# CORRELATE STUDY ON KI-67 ANTIGEN EXPRESSION IN GASTRIC CARCINOMAS

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**Key words:** gastric cancer, KI-67, immunoreactivity, prognostic

**Abstract.** Despite their importance, prognostic factors in gastric cancer are not well known, yet. The study included surgical specimens from 40 patients with gastric carcinoma. All specimens were fixed in 10% buffered neutral pH formaldehyde and paraffin embedded. Histological sections were stained using current techniques: haematoxylin-eosin, tricromic van Gieson, and Alcian blue. We used Laurén histological classification with two main types of gastric carcinoma: intestinal and diffuse. We assessed the proliferative activity by determining the percentage of immunoreactive cells for the Ki-67 antigen (AM297 antibody Biogenex) and comparing the KI-67 index with the histological type. Immunoreactivity was positive for KI-67 in all the cases: 12 cases of intestinal gastric carcinoma - low to moderate index, 26 cases of diffuse gastric carcinoma – moderate to high index and 2 cases of mixed gastric carcinomas – low to moderate index. The positive rates for KI-67 along with histological differentiation give us useful information regarding the evolution and the prognosis of gastric cancer. These observations prove that a high proliferative activity is prognostic for a highly malignant tumour phenotype.

## INTRODUCTION

Gastric adenocarcinomas make up 90-95% of all malignant stomach tumours (1). Due to endoscopic investigations correlated with histopathological examinations, early adenocarcinoma has been having increasingly good prognosis, contrary to advanced gastric carcinoma. Epidemiological, immunohistochemical (IHC) and progressive data also confirm the two types of most common histological variants, that is intestinal and diffuse gastric carcinomas (Lauren 1965).

Numerous studies dwelt on IHC used for prognosis determination, together with protein gene products. Ki-67 antigen is a cell cycle and cell proliferation marker used to estimate the proliferation coefficient in a cell population (2). Ki-67 antigen is a nuclear antigen expressed throughout all the phases of the cell cycle, except for the G0 phase. The Ki-67+ tumour cell percentage may be correlated with the tumour aggressiveness or progress parameters.

There is little information in literature about the prognostic value of the Ki-67 proliferation index in gastric carcinoma. Therefore, this study is designed to carry out an IHC analysis of the proliferative activity expressed by the percentage of immunoreactive cells for the Ki-67 antigen, and the latter's comparison with the histological type.

## MATERIAL AND METHODS

The study material consisted of surgical specimens from 40 patients diagnosed with gastric neoplasm. All specimens were fixed in 10% buffered neutral pH formaldehyde and paraffin embedded. Histological sections were stained using current techniques: haematoxylin-eosin, van Gieson, Alcian blue, and PAS, which enabled us to classify injuries and determine their differentiation. We employed Lauren and WHO 2000 classification to assess the histological types. (3)

Immunohistochemical stain using the Avidin-Biotin Complex method was performed by means of the Optimax autostainer manufactured by Biogenex. Pretreatment consisted of 12-15min. boiling in citrate solution followed by 20 minutes incubation with Ki-67 antigen (AM 297 Biogenex – Ready to use). DAB viewing followed by a slight haematoxylin stain (1 min) revealed Ki-67 under the form of a brown reaction product with nuclear location (4,5,6).

On quantification, we avoided necrosis and hemorrhage foci, as well as the inflammation areas, and we used a 3-rate classification:

- *low rate (low malignity)* between 5 and 20% of the tumour cell nuclei were stained;
- *intermediate rate (intermediate malignity)* between 20 and 70% of the tumour cell nuclei were stained;
- *high rate (high malignity)* over 70% of the tumor cell nuclei were stained (7).

Statistical processing was performed using the Spearman Rank R Correlation Test, Chi-Square Anova tests.

## RESULTS

Ki-67 antigen marking revealed both punctiform and intense and diffuse stains in the whole nucleus. Ki-67 immunoreactivity was identified in 40 of the 40 gastric carcinomas, and the reactive nuclei percentage is shown in fig. 1, table I.

