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Correlation of HER2 Protein According to Histological Grade and Depth Invasion in Gastric Cancer

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Abstract
The aim of this study was to determine the correlation of HER2 protein with histological grade and depth of invasion of the primary tumor with gastric cancer. Method: were used archival tissue samples of 60 gastric cancer patients who are treated by immunohistochemistry antibody to HER2 per the manufacturer's protocol and evaluated a modified scoring system HER2 positivity surgical resection sample obtained. Data were processed using the descriptive statistics, chi square test and one sample T-test, and Pearson's correlation test. Results: Statistical analysis of HER2 protein according to histological grade gastric adenocarcinoma made using chi-square test does not show a statistically significant correlation between these two variables ($\chi 2 = 29.400$), the analysis using one sample t-test showed no statistically significant difference ($t = 2.850$) and analysis with Pearson correlation test shows a positive correlation that is statistically significant ($r = 0.214$). Statistical analysis of the HER2 protein according to the depth of invasion of gastric cancer was performed using chi-square test does not show a statistically significant association ($\chi 2 = 59.700$), the analysis using one sample t-test showed no statistically significant difference ($t = 2.900$), and analysis by Pearson-this test correlation shows a positive correlation that was not statistically significant ($r = 0.021$). Conclusions: There is a weak positive correlation of HER2 protein according to histological grade carcinoma ($r = 0.214$), and was recorded negligible positive correlation of HER2 protein according to the depth of invasion of cancer ($r = 0.021$).

Keywords: HER2, Histological Grade, Depth of Invasion, Gastric Cancer, Correlation

1. Introduction

Gastric cancer is a malignant epithelial tumor of the gastric mucosa with glandular differentiation, and one of the most common tumors, and the second leading cause of cancer death in the world, although its incidence has been decreasing, especially in developed countries, since the middle of the 20th century (Kelley and Duggan, 2003). The incidence of gastric cancer depends largely on the geographical area. In Croatia, this number ranges from 35 for men and about 20 for women, and it is the third most common malignancy (Kumar et al, 2010). Generally speaking, the CFR (case fatality ratio) for gastric cancer is 0.75 and is much higher than for other malignancies, e.g. colon cancer (CFR 0.52), breast cancer (CFR 0.36), or prostate cancer (CFR 0.33). The assessment of the tumor stage is based on the basic principles of tumor growth and spread. In the initial phase of growth, the tumor

spreads within the organ in which it originated, and with further growth it can directly spread to neighboring organs. During tumor growth, tumor cells enter blood and lymphatic vessels and metastasize to lymph nodes and distant organs. Therefore, the most common elements used in tumor stage assessment are:
1. location of the tumor inside the stomach (there is a special scheme that is used to assess the expansion of the tumor),
2. the depth of tumor invasion in the stomach wall,
3. the presence of tumors in the lymph nodes and the number of affected lymph nodes,
4. presence of distant metastases.

When analyzing gastric adenocarcinoma, in addition to determining the histological type according to Lauren and the macroscopic type according to Borrmann, it is also necessary to determine the degree of differentiation and Goseki grade (Gullick, 2001). Gastric adenocarcinomas can be well / medium / poorly / undifferentiated or grade (G) 1 / 2 / 3 / 4. The depth of invasion is the morphological characteristic that has the greatest importance for the clinical outcome (Hynes and Stern, 1994). Dysplasia of the epithelium of the gastric mucosa is considered to be a precursor lesion from which "early" cancer arises, which can then progress to advanced lesions. "Early" gastric cancer is defined as a lesion limited to the mucosa and submucosa regardless of the presence or absence of metastases in the perigastric lymph nodes. Advanced gastric cancer is a neoplasm that has spread below the submucosal layer into the muscular part of the wall or even deeper. Gastric cancer is considered as "early" (pT1) or advanced (≥ pT2), based on this, a prognostic discrepancy is observed between these two levels of invasion, at the level of the TNM classification, which is currently the only prognostic factor (Dako Connection, 2010; DAKO Catalog, 2010).

1.2. Molecular prognostic parameters

Overexpression of HER 2 protein in gastric cancer (which occurs in about 20% of gastric cancer cases) has been shown to be an indicator of poor prognosis in gastric cancer (Jorgensen, 2010). However, such cancer cases may respond favorably to combined chemotherapy and trastuzumab (targeted therapy) (Hofmann et al, 2008). In contrast to breast cancer, basolateral membrane staining instead of circular membrane staining is sufficient to qualify positive c-erbB-2 immunostaining in gastric cancer (Hetzel et al, 1992).

HER2 is a transmembrane glycoprotein essential in growth factor signaling. HER2 expression has also been found in colon, bladder, ovarian, endometrial, lung, cervical, head and neck, esophageal, and gastric cancers. Increased expression correlates with clinical outcome, conferring a poor prognosis, and is also a predictive factor for poor response to chemotherapy and endocrine therapy. The greatest value of determining HER2 status lies in predicting response to HerceptinR -Trastuzumab therapy (HERCEPTIN F.Hoffmann-La Roche Ltd, Basel, Switzerland and Genetech, Inc., South San Francisco, CA), in that the efficacy of anti-HER2 therapy correlates with the degree of HER2 positivity, and Herceptin therapy has revolutionized the treatment of breast cancer (Hetzel et al, 1992; Hirashima et, 2001; Douglass and Nava, 1985).

The TNM classification is currently the most important prognostic factor for gastric cancer. However, the prognosis in different patients with the same stage of the disease is not the same. Therefore, it is necessary, along with the TNM classification and classic pathological characteristics of the tumor, to identify biological prognostic factors, which are often derived from genetic processes and which are considered to be a key step in the prognosis of gastric cancer.

There are opinions that the expression of HER2 plays a key role in the development of gastric cancer, its progression and metastasis. However, little is known about its expression, because a limited number of papers have been published on this topic in the world. Given that the majority of stomach cancers, nowadays, unfortunately, are diagnosed at an advanced stage of the disease, it is still unclear whether the expression of the HER2 protein in this cancer is related to any of the proven prognostic factors, and whether it can have a potential role in the prognosis of the disease and possibly provide guidelines for therapeutic treatment.

The aim of the work was to determine the correlation of HER2 protein expression with the histological grade and the depth of invasion of the primary tumor in gastric cancer.

2. Material and methods

The research conducted and presented in this paper represents a fundamental or basic type of research with an observational method of obtaining data used in the study.

2.1. Material

This study used archival tissue samples of gastric carcinoma from 60 patients obtained after subtotal or total gastrectomy with regional lymphadenectomy of the lymph nodes of the perigastric fatty tissue along the lesser and greater curvature of the stomach, then the lymph nodes of the celiac tripus region and the hepatoduodenal ligament region. Pathohistological diagnostics of all gastric carcinoma samples included in this study was performed at the Department of Pathology and Cytodiagnostics of the Travnik Hospital, in the period from the beginning of 2022 to the end of 2025. In the prospective part, a pathohistological profile of HER2 amplifying gastric carcinomas was constructed based on a retrospective study, which was applied in the selection of pathohistologically verified gastric carcinomas in which immunohistochemical detection of HER2 protein was performed. Data on clinicopathological parameters of histological grade and depth of invasion of the primary tumor were obtained by individual microscopic observation of tissue samples of cancer tissue from all samples.

