NJSC «WEST KAZAKHSTAN MARAT OSPANOV MEDICAL UNIVERSITY»

Abstract of the dissertation
for the degree Doctor of Philosophy (PhD)

«Epidemiological features of gastric cancer, in relation to the measurements of cellular biomarkers and the reparation activity of double-stranded DNA breaks"

Specialty: 6D110100 –«Medicine»

TULYAEVA ANAR BALKASHOVA

Scientific supervisor:
Candidate of medical sciences, IztleuovYe.M.
Scientific adviser:
Doctor of Medical Sciences, Professor, BekmukhambetovYe.Zh.
Foreign adviser:
Professor, PhD, Irakliy Kokhreidze
Tbilisi State Medical University.
Tbilisi, Republic of Georgia

Republic of Kazakhstan
Aktobe, 2023

ABSTRACT

Of Tulyayeva Anar Balkasheva on the topis «Epidemiological features of gastric cancer, in relation to the measurements of cellular biomarkers and the reparation activity of double-stranded DNA breaks" , PRESENTED FOR THE DEGREE DOCTOR OF PHILOSOPHY (PHD) IN THE SPECIALTY 6D110100 –«Medicine».

Scientific supervisor: Candidate of medical sciences, IztleuovYe.M.
Academic adviser: Doctor of Medical Sciences,Professor,BekmukhambetovYe.Zh.
Foreign consultant: Doctor of Medical Sciences,Professor, Kokhreidze I.

Background:

Gastric cancer is one of the most important public health problems. It takes the fourth place worldwide among causes of mortality from oncology diseases and belongs to the most common localization (according to Globocan reports, 2022). In developing countries, gastric cancer occurs in more than 70% of cases, and in East Asian countries about 50%. In recent decades, it has been noted that the incidence in the East Asia has increased markedly, for example, in Mongolia, Japan, Korea, while in North America and Northern Europe, in some regions of Africa, the incidence rate was significantly lower [Bray F.et al., 2018]. In Kazakhstan, gastric cancer takes the third place among the diagnosed oncological pathology, with incidence rate of 15.8/100,000 cases among both genders, and third place in mortality, with 11.4/100,000 cases of mortality from gastric cancer [https://gco.iarc.fr /].

Having a multivariant etiology, with geographical features and heterogeneous structure, despite recent achievements in improvement of treatment options, gastric cancer remains one of the most difficult types of cancer to treat. The median overall survival is 10-12 months, and the 5-year overall survival is approximately 5-20% [Thrift A.P.et al., 2020].

Thus, there is a huge need to search for practical and prognathic factors to identify therapeutic targets, with increase in response to treatment and prolongation of overall survival. Among them, the area of usage of biomarkers has a high potential to predict and improve monitoring and to choose right treatment option for patients with gastric cancer [Abbas M.et al., 2017].

The Ki67 marker is a well-known marker of proliferation in the evolution of cellular proliferation. It has been widely investigated as a potential prognostic marker of proliferation in retrospective studies of malignant diseases [YangC.et al.,2018]. It has been clinically proven in many studies that Ki67 correlates with metastasis and the clinical stage of tumors. It was also found that the expression of Ki67 is significantly higher in malignant tissues with poorly differentiated tumor cells, compared with normal tissue. In accordance with its prognostic role, Ki67 expression can identify subpopulations of patients who are more likely to respond to a particular treatment therapy [Li L.T. et.al.,2015].

Apoptosis is one of the main mechanisms, which appears in response to cell death and induction of apoptosis in the tumor cells means a potential target for cancer therapy. Apoptosis evasion is one of the main signs of cancer which in turn both increases resistance to anticancer therapy and contributes to development of cancer progression. One of the main mechanisms based on resistance to anticancer drugs is the altered expression of proteins of the Bcl-2 family. The proteins of the Bcl-2 family are largely identified in normal cells and plays a critical role in cell death and survival. Its anti-apoptotic role and overexpression in cancer cells is widely studied today [Suvarna V.et al.,2019].

The "personalized treatment approach" currently became a frequent practice. In the era of molecular medicine, the Lauren Classification, published half a century ago, is one of the most relevant morphological classifications. Macro and microscopic differences of Gastric cancer according to the Lauren classification, with the epidemiological and epigenetic varieties are of great interest in the study of selection of the most effective therapy for a specific tumor, based on genetic analysis and level of expression of "special" proteins [Gullo I.et al., 2018].

