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# Clinical and morphological features of neuroendocrine tumors of gastrointestinal tract

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# CONTENTS

# INTRODUCTION

## Relevance of the research topic

The world statistics show a rapid increase in the incidence of neuroendocrine tumors over the past 40 years (Kojima M. et al., 2016). According to the SEER Registry (Dasari A. et al., 2017), in the United States, the incidence of neuroendocrine neoplasia increased 6.4 times in the period from 1973 to 2012.

In the Russian Federation, the incidence of neuroendocrine tumors is also increasing, so in 2001 the incidence was 0.03 cases per 100,000 population, and in 2019 it increased to 5.19 cases per 100,000 population (Yastrebova E.S. et al., 2021).

Due to the fact that the algorithm for the treatment of neuroendocrine tumors of the gastrointestinal tract is determined only by the localization of neoplasia and is focused on classification, the search for additional prognostic markers is an urgent task.

We conducted a series of studies that evaluated various factors and their possible prognostic value in the structure of neuroendocrine tumors of the gastrointestinal tract.

At the initial stage, the clinical and morphological features of 298 patients with neuroendocrine tumors of the gastrointestinal tract were analyzed. At the next stage, the influence of peripheral blood parameters, systemic inflammation factors and proliferation index (Ki-67) on the course of neuroendocrine tumors of the gastrointestinal tract was studied. The prognostic significance of type 2 diabetes mellitus, glucose levels before treatment, body mass index and carcinoid syndrome were also evaluated.

Expanding knowledge about the molecular and genetic nature of neuroendocrine tumors of the gastrointestinal tract is an extremely important task, the solution of which will improve existing algorithms for the diagnosis and treatment of this nosology. In this regard, at the next stage, genomic sequencing of a new

generation was performed in 40 patients in order to determine the frequency of pathogenic somatic mutations. At the final stage, an assessment of the influence of unfavorable prognosis factors on the time without progression of patients with neuroendocrine tumors of the gastrointestinal tract was carried out and a scale for evaluating unfavorable prognosis factors was developed.

## The degree of elaboration of the research topic

Given the extreme specificity of such nosology as neuroendocrine tumors of the gastrointestinal tract, there are not so many works devoted to this topic. However, in the last decade the number of works has been increasing. Of course, such an increase in interest in neuroendocrine tumors is associated with an increase in morbidity, which in turn is associated with a wider introduction of immunohistochemical examination of tumor material into the diagnostic algorithm.

S.B. Polikarpova defended her dissertation in 2010 on the topic: "Neuroendocrine tumors of the abdominal cavity and retroperitoneal space (clinic, diagnosis, treatment, prognosis)", in which for the first time a retrospective analysis was carried out on a large sample of patients with NET of the abdominal cavity and retroperitoneal space, tactics for the treatment of patients with certain localizations were developed and justified neuroendocrine tumors. However, in this work there was no analysis of such an available factor as factors of systemic inflammation. Also, in this work, the effect of the parameters of the metabolic syndrome on neuroendocrine tumors of the gastrointestinal tract was not evaluated.

I.G. Gafton's dissertation (2014) "Neuroendocrine tumors of the gastrointestinal tract", in which the author analyzed the expression levels of the BRCA1, c-kit, EGFR, ALK translocation genes and studied their effect on prognosis. An associative relationship was established between a decrease in the degree of BRCA1 expression and an unfavorable prognosis. This work is devoted to molecular and genetic aspects in the structure of neuroendocrine tumors of the gastrointestinal tract. Considering that in our study we also touch on this issue, we can say that an

increase in the amount of information in this direction may allow us to identify the factors of the unfavorable course of the disease, as well as make the treatment more personalized.

M.V. Lysanyuk's dissertation research (2020) "Optimization of the diagnosis and treatment of neuroendocrine tumors of the gastrointestinal tract and pancreas" was devoted to the personalization of patient treatment by creating a scale (the age of the patient, the localization and degree of malignancy of the tumor, the prevalence of the oncological process, the method of treatment were evaluated in points). However, this study also did not take into account the factors of systemic inflammation and the parameters of the patient's metabolic syndrome.

In 2022, A.Z. Isyangulova's dissertation research "Morphological and molecular genetic features of neuroendocrine tumors" was published. In this work, a new generation of sequencing was performed in tissue samples of neuroendocrine tumors in patients with a burdened hereditary history.

Dissertation research by M.Yu. Meshcheryakova (2022) "Clinical, genetic and epigenetic features of neuroendocrine neoplasms of the colon", in which the molecular genetic and epigenetic status of samples of neuroendocrine tumors of the colon of various degrees of differentiation was studied, "A method for predicting the clinical course of neuroendocrine neoplasms of the colon, consisting in determining the probability of favorable and unfavorable outcomes of the disease based on methylation indicators, was developed genes RASSF1A, MGMT, DAPK, RUNX3, P16, APC1, MLH1, AHR repressor and retrotransposon LINE1, a prognostic model of the unfavorable clinical course of neuroendocrine neoplasms of the colon has been created. However, in this work, only neuroendocrine tumors of the colon were analyzed,6 which in the structure of neuroendocrine tumors of the gastrointestinal tract accounts for 5-7% of all neuroendocrine tumors of the gastrointestinal tract.

A special contribution to the study of neuroendocrine tumors was made by V.S. Trifanov's dissertation work "Neuroendocrine tumors of the pancreas. Clinical opportunities and prospects" (2022). However, this work is also limited to only one localization – neuroendocrine tumors of the pancreas.

All of the above works have made a great contribution to the development of neuroendocrine neoplasia. However, as before, the factors determining the prognosis and the treatment algorithm are based on the classification of neuroendocrine tumors and the localization of the primary focus. In this regard, the work aimed at finding additional predictive markers is extremely relevant at the present time.

## The purpose of the study

Determination of unfavorable prognosis factors in patients with neuroendocrine tumors of the gastrointestinal tract to improve the effectiveness of treatment of cancer patients

## Research objectives

1. To evaluate the influence of peripheral blood parameters, systemic inflammation factors, Ki-67 levels as prognostic factors in gastrointestinal NET.
2. To evaluate the effects of the presence of type 2 diabetes mellitus, glucose levels at the initial stage and body mass index as prognostic factors in gastrointestinal NET.
3. To assess the frequency of pathogenic somatic mutations and heterogeneity of neoplasias in the structure of the gastrointestinal NET.
4. To develop a scale for assessing the factors of unfavorable prognosis of gastrointestinal NET.

## Practical significance of the study

The results obtained by us will allow us to identify additional prognostic markers for optimal treatment planning of patients with gastrointestinal NET.

## Material and methods of research

It is planned to conduct an open retrospective study – analysis of 298 case histories of patients diagnosed with gastrointestinal NET, which have been collected on the basis of the *SPb GBUZ GKOD* since 2015 (NET registry).

**Stages of research:**

1. Retrospective study of the clinical and morphological features of group c of the disease in patients with gastrointestinal NET (it is planned to investigate the following factors – gender, age, primary localization of the tumor, localization of metastases, carcinoid syndrome, treatment).

2. Based on medical histories, calculate the indicators of relapse-free survival and overall survival in patients with gastrointestinal NET.

3. A retrospective study of the factors of systemic inflammation will confirm or refute their influence on the prognosis in gastrointestinal NET.

4. To determine the frequency of pathogenic somatic mutations in the structure of neuroendocrine gastrointestinal tumors by sequencing a new generation (it is planned to sequence a new generation of 40 samples of tumor tissue of patients with gastrointestinal NET).

5. A retrospective study of the absence or presence of diabetes mellitus, blood glucose levels at the initial stage and BMI of patients with gastrointestinal NET will confirm or refute their influence on the prognosis for gastrointestinal NET.

6. Based on the results of the analysis to identify the factors of unfavorable prognosis

7. Taking into account the identified factors of unfavorable prognosis, it is planned to create a scale of unfavorable prognosis for patients with gastrointestinal NET.

**Scientific novelty of the study**

In the dissertation work:

– for the first time in the Russian Federation, a study was conducted on the influence of peripheral blood parameters and systemic inflammation factors in patients with neuroendocrine tumors of the gastrointestinal tract;

- for the first time, the prognostic significance of type 2 diabetes mellitus, glucose levels before treatment and body mass index were evaluated;
- for the first time, genomic sequencing of a new generation was performed in 40 patients in order to determine the frequency of pathogenic somatic mutations;
- for the first time, a prognostic scale of unfavorable clinical course of neuroendocrine tumors of the gastrointestinal tract has been created.