2.2. Methods

This is a retrospective-prospective (ambispective) study. The condition for including samples in this study is that the patients had an operable tumor with a known status of regional lymph nodes and without evident distant dissemination at the time of diagnosis. The same number, i.e. 60 samples of normal stomach tissue, were used as a control group.

Pathohistological processing of biopsy material obtained by subtotal or total gastrectomy with regional lymphadenectomy involved taking tissue samples according to dissection rules, and their standard histochemical processing and, in the first act, staining with the standard hematoxylin-eosin method as well as with the special histochemical method of staining PAS (Periodic Acid Schiff) for mucin. Microscopic interpretation of tissue samples stained with these methods resulted in clinical-pathological parameters: histological grade and depth of invasion of the primary tumor. In the following procedure, the most representative tissue samples of gastric carcinoma tissue that were embedded in paraffin blocks were re-cut on a microtome and immunohistochemically stained, i.e. treated with an antibody to HER2 according to the manufacturer's protocol, and then evaluated by light microscopy and scored using a modified scoring system for HER2 positivity of surgical resections.

2.3. Guide to HercepTest scoring in gastric carcinoma

Tests to examine the consistency, i.e. validity, of results between immunohistochemical staining and in situ hybridization pre-Toga studies showed that the HER2 IHC test is suitable for staining gastric carcinoma cells, but differences appeared in relation to breast carcinoma, and changes were required in the scoring system for gastric carcinoma. Pre-ToGA and ToGA (Trastuzumab for Gastric Cancer) studies have shown that a modified HER2 positivity scoring system should be used for gastric cancer samples.

2.4. Ethical aspects of the study

The use of archival gastric cancer tissue samples in this study was approved by the Ethics Committee of the Travnik Hospital, the institution where the study was conducted, for the purpose of using the data for scientific purposes.

2.5. Statistical data processing

After microscopic processing of the material, the obtained data were entered into MS Excel 2007. The stored data were transported to the SPSS 16.0 software package for statistical processing. Descriptive statistics, non-parametric significance tests, namely Chi square test and One sample T-test, as well as correlation tests, namely Pearson's correlation test, were used in data processing.

3. Results

Out of the total number of cancer tissue samples in the experimental group, none of the samples had histological grade I, or the grade of well-differentiated cancer. A total of 10 samples had moderate differentiation, or histological grade II, which is 16.7% of the total number of samples in the experimental group, while the largest number of samples, 50 of them, had histological grade III, or poor differentiation, which is 83.3% of the total number of samples in the experimental group, which is shown numerically and in percentages in Chart 1. Since no cancer tissue sample in the experimental group was well-differentiated, or had histological grade I, the slice for this group of samples in the pie chart is not shown, but the data on this group are given numerically and in percentages in order to complete the analysis of the stage of differentiation of cancer tissue in the samples of the experimental group.

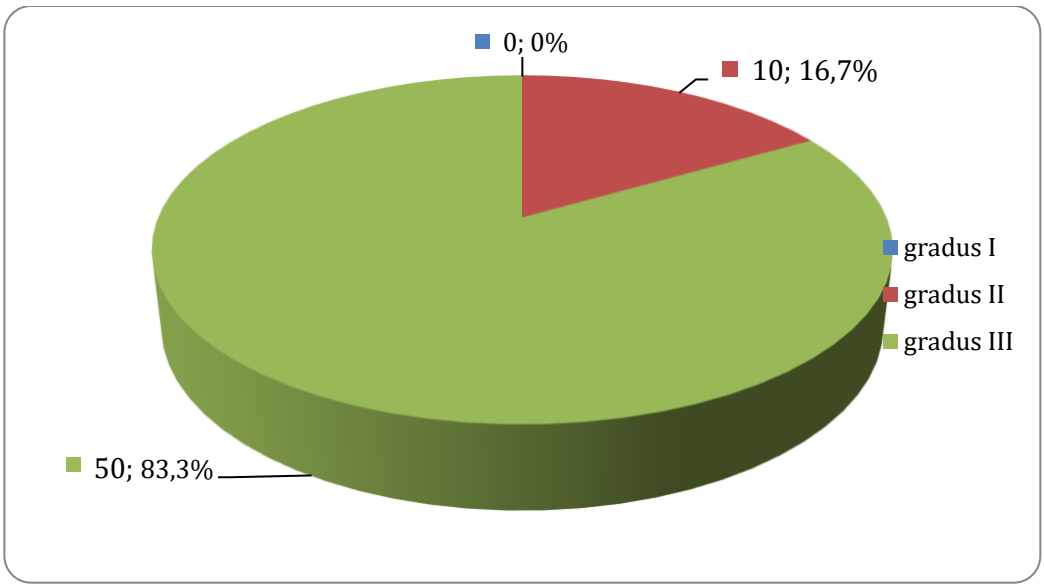


Chart 1: Structure of the differentiation stage the cancer tissue samples of experimental groups

Microscopic analysis of the depth of invasion of each sample of cancer tissue in the experimental group revealed that no cancer sample had a pT1 stage, i.e. was not limited to the mucosa or submucosa. A total of 9 samples, i.e. 15% of the total number of samples in the experimental group, had a pT2 stage, and in these samples, cancer cells infiltrated and remained limited to the muscular layer of the stomach wall, while in 48 samples, i.e. 80% of the total number of samples in the experimental group, infiltration of cancer tissue into the subserosa of the stomach wall, i.e. pT3 stage, was recorded. The remaining 3 samples, i.e. 5% of the total number of samples in the experimental group, had a pT4 stage, and perforation of the stomach wall serosa and/or infiltration of stomach cancer cells into surrounding structures was present. Data on the depth of invasion of cancer tissue samples of the experimental research group classified by pT classification are numerically and percentageally shown in chart 2. Given that none of the cancer tissue samples of the experimental group were limited to the mucosa or submucosa, i.e. did not have a pT1 stage, the section for this group of samples is not shown in the pie-graph, but the data on this group are listed numerically and percentageally in order to complete the whole analysis of the depth of invasion of cancer tissue samples of the experimental group.

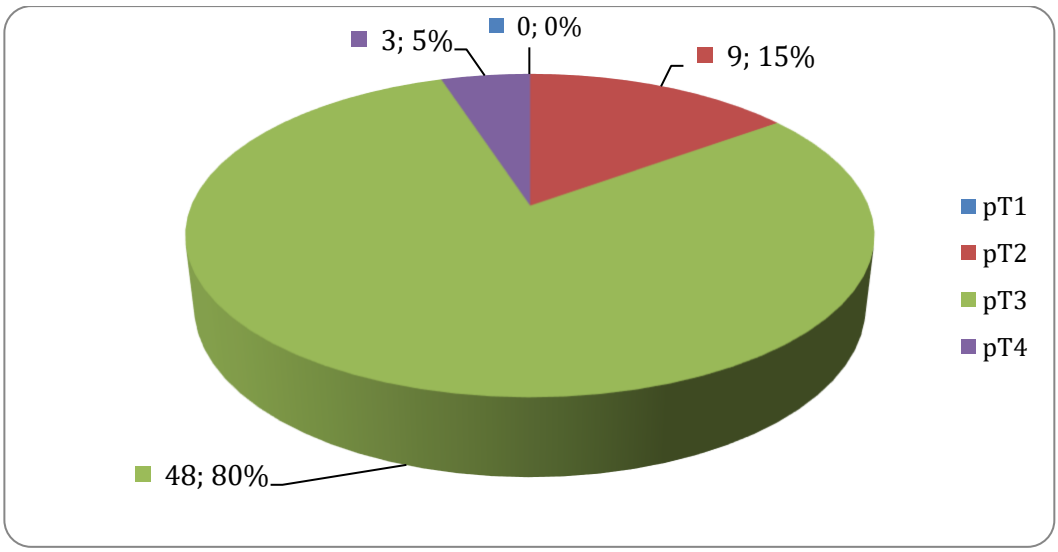


Chart 2. Structure of the depth of invasion and pT classification of the cancer tissue samples of the experimental group research

After processing and analyzing the characteristics of the cancer samples of the studied group of patients, such as the histological grade or degree of differentiation of the cancer and the depth of invasion of the primary tumor, immunohistochemical staining and analysis of all samples of the experimental and control groups of samples are performed.