One of the advanced directions of improvement of the effectiveness of non-surgical methods of treatment of oncologic patients is the development of methods to increase the sensitivity of tumor cells to chemotherapy provided, which are based on the use of defects in the repair system to achieve the most effective antitumor effect.

Various endogenic and exogenetic agents that damage DNA lead to single- and double-stranded DNA breaks, including non-conformity of repair. If the inter-chain and intra-chain relationships of two DNA strand breaks are damaged, it leads to genomic instability and mutation, which is one of the signs for cancer cells. [Hanahan D.et al., 2011]. To prevent this process, a number of complex mechanisms called DNA damage response (DDR) are activated in the cellular system. When double-stranded DNA breaks, serine 139 catalyzes, which later leads to the phosphorylation of H2AX with linked proteins, and accumulates at the site of breaks of double-stranded DNA [Palla V.V. et.al.,2017].

gH2AX also plays a critical structural role, contributing to the modern and effective preservation of remodeling factors at the restoration place, thereby helping to hold the broken ends together, provides time for repair and minimizes the risk of mismatch repair [Okabe A.et al.,2019].

DNA double-stranded break repair (DSB) plays a critical role in maintaining genome integration [Gillyard T.et al., 2021]. When DNA repair mechanisms are interrupted or deregulated, this can increase the rate of mutagenesis and genomic instability and thus lead to the progression of cancer [Li L.Y.et al., 2021]. The analysis of the dynamics of the level of phosphorylated histone γH2AX is one of the modern methods of study of DNA repair systems in the cell.

Taking into consideration the peculiarities of genetic transformations in gastric cancer and their reflection on the long-term effects after surgical interventions and chemotherapy, we focused on a comprehensive clinical and morphological study of the gastric cancer.

Goal of the study - to study correlative interconnections of one-year and five-year

survival rate in various types of gastric cancer with content of cellular biomarkers and reparation activity of DNA strand breaks.

Objectives of the study:

1. To analyze the trend of gastric cancer disease in the Aktobe region for the period of 2009-2018 (10 years), and five-year survival rate for 2014-2018. (5 years).

2. To examine biomarkers Ki67, Her2 and BCL 2 in various types of gastric cancer (according to Lauren), and to establish a link with five-year survival rate data.

3. To examine the interconnection between breaks and repairs of DNA strands (γH2AX, 53BPI) in lymphocytes, with cellular biomarkers and one-year survival rate at the patients with gastric cancer.

Scientific novelty:

1. Regional features of gastric cancer disease have been established in a scientific first in the Aktobe region.

2. Correlative interconnection between the proliferation marker, apoptosis regulator and Lauren Classification with one-year and five-year survival have been established in gastric cancer.

3. The interconnection between double-stranded DNA breaks and DNA repairs of the strands with cellular biomarkers and one-year survival rate has been established at the patients with gastric cancer.

Practical significance:

1. The application of the Classification of gastric cancer according to Lauren in clinical practice, will make it possible to more accurately predict states and outcomes of the disease, since a correlative interconnection between histotypes and survival has been established.

2. The obtained data reveal the mechanisms of carcinogenesis of gastric cancer, indicate the interconnection of markers of proliferation and apoptosis with survival. Therefore, the determination of biomarkers will allow to indicate in patient-specific way the tactics of patient management in terms of the scope of diagnosis and treatment, which will affect the outcomes of the disease.

3. The results of the study of double-stranded DNA breaks and DNA repairs in the combined treatment of gastric cancer (surgery + chemotherapy) proved the relationship of repair with biomarkers. Thus, this study is recommended to determine the purpose of targeted chemotherapy and monitor the effectiveness of the treatment.

Main provisions, provided for defense:

1. In the Aktobe region the trend of disease is aAPC 3,2%, with potential of further growth ($p<0,001$). In the retrospective study the five-year survival is 28,4%. The total median survival is 8,0 months, the worst median survival of young people was 5,0 months, $p=0,008$. Timely seek of medical advice and relevant treatment have meanings in the total five-year survival (I)50% against (IV)7% long-rank test, $p<0,001$. A significant difference of five-year survival was identified among subtypes of Lauren classification. Interstitial type - 30.7%, Diffuse type - 25.9%, Mixed type - 19.5%, $p <0.047$.