## Implementation of research results in healthcare practice

The results of the study were introduced into the practice of the work of the departments of antitumor drug therapy of St. Petersburg State Medical Institution "City Clinical Oncological Dispensary". Fundamental theoretical provisions have been introduced into the educational process of teaching at the Department of Oncology of the St. Petersburg State University.

## Methodology and methods of dissertation research

The dissertation work was carried out in the design of a comparative study using general clinical, anamnestic, laboratory and statistical research methods.

## Provisions submitted for protection

1. Independent adverse factors increasing the risk of disease progression were: baseline relative neutrophil count >58.30% (p=0.0336, HR 1.05: 95% CI 1.01-1.09), baseline relative lymphocyte count <30% (p=0.0443, HR 1.03: 95% CI 1.01-1.06) and NLI >1.85 (p=0.0228; HR 1.17: 95% CI 1.02-1.34).

2. In the presence of type 2 diabetes and the same tumor localization, an increase in blood glucose by 1 mmol/l increases the risk of death by 3 times (95% CI 1.6-5.7, p<0.01). In the presence of type 2 diabetes and a fixed level of glucose in the blood, localization of the tumor without a primary identified focus increases the risk

of death by 608.7 times (95% CI 8.96-41370.8, p<0.01) compared with localization in the stomach.

3. pathogenic mutations were detected in 9 samples out of 40 (22.5%): PTEN (2.5%/1) (in combination with BRCA 1), PIK3CA (2.5%/1), RB1 (2.5%/1) (in combination with BRCA 2), CHEK2 (2.5%/1) (in combination with POLE), MLH1 (2.5%/1) (in combination with BRCA 1). The most frequent mutations were BRCA 1 (3/7.5%) and BRCA 2 (3/7.5%).

4. According to the results of multivariate analysis, it was possible to identify factors of unfavorable prognosis of the course and early progression of gastrointestinal NET – Ki-67 >5%, the relative number of neutrophils >58.30%, the relative number of lymphocytes ≤ 30%, neutrophil-lymphocytic index >1.85. The optimal threshold value of the number of unfavorable prognosis factors at the cut-off point was 2: the presence of 2 or more unfavorable prognosis factors at the time of the initial assessment negatively affected the patients' PFS. The presence of >2 factors of unfavorable prognosis of the disease increased the risk of disease progression by 67%: p=0.0013; HR=1.67, 95% CI 1.05-1.78.

## The degree of reliability of the results of the work and their approbation

The author analyzes foreign and domestic literature sources devoted to the study of the characteristics of neuroendocrine tumors of the gastrointestinal tract. The author has developed the design of the study. The work with the analysis of the obtained research results, their interpretation, as well as the implementation of statistical data processing were carried out by the author personally.

The results of the work were tested within the framework of the conferences "Three Pillars of Clinical Oncology", 2022 and 2023 (presentation on the results of the study), within the framework of the conference "Fundamental Science and Clinical Medicine – 2023" (presentation on the results of the study).

The main provisions of the dissertation and the results of the work were reported at the All-Russian Scientific and Practical conference "Neuroendocrine

tumors: treatment issues" (Rostov-on-Don, December 6, 2021); at the conference "Three Whales of clinical Oncology" (St. Petersburg, 2022).

## Publications

On the topic of the dissertation published: 4 printed works, 4 of them in the journal recommended by the Higher Attestation Commission of the Ministry of Education of the Russian Federation for the publication of the main results of dissertations for the degree of Candidate of Medical Sciences. 10 abstracts in the framework of both leading Russian conferences with international participation, as well as in the framework of European and American conferences.

The results of the work carried out were accepted as publications in the form of posters within the framework of the conferences ASCO GI – 2023 (1 poster), ENETs – 2022, 2023 (5 posters), RUSSCO 2022 (1 poster).

## Structure and scope of the dissertation

The dissertation work is presented on 167 pages of typewritten text and consists of an introduction, a literature review, a description of research materials and methods, the results of own research and their discussion, conclusions, practical recommendations and a list of references, including 12 domestic and 99 foreign sources. The work is illustrated with 39 tables and 39 figures.

# Chapter 1
# CLINICAL AND MORPHOLOGICAL FEATURES OF NEUROENDOCRINE TUMORS OF THE GASTROINTESTINAL TRACT (literature review)

## 1.1 Clinical and demographic features of neuroendocrine tumors of the gastrointestinal tract

### *1.1.1 Morbidity*

Neuroendocrine tumors (NET) are an extremely heterogeneous group of neoplasms originating from neuroendocrine cells of the embryonic intestine and possessing biologically active properties.

Up to now, there are no statistical data on the incidence of neuroendocrine tumors in the Russian Federation. According to the SEER registry (Surveillance, Epidemiology, and End Results) in the USA, the incidence of NET as of January 1, 2004 was 5.25 cases per 100 000 population. There has been a significant increase in the incidence of NET of all localizations over the past 30 years. Thus, taking into account the size of the US population, 7 350 patients with neuroendocrine tumors should be registered in our country annually (the population of Russia is 140 000 000 people as of 2012). The most frequent localization (66%) is the gastrointestinal tract, the predominant location is the cecum (17.1%), the rectum (16.3%). About 30% of NETs occur in the bronchopulmonary system. NETs are often diagnosed at a common stage. Thus, according to SEER, 50% of patients at the time of diagnosis already have locoregional or distant metastases [63] It is also worth noting that neuroendocrine tumors occupy from 2 to 8-10% of all malignant neoplasms of the pancreas, with an annual incidence of 0.32-2.23 cases per 100 thousand population [7, 90].

Currently, there are several classifications of NET. Classification by embryogenesis [111]: from the anterior intestine (FHREGUT) – bronchi, stomach, pancreas (pancreas), duodenum; from the middle intestine (MIDGUT) – small intestine, caecum, appendix; from the posterior intestine (HINDGUT) – colon, rectum. The WHO classification of neuroendocrine tumors [65] is based on the criteria of the WHO classification of tumors of the digestive system, published in 2019. Highly differentiated gastrointestinal NETs have three degrees of malignancy (G1, G2, G3). Group G3, which was first isolated for pancreatic NETs in the WHO classification of endocrine organs in 2017, combines highly differentiated NETs of high malignancy with increased proliferative activity. NEC gastrointestinal tract and pancreas refers to low-grade and, in turn, are divided into small-cell large-cell neuroendocrine cancer. Also, there is a group of mixed neoplasias – MINEN.

Functional activity distinguishes functioning and non-functioning neuroendocrine tumors. Biologically active gastrointestinal NETs are clinically most often manifested by carcinoid syndrome, which is a combination of symptoms of diarrhea, emotional lability, bronchospasms, hot flashes, etc. However, in its pure form, carcinoid syndrome occurs in about 10% of patients. Other gastrointestinal NETs are a rather heterogeneous group of diseases that occur with variable and often poorly distinguishable symptoms. Such mimicry of symptoms makes it extremely difficult to diagnose the tumor early and, accordingly, receive the necessary treatment. It is interesting to note that the calculated time before diagnosis is 5-7 years [78].

NETs often produce several hormonally active substances at once. In this regard, the diagnosis is based not only on the presence of a characteristic hyperfunctional syndrome, but also on the identification of the dominant population of endocrine cells, which makes up more than 50% of tumor cells. In accordance with this, BUT are verified as insulinomas, glucagonomas, gastronomas, somatostatinomas, vipomas, calcitoninomas, carcinoids (serotonin-producing), etc. [1].

Non-functioning BUT long-term asymptomatic and can reach large sizes, and at the time of primary diagnosis, in 32-73% of cases, patients with NETP have metastatic liver damage [4, 7].