The immunohistochemical analysis of the application of Herceptest on the experimental group of gastric cancer biopsy samples yielded results showing that the largest number of gastric cancers showed HER2 negative immunoreactivity, that is, that the largest number of gastric cancers did not show immunoreactivity in the expression of the HER2 protein, and out of a total of 60 samples that made up the experimental group, 36 of them, or 60%, showed HER2 negative immunoreactivity (Figure 1). Furthermore, weak or barely visible membrane activity, some of which are only in parts of the cell membranes of the cancer cells, is shown by a total of 9 samples, or 15% of the total number of samples in the experimental group (Figure 2).

Ambiguous membrane activity was shown by 8 samples of the experimental group, i.e. 13.33% of the total number of samples of the experimental group (Figure 3), and these samples in further work should be retested by the method of in situ hybridization in the light microscopic field (CISH).

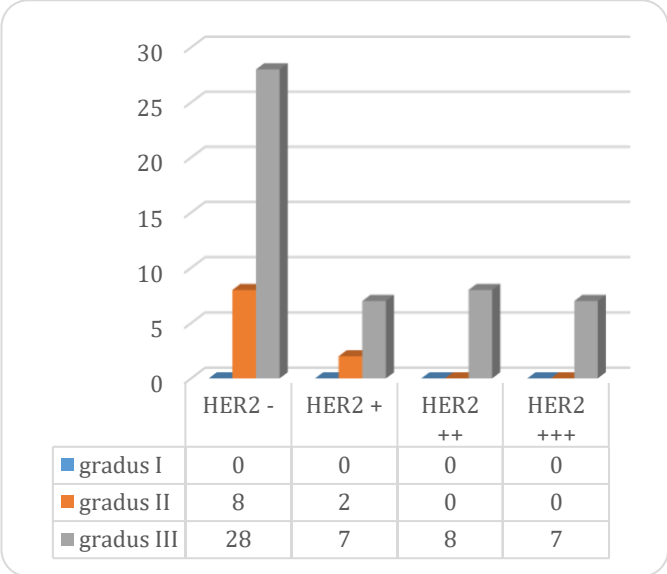
And finally, what is most important and why the research was done, hyperreactivity, i.e. baseolateral or lateral membrane reactivity in HER2 protein expression, was shown by 7 samples, i.e. 11.67% of the total number of samples of the experimental group (Figure 4).

One sample showed apical membranous activity, with no evident baseolateral or lateral membranous activity in HER2 protein expression, and that sample was considered a HER2-negative immunoreactivity sample (Figure 5). All samples of the control group of samples represented by samples of histologically regular stomachs showed HER2 negative immunoreactivity (Figure 6).

Analyzing the histological grading of gastric cancer, where in the experimental group none of the samples had the histological grade of well-differentiated cancer, and even 50 of them had the histological grade of poorly differentiated gastric cancer, and putting them in relation to the reactivity of cell membranes in the expression of the HER2 protein, the result was that hyperreactivity in the expression of the HER2 protein was shown exclusively by poorly differentiated gastric cancers, i.e. all 7 samples with baseolateral or lateral membrane activity in with HER2 protein expression belonged to the group of poorly differentiated cancers, which is 11.7% of the total number of samples of the experimental group, while pathohistologically verified cancers of moderate and good differentiation did not show hyperreactivity in the expression of HER2 protein. An almost identical result was

obtained with ambiguous membrane activity, where moderately and well-differentiated gastric carcinomas did not show this kind of membrane activity, while 8 samples of poorly differentiated gastric carcinomas, i.e. 13.3% of the total number of samples of the experimental group, showed ambiguous membrane activity in the expression of HER2 protein, which is numerically and percentageally shown in chart 3.

The control group of samples is without pathohistologically verified cancer tissue, so their histological grade was not determined either.



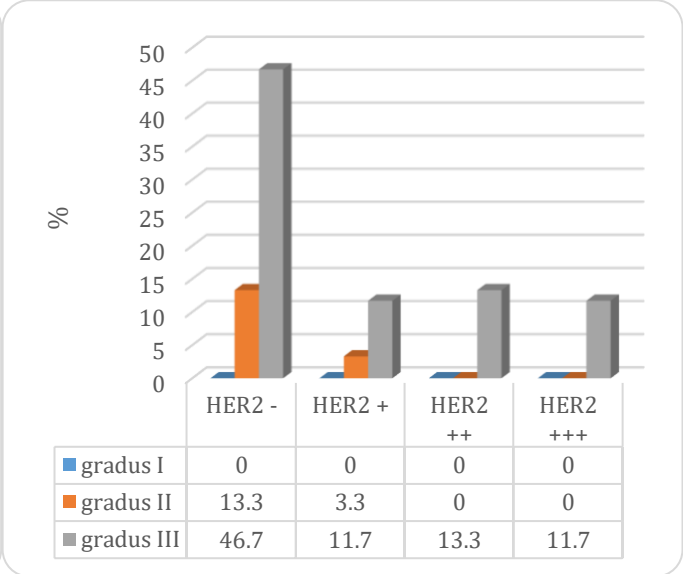


Chart 3: The expression of HER2 protein compared histological grade of gastric cancer studied groups