Rare positive nuclei dispersed in the normal gastric epithelium and in the adjacent glands have been revealed. We have identified 1-5% of Ki-67 positive nuclei in the gastric mucous membrane with intestinal metaplasia, especially incomplete colonic metaplasia, as well as in areas with intraepithelial neoplasia.

The percentage of reactive nuclei widely varied, differing depending on the histological type. By using Lauren's classification, we obtained the following results:

➢ Of the 12 cases of intestinal ADK, all were positive and registered a percentage of reactive nuclei which varied between 10 and 25%, enabling the classification of this group as having a low malignant rate. The reaction type was predominantly punctiform. (fig. 2, 3).

➢ Of the 26 cases of diffuse gastric carcinoma, all were positive, and registered a percentage of reactive nuclei which varied between 30% and 60%, enabling the classification of this group as having an intermediary malignant rate. The type of IHC reactions was predominantly intense and diffuse for the entire nucleus (fig. 4, 5).

➢ The two cases of mixed gastric carcinoma were positive for Ki-67, with a score of 25%, respectively 30%. Thus, one case fit the group with a low malignant rate and the other the group with the intermediate rate. The type of IHC reaction was predominantly intense and diffuse for the entire nucleus.
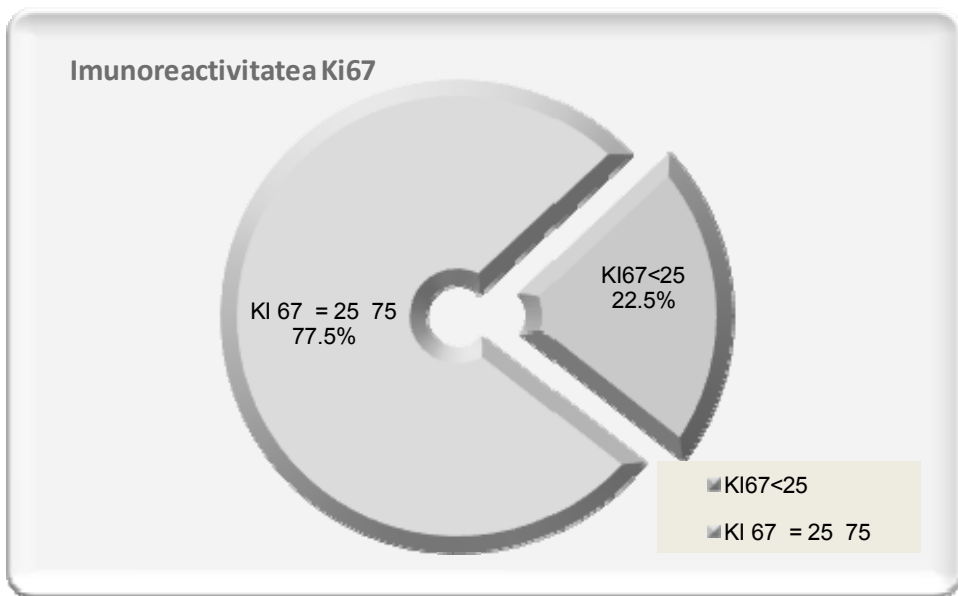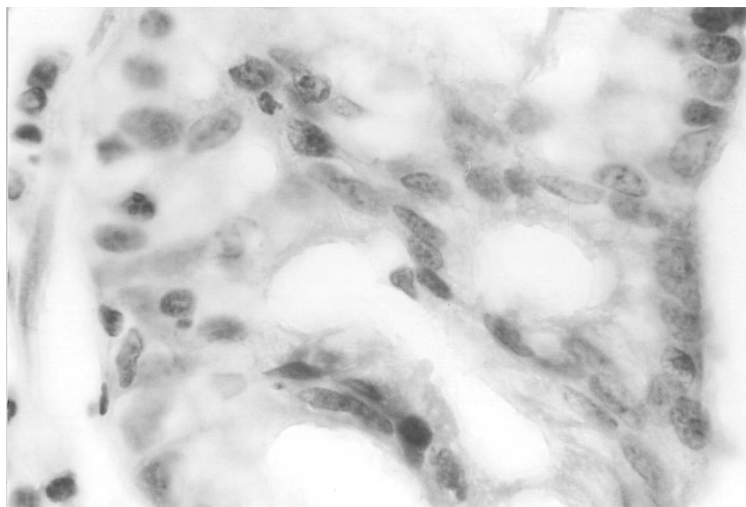