For the diagnosis of NET, a wide range of laboratory and instrumental diagnostics are used (ultrasound, CT, MRI, PET (with In111-octreotate, based on the expression of somatostatin receptors, mainly type 2 [57], PET CT with Ga68-DOTA-octreotide, and for NET Gr3 – PET CT with 18 FDG)). In the diagnosis of NET, it is not enough to conduct a histological examination of tumor material, immunohistochemical examination (determination of the expression of Ki-67, synaptophysin, chromogranin A) is of particular importance. There are also a number of biochemical markers of the metabolism of biologically active substances specific for the diagnosis of NET: for example, the determination of the level of 5-hydroxyindolacetic acid (5-GIUK) in the daily urine, the level of serotonin in the blood, the level of neuron-specific enolase (NSE) in the blood, the level of gastrin in the blood, etc. Also, in some situations, a genetic study is required for the presence of multiple endocrine neoplasia syndrome (ME-1, ME-2A, ME-2B, Hippel–Lindau syndrome, Carney syndrome).

### *1.1.2 Principles of treatment*

The main method of treatment of NET is surgical (both with a radical and cytoreductive purpose).

Unfortunately, due to the late detection of the tumor, this method often turns out to be irrelevant, since the disease is diagnosed in the metastatic stage.

The choice of systemic treatment tactics depends on the degree of differentiation of the tumor. Thus, patients with inoperable highly differentiated NETs (G1, G2) with a positive status of somatostatin 2A and/or type 5 receptors are shown to be prescribed somatostatin analogues [67, 68, 96]. In inoperable highly differentiated NETs (G1, G2) in the absence of somatostatin receptors, the use of interferon alpha-2b as the first line is recommended [47, 62, 87]. Patients with inoperable highly differentiated pancreatic NETs (G1, G2) in the second line after

treatment with somatostatin analogues or in the first line of therapy in the absence of somatostatin receptors are recommended to prescribe targeted therapy: sunitinib (only with pancreatic NETs) together with somatostatin analogues [36, 38, 94]. Also in the second line, the appointment of everolimus together with somatostatin analogues is used for all NET localizations (G1, Ki-67≤2%) [9, 37, 88, 91].

It is important to note that in the presence of a clinical or biochemical picture (subclinical syndrome) of hypersecretion of metabolically active substances, the use of somatostatin analogues is indicated for all NETs, regardless of the degree of malignancy of the secreting tumor. In this case, somatostatin analogues are used to control symptoms and can be used in conjunction with other types of systemic therapy.

The following chemotherapy regimens are used as the first line of treatment in patients with inoperable low-grade G3 NET: EP, EC, XELOX, TemCap, FOLFIRI, GEMOX, FOLFOX, Temozolomide, Capecitabine [9], and as the second line of treatment, drugs such as Everolimus and Sunitinib can be used. To control the carcinoid syndrome, it is recommended to prescribe somatostatin analogues (Octreotide depot, Lanreotide).

In recent years, international efforts have been made to improve the understanding of the factors that affect the prognosis of GI NET. It is very important to classify tumors correctly in order to individualize the approach to treatment. A comprehensive analysis of the currently available input data is necessary for the formation of forecast factors for NET [5].

### *1.1.3 Patient's age as a possible predictive marker*

When assessing the main factors of the possible prognosis of gastrointestinal NET, it is worth paying attention to the relationship with the age characteristics of patients. There are certain contradictions here that we encounter when analyzing the

American retrospective study of the National Database [76]. The data of the world literature in most cases indicate a more aggressive course of oncological diseases in "young adults". However, the source [76] analyzed 31,983 patients diagnosed with gastrointestinal NET, and only 5% of patients were younger than 35 years old. It was found that in young patients, localized forms of gastrointestinal NET with a high degree of differentiation are found for the most part. In multivariate analysis, young age was associated with a lower risk of mortality.

An analysis of the world literature, which is devoted to the relationship between the age characteristics of patients and the prognosis of gastrointestinal NET, allows us to conclude that there are very few studies devoted to this topic. The age factor deserves further research.

### *1.1.4 Localization of the primary focus and race of a patient with neuroendocrine tumors of the gastrointestinal tract*

In the American study "One hundred years after the "carcinoid" [63]: epidemiology and prognostic factors of neuroendocrine tumors in 35,825 cases in the USA", it was concluded that the localization of the primary tumor depended on the patient's race: the lungs are most common in European patients, and the rectum – in Asian/Pacific islanders, American Indians/Alaska natives and African Americans. In addition, the duration of survival varied depending on the histological type. In the multivariate analysis of patients, the stage of the disease, the location of the primary tumor, histological class, gender, race, age and year of diagnosis were predictors of outcome ($p<0.001$).

A significant factor affecting the unfavorable prognosis of gastrointestinal NET is the localization of the primary tumor. According to the SEER database, the lungs/bronchi are the most frequent localization of NET and account for 30.6%, followed by the small intestine (22.2%), rectum (16.2%), colon (13.4%), pancreas

(10.8%) and stomach (6.8%) in terms of the frequency of lesions. The overall survival rate for 1, 3, 5 and 10 years for patients with NET was 72.8%, 52.7%, 39.4% and 18.1%, respectively. The best prognosis was in patients with NET of the rectum and small intestine (HR 1,660, 95% CI, 1,579, 1,744), lungs and bronchi (HR, 1,786, 95% CI, 1,703, 1,874), stomach (HR, 1,865, 95% CI, 1,755, 1,982) and colon (HR, 1,896, 95% CI, 1,799, 1,999). Patients with pancreatic NET had the highest risk of death (HR 2,034, 95% CI 1,925, 2,148) [79].

### *1.1.5 Metabolic syndrome and neuroendocrine tumors of the gastrointestinal tract*

A highly relevant issue at the moment is the study of such factors as the patient's lifestyle, the patient's eating habits and metabolic syndrome [16, 20, 31, 35, 55, 56, 86, 98, 99, 102].

In March 2021, Italian authors published the results of a study evaluating the possible relationship between metabolic syndrome and the occurrence of neuroendocrine gastrointestinal tumors [19]. Obesity, mainly visceral obesity, and metabolic syndrome are the main risk factors for the development of type 2 diabetes, cardiovascular diseases and cancer. There are no data analyzing the association of obesity and metabolic syndrome with gastroenteropancreatic neuroendocrine tumors. The Fatty Liver Dystrophy Index (FLI) is a non-invasive tool for identifying individuals with non-alcoholic fatty liver disease. The Visceral Obesity Index (VAI) has been proposed as a gender-specific indicator of fat dysfunction. Both indices have been proposed as early predictors of metabolic syndrome. The aim of this study was to study the association of FLI VAI as early predictors of metabolic syndrome with gastroenteropancreatic neuroendocrine tumors. The study included 109 patients with histologically confirmed G1/G2 NET gastrointestinal tract (53 M; 57.06±15.96 years), as well as 109 healthy individuals comparable in age, gender and body mass

index. 44 patients with gastrointestinal NET were G2, of which 21 were with progressive disease, and 27 patients had metastases. Patients with gastrointestinal NET had a higher value of VAI ($p<0.001$) and FLI ($p=0.049$) and a higher presence of metabolic syndrome (p Values of VAI and FLI and the presence of metabolic syndrome were higher in G2 patients than in G1 patients ($p<0.001$), in patients with progressive disease, and in metastatic versus patients without distant metastases (p), in addition, higher values of VAI and FLI and a more pronounced metabolic syndrome significantly correlated with the worst clinical manifestations of gastrointestinal NET. The cutoff values for FLI and metabolic syndrome were also presented to predict a high gradation of gastrointestinal NET and the presence of metastasis.

In August 2018, a group of Portuguese scientists published the results of their own research [108]. In this study, patients with gastrointestinal NET (n=96) were compared by age, gender and area of residence with the control group (n=96) obtained from the general population in the case-control study. Patients had tumors of the gastrointestinal tract (75.0%) or pancreas (22.9%), grade G1 (66.7%) or G2 (27.1%) with localized disease (31.3%), regional metastasis (16.7%) or distant metastasis (43.8%) at diagnosis and 45.8% had clinical carcinoid syndromes. The metabolic syndrome was determined in accordance with the criteria of the Joint Interim Statement (JIS). Gastrointestinal NETs were associated with the criteria of metabolic syndrome, as well as with the waist circumference of individual subjects, fasting triglycerides and fasting plasma glucose ($p=0.003$, $p=0.002$, $p=0.011$ and p). The probability of association was higher when the number of individual components of the metabolic syndrome was more than four.

Few studies have examined the alleged relationship between glucose disorders and gastrointestinal NET, and most relate to pancreatic NET. Diabetes is a distinctive feature of some rarely functioning gastrointestinal NETs, such as glucagonomas, vasoactive tumors secreting intestinal polypeptides (vipomas), and somatostatinomas and is present in 70% of non-functioning pancreatic NETs [107].