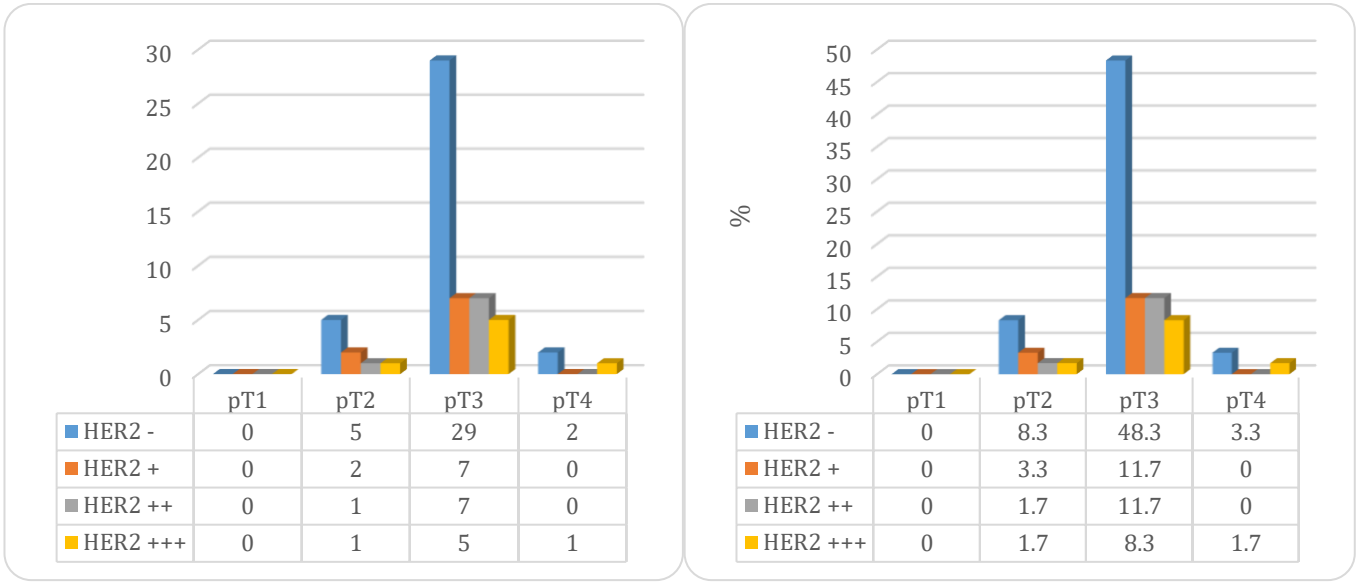
Statistical analysis of HER2 protein expression according to the stage of differentiation of gastric adenocarcinoma performed using the chi-square test does not show a statistically significant relationship between these two variables ($\chi 2=29.400$; statistically significant relationship is if $p<0.05$), analysis using the one sample T-test does not show a statistically significant difference ($t=2.850$; the difference is not statistically significant if $p>0.05$), and analysis using the Pearson correlation test shows a positive correlation that is not statistically significant ($r=0.214$) between these two examined parameters.

Taking into account the depth of invasion of gastric cancer in the samples of the experimental group, in which as many as 48 samples, or 80% of the total number of samples, have the pT3 stage, i.e. the cancer cells in these samples infiltrate the subserosa, and no sample has the pT1 stage, and comparing them with the membrane immunoreactivity in the expression of the HER2 protein, the result was that the highest number of hyperreactivity is shown by cancers with the pT3 stage, as many as 5 of them, or 8.3% of the total number of samples of the experimental group, while one sample each showing hyperreactivity in the expression of the HER2 protein has the pT2 and pT4 stages. Ambiguous membrane activity was shown by 7 samples, or 11.7% of the total number of samples in the experimental group with the pT3 stage, and 1 sample, or 1.7% of the total number of samples in the experimental group with the pT2 stage, which is numerically and in percentages shown in chart 4.

The control group of samples consisted of samples of histologically regular gastric mucosa without pathohistologically verified cancer cells, and they were in the pT0 stage, and were not taken into account in relation to membrane immunoreactivity in the expression of the HER2 protein.

Statistical analysis of HER2 protein expression according to the depth of gastric cancer invasion performed using the chi-square test does not show a statistically significant relationship between these two variables ($\chi 2=59.700$; a statistically significant relationship is if $p<0.05$), analysis using the one sample T-test does not show a statistically significant difference ($t=2.900$; the difference is not statistically significant if $p>0.05$), and analysis using the

Pearson correlation test shows a positive correlation that is not statistically significant (r=0.021) between these two parameters examined.



	pT1	pT2	pT3	pT4
HER2 -	0	5	29	2
HER2 +	0	2	7	0
HER2 ++	0	1	7	0
HER2 +++	0	1	5	1

	pT1	pT2	pT3	pT4
HER2 -	0	8.3	48.3	3.3
HER2 +	0	3.3	11.7	0
HER2 ++	0	1.7	11.7	0
HER2 +++	0	1.7	8.3	1.7

Chart 4: The expression of HER2 protein in relation to the depth of invasion of gastric cancer studied groups

4. Discussion

The aim of the study was to qualitatively and quantitatively determine the possible expression of HER2 protein in gastric cancer, and to examine the correlation of HER2 protein expression with the examined clinical-pathological parameters of gastric cancer: histological grade and depth of invasion of the primary tumor.

Before discussing the main objectives of this study, we need to discuss the basic characteristics, i.e. clinical-pathological parameters of the experimental research group.

When we talk about the histological grade, i.e. the stage of differentiation of gastric cancer in our experimental research group, out of the total number of cancer tissue samples, not a single sample had histological grade I, i.e. the grade of well-differentiated cancer. A total of 10 samples had moderate differentiation, i.e. histological grade II, which is 16.7% of the total number of samples in the experimental group, while the largest number of samples, 50 of them, had histological grade III, i.e. poor differentiation, which is 83.3% of the total number of samples in the experimental group. The depth of invasion of cancer cells in the stomach wall of each individual sample of the experimental group of the study was determined by detailed and decisive microscopic analysis, and we obtained data that no cancer sample had the pT1 stage, that is, it was not limited only to the mucosa or submucosa. A total of 9 samples, or 15% of the total number of samples of the experimental group, had the pT2 stage and in these samples the cancer cells infiltrated and remained limited to the muscular layer of the stomach wall, while in 48 samples, or 80% of the total number of samples of the experimental group, infiltration of cancer tissue into the subserosa of the stomach wall was recorded, that is, the pT3 stage. The remaining 3 samples, or 5% of the total number of samples of the experimental group, had the pT4 stage and they had perforation of the serosa of the stomach wall and/or infiltration of cancer cells of the stomach into the surrounding structures.

Analyzing the above-mentioned discussion on the basic characteristics, that is, clinical-pathological parameters of the experimental research group, it can be concluded that the experimental group of our research is objectively variegated in all parameters, which is important in a studious approach to the given research goals.

The results of our study, which was conducted on an experimental group of 60 samples of pathohistologically verified gastric cancer, showed hyperexpression of HER2 protein on the cell membranes of cancer cells in 7 samples, which is

11.67% of the total number of samples, while ambiguous membrane activity was shown by a total of 8 samples, which is 13.33% of the total number of samples of the experimental group. Also, the results of our study show weak membrane activity in 9 samples, which is 15% of the total number of samples of the experimental group, and negative HER2 immunoreactivity was found in the largest number of samples, i.e. in 36 samples, which is 60% of the total number of samples of the experimental group.

So far, HER2 expression has been investigated in studies with a relatively small number of samples and the results of these studies are very controversial, as can be seen from a recent review by Gravalos et al. (Gravalos and Jimeno, 2008). The frequencies of HER2 protein expression in published studies vary enormously from 8% to 91%. One of the first studies on this topic was conducted in 2000 when Allgayer et al., examining the frequency of HER2 protein expression in gastric cancer, obtained an extremely high expression of 91%. However, both in this and in other initially conducted studies on this topic, tumor heterogeneity was neglected, which is extremely pronounced in gastric cancer compared to breast cancer (4.80%:1.40%), and the malignant cell scoring system used was for breast cancer. These data can be taken as the reason for such a high percentage of HER2-positive gastric cancers (Van Cutsem et al, 2009).