2. In the cross-sectional study: Diffuse type was 61% and was detected in advanced forms of gastric cancer. Whereas the interstitial type was 39%, with overall low 5-year survival rate of 21%, which in turn underlines the epidemiological and epigenetic differences of the Lauren classification. High Ki67 index>50% is associated with low tumor differentiation p=0.005, Her2+ with lymphogenic metastasis, p=0. 027.

For BCL2+, high 1-year survival rate (61%), 5-year survival rate – 25.4%, p<0.0001 was revealed.

With positive Ki67 index<50%/BCL2, the risk of rapid onset of unfavorable outcome is 50% lower compared to Ki67>50%/BCL2 (negative index), p=0.0002.

3. In the pilot study: after gastrectomy with lymph dissection in the volume of D2, when comparing the quantitative indicators of the parameters of double-stranded DNA breaks in lymphocytes (yH2AX), positive inversely proportional interconnection r=-0.5046, with the number of breaks and immunoreactivity of BCL2, p=0.003, was noted. Whereas 53BP1 (repair) in the parameter "the amount of repair per cell" positively correlates with BCL2, p=0.028.

After combined treatment, increase of repair activity of two DNA strand breaks (53BP1) is noted at the BCL2 positive patients (r =0.2634), p =0.0454. With a high one-year survival rate of 51.5% compared to other biomarkers (p=0.019).

Approval of the work

The main provisions of the thesis work are presented and discussed at the following events:

- VI International Scientific Conference of young scientists and students initiated by the Foundation of the First President of Kazakhstan – Elbasy and South Kazakhstan Medical Academy, "Prospects for the development of biology, medicine and Pharmacy" (December 7-8, 2018, Shymkent, Kazakhstan).

- VIII Annual International Scientific and Practical Conference "Topical Issues of Medicine" and "Satellite Forum on Public Health and Health Policy" (April 10-12, 2019, Baku, Azerbaijan).

-III International Scientific and Educational Conference "Internationalization of continuing medical education. Future outlook" (April 25-26, 2019, Aktobe, Kazakhstan).

-The Second International Scientific-Practical Virtual Conference-Modern Medicine: Problems, Prognoses and Solutions. (December 18-20, 2020, Almaty, Kazakhstan)

- XVII International Symposium on Morphological Sciences - ISMS 2021 (May 27-30, 2021, Almaty, Kazakhstan).

Thesis related publication: 8 publications have been published on the topic of the thesis. Including 3 publications in the materials of international scientific and practical conferences, 1 article in the international journal, 3 articles in the scientific publications recommended by the Quality Assurance Committee in Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan. (Astana Medicine journals, Science and Healthcare, VestnikKazNMU). Publications at the international conferences – 4 (2 of them in

the journal indexed in the Scopus information database, 1 in the journal indexed in the Web of Since information database).

Implementation of study results:

Thesis materials are used in the educational process at the Department of Oncology in NJSC «West Kazakhstan Marat Ospanov Medical University». (Appendix A)

Methodological guidelines "The main diagnostic values of using the classification of gastric cancer by Lauren". Academic Council dated 31.01.23 No.5(802), ISBN9786017650629, (Aktobe, 2023)

Author'spersonal contribution:

Author took part in all stages of the study: from setting the study goal, tasks, design development, protocol. Obtained informed consent, active participation in the study. The author conducted a literary search, developed of a database, statistical processing with subsequent interpretation.

The scope and structure of the thesis: The thesis work is presented on 152 pages and consists of introduction, literary review, materials and study methods, and study results under tasks set. The thesis contains 21 tables, 27 figures, 2 diagrams, 23 graphs. The list of references includes 339 sources, of which 8 are in Russian, 2 are in Kazakh, 329 - in foreign languages.

Study design:

Retrospective, cross-sectional and pilot studies.

The study was conducted on the basis of Medical Center of NJSC «West Kazakhstan Marat Ospanov Medical University» in the Aktobe city in the period from 2017 to 2021. All patients are residents of the Aktobe region. The study included patients examined and discussed by the multidisciplinary group according to the protocol of diagnosis and treatment of gastric cancer of the Ministry of Health of the Republic of Kazakhstan dated 2017 No. 56. All patients were prescribed surgical treatment. A total of 1,617 patients were included in the study:

1. Retrospective study: 1,454 newly diagnosed patients with gastric cancer of both genders.

2. Cross-sectional study: 159 male and female patients aged from 18 to 78 years with diagnosed gastric cancer who have undergone and are being prepared for surgery.