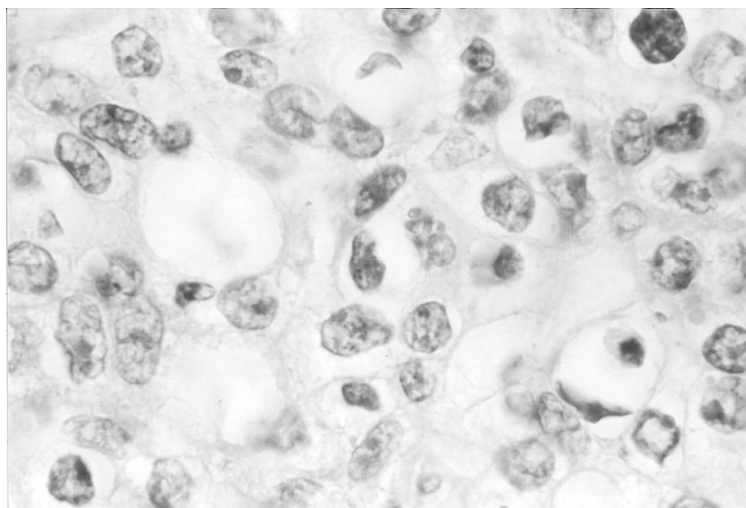**Fig 1**. Case distribution depending on Ki-67 immunoreactivity

**Table 1**. Case distribution depending on Ki-67 immunoreactivity
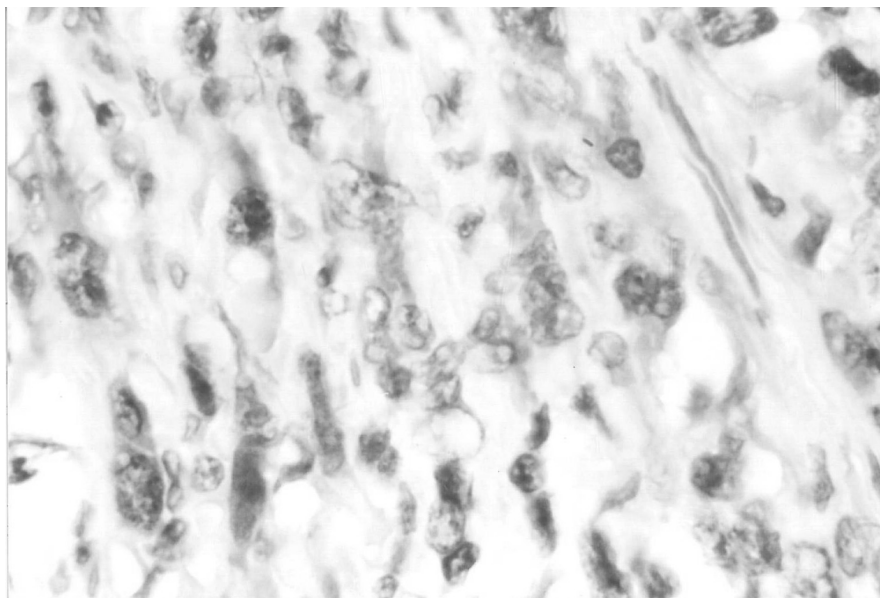
| Gastric carcinomas - p53 | Case np. | % |
|---|---|---|
| **<25** | 9 | 22.5% |
| **25-75** | 31 | 77.5% |

| Gastric carcinomas - p53 | Case np. | % |
|---|---|---|
| >75 | - | - |
| Total | 40 | |