Newer studies that were conducted after the pre-ToGa and large multicenter ToGa studies, took into account the heterogeneity of gastric cancers, as well as a modified tumor cell scoring system adapted for gastric cancer. In our study, these two parameters were also taken into account and we obtained 11.67% of HER2-positive cancers, which is similar and different from the findings of previous studies: the large multicenter ToGa (Trastuzumab for GAstric Cancer) study in which there were 22.10%, the results of the Chinese study conducted by Guan Zhen Yu et al. which had overexpression of HER2 protein in 28% of the primary gastric adenocarcinomas examined, as well as the results of the largest, most recent study conducted to date on 924 patients in which there were 7% HER2 positive cancers (Heike et al, 2010).

The results of the comparative analysis of HER2 protein expression and cancer differentiation stage in our study showed that hyperreactivity in HER2 protein expression is shown only by poorly differentiated gastric cancers, i.e. all 7 samples with baseolateral or lateral membrane activity in HER2 protein expression belonged to the group of poorly differentiated cancers, which is 11.7% of the total number of samples of the experimental group, while pathohistologically verified cancers of moderate and good differentiation did not show hyperreactivity in HER2 protein expression. An almost identical result was obtained with ambiguous membrane activity, where moderately and well-differentiated gastric carcinomas did not show this kind of membrane activity, while 8 samples of poorly differentiated gastric carcinoma, i.e. 13.3% of the total number of samples of the experimental group, showed ambiguous membrane activity in the expression of HER2 protein. Weak membrane activity in the expression of HER2 protein was obtained in 2 samples of moderately differentiated carcinomas, which is 3.3% of the total number of samples, and in 7 samples of poorly differentiated carcinomas, which is 11.7% of the total number of samples in the experimental group. Negative HER2 immunoreactivity was shown by 8 samples of moderately differentiated carcinomas, which is 13.3% of the total number of samples, and 28 samples of poorly differentiated carcinomas, which is 46.7% of the total number of samples in the experimental group of our study. Statistical analysis of HER2 protein expression according to the stage of differentiation of gastric adenocarcinoma performed using the chi-square test does not show a statistically significant relationship between these two variables ($\chi 2=29.400$; a statistically significant relationship is if $p<0.05$), analysis using the one sample T-test does not show a statistically significant difference ($t=2.850$; the difference is not statistically significant if $p>0.05$), and analysis using the Pearson correlation test shows a positive correlation that is not statistically significant ($r=0.214$) between these two examined parameters.

The results of our study regarding the depth of invasion of gastric cancer showed that no sample of cancer with stage pT1 showed hyperreactivity of HER2 protein expression, while 1 sample of cancer with stage pT2 and pT4 showed such reactivity, which is 1.7% of the total number of samples, and 5 samples of gastric cancer with stage pT3, which is 8.3% of the total number of samples. Ambiguous membrane activity was obtained in one pT2 stage gastric cancer sample, which is 1.7% of the total number of samples, and in 7 pT3 stage cancer samples, which is 11.7% of the total number of samples. Almost identical results with ambiguous membrane activity were obtained by the samples of our experimental group with weak membrane activity, so 2 samples of cancer stage pT2, which is 3.3% of the total number of samples, and 7 samples of cancer stage pT3, which is 11.7% of the total number of samples, had this activity, and 5 pT2 stage cancer samples, which is 8.3% of the total number of samples, had negative HER2 immunoreactivity, followed by 29 pT3 stage cancer samples, which is 48.3% of the total number of samples, and 2 pT4 stage cancer samples, which is 3.3% of the

total number of samples of the experimental group. Statistical analysis of HER2 protein expression according to the depth of gastric cancer invasion performed using the chi-square test does not show a statistically significant relationship between these two variables ($\chi 2=59.700$; statistically significant relationship is if $p<0.05$), analysis using the one sample T-test does not show a statistically significant difference ($t=2.900$; the difference is not statistically significant if $p>0.05$), and analysis using the Pearson correlation test shows a positive correlation that is not statistically significant ($r=0.021$) between these two examined parameters.

HER2 protein expression is also correlated with the control group, which in absolutely all samples showed a completely negative immunoreaction to HER2 protein expression.

4.1. Limitation of the study

Until now, HER2 expression has been examined through studies with a relatively small number of examined samples and the results of those studies are very controversial, and the frequencies of HER2 protein expression through published studies vary enormously from 8% to 91%.

Our study was also conducted on a relatively small number of samples, due to the smaller number of gastric cancer samples that met the given conditions, as well as the costs of the examination, which came from personal financial resources.

5. Conclusions

The results of the correlation of HER2 protein expression with the examined clinicopathological parameters show that there is only a weak positive correlation of HER2 protein with the histological grade or stage of differentiation of the cancer ($r=+0.214$), and a negligible positive correlation of HER2 protein with the depth of cancer invasion ($r=+0.021$).

Therefore, the expression of HER2 protein correlated with other clinicopathological parameters in our study did not show statistical significance, which is the same as in the study by Heike Grabasch et al. [15] who correlated HER2 protein expression with the degree of differentiation, depth of tumor invasion, lymph node status, and lymphovascular invasion. The results of the study by Guan Zhen Yua et al. are similar. which obtained a non-significant relationship with gender, depth of tumor invasion, tumor grade, lymph node status (Guan et al, 2009).

A weak positive correlation without statistical significance in our study was found between HER2 protein expression and histological grade of gastric cancer. Namely, all samples that showed hyperexpression of HER2 protein.

A weak positive correlation without statistical significance was found in our study between HER2 protein expression and histological grade of gastric cancer. Namely, all samples that showed HER2 protein hyperexpression were poorly differentiated carcinomas, i.e. carcinomas of histological grade III, while none of the samples of histological grades I and II showed either HER2 protein hyperexpression or ambiguous membrane activity.

HER2 protein expression showed a negligible positive correlation without statistical significance with the depth of invasion of gastric cancer, i.e. its pT stage, using the univariate statistical method according to Pearson, where 71.43% of all samples with HER2 protein hyperexpression had the pT3 stage, which is again 8.3% of the total number of samples in the experimental group.

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Conflict of Interest: The authors declare no conflict of interest.

Informed Consent Statement/Ethics Approval: Not applicable.

Declaration of Generative AI and AI-assisted Technologies: This study has not used any generative AI tools or technologies in the preparation of this manuscript.