3. Pilot study: 30 healthyconditioned patients and 30 patients over 18 years of age, both genders with diagnosed gastric cancer who received combined treatment.

The study was conducted in 3 stages. Before surgery, after surgery, after 1 course of chemotherapy.

Ethical Approval: The study was approved by the Bioethical Commission of NJSC «West Kazakhstan Marat Ospanov Medical University» Protocol No. 24 of 03.10.2017. The protocol of study and ethical documents were approved: the form of informed consent of the patient (Appendix B). The work with the data of the electronic register of oncologic patients was carried out in compliance with the rules of biotic norms. The examination of patients was conducted after voluntary informed consent, in compliance with all the principles of the Helsinki Declaration.

The design and Protocol of the study were approved at the meeting of the local bioethical expert commission of the Marat Ospanov Medical University (Protocol No. 24 of 03.10.17).

The thesis work was carried out within the framework of the intramural scientific and technical program "Analysis of the epidemiological situation and monitoring of cancer treatment by the method of repair of double-stranded DNA breaks in lymphocytes on the example of gastric and breast cancer" state registration No. 12/4-1-17/163 dated 30.01.2018.

Study design of the first objective: Historical Cohort Study

Continuous sampling. A retrospective study of gastric cancer disease dynamics in the Aktobe region was conducted based on the analysis of the electronic register of cancer patients for 2009-2018. The study of disease dynamics included 1454 newly diagnosed cases of Gastric Cancer among both genders. The study of survival analysis included 762 medical records of patients treated for gastric cancer.

The Excel database was developed, including: passport data of the patient, grouping of patients by type of treatment, the period from the beginning of the disease, diagnosis and date of death, histological type of tumor. The study was conducted according to the inclusion and exclusion criteria

Statistical analysis: The population of the Aktobe region for statistical calculation was taken from the base of the Statistics Committee of the Ministry of National Economy of the Republic of Kazakhstan: "The population of the Republic of Kazakhstan by regions, cities and districts for 01.01.2009-01.01.2018". Calculations were carried out in the Statistica.10 program (Dell Technologies, Round Rock, Texas, USA), as well as in SPSS.v.25 program. For all tests, two-sided error of the type I p=0.05 with 95% confidence interval (CI) was considered statistically significant.

The rough incidence rate of gastric cancer (per 100,000 people) is calculated by generally accepted statistical methods.

The trends of disease are determined by the least square method. The average annual growth rate, percentage changes were estimated by linear regression analysis, including predictive index for 2019-2020. Changes in incidence rates were presented as a summary indicator of tendency over a fixed period of time and expressed as an average annual percentage change. The five-year survival rates were assessed using generally accepted Kaplan-Meier analyses to determine differences between groups using long-rank test with 95% confidence intervals.

Study design of the second objective: Cross sectional study

Random sample. The study included 159 patients aged from 18 to 78 years. Residents of the Aktobe region with newly diagnosed gastric cancer. All patients underwent surgical treatment in the period from 01.01.2018 to 01.06.2021 at the "West Kazakhstan Marat Ospanov Medical University". The study was conducted according to the inclusion and exclusion criteria.

Statistical analysis:

The representative sample included 151 people + 30%. Online Calculator http://www.raosoft.com/. According to registration documents of the form 07/u (histologically verified newly identified), 164 patients with gastric cancer diagnosis

were identified in the Aktobe region for 2018, that is 11% of all newly identified oncological pathology for 2018.

Continuous variables were presented as an average value ± standard deviation; median (interquartile range) and categorical variables as a quantity (percentage), with calculation of 95% confidence interval. Clinical and pathological factors in different subgroups of combined expression of BCL2, Ki-67% Her2 were compared using nonparametric criterion χ2(Pearson X) or analysis of the dispersion test depending on the type of data.

According to Sharashov Ye. and co–authors, the survival analysis is determined by two methods: the use of Life tables, Kaplan–Meier analysis. Kaplan–Meier survival curves stratified by marker expression were developed, and prognostic differences between the groups were studied using a long-rank test. The Long rank test is able to determine intergroup differences in the same way, compared to the Breslow criterion, which determines earlier intergroup differences, and the TaronWare criterion, which will determine later intergroup differences.