**Fig. 2.** Punctiform nuclear stain for Ki-67 (10%) in an intestinal, well-differentiated ADK. Col IHC x 40.
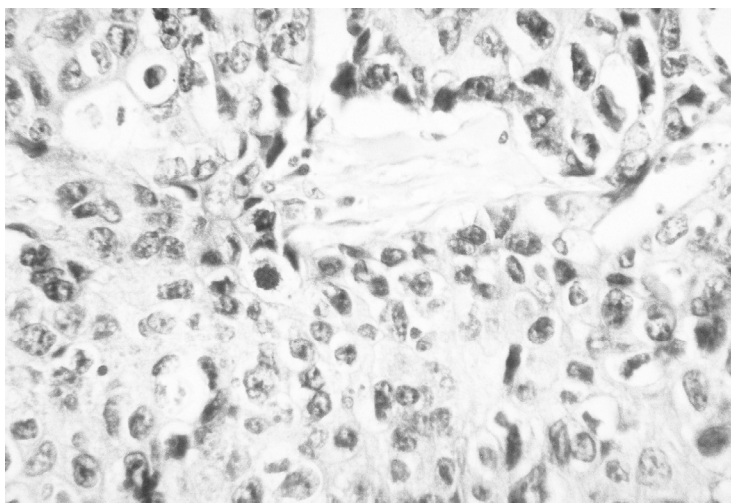


**Fig. 3.** Punctiform and homogenous nuclear stain for Ki-67 (30%) in a moderately differentiated, intestinal ADK. Col IHC x100

**Fig. 4.** Punctiform and homogenous nuclear stain for Ki-67 (60%) in a diffuse gastric carcinoma with signet ring cells carcinoma. Col IHC x100
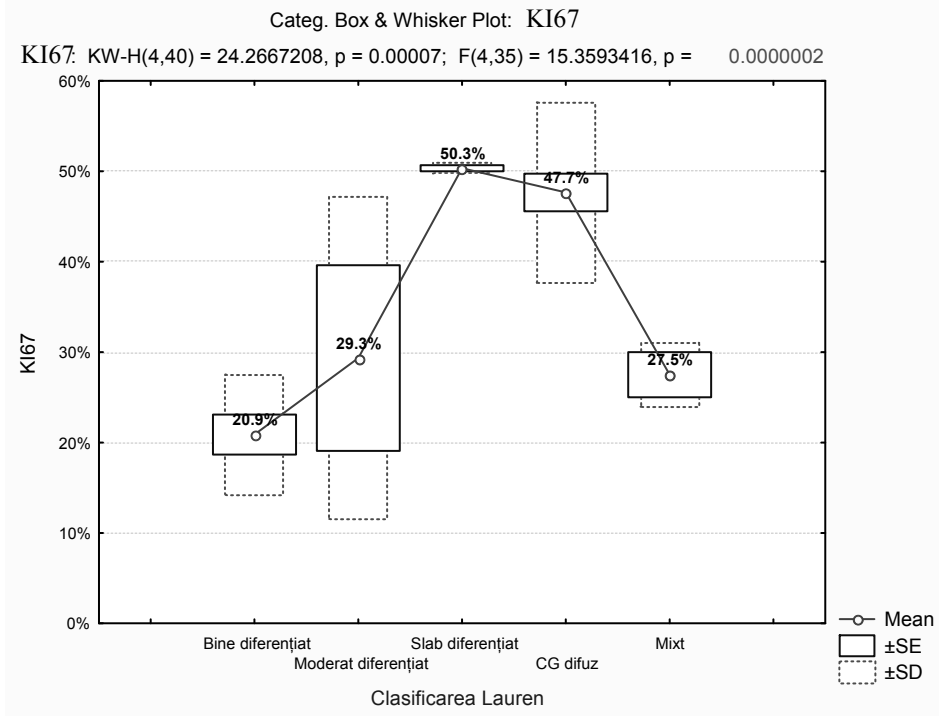
The statistical analysis of KI-67 immunoreactivity based on Lauren's classification revealed the following aspects (Fig.6,7 , Table 2,3,4)