In December 2021, Italian authors conducted a post-hoc analysis of the CLARINET study, which assessed the prognostic role of diabetes mellitus in progressive neuroendocrine tumors of the gastrointestinal tract [43]. Diabetes mellitus according to the results of this study was not a negative prognostic factor. The potential antitumor effect of metformin was observed in patients receiving placebo.

The relationship between the metabolic syndrome and both the occurrence of gastrointestinal NET and its further course is an extremely promising subject for further study. There are a large number of "blind" zones in this matter. The prognostic significance of insufficiently compensated diabetes mellitus, obesity (both hereditary and acquired) requires mandatory further study. It is especially worth noting that the factors of systemic inflammation are closely related to the metabolic syndrome. According to modern research, the progressive development of obesity and the formation of metabolic complications are the result of chronic inflammation of adipose tissue and its dysfunction as an endocrine and immunologically active organ. In obesity, as in any chronic inflammatory process, in the early stages, infiltration of adipose tissue by macrophages occurs, which are mainly localized around hypertrophied and/or dead adipocytes, contribute to adipocyte hypertrophy, increase cytokine synthesis by fat cells and increase the inflammatory response. With inflammation in adipose tissue, blood flow slows down, capillary permeability increases, endothelial dysfunction is detected, accompanied by vasodilation. These disorders lead to the formation of hypoxia of adipose tissue. Fibrosis develops in adipose tissue, characterized by the accumulation of connective tissue cells and extracellular matrix, the components of which are produced by adipocytes under the influence of activated macrophages and localized in the form of an amorphous zone around fat cells [11].

The study of sporadic gastrointestinal NET deserves special attention. A study aimed at studying the risk factors of gastrointestinal NET was published by a group of Italian authors in January 2022 (data from three referral centers were analyzed) [89]. A retrospective case-control study was conducted, including 148 consecutive

sporadic gastrointestinal NETs and 210 people from the control group of the same age and gender. Data on clinical features, family history of cancer and other potential risk factors were collected. Independent risk factors for gastrointestinal NET were: family history of neuroendocrine gastrointestinal cancer (HR 2.16, 95% CI 1.31-3.55, p=0.003), type 2 diabetes mellitus (HR 2.5, 95% CI 1.39-4.51), p=0.002) and obesity (HR 1.88, 95% CI 1.18-2.99, p=0.007). In patients with type 2 diabetes mellitus, metformin intake was a protective factor (HR 0.28, 95% CI 0.08-0.93, p=0.049). Type 2 diabetes mellitus was also associated with a more common (HR 2.39, 95% CI 1.05-5.46, p=0.035) and progressive course of the disease (HR 2.47, 95% CI 1.08-5.34, p=0.03). When stratifying cases by primary localization, the independent risk factors for pancreatic NET were type 2 diabetes mellitus (HR 2.57, 95% CI 1.28-5.15, p=0.008) and obesity (HR 1.98, 95% CI 1.11-3.52, p=0.020), while for intestinal NET family history of neuroendocrine gastrointestinal cancer (HR 2.46, 95% CI 1.38-4.38, p=0.003) and obesity (HR 1.90, 95% CI 1.08-3.33, p=0.026).

Further research in this direction is extremely promising, since, if confirmed, such research results can have a significant impact on the prevention strategies of gastrointestinal NET.

The chronotype is defined as a sign that determines the circadian preference of the subject in behavioral and biological rhythms relative to the external light-dark cycle. Although individual differences in the chronotype have been associated with an increased risk of developing certain types of cancer, no studies have been conducted with gastrointestinal NET.

A study by Italian authors, published in July 2021, determined the differences in chronotype between 109 patients with gastrointestinal NET and 109 healthy volunteers, comparable in gender, age and BMI, as well as its correlation with tumor aggressiveness [24].

Patients with gastrointestinal NET had a lower chronotype index (p=0.035) and a higher percentage of evening chronotype (p=0.003) than the control groups. Patients with the morning chronotype had a lower BMI, waist circumference and a higher percentage of metabolic syndrome. Interestingly, taking into account the

clinical and pathological features, patients with metastases, G2 gradation and progressive disease presented a lower assessment of the chronotype. The chronotype score was negatively associated with anthropometric measurements, metabolic profile, percentage of metabolic syndrome and Ki67 index. Thus, patients with gastrointestinal NET have an unhealthy metabolic profile and are more likely to present an evening chronotype. These results confirm the importance of including the chronotype assessment as an additional tool for the prevention of metabolic changes and tumor aggressiveness of the gastrointestinal NET.

It remains an open question to clarify the effect of BMI in patients with gastrointestinal NET at initial diagnosis and further course of the disease. All these data allow us to conclude that further research in this direction is extremely promising. Also, the influence of the presence of bad habits (alcohol, smoking) on the course of the disease is extremely poorly studied.

### *1.1.6 Prognostic role of vitamin D3 deficiency in patients with neuroendocrine tumors of the gastrointestinal tract*

Vitamin D3 deficiency may also be a risk factor, as well as the most aggressive course of gastrointestinal NET. This assumption is supported by data published by Italian authors in February 2022 [109]. A retrospective study was conducted, including 75 patients with gastrointestinal NET (G1-G2) and 123 healthy volunteers corresponding to age, gender and body mass index. Patients with gastrointestinal NET had significantly lower levels of 25(OH)D compared to the control group (17.9±7.8 vs. 24.2±7.7 ng/ml, $p<0.0001$). Patients with ileal NET had lower levels of 25(OH)D compared with other primary tumor localities ($p=0.049$), and patients who had a history of small bowel resection presented a significant risk of severe vitamin D3 deficiency (HR=2.81, 95% CI 1.25-3.37, $p=0.018$). There was no correlation with treatment with somatostatin analogues. Levels 25(OH)D were significantly lower in

G2 compared to the GI NET1 (15.6±7.8 vs. 19.9±7.4 ng/ml, p=0.016) and in patients with progressive disease (12.6±5.7 ng/ml) compared with those with stabilization of the disease (on average 21.5±8.2 ng/ml, p=0.001) or after cytoreduction (19.6±7.3 ng/ml, p=0.002).

Patients with vitamin D3 deficiency also had a low survival rate without disease progression compared to patients without vitamin D3 deficiency (p=0.014), while no correlation with disease-specific survival was found.

## 1.2 The role of indicators of systemic inflammation in the prognosis of neuroendocrine tumors of the gastrointestinal tract

Chronic systemic inflammation is a predisposing factor for many processes that are characteristic of cancer, such as proliferation, progression and evasion of immune defense mechanisms. Many researchers have devoted their works to the factors of systemic inflammation [27, 51, 59, 60, 72, 74, 75, 83, 101]. Thus, the relevance of the search for inflammatory biomarkers has been identified as critical for many types of tumors, including gastrointestinal NET. Inflammatory biomarkers are understood as the following indicators:

- peripheral blood leukocytes;
- peripheral blood neutrophils;
- peripheral blood lymphocytes.

1. Peripheral blood monocytes.
2. Peripheral blood eosinophils.
3. Peripheral blood platelets.

Based on the following indexes were calculated:

1. Neutrophil-lymphocytic index (NLI): the ratio of the absolute number of neutrophils to the absolute number of lymphocytes.

2. Platelet-lymphocyte index (TLI): the ratio of the absolute number of platelets to the absolute number of lymphocytes.

3. Lymphocyte-monocyte index (LMI): the ratio of the absolute number of lymphocytes to the absolute number of monocytes.

4. Derivative of NLI (dNLI): the calculation formula is as follows: dNLR=Absolute number of neutrophils / (Leukocytes – Absolute number of neutrophils).