We used Univariable analysis and Multi variable analysis of regression of proportional Cox risks [Bradburn MJ et al., 2003]. The data were analyzed using statistical software R (v. 4.1.0, Vienna, Austria). (https://www.r-project.org/). The results were statistically significant at $p<0.05$.

Study design of the third objective: Pilot study.

Convenience sample. The study included 30 patients aged from 20 to 76 years, with newly diagnosed gastric cancer of any severity (N30), examined and discussed by the multidisciplinary group, having indications for treatment according to the protocol of diagnosis and treatment of oncologic patients of the Republic of Kazakhstan dated 01.03.2019 No. 56. The study was conducted in the period from 09.2020 to 08.2021 in the MC of the West Kazakhstan Marat Ospanov Medical University. The study was conducted according to the inclusion and exclusion criteria.

Method of study:

The first stage of the pilot study involved No.30 conditionally healthy and No.30 patients with morphologically confirmed diagnosis of gastric cancer. The peripheral blood sample was taken into a vacutainer with EDTA2 from all patients after informed consent.

For the second stage the study of double-strand breaks and DNA repair activity in lymphocytes (yH2AX,53BPI) was carried out in response to treatment on the AKLIDES apparatus at the patients with gastric cancer.

In the control group of conditionally healthy patients, blood sampling was taken once. In the main group, the study was conducted in 3 stages. 1 point the day before the surgery. 2 point after surgery on the 9th day (taking into account earlier postoperative complications) [209].3 point on the 7th day after the 1st course of adjuvant chemotherapy.

The main technique at this stage is to evaluate the repair of DNA double-stranded breaks in the blood lymphocytes by indirect immunofluorescence analysis using yH2AX, 53BPI foci system using AKLIDES® device (Germany/Medipan). Immunohistochemical studies of gastric tissue were carried out only in the study

group. The cutting, tissue preparation and staining protocols, microscopy are the same as in the prospective study.

Statistical processing of the results was carried out in the Statistica.10 program (Dell Technologies, Round Rock, Texas, USA), as well as in SPSS.v program.25. Quantitative indicators are presented in the form of M (CO), where M is the average value, 95% CI, SD is the standard deviation. For indicators characterizing qualitative characteristics, the absolute number and relative value in percentages (%) are indicated. To test two unrelated groups, we used the Mani Whitney test. (The control group of conditionally healthy No. 30, the main group of patients newly identified with verified diagnosis of gastric cancer No. 30). To determine the sensitivity and specificity of the test, we used ROC analysis.

To check the normality of the distribution of the studied quantitative indicators in the groups, the Kolmogorov-Smirnov consent criterion was used. If the distribution of the studied numerical indicators differed from normal, then the significance of the differences for related samples was checked using a nonparametric analogue of the dispersion analysis of repeated measurements - Friedman's rank dispersion analysis using Kendal concordance coefficient.

Statistical power of sample No.30 was 80% with confidence level of 95%, correlation coefficient is 0.5 [Грыжибовский А.М., 2020]. To determine the existence of links between the qualitative rank parameter of the BCL2 biomarker and the quantitative parameters yH2AX,53BP1, we calculated the Kendall Tau correlation coefficient.

To determine the existence of relationships between the qualitative parameters of Her2, Ki67% biomarkers and quantitative parameters yH2AX,53BP1 foci for a small sample among 30 patients with Gastric Cancer, we calculated the nonparametric criterion of Wald-Wolfowitz. The differences were considered significant at $p < 0.05$. Kaplan–Meier survival curves stratified by marker expression were developed, and prognostic differences between groups were studied using a logarithmic criterion. The differences were considered significant at $p < 0.05$. To identify the survival effect in the parameters of DNA repair and breaks, we used a multivariate mixed analysis of Proportional hazard mixed Cox model [Husain H,et al.,208]. The data were analyzed using statistical software R (v.4.1.0., Vienna, Austria). (https://www.r-project.org/). Medcalc.

