinomas with metastases (46.8%) and without synchronous distance metastases (42.9%). The index of tumor proliferation calculated in our study does not indicate the existence of a relation between the Ki-67 score and the pTNM stage.

Patients with carcinomas characterized through low Ki-67 scores presented a survival rate at the end of the 5-year interval of 17.9% (six patients being alive). For cases with high Ki-67 scores, we observed a slightly lower survival rate (15.2%, four patients alive). The Ki-67 values noted in the two categories do not present significant differences (p>0.05 NS) (Figure 9).

In our study, the proliferative activity in gastric cancers does not constitute a prognosis factor and does not influence the survival of patients. Calculating the average survival rate (in months) in patients showed relatively close values: 18.8 months for carcinomas associated with low Ki-67 scores, and 16.2% for carcinomas associated with high Ki-67 scores (Figure 10).



	1 year	2 years	3 years	4 years	5 years
Ki-67 ↑	36.4%	21.2%	18.2%	15.2%	15.2%
Ki-67 ↓	39.3%	25%	21.4%	17.9%	17.9%

Figure 9 – ***Correlation between survival at 5 years and Ki-67.***

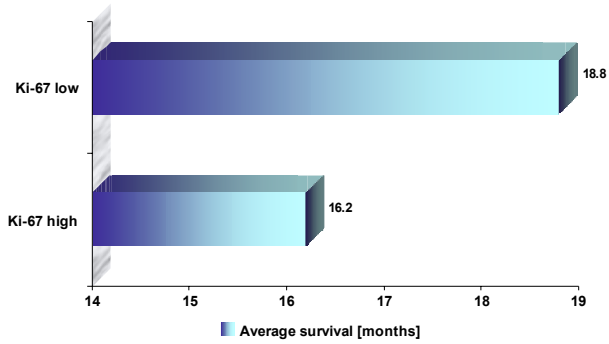


Figure 10 – ***Average survival [months] in relation with Ki-67.***

Discussion

The prognosis of patients with gastric cancer can be influenced by the alteration of oncogenes or tumoral suppressor genes, determining alterations of the kinetics of cell proliferation. The characteristics of cellular kinetics reflect the aggressiveness of tumors and even their prognosis, a series of studies demonstrating the correlation between the marked proliferative activity and an unfavorable prognosis in a variety of neoplasias [1–3].

Ki-67 monoclonal antibodies detect a nuclear antigen expressed exclusively at the level of cells in the proliferation phase (phases G1, S, G2 and mitoses), but not in the G0 phase. Therefore, Ki-67 antibodies allow for the imunohistochemical determination of the tissular growth fraction [4–6].

Correa postulated that gastric cancer develops through a complex sequence of events from normal mucosa to superficial gastritis, chronic atrophic gastritis, IM, dysplasia, and finally to intestinal type gastric carcinoma. In these premalignant stages, the apoptotic activity of the cells is lower than their proliferation rate, and this difference grows along the multi-step gastric carcinogenesis [7–9].

In the gastric carcinomas studied, we noted varied Ki-67 scores (10.2–92.5%), indicating different proliferative activities, in concordance with data from literature [10]. MI Ki-67 presented an average value of 46.4%. For a better systematizing of the results we classified gastric carcinomas into two great categories: carcinomas with high MI Ki-67 (≥45%) – 33 cases (54.1%); carcinomas with low MI Ki-67 (<45%) – 28 cases (45.9%).

We noted a significantly greater frequency of carcinomas with high Ki-67 in male patients (51.2%). In females, carcinomas with low MI Ki-67 were predominant (66.7%). Elderly patients developed more frequently tumors with high proliferative index (in a percentage of 59.4% of cases). This observation concurs with the data obtained by de Manzoni G *et al.* [1], who observed a significant interaction between MI Ki-67 and age, an MI Ki-67 of >40% being associated with an unfavorable prognosis in patients over 68 years of age. This study suggests that determining of Ki-67 might be useful in the pre-operatory defining of the subgroup of elderly patients with unfavorable clinical evolution, recurrences and early death.

Cancers developed at the level of the cardia and those extended in the entire stomach proved to be particularly aggressive neoplastic proliferations, with high MI Ki-67 in percentages of 100% and 60% of cases. We noted 41.9% antral carcinomas, 40% carcinomas of the gastric body, and 33.3% carcinomas developed on the gastric stump with high values of MI Ki-67.

Some studies have shown that gastric neoplasm of intestinal type presents a significantly higher MI Ki-67 [11, 12]. Others, however, did not demonstrate the existence of a correlation between the proliferation of neoplastic cells and the histological type of tumors, according to the Lauren classification [13, 14].