**Fig. 5.** Punctiform and homogenous nuclear stain for Ki-67 (50%) in a diffuse gastric carcinoma.  Col IHC x40

**Table 2.** Statistical indices of Ki-67 immunoreactivity based on Lauren's classification

| | Mean KI 67 | Mean | | Dev.std | Er. std | Min | Max | Q25 | Median | Q75 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | -95% | +95% | | | | | | | |
| Well differentiated | 20.9% | 15.8% | 26.0% | 6.6% | 2.2% | 10.0% | 34.0% | 20.0% | 21.0% | 21.0% |
| Moderately differentiated | 29.3% | -14.8% | 73.5% | 17.8% | 10.3% | 10.0% | 45.0% | 10.0% | 33.0% | 45.0% |
| Slightly differentiated | 50.3% | 48.9% | 51.8% | 0.6% | 0.3% | 50.0% | 51.0% | 50.0% | 50.0% | 51.0% |
| Diffuse GC | 47.7% | 43.3% | 52.0% | 10.0% | 2.1% | 30.0% | 75.0% | 40.0% | 48.0% | 53.0% |
| Mixed | 27.5% | -4.3% | 59.3% | 3.5% | 2.5% | 25.0% | 30.0% | 25.0% | 27.5% | 30.0% |
| **Total** | 39.4% | 34.7% | 44.2% | 15.0% | 2.4% | 10.0% | 75.0% | 28.0% | 40.5% | 50.0% |

Categ. Box & Whisker Plot: KI67

KI67: KW-H(4,40) = 24.2667208, p = 0.00007;  F(4,35) = 15.3593416, p =      0.0000002



**Fig 6.** Average values of Ki-67 immunoreactivity based on Lauren's classification

**Table 3.** Test for comparing the values of Ki-67 immunoreactivity based on Lauren's classification

| % mucus | F (95% confidence interval) | p |
|---|---|---|
| ANOVA test | 15.35934 | 0.000000 |

For the moderately and the well differentiated gastric carcinomas, the values of the Ki-67 immunoreactivity are significantly smaller in comparison with the mean registered values for slightly differentiated or diffuse gastric carcinoma (F=15.35, p<<0.01, 95%CI).

**Table 4.** Findings of the  Ki-67correlation test vs. Lauren classification

| | r- correlation index (95% confidence interval) | p |
|---|---|---|
| Spearman Rank R Test | 0.76486 | 0.000 |
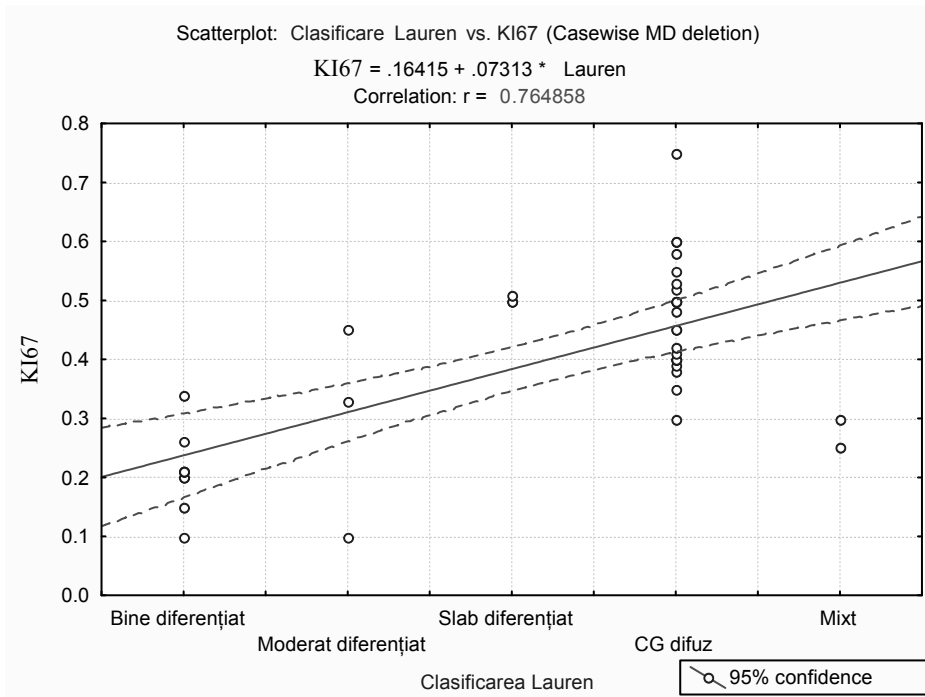