Results of the study:

Historical cohort study

The overall incidence of gastric cancer increased from 19.2 to 29.3 and averaged 25.8 (R2 0.65) with aAPC of 3.2% and with the potential for further growth (30.4 by 2020, p<0.001). Non-cardiac localization (17.8, p<0.001, aAPC 6.4%) and intestinal tumor type (17.0, p<0.001, aAPC 7.35%) prevailed. The observed overall five-year survival was 28.4% [95% CI 24.5;32.3] with a median survival of 8.0 months [95% CI 6.6;9.4]. Groups aged 40-49 years and 70+ had the lowest rates (24.4% and 22.1%, respectively, long-rank test p=0.008), but the youngest people (18-39 years) showed the worst median survival - 5.0 months after diagnosis with a survival rate of 29.4%. Resection surgery made a significant contribution to the median survival rate - 23.0 months versus 6.0 at non-operated patients (long-rank

test p<0.001). Timely seeking medical help and receiving appropriate treatment for patients with gastric cancer is undoubtedly significant in the overall five-year survival rate (50% vs. 7% long-rank test p <0.001).According to the morphological type according to the results of the classification of Lauren, which in turn epidemiologically and epigenetically different by types, affected the overall five-year survival rate (30.7% vs. 25.9% and 19.5% long-rank test p<0.047).

Cross sectional study: According to our cross-sectional study (159 patients), the average age was 59 years [95Cl57.43:60.29] (rank: 29-80 years). The incidence rate of men is 2 times more frequent than of women. 98 patients (61%) had locally advanced gastric cancer. The ratio of locally advanced cancer and advanced gastric cancer was (0.79; 70/89). The majority of patients participating in the study had stage III (68.55%) with a low differentiated tumor - 66%.

According to the Lauren classification, diffuse and mixed gastric cancers were detected 2 times more than the intestinal type of gastric cancer (61% vs. 38.36%).One third of the patients had the highest activity of the Ki proliferation biomarker 67%: index 76-100% (38.36%), and the lowest proliferative activity was - 6.92%.Her2 positive tumors accounted for 15% of all 159 patients with gastric cancer. The biomarker of apoptosis BCL 2 was negative at 52%, and positive at 48%.

Clinicopathologic characteristics of Ki67% marker:
Positive expression of K67% was detected at all 159 patients with gastric cancer. According to the data, the Ki proliferation index of 67% has no statistical relationship with age, gender, pTNM, classification, biomarkers BCL-2 and Her2. However, there is a strong statistical relationship with low-grade tumors - 29% (p=0.005).

Clinicopathologic characteristics of BCL-2 marker:
Positive expression of BCL-2 was detected in 49% (77/159) of gastric tumor tissues and in 82 cases of negative expression of immunohistochemical staining. There was no statistically significant dependence on the stage of tumor, classification of Lauren, age and gender, localization of tumor, spread of tumor along the lymphatic pathways, with markers Ki 67, Her2.

Clinicopathologic characteristics of Her2 marker:
Positive expression of Her2 was detected in 14% (159/25) of patients with gastric cancer. According to Her2 data, there is a statistical relationship between lymphogenous nodal involvement, p = 0.027.

One-year and five-year survival in patients with gastric cancer:
Overall one-year survival was 48% [95%CI: 0.45-0.52], five-year survival was 18% [95%CI: 7.38-14.65]. The median survival rate is 11 months.

Survival due to the amount of BCL-2 marker:
In the main group of patients with low BCL-2 immunoreactivity, the overall 1-year survival of patients was 30% [95%CI: 21.03-43.30], 5-year survival was 12.49% [95%CI: 14.60-25.67] median survival was 12 [95%CI: 8.69-15.30] months. In the second group of patients with high BCL-2 immunoreactivity, the overall 1-year survival was 61% [95%CI: 50.0-74.5], 5-year survival was 25.40% [95%CI: 20.61-22.31] median survival was 23 [95%CI: 7.95-39.04] months. There is a high

survival rate at the patients with increased BCL-2 immunoreactivity compared to low BCL-2 immunoreactivity, with a strong statistical relationship (p<0.0001)

Survival due to ratio of the number of markers Ki67%/BCL2: For patients of the first group with Ki67>50% / BCL2neg, the total 1-year survival of patients was 37% [95%CI: 26-54], 5-year survival was 11.75% [95%CI: 8,9-14,6] median survival was 9 [95%CI: 6,23-11,77] months.

For patients with Ki67<50% / BCL2 positive, overall 1-year survival was 71% [95%CI: 54-92], 5-year survival was 31.32% [95%CI: 23.41-39.23] median survival was 33 [95%CI: 18.68-47.32] months.

For patients with Ki67>50% / BCL2 positive, overall 1-year survival was 55% [95%CI: 41-73], 5-year survival was 20.8% [95%CI: 15.53–26.06] median survival was 15 [95%CI: 1.6-28.4] months.