The influence of systemic inflammation factors on the prognosis of the course of the tumor process has been proven in oncological diseases and other localizations. So, in 2021, a study was published in which the prognostic value of systemic inflammation factors in breast cancer was studied [81]. The level of NLI was analyzed in 168 patients with luminal breast cancer. The study population was divided into NLLOW or NLLIDH according to a threshold value <2.12 (AUC: 0.645, 95% CI: 0.57-0.72, p=0.021) established by ROC analysis. Patients with NLOW before treatment showed significantly shorter BV (HR: 6.97, 95% CI 1.65-10.55, p=0.002) and OS (HR: 7.79, 95% CI 1.25-15.07, p=0.021) compared to those for whom NLI-high was registered. The prognostic value of systemic inflammation factors has also been studied in patients with squamous cell carcinoma of the oral mucosa [12]. The data obtained by a group of Russian authors and published in 2021 show that the relative number of neutrophils >61.18% (HR=0.66; p=0.0280), the absolute number of lymphocytes $\leq 2.12 \times 10^9$/l (HR=0.65) have a negative impact on OS; p=0.0025), the relative number of monocytes >9.1% (HR=0.67; p=0.0313), NLI >2.30 (HR=0.63; p=0.0130) and LMI ≤3.47 (HR=0.64; p=0.0157). The relative number of monocytes 6.96% and the absolute number of eosinophils in peripheral blood $\leq 0.09 \times 10^9$/l had a significant negative effect on the results of multifactorial analysis on PFS. In March 2021, a study was published that examined the prognostic value of the dNLI indicator for predicting the course of non-small cell lung cancer [84]. This meta-analysis (8 studies, which included a total of 2,456 patients) showed that elevated dNLR levels before treatment may be a factor in an unfavorable prognosis for patients with non-small cell lung cancer who received immunotherapy.

The results show that a higher dNLI significantly predicted poor OS (HR=1.65, 95% CI from 1.46 to 1.88; p<0.001) and IBP (HR=1.38, 95% CI from 1.23 to 1.55, p<0.001). In 2020, a group of scientists published the results of a study in which preoperative levels of NLI and aphids were studied in patients with adrenocortical cancer [29]. 57 patients were included in the study. Increased preoperative levels of NLI and TL correlated with lower overall survival rates, while higher PLR was also associated with poorer relapse-free survival for patients undergoing surgical treatment for adrenocortical cancer. Also in the work under the guidance of *S.I. Kutukova* showed that the overall survival of patients with gastric adenocarcinoma with a low neutrophil-lymphocytic index was significantly higher than in the rest of the cohort of patients: 16 months versus 8 months (95% CI from 12 to 23 months, p=0.0382) [12]. In the same study, it was proved that the overall survival of patients with a low aphid index was higher: 16 months versus 8 months (95% CI from 11 to 24 months, p=0.0026).

Studies of the influence of systemic inflammation factors on the prognosis of the disease were also conducted in neuroendocrine tumors of the gastrointestinal tract. So, in March 2020, the results of a study were published that showed a correlation between the stage of the disease (according to ENETs, TNM) in patients with neuroendocrine tumors of the pancreas and the level of NLI and aphids [30]. The values of the studied factors of systemic inflammation increased in direct proportion to the increase in the TNM stage (p=0.0001 and p=0.0001, respectively). In addition, it was found that the values of NLI are higher in patients with metastatic lesions of regional lymph nodes than in patients without metastases in lymph nodes (p=0.001). A meta-analysis of retrospective studies, which was carried out by Chinese colleagues led by Yu Zhou and published in May 2018, suggests that increased NLI may be a factor in an unfavorable prognosis for gastrointestinal NET [77]. This conclusion should be applied only for pancreatic NET, since most of the included patients were with this localization. In June 2021, the data of their own study were published, in which the prognostic value of the NLI was proved [48]. 144 patients with pancreatic NET were included in this study. The level of NLI ≥4

was associated with worse overall survival in both single-factor analysis (HR=3.53, 95% CI 1.50-8.31, p=0.004) and multivariate analysis (HR=2.57, 95% CI 1.061-6.216, p=0.036). Also in this study, it was proved that the presence of synchronous liver metastasis was determined as a prognostic factor in multivariate analysis (HR=3.35, 95% CI 1.411-7.973, p=0.006). Interestingly, the absolute number of tumor-associated neutrophils was higher in liver metastases compared to the primary tumor (p=0.048).

In 2017, data from a Japanese retrospective study were published, which included 58 patients with locally advanced pancreatic NET [60]. This group of patients underwent radical surgical treatment. The observation period is from 2001 to 2015. In this work, the dependence of the preoperative level of NLI and clinical and pathological parameters, such as the clinical characteristics of the patient, tumor progression and postoperative oncological outcome, was demonstrated. The results of this study demonstrate that the high preoperative level of NLI (≥2.4) was largely associated with a large tumor size (p=0.0015). The overall and relapse-free survival of patients with high NLR (≥2.4) was significantly worse than in patients with low NLR (<2.4, p=0.0481 and p<0.0001, respectively). Multivariate analysis showed that the LLI ≥2.4 and tumor size ≥2 cm were independent predictors of postoperative relapse (risk ratio 6.012, p=0.0035 and 6.760, p=0.0049, respectively). Interestingly, a high level of NLI independently predicted postoperative metastasis to the liver, but not to the lymph nodes.

In 2017, a study conducted at Shanghai Renji Hospital was published [25]. 119 patients with gastrointestinal NET were followed up: 83 cases (69.7%) of men and 36 cases (30.3%) of women. The age of the patients ranged from 24 to 86 years. Additionally, the following factors were taken into account: the ratio of platelets/lymphocytes (aphids) and the ratio of neutrophils/ lymphocytes (NLI). Tumor localization: stomach (n=70, 58.8%), duodenum (n=10, 8.4%), small intestine (n=2.1.7%), appendix (n=3, 2.5%), colon (n=12, 10.1%) and rectum (n=22, 18.5%). The diameter of the tumor ranged from 0.6 to 20.0 cm, the average diameter was 5.4 cm. According to the degree of differentiation, the following distribution was

noted: 25 cases of GI NET G1, 7 cases – GI NET G2 and 87 cases of GI NET G3. 113 patients underwent complete follow-up, and the average follow-up period was 75 (from 1 to 112) months. The overall 5-year survival rate was 58.4%. The survival rate of patients with degrees of differentiation G1, G2 and G3 was 100%, 71.4%, 44.4%, respectively. The analysis of the clinical and morphological features of the tumor showed that age ≥61 years (p=0.000), tumor located in the stomach, duodenum and colon (p=0.041), tumor size ≥4 cm (p=0.002), degree of differentiation G3 (p=0.000) late stage TNM (p=0.000) and blood aphids ≥133 (p=0.017) were associated with lower 5-year survival. Multivariate analysis showed that the patient's age (HR=3.036, 95% CI from 1.548 to 5.956, p=0.001), pathology classification (HR=1.852, 95% CI from 1.099 to 3.122, p=0.021), lymph node metastases (HR=2.635, 95% CI from 1.198 to 5,797, p=0.016) and distant metastases (HR=2,685, 95% CI from 1,383 to 5,214, p=0.004) were independent risk factors affecting the prognosis of patients, but the level of blood aphids was not associated (HR=1,735, 95% CI from 0.947 to 3.176, p=0.074) [94].

The work of researchers led by Tarik Zalman, which was published in 2016 [85] analyzes a study that included 132 patients with a diagnosis of gastrointestinal NET. Results: NLI and HR increased as the prevalence of gastrointestinal NET increased. Analysis of embryonic origin revealed higher rates of NLI and HR in the NET, which originates from the anterior intestine. NLI and aphids were also higher in patients with pancreatic NET compared to patients with gastrointestinal NET. Analysis of the TNM stage showed that the metastatic stage was accompanied by significantly higher UFOs and aphids. Also, this study revealed a strong negative correlation between progression-free survival and levels of NLI and aphids.

In the work of the author Takayuki Miura, which was published in 2021, the ratios of circulating/systemic neutrophils-lymphocytes, monocytes-lymphocytes, platelets-lymphocytes and platelets-leukocytes were evaluated in 120 patients who underwent surgical treatment for highly differentiated neuroendocrine tumors of the pancreas without synchronous distant metastases in the period from 2001 to 2018. source [45]. Univariate or multivariate analysis using the Cox proportional risks model

was used to calculate the risk ratio with 95% confidence intervals. One-dimensional analysis showed that the preoperative ratio of neutrophils and lymphocytes, tumor size, TMN classification of the European Neuroendocrine Society for the Study of Tumors, the classification of the World Health Organization 2017 and venous invasion were associated with relapse. The optimal preoperative threshold value of the ratio of neutrophils to lymphocytes was 2.62, based on the ROC analysis. In multivariate analysis, a higher ratio of neutrophils to lymphocytes before surgery (OS=3.49, 95% CI 1.05-11.7; p=0.042) and the classification of the World Health Organization 2017 (OS=8.81, 95% CI 1.46-168.2, p=0.015) were independent prognostic factors of relapses.