Fig 7. The regression line in the Ki-67 correlation vs. Lauren classification

The high value of the correlation index, as well as the slope of the regression line, proves the significant correlation between the Ki-67 values and Lauren's classification (r=0.76, p<<0.01, 95%CI).

## DISCUSSIONS

In several malignant neoplasias the percentage of the positive Ki-67 cells may be correlated to the aggressiveness parameters or tumoral progression parameters; therefore its practical interest and function are still little known.

As in gastric cancer the data regarding the prognostic value of the proliferation index Ki-67 are reduced, and the relationship between the positivation rate Ki-67 and the prognostic remains unclear, we analyzed the proliferative activity on a group of 40 gastric carcinomas. Studying the expression of the Ki-67 antigen on the 40 studied cases, we noticed a heterogeneity of the expression and positive immunoreactions in all 40 cases (100%) subject to AM immunocoloration.

The IHC detection of the Ki-67 antigen in combination to other clinical morphological and biological parameters proved to be highly relevant in appreciating the prognostic of patients with gastric cancer. In the specialized literature, there are few data on the prognostic value of the marking index Ki-67 and the relationship between the positivation rate Ki-67 and the prognostic remains unclear. Bouzubar and collaborators (1989) (8) sustain that the tumours with Ki-67 marking over 20% run a higher recurrence risk. Wintzer and collaborators (1991) (9) reported a more severe prognostic in patients with tumours having values of Ki-67 >16%, while Sabion (1991) notes a less probability of survival for 5 years in patients with values of Ki-67 > 13% (4).

It may be noted a significant relationship between the Ki-67 correlation and other prognostic indices, the positive cell rate Ki-67 (the score Ki-67 respectively) appears thus different on histological subtypes of gastric cancer, depending on the stage and the differentiation degree of the tumour, which confirms its prognostic validity.

Correlating the score groups Ki-67 with the histological types of Lauren's classification, we noticed the following:

In Lauren's classification the 12 cases of intestinal ADK have got <25% reactive nuclei Ki-67 with a low malignity score, while the 26 cases of diffuse gastric carcinomas have got >25% reactive nuclei Ki-67 with an intermediary malignity score. The 2 cases of mixed gastric carcinomas were at the limit between the low score and the intermediary score of the malignity.

In Thaler's study (1987) the detection of the Ki-67 antigen proved superior to the histological differentiation or mitotic index determination methods and he believes that irrespective of the histological type, all tumors with over 5% positive cells Ki-67 have an unfavorable clinical course (10).

Nakano and Oka's results (1993) sustain that high proliferative activity is correlated to high malignity of the tumour, with the recurrences and the high rate of metastasis. The greater the proliferative activity of a tumor, the lower its malignant potential, and the lower the survival (at the time of the diagnosis).

In all the 5 cases we have got data on the later evolution of the patients in the studied group. All were dead in less than 5 years, the rate of reactive nuclei Ki-67 varying in these cases from 10% to 50%.

## CONCLUSIONS

The presented results enables us to draw the following conclusions:

Well or moderately differentiated intestinal gastric carcinomas presented low to medium values of the proliferative activity (Ki-67 at a rate of 25-30%), while diffuse gastric carcinomas demonstrated a medium to high immunoreactivity with a Ki-67 from 25 to 75%. The obtained results enable us to state that the reduction of the tumoral differentiation is associated to a high rate of positiveness for Ki-67 and thus to an unfavorable prognostic.

Therefore, the detection of the antigen Ki-67 may be a better determination method than the histological differentiation or mitotic index determination methods.

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