For patients with Ki67%<50%/ BCL2 negative index, the overall 1-year survival was 51% [95%CI: 33-76], 5-year survival was 11.76% [95%CI: 4.81–18.71] median survival was 7 [95%CI: 3.63-10.36] months. p<0.0001

Univariant and multivariant analysis of proportional Cox risks:

In group Ki67% <50%/BCL2 positive: the risk of rapid adverse outcome is 74% lower, compared with group Ki67% >50%/ BCL2 negative, with a strong statistical relationship [HR 0.26 95%CI: 0.13-0.53 p=0.0002]. During multivariant analysis, the risk of rapid adverse outcome is also 66% lower, but there is no statistical relationship [HR 0.35 95%CI: 0.01:8.20 p=0.514].

In group Ki67% >50%/ BCL2 positive: the risk of rapid adverse outcome is 48% lower, compared with group Ki67% >50%/ BCL2 negative, with a strong statistical relationship [HR 0.52 95%CI: 0.31-0.85 p=0.00985]. During multivariant analysis, the risk of rapid adverse outcome is also 66% lower, with a strong statistical relationship [HR 0.29 95%CI: 0.10-0.80 p=0.017].

In group Ki67% <50%/ BCL2 negative: the risk of rapid adverse outcome is 16% higher, compared with group Ki67% >50%/ BCL2 negative [HR 1.16 95%CI: 0.67-2.02 p=0.581]. During multivariant analysis, the risk of rapid adverse outcome is also 2% higher, but there is no statistical relationship [HR 1.02 95%CI: 0.04-24.01 p=0.990].

Pilot study:

The study included 30 patients who received treatment from Gastric Cancer from September 2018 to August 2021; the average age was 62.13 years (from 20 years to 76 years). The international TNM classification, histological classification of gastric cancer according to Lauren, was used to categorize tumors. The majority of patients had the most aggressive form of gastric cancer (Diffuse type of gastric cancer was 53.33%). According to pTNM classification, the Local gastric cancer was 60% (IA, IB, IIA, IIB). Lymphangitic metastasis was observed at 56.67% of patients. About 60% of patients underwent Standard gastrectomy with lymphodissection in the volume of D2. Non-cardia gastric cancer prevailed in comparison with cardiac gastric cancer (56.66% vs. 43.33%). In the immunohistochemical study, positive immunoreactivity of BCL2 biomarker was noted at 63.33% of patients. Also, the positive immunoreactivity of HER2

biomarker at 13.33%. The proliferative activity of gastric cancer was very aggressive by 2/3 (The biomarker Ki 67% was more than 50% of co19 people).

Results of correlation relationship with the Ki67% biomarker with all parameters of double-stranded DNA breaks(yH2AX):
According to the data, one of the main parameters, the average value of all foci of ruptures in the cell has statistical differences between the two groups p= 0.04, With subsequent surgical treatment, there are also statistical differences p= 0.001. Statistically significant differences with the biomarker Ki67% were revealed in the nuclei of increased cross-section intensity (between group No. 1 with low proliferative activity >50% and the second group <50%) p = 0.01. and the average value of the intensity of the glow of the ruptures in conventional units, after surgical treatment (p= 0.023). After surgical treatment, there is an increase in indicators in the Ki group of 67% >50, on average, the intensity of the glow of the ruptures in conventional units, p=0.04. There were also differences in the average number of ruptures per cell before surgery, p= 0.04. After surgery, there is a decrease in ruptures in patients with Ki67%<50%, p=0.001.

Results correlations with Ki67% biomarkers with all parameters of double-stranded DNA repair (53BP1): According to the data, there was no statistical relationship between the repair activity of two DNA strand breaks and the correlation between the biomarker biomarker Ki67% (between group No. 1 with low proliferative activity >50% and the second group <50%).

Results correlations with the Her2 biomarker with all parameters of double-stranded DNA breaks (yH2AX): According to the data, differences in the number of counted nuclei of 2-strand DNA rupture were revealed after combined treatment and correlation between the Her2 positive and Her2 negative groups, p=0.04.There are also differences in the average value of all low foci of rupture in conventional units after combined treatment between the two groups p= 0.04. In the group of Her2 positive patients, much more than the average value of low intensity of all foci of rupture in the cluster after combined treatment p=0.04 was revealed. Also, tumor-damaged cells in the group of Her2 positive patients with rzd have more ruptures after combined treatment compared to the group of Her2 negative patients, p=0.04

Results correlations with the Her2 biomarker from all parameters of double-stranded DNA repair (53BP1): Between group No.1 positive and group No. 2 negative immunoreactivity, there are statistically significant differences in the average value of low intensity of all foci of repair of double-stranded DNA breaks in the cluster, in conventional units after combined treatment, p=0.013.