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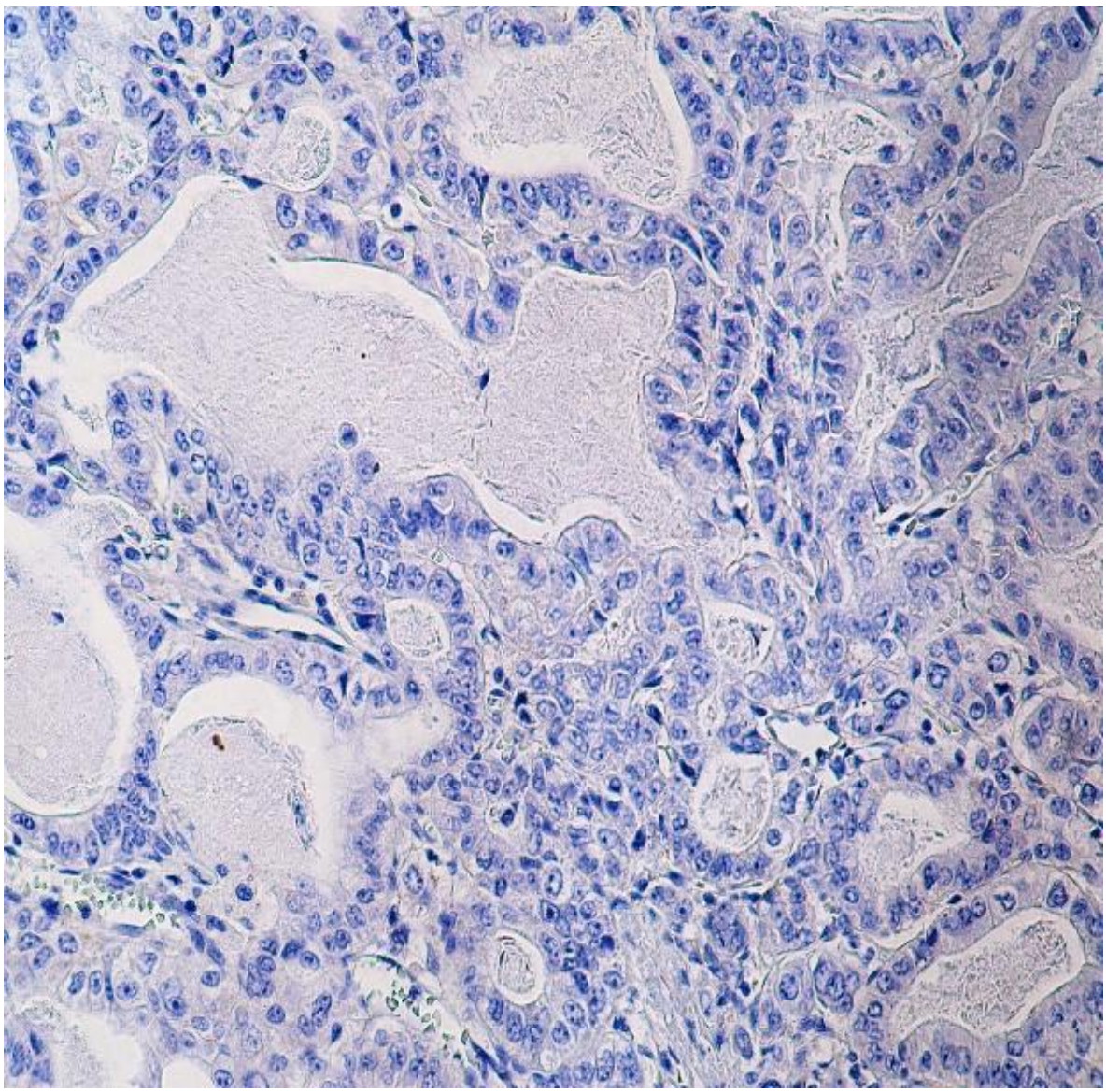

Figure 1: The negative reactivity of the HER2 protein (0) (40x)

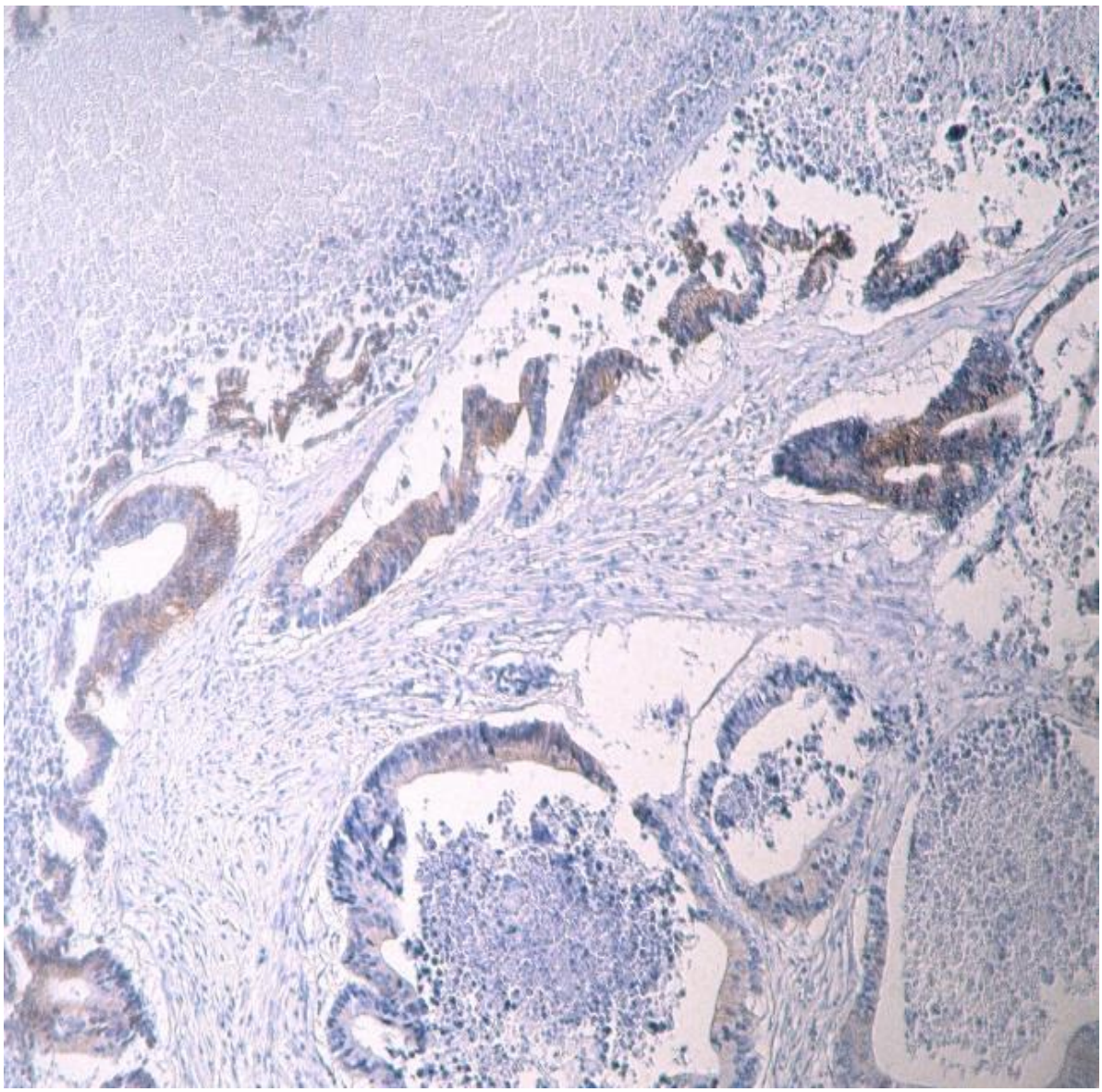

Figure 2: Light HER2 expression (+) (40x)