A group of authors led by researcher Norifumi Harimoto retrospectively collected data on patients with pancreatic NET who underwent pancreatic resection for therapeutic purposes in the period from January 2008 to December 2017 in six institutions [82]. Data on clinical and pathomorphological factors, features of the course of the disease and the results obtained by immunohistochemical staining of tumor-associated macrophages (OAM) were analyzed in a total of 55 patients in this study. High NLI (>3.41) in patients was largely associated with a higher number of leukocytes in the blood of patients, a higher Ki-67 index, a higher number of mitoses, a higher degree, a higher frequency of metastasis to the lymph nodes, a higher frequency of lymphatic and nerve invasion, massive blood. loss and a large number of OAM expressing CD163. Relapse-free survival in patients with high NLI was significantly lower than in patients with low NLI. Multifactorial analysis revealed that such indicators as high NLI, the degree of differentiation of NET G2 or G3 and the presence of a history of synchronous liver resection disease are independent risk factors for relapse after therapeutic resection.

Taking into account the above data, it can be concluded that such an extremely accessible parameter in routine practice as a clinical blood test can be a factor in predicting the course of gastrointestinal NET. Which makes further study of systemic inflammation indicators an extremely promising direction.

## 1.3 Morphological features of neuroendocrine tumors of the gastrointestinal tract

### *1.3.1 Immunohistochemical characteristics of neuroendocrine tumors of the gastrointestinal tract*

An important factor affecting the unfavorable prognosis of the disease is the exact determination of the stage of NET. For example, a number of researchers conducted an analysis for the period from 2000 to 2018 of the SEER surgical database in order to identify incorrect classification of NET from successfully performed resections of pancreatic NET of various stages, which were revised in accordance with the new WHO classification of 2017. Overall survival, including relapse-free survival, was assessed using the Kaplan-Meyer method for the original and new assessment systems, respectively.

In total, 176 cases were identified for the revision of the NET qualification. The result was as follows: 17/64 (26.6%) G1 neoplasms were classified as G2; 12/95 (12.6%) G2 as G1; and 1/11 (9.1%) G3 as G2. Experts agreed with 97% of the classified cases [13]. It can be concluded that the incorrect classification of the degree of BUT is not uncommon, but it should be eliminated by professional development, a more detailed study of this nosology and compliance with the recommendations.

In the journal Diseases of the Colon & Rectum, published in the official publication of the American Society of Surgeons, a study was published to identify features in patients diagnosed with rectal NET. 91 patients (average age 58 years) with a diagnosis of rectal cancer were followed up in the period from 1999 to 2011. Of these, 35 patients are men, 56 are women. Neuroendocrine tumors were classified as G1 and G2 tumors with Ki-67≤20% and/or mitotic number ≤20 [80]. At the initial stage, the following clinical and pathological data were determined, including the stage according to the TNM system, the level of invasion; tumor size; previous treatment methods and results, including survival data.

The average duration of follow-up was 58.1 months, while 3 patients had stage IV of the disease. The following treatment methods were used: radiofrequency ablation (5 patients), local excision (79 patients), surgical resection (4 patients) and radiation therapy (1 patient; T3N1 tumor). A positive surgical edge of resection was noted in 17 cases (%). Local recurrence occurred in 8 cases and 1 recurrence in the bone 13 months after removal of the tumor T3 N1. During the study, a relationship was revealed between local relapse and the marker Ki-67, mitotic number, degree and lymphovascular invasion ($p<0.01$). A larger tumor size was associated with a decrease in progression-free survival. Determination of the Ki67 proliferation index of NET gastrointestinal tract and pancreas in accordance with ENETs recommendations is the gold standard in determining the risk of progression of NET pancreas.

In a study of Japanese colleagues [40], 601 cases of rectal NET were analyzed (515 cases were with G1 degree and 86 cases with G2 degree). The average size of the tumor was 0.7 cm. Compared with NET G1, G2 tumors had significantly larger tumor size (0.8 vs. 2.2 cm, $p<0.001$), a smaller percentage of patients with tumors limited by the submucosa (92.6 vs. 42.8%, $p<0.001$), more frequent presence of microvascular invasion (MVI) (3.6 vs. 16.9%, $p<0.001$) or perineural invasion (PNI) (2.0 vs. 24.1%, $p<0.001$). The frequency of metastasis to lymph nodes and distant metastases was 5.2 and 2.1% in G1 NET compared with 44.2 and 31.4% in G2 tumor, respectively ($p<0.001$). For tumors 1-2 cm in size, limited by the submucosa, the frequency of metastasis to the lymph nodes was 6.1% for NET G1 compared with 21.1% for NET G2. The status of MVI/PNI was a prognostic factor of metastasis to the lymph nodes of the G2 tumor, and not G1 NET in this subgroup. Thus, it can be concluded that the NET of the rectum G2 was much more invasive with a significantly higher frequency of metastasis to the lymph nodes compared to the tumor G1.

A group of authors from Denmark published a study [66] aimed at finding out the expression and prognostic value of three markers (SSTR-2a, CgA and synaptophysin) in 163 patients with gastrointestinal NET with an index Ki67>20%.

The expression of SSTR-2a, CgA, and synaptophysin was analyzed in tumor samples by immunohistochemistry and semi-quantitatively evaluated as negative (<5%), heterogeneously positive (5-30%) or strongly positive (>30%). P53 was defined as normal when evaluated as heterogeneously positive (1-30%), and abnormal when negative (0%) or strongly positive (>30%). In a multivariate analysis, better survival was observed among patients with heterogeneously positive p53 compared to strongly positive. With dichotomization, tumors with heterogeneously positive p53 compared with negative and strongly positive p53 also showed significantly better survival (p=0.002). Survival was significantly worse for negative CgA compared to heterogeneously positive CgA (p=0.02). Strongly positive expression of SSTR-2a was found in 26% of the 163 included patients. Well-differentiated morphology correlated with strong expression of SSTR-2a and CgA, as well as heterogeneously positive p53 staining and was more common in primary pancreatic cells. In primary pancreatic cells, strongly positive SSTR-2a was associated with longer survival (one-dimensional analysis, p=0.02). Significantly lower Ki67 proliferation index was found in patients with heterogeneously positive expression of p53, positive SSTR-2a and CgA. These results indicate that abnormal expression of p53 is an independent negative prognostic marker in gastrointestinal NET with an index of Ki67>20%. Patients with heterogeneously positive p53 had the best prognosis. SSTR-2a was a positive prognostic marker for pancreatic NET. Negative CgA was associated with significantly worse overall survival compared to heterogeneously positive CgA expression in multivariate subanalysis. The lower Ki67 index significantly correlated with the heterogeneously positive expression of p53, positive SSTR-2a and CgA.

In 2017, a group of Italian authors proposed a formula for assessing the further course of GI NET [93]. A retrospective analysis of stage IV gastrointestinal NET was performed, where two hundred and eighty-three stage IV gastrointestinal NET were evaluated, including 93 neuroendocrine tumors G1 (32.9%), 153 neuroendocrine tumors G2 (54%) and 37 neuroendocrine carcinomas G3 (13.1%). Independent risk factors for disease progression were Ki67, the proportion of metastatic liver damage and the presence of extraabdominal metastases. The risk score was calculated

as follows: (0.025×Ki67) + [(0 in the absence of liver metastases or liver involvement <25%) HR (0.405 with liver involvement 25-50%) HR (0.462 with liver involvement >50%)] + [(0 in the absence of extraabdominal metastases) HR (0.528 in the presence of extraabdominal metastases)]. The accuracy of the risk assessment for predicting the progression of the tumor process was higher compared to the G assessment system (area under the curve: 0.705 and 0.622, respectively). Three subgroups of patients with low, medium and high risk of disease progression were identified on the risk score scale, the median progression-free survival was 26 months, 19 months and 12 months, respectively.

The introduction of such formulas and scales into clinical practice can help distinguish patients with different levels of risk of progression for planning individual therapeutic approaches and follow-up programs. Further work in this direction is extremely promising and relevant to improve the results of treatment of patients with gastrointestinal NET.