Results correlations with the BCL2 biomarker with all parameters of two DNA strand breaks (yH2AX): According to the data, a weak relationship was found with the biomarker BCL2 and the average value of the intensity of the glow of the gaps in conventional units, p = 0.02.When comparing the quantitative parameters of the parameters of double-stranded DNA breaks in lymphocytes (yH2AX), an inversely proportional relationship r=-0.5046 with the number of breaks and the immunoreactivity of BCL2, p=0.003 is noted.

The results are correlations with the biomarker BCL2 from all parameters of repair of double-stranded DNA breaks (53BP1):

There was a weak correlation with statistical significance between the BCL2 biomarker and the average amount of repair per cell after surgery, p=0.029. After 1 course of chemotherapy, there was a weak correlation with statistical significance between the BCL2 biomarker and the average amount of repair per cell, p=0.04. Also, there is a weak correlation (r-0.357) with the total amount of repair and immunoreactivity of BCL2, p=0.05.

The survival analysis was carried out in accordance with the Kaplan-Meier method. One-year survival in the study group was 65.5% [95Cl% CI: 49.1-87.5]. In one-year Kaplan-Meier survival, a statistically significant difference was shown for the BCL2 biomarker (p = 0.019)

CONCLUSION

Thus, based on the results of our study, we can draw the following conclusions:

1.The analysis of the morbidity trend showed an increase rate from 19.2 to 29.3 and averaged 25.8 (R2 0.65) with a Tpr of 3.2% and had the potential for further growth (p<0.001). The observed overall five-year survival was 28.4% [95% Cl 24.5;32.3] with a median survival of 8.0 months [95% Cl 6.6;9.4]. Patients under 50 years of age had the lowest five-year survival rates of 24.4% (p=0.008). Especially young patients (18-39 years old), of whom 50% did not live up to 5 months after diagnosis.TNM classification is one of the main biomarkers of survival prognosis (p<0.001). Retrospectively, the use of the Lauren classification revealed significant differences in survival in patients with gastric cancer. (p=0.05)

2. A correlation was established with the Lauren classification: diffuse type of Gastric Cancer (61%) is more common in advanced forms (stage 3 and 4) (p=0.0001)In gastric tumors, there is a high content of the Ki67 proliferation marker (50-100%) (107/159), while the Ki67 index (50-75%) correlates with a low differentiated tumor (p=0.005) (high aggressiveness). The apoptosis marker BCL-2 does not correlate with the clinical and pathological characteristics of Gastric Cancer. The Her2 new marker correlates with lymphogenic metastasis (p=0.027). With positive BCL-2, there is a high 1-year and 5-year survival rate (p<0.0001), the risk of a rapid onset of an adverse outcome is 50% lower compared to negative BCL-2 (p=0.0434).The Ki67/Bcl-2 ratio index has a statistical relationship with 1-year and 5-year survival. The ratio of Ki67 neg/Bcl-2 pos markers reduces the risk of a rapid onset of an unfavorable outcome by 74%, compared with the Ki67pos/Bcl-2neg group (p= 0.0002).

3. The analysis of DNA repair mechanisms in lymphocytes (breaks and repair) between conditionally healthy and patients with gastric cancer showed statistically significant differences. When monitoring treatment (point 1, 2 and 3), a statistically reliable indicator is the diameter of the DNA break (p=0.03568). Biomarker Ki67 has no correlation with DNA repair mechanisms. The Her2 biomarker correlates statistically significantly with the number of repair foci in the nuclei (p=0.026). And also, after surgical treatment (point 2) with Her2, positive differences in the diameter of rupture repair are noted (p=0.03).

At the second outcome measure (after operative therapy), a positive correlation of BCL-2pos with the average amount of repair per nucleus (p=0.029) was revealed, which is also observed at the third point (after 1 course of chemotherapy) (p=0.04). Of the total number who have achieved one-year survival in gastric cancer, 51,5% have BCL-2 pos (p=0.0019).