Our results do not show a relation between Lauren's Classification of gastric carcinomas and the Ki-67 score. High values of MI Ki-67 were observed in 47.4% of intestinal-type carcinomas and 47.1% of diffuse-type carcinomas.

Histological forms associated with high Ki-67 values are represented by the anaplastic carcinomas (100% cases) and papillary adenocarcinoma (60% of cases). We observed cases with high Ki-67 scores in 42.8% of tubular adenocarcinomas and in 47.15% of signet ring cell carcinoma. Other studies reveal lower levels of proliferation and apoptosis in poorly-differentiated and signet ring cell carcinomas [15, 16].

We observed a tight correlation between the degree of tumor differentiation and the Ki-67 score. Well-differentiated carcinomas (G1) presented lower Ki-67 values than the mean value. Among moderately differentiated carcinomas, 35% presented high Ki-67 values. In a significantly greater percentage (53.2%), poorly differentiated carcinomas were characterized through intense proliferative activities, with high Ki-67 scores.

Although in several studies we observed a significant correlation between the expression of Ki-67 and the depth of tumor invasion [17], most authors did not demonstrate a relation between MI Ki-67 and the clinicopathological factors in gastric cancer, such as lymph node metastases, invasion of the serosa, or venous invasion [18–21].

The values obtained do not indicate the existence of a correlation between the lymphovascular invasion and the Ki-67 score. Among the carcinomas with high MI Ki-67, 44.7% presented lymphovascular invasion, and 47.8% did not associate intravascular tumoral emboli.

We did not note a correlation between the level of tumor invasion and the Ki-67 value. We noted high scores for Ki-67 in 50% of pT1 carcinomas, 55.6% of pT2 carcinomas, 41.2% of pT3 carcinomas, and 46.7% of pT3 carcinomas.

Also, the level of lymph node invasion does not correlate in our study with the value of Ki-67. For pN2 and pN3 carcinomas, we noted high Ki-67 scores in only 39.1% and 25% of cases.

High Ki-67 values were obtained in very close percentages in carcinomas with metastases (46.8%) and without synchronous distance metastases (42.9%).

The index of tumor proliferation calculated in our study does not indicate the existence of a relation between the Ki-67 score and pTNM stage [22–24].

Some studies showed that patients with rapidly proliferative tumors present a clinically unfavorable evolution, with early recurrences and death [25–27]. Numerous authors have observed, however, that immune stain with Ki-67 has a limited independent value in predicting the prognosis of patients with gastric cancer [1, 11, 28–30].

Concerning our study group, patients with gastric carcinomas characterized through low Ki-67 scores presented a survival rate at the end of the 5-year interval of 17.9% *vs*. 15.2% for cases with high Ki-67 scores. The Ki-67 values noted in the two categories do not present significant differences. Calculating in months the average survival of patients showed relatively close values: 18.8 months for carcinomas associated with low Ki-67 scores, and 16.2 months for carcinomas associated with high Ki-67 scores.

In our study, the proliferative activity in gastric cancers does not constitute a prognostic factor and does not influence the survival of patients.

Conclusions

In the gastric carcinomas studied, we noted varied Ki-67 scores (10.2–92.5%), indicating different proliferative activities. MI Ki-67 presented an average value of 46.4%.

We noted a significantly higher frequency of carcinomas with high Ki-67 scores in elderly patients (59.4%).

Cancers developed at the level of the cardia and the ones extended in the entire stomach proved to be particularly aggressive neoplastic proliferations, with high MI Ki-67 in proportions of 100% and 60% of cases.

Our results do not show a relation between the Lauren's Classification of gastric carcinomas and the Ki-67 score.

The histological forms associated to high Ki-67 values are represented by the anaplastic carcinoma (100% of cases) and papillary adenocarcinoma (60% of cases).

We observed a close correlation between the degree of tumor differentiation and the Ki-67 score. Well-differentiated carcinomas (G1) presented Ki-67 values smaller than the average value. Among the moderately differentiated carcinomas, 35% presented high Ki-67 values. In a significantly greater percentage (53.2%), poorly differentiated carcinomas were characterized through intense proliferative activities, with high Ki-67 scores.

There is no correlation between the lymphovascular invasion, the depth of invasion, the presence of lymph node and distance metastases, pTNM stage, and the Ki-67 score.

Immunohistochemical evaluation of tumor proliferation does not constitute a prognostic factor in our study, but it is useful in identifying a group of patients with aggressive tumors, having an indication of postoperative adjuvant chemotherapy.

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