### *1.3.2 Molecular and genetic characteristics of neuroendocrine tumors of the gastrointestinal tract and their effect on prognosis*

The role of genes in the development of tumors in familial tumor syndromes has been most studied. About 10% of the NET of the lung and gastrointestinal tract is diagnosed in patients with hereditary burden. These syndromes include multiple endocrine neoplasia type 1 (MEN1) and von Hippel–Lindau syndrome, as well as the less common neurofibromatosis syndrome [3, 15, 52, 61, 73, 103].

In most cases, the type of inheritance of MEN is autosomal dominant. In half of the cases, the syndrome occurs sporadically [58]. The gene that is known to be associated with this syndrome is the MEN1 gene, identified in 1997 and located on chromosome 11q13 [73]. This gene consists of 10 exons encoding a new protein of 610/615 amino acids and is called menin [14]. Very rarely, the mutation of the

p27Kip1 (p27)/CDKN1B gene is associated with MEN1 syndrome [73]. More than 500 mutations have been identified in this gene [11, 89]. It has been reported that the MEN gene is changed in a significant part (44%) of sporadic NETs of the pancreas [28, 54].

Von Hippel–Lindau syndrome is manifested by the development of NET, including NET pancreas in 8-17% of patients, in particular mutations in exon 3 of the gene are associated with NET pancreas [81, 109]. The syndrome also has an autosomal dominant type of inheritance and is caused by inactivation of the germ line in the VHL gene. The gene product (pVHL) is a negative regulator of HIF, a set of transcription factors activated by PI3K/mTOR and controlled by pVH [49]. The VHL mutation is rarely found in sporadic pancreatic NETs, but its inactivation by gene deletion (18%) or promoter hypermethylation (6%) leads to similar effects [106].

Approximately 10% of patients with neurofibromatosis type 1 develop NET of the gastrointestinal tract, usually periampular or duodenal somatostatinoma. As a consequence, 40% of these rare tumors are detected due to changes in the NF1 germ line [53, 71, 110]. Patients suffering from type 1 neurofibromatosis inherit mutations of the NF1 gene, which inactivates the germ line and causes a profound violation of Ras/MAPK regulation and PI3K/mTOR signal transmission. Tuberous sclerosis is characterized by a direct violation of the regulation of the PI3K/mTOR signal transmission network, indirectly involved in previous syndromes. In fact, the disease is caused by inactivating mutations in one of two genes, TSC1 and TSC2 [25, 60]. Gastrointestinal NETs rarely develop as a consequence of this syndrome. However, recent studies have reported suppression and mutation of TSC1/TSC2 in sporadic NETs of the pancreas [13]. Data on the genetic background of sporadic NETs in the literature are limited due to the rare incidence.

Although a number of genes, including MEN1, RAR-β, hMLH1, RASSF1, Her2/neu, Cyclin D1, p16 INK4a/P14 ARF, p18 INK4c, p27 Kip1, p53 and coding tyrosine kinase receptors, have been implicated in the pathogenesis of NET.But the genetic mechanisms of tumor development are poorly understood [80]. Despite the low frequency of background mutations, several studies have revealed that driver

mutations in the genes of the pathway MEN1, DAXX/ATRX and mTOR (PTEN, TSC1/2) are involved in the development and progression of the disease [40].

In the EXOME study, where sporadic pancreatic NETs were studied in 68 patients. Yuchen Jiao et al. It was found that 44% of these tumors carried mutations in the MEN1 gene, 43% – in two chromatin transcription remodeling subunits DAXX/ATRX, TSC2 and PIK3CA – in ~16% of tumors and 14% of mutations in the mTOR pathway [28]. Of these, mutations in PTEN and TSC2 are mutations with loss of function, whereas PIK3CA mutations are present in the previously described oncogenic "hotspot" residue, which activates the kinase domain of the encoded protein [66]. It should be noted that the detection of mutations in the mTOR pathway in the pancreatic NET has significant clinical therapeutic significance for the use of targeted therapy in the treatment of patients with such a disease. The discovery of the association of NET with two somatic mutations that were not previously associated with cancer, DAXX and ATRX, was extremely significant. These genes were mutated in 25% and 18%, respectively. Changes in ATRX or DAXX are mutually exclusive, which indicates that the encoded proteins function along the same pathway. Patients with NET who had altered ATRX or DAXX genes had significantly longer survival than patients with wild-type tumors [93].

The molecular profile in tumors of different degrees of differentiation is different. Highly differentiated NETs differ from low-differentiated ones in prognosis, the number of mitoses and the level of Ki-67. Moreover, they are essentially devoid of TP53 and RB1 mutations, which are instead the main drivers in low-grade tumors of any localization [39, 34, 42, 97].

In one study of whole exome sequencing, it is reported that low-differentiated gastric NETs and adenocarcinomas have common TP53 mutations, affecting, according to the literature, from 53 to 100% of cases [65, 69, 95, 111], and rare mutations of SYNE1. But at the same time, they do NOT demonstrate a higher frequency of mutations than gastric adenocarcinoma [69].

In low-grade tumors, promoter hypermethylation was detected for such genes as DAPK1, TIMP3, PAX5, HIC1, CADM1 and many others [45].

The molecular profile of NET G3 of the colon is similar to adenocarcinoma of the colorectal tract with mutations APC, KRAS, BRAF and TP53 [12, 28, 45, 70, 82, 85]. The occurrence of microsatellite instability was also described, loss of heterozygosity (LOH) was detected at the TP53 and SMAD4 loci and in chromosome 6q [12, 52].

In highly differentiated NETs, methylation in the RASSF1A gene was observed, observed in other tumors of the pancreas, lungs and gastrointestinal tract [15, 47]. KRAS mutations present in almost all pancreatic adenocarcinomas and up to 50% of colon tumors have been described in low-grade NETs of the stomach, pancreas and intestine (frequency range from 8 to 60%; median 30%). At that time, BRAF mutations (frequency range from 13 to 59%; median 17%) were detected only in colorectal NETs with low G3 differentiation [3, 58, 61, 73, 103]. It should be noted that these mutations were practically not observed in highly differentiated NETs, but they have methylation in the RASSF1A gene, observed in tumors of the pancreas, lungs and gastrointestinal tract [14, 15, 47, 49, 54, 106]. In tumors with a low degree of G3 differentiation, promoter hypermethylation was more often detected for such genes as DAPK1, TIMP3, PAX5, HIC1, CADM1 and many others [45].

Several studies have reported the presence of BRCA2 germ line mutations in pancreatic NET [36, 53, 71]. Other rare mutations were found in auxiliary HR DNA repair genes, such as RAD50, RAD51AP2, and BRIP1 (from 3 to 5%) [110]. Three repair genes (MSH3, MSH4 and MSH6) were also found in up to 1% of tumors. Although the effect of these mutations on the development and aggressiveness of the tumor has not been determined. In addition, it should be emphasized that EGFR was detected in 18 (13%) patients, HER2 – in 3 (2%), KIT – in 16 (11%) and PDGFRalpha – in 135 (96%) [110].

It is also worth noting that NET has a great need for vascularization to deliver nutrients to a growing tumor. Neuroendocrine tumor G3, is highly vascularized, due to significant activation of HIF1a. Activation of HIF1a is caused by genetic inactivation of the VHL protein and stimulating hypoxic conditions that are usually present in the vascular environment of the NET environment.

Pancreatic NETs G3 express high levels of VEGF, VEGFR-2 and 3, as well as PDGFR α and β. When the tumor differentiates, VEGF expression is lost and the density of vascular vascularization decreases, which is a paradox found in pancreatic NETs. However, no correlation between VEGF expression and overall survival has been proven [39].

Despite the proven efficacy of sunitinib in NET, there have been many reports of early progression, as well as the presence of tumor recurrences immediately after the response, which suggests the presence of both primary and acquired resistance, which may jeopardize the use of this therapy and represent a clinical problem.

At the moment, various mechanisms of resistance to sunitinib have already been identified. To date, no selective HIF-1a inhibitor has been approved as an antitumor therapy.

Several multirosine kinase inhibitors with combined activity against VEGF and against MET have shown increased inhibition of angiogenesis and suppression of invasion and metastasis of neuroendocrine tumors.

There are also alternative methods of vascularization that do not depend on the stimulation of the VEGF pathway. The appearance of the tumor vascular network also depends on the Ang2 receptor.

Pericytes also play an important role in the progression of the tumor, since an increase in the number of these cells during treatment with sunitinib may be associated with the emergence of resistance to it.