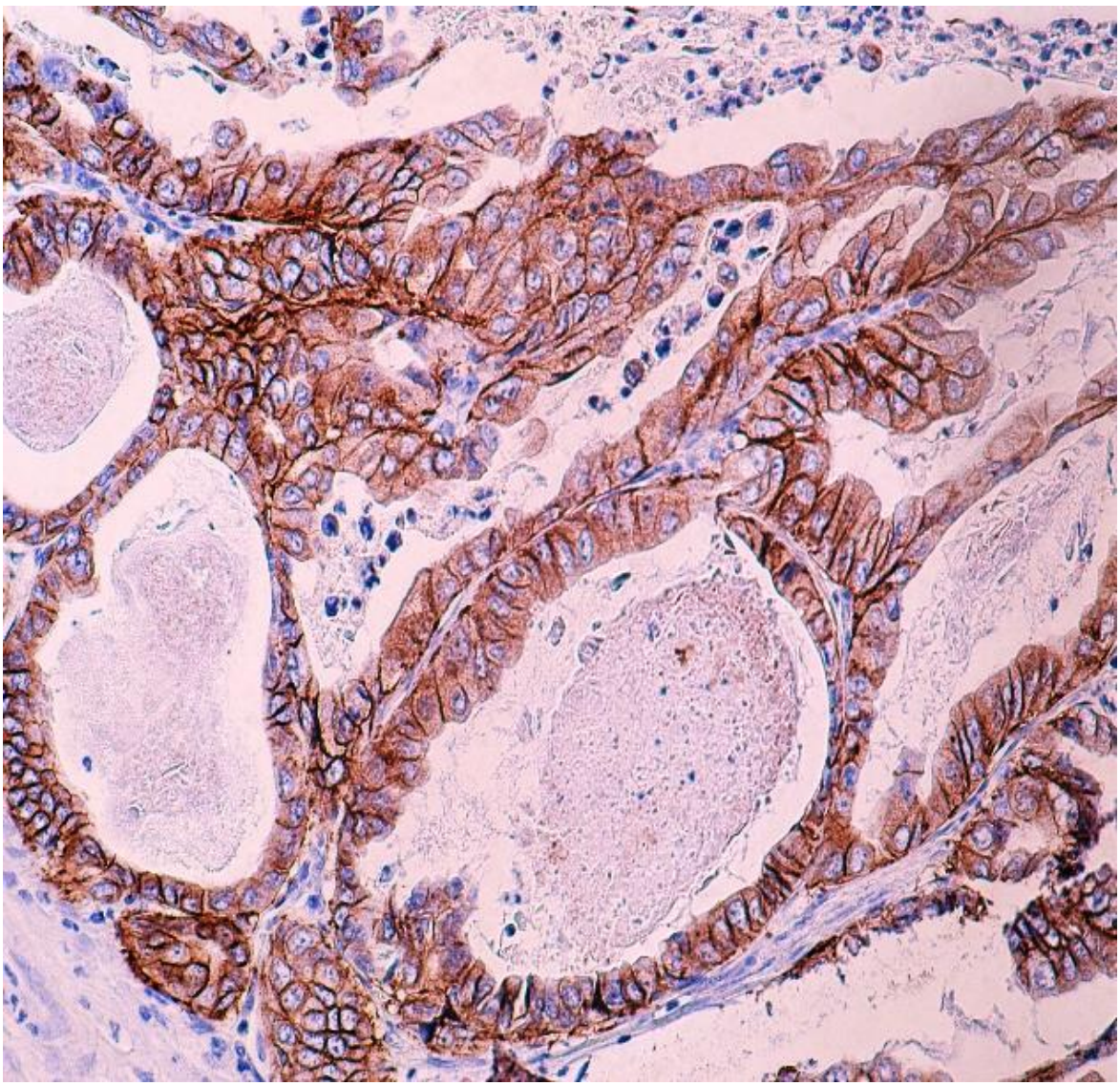

Figure 3: Ambiguous HER2 positivity (++) (40x)

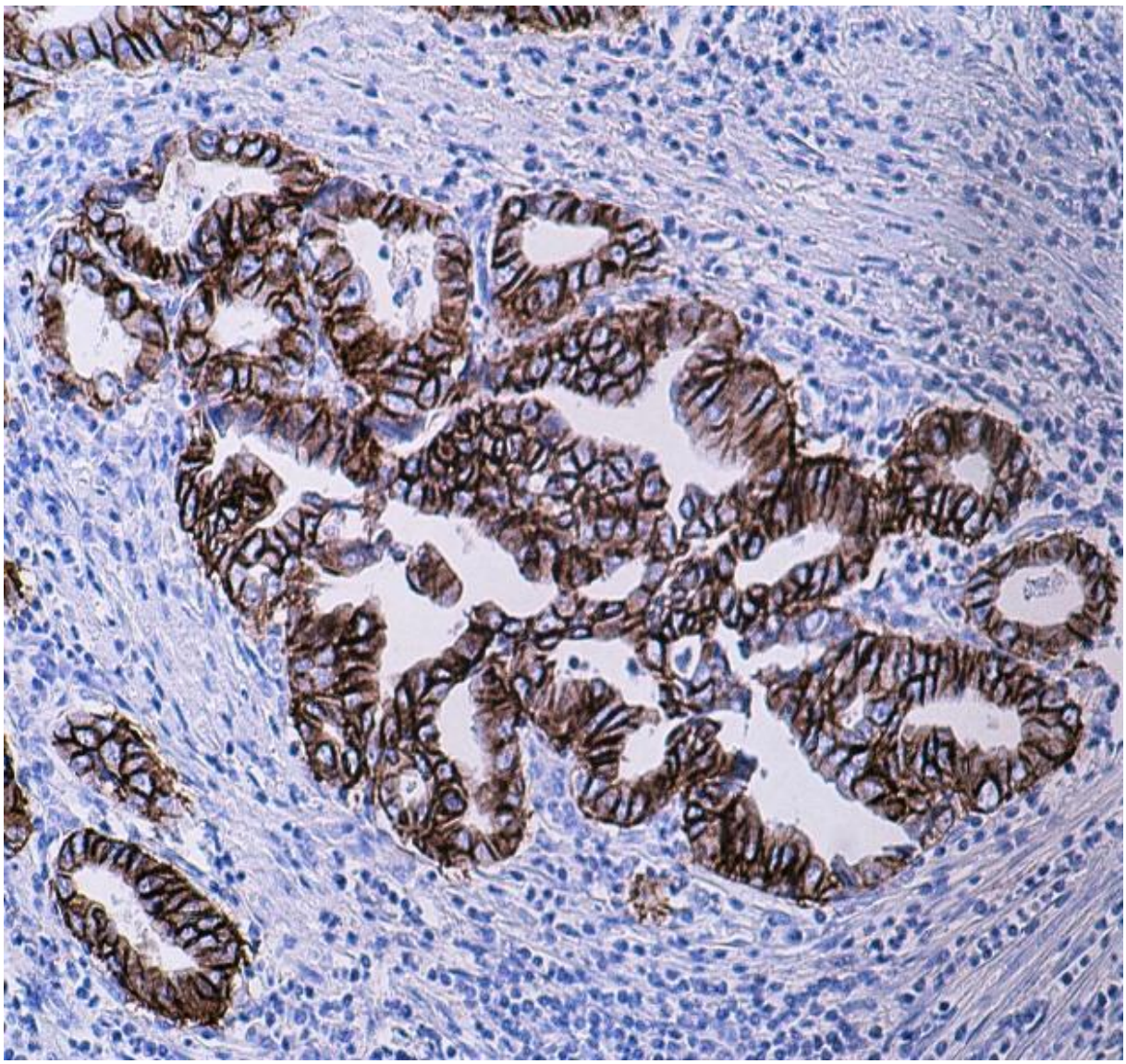

Figure 4: A strong expression of HER2 protein or hyperreactivity in the expression of HER2 protein (40x)