The use of PDGFRß inhibitors for targeted effects on tumor-associated pericytes, along with standard antiangiogenic therapy, can help achieve an adequate balance in therapy.

Another interleukin associated with antiangiogenic treatment is IL-8, which is associated with an extremely unfavorable prognosis. It was proposed to use neutralizing IL-8 antibodies in the NET of the pancreas.

Lysosomal sequestration is another well–known mechanism of resistance to antiangiogenic agents that can be overcome with the help of P-gp inhibitors.

Overexpression of EZH2 and its resistance to associated antiangiogenic agents demonstrate the dynamics of tumor behavior, since it can be overcome by increasing the dose. PlGF is elevated in patients with pancreatic NET, which is associated with a worse prognosis.

Analysis of the source [39] and [17] shows that EZH2 can play an important role in regulating the biological behavior of neuroendocrine tumors.

There is also an association between the p53 and EZH2 pathways in NET. EZH2 represents a potential target antigen in cancer therapy based on unobserved expression in normal tissues and the important role of EZH2 in oncogenesis.

There is a large number of works devoted to the correlation of endocan expression and the prognosis of malignant neoplasms, in particular neuroendocrine tumors [32, 33, 41, 64, 105].

In another study, in which 73 patients with pancreatic NET participated, the following conclusion can be drawn – a high level of MVD in pancreatic NET is associated with a favorable prognosis [34].

The prognostic significance of MVD was determined using 55 tumor blocks of patients with pancreas. Patients with higher levels of MVD demonstrated higher progression-free survival.

Endocane expression levels correlated with low MVD and low Ki-67 index.

Thus, positive expression of the endocan is associated with a high potential for malignancy [44]. Endocan expression is an independent risk factor for the progression of pancreatic NET.

Subsequently, studies were conducted to study the relationship between clinical and pathological characteristics, levels of tumor endocan expression and MVD, and the risk of tumor recurrence. Analysis of the data obtained showed that positive endocan expression, lymph node lesion and tumor metastasis demonstrate an increased risk for tumor recurrence.

Studies show a link between the presence of a PD-L1 tumor reaction and the response to anti-PD-1 therapy [42]. However, there are data on patients with PD-L1-positive tumors that do not respond, and patients with PD-L1-negative tumors that

respond. When analyzing the studies, it was demonstrated that a total of 8.7% of tumors showed PD-L1 expression. A phase 1b multicoort study evaluated the effect of pembrolizumab on patients with PD-L1-positive tumors. Treatment with pembrolizumab resulted in an objective response rate of 12%. Stabilization of the disease was noted in 60% of patients [70].

It can be concluded that a better understanding of the carcinogenesis of NET will lead to the discovery of prognostic biomarkers that can help individualize treatment and develop new drugs.

## 1.4 Biochemical markers of neuroendocrine tumors of the gastrointestinal tract

### *1.4.1 Chromogranin A*

Chromogranin A (CgA) is a non–specific biomarker secreted by neuroendocrine tumor cells. An increase in the level of circulating chromogranin A can be detected in patients with gastrointestinal NET and has been shown to correlate with tumor load. The prognostic roles of the level of chromogranin A and changes in the level of chromogranin A are contradictory. There are numerous works devoted to the prognostic role of Chromogranin A [21-23, 46, 50, 92].

The study [100] retrospectively analyzed 102 grade 1/2 NET gastrointestinal patients with available baseline or sequential levels of chromogranin A from the National Cheng Kung University Hospital to assess the relationship between the level of circulating chromogranin A and the degree of tumor differentiation, overall survival and prognosis of tumor response. Baseline levels of chromogranin A were associated with stage and sex. Higher baseline levels of chromogranin A were associated with poorer overall survival. The results of this study show that

chromogranin A can be a prognostic marker of tumor load, overall survival and tumor progression in patients with gastrointestinal NET.

Correlation of survival of chromogranin A level and prognosis of patients was investigated and in the Asian patient population [69] 60 patients with advanced gastrointestinal NET treated at the medical center in the period from April 2010 to April 2013 were retrospectively included. The level of chromogranin A in plasma was analyzed for correlation with the patient's clinical outcome and tumor response. Percentage changes in paired chromogranin A (ΔCgA) tests of more than 17% can predict a partial response or stabilization of the disease from a progressive disease with a sensitivity of 91.2% and a specificity of 82.9%.

A group of authors from the Netherlands studied the factors of unfavorable prognosis of NET of the small intestine [95]. In the period from January 2000 to June 2016, 400 patients with G1 and G2 NET of the small intestine were included. The analysis of negative prognosis factors allowed us to conclude that the Ki-67 index≥10, an unknown primary tumor, chromogranin A >6hVGN and elevated liver tests were identified as independent predictors of deterioration of disease-specific survival.

# Chapter 2
# MATERIAL AND RESEARCH METHODS

## 2.1 General characteristics of patients

We observed 298 patients with a diagnosis of "Neuroendocrine tumor of the gastrointestinal tract (gastrointestinal tract)" who were treated at the St. Petersburg State Medical Institution "City Clinical Oncology Dispensary" in the period from January 2015 to December 2021. The date of the final evaluation of the database (slice) is 01.04.2021.

Criteria for inclusion of patients in the study:

1. The opportunity to sign a form of voluntary informed consent to participate in this study.
2. Age – over 18 years.
3. Verified neuroendocrine tumor of the gastrointestinal tract.

Criteria for non-inclusion of patients in the study:

1. The presence of decompensated or any other concomitant disease that has a significant impact on the patient's survival and limits the choice of treatment method.

The distribution of patients by gender and age (according to the WHO classification of age groups, 2016) is presented in Table 1.

In the studied cohort of patients, there were significantly more women – 182 (61.08%) than men – 116 (38.93%) ($p<0.0001$). When analyzing the age of patients, it should be noted that among all age groups, elderly patients predominated (60-74 years according to the WHO classification, 2016) – 136 (45,64%) ($p<0.0001$).

Table 1 – Distribution of patients with neuroendocrine gastrointestinal tumors by gender and age

| Age | Gender | |
|---|---|---|
| | men, abs. (%) | women, abs. (%) |
| 18-44 years old (young age) | 18 (6,04%) | 25 (8,39%) |
| 45-59 years old (middle age) | 25 (8,39%) | 50 (16,78%) |
| 60-74 years old (elderly age) | 54 (18,12%) | 82 (27,52%) |
| 75-90 years old (senile age) | 16 (5,36%) | 25 (8,39%) |
| over 90 years old (centenarians) | 3 (1,01%) | 0 |
| Total: | 116 (38,93%) | 182 (61,08%) |

Neuroendocrine character of malignant lesions of the gastrointestinal tract was verified in all patients at the stage of primary diagnosis. In the majority of patients – 98 (32.89%) – the primary tumor focus was localized in the pancreas (p= 0.0081), in 69 (23.15%) patients the stomach was primarily affected, in 68 (22.82%) – the small intestine. Other parts of the gastrointestinal tract were affected much less frequently: the rectum was primarily affected in 16 (5.37%) patients, the duodenum – in 13 (4.36%) patients, the appendix – in 12 (4.03%) patients, the colon – in 11 (3.69%) patients. Separately, it should be noted that in 11 (3.69%) patients, the localization of the primary tumor focus could not be determined.

All patients, after verification of the process and instrumental examination of all systems and organs, underwent staging of the tumor process according to the TNM classification, 7th edition. Taking into account the existing clinical recommendations, staging according to the TNM system was carried out depending on the localization of the primary tumor focus.

The distribution of patients for each descriptor is shown in Table 2.

Table 2 – Distribution of patients with neuroendocrine gastrointestinal tumors according to the TNM system (7th edition)

| T | Quantity, abs. (%) |
|---|---|
| 1 | 90 (30,2%) |
| 2 | 88 (29,53%) |
| 3 | 55 (18,46%) |
| 4 | 40 (13,42%) |
| Not defined | 25 (8,39%) |
| N | Quantity, abs. (%) |
| 0 | 151 (50,67%) |
| 1 | 93 (31,21%) |
| 2 | 43 (14,43%) |
| 3 | 1 (0,34%) |
| X | 10 (3,36%) |
| M | Quantity, abs. (%) |
| 0 | 187 (62,75%) |
| 1 | 111 (37,25%) |

At the