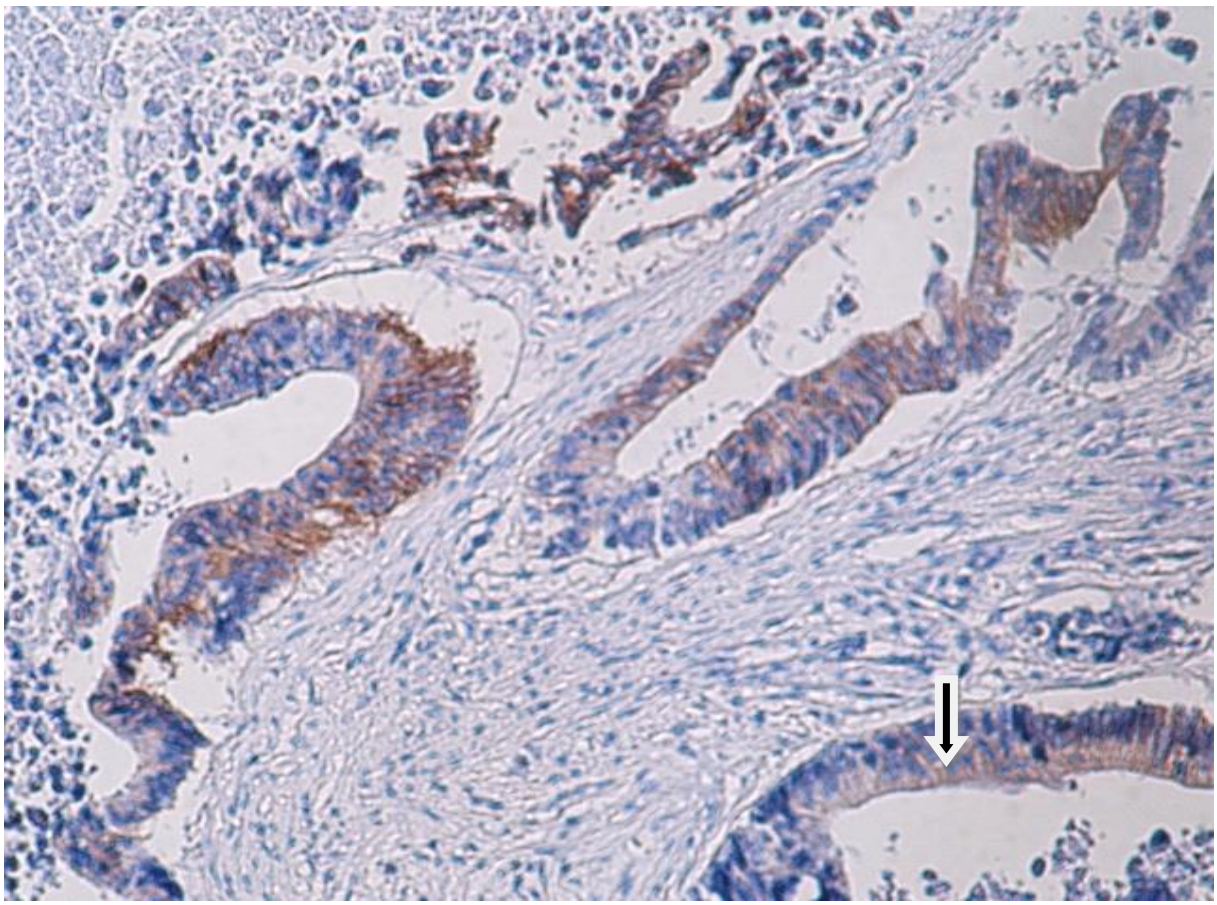

Figure 5: Apical membrane activity in HER2 protein expression (40x)

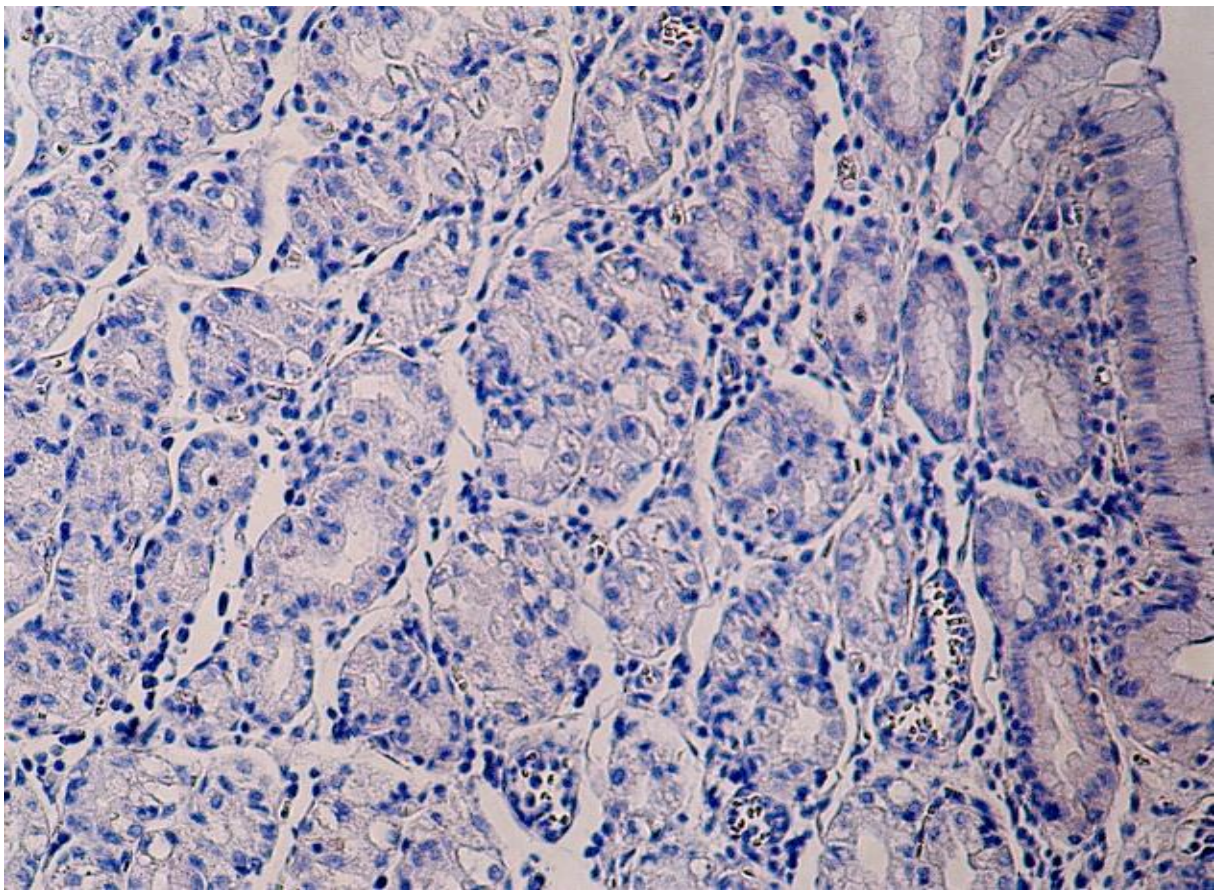

Figure 6: Negative expression of HER2 protein in control samples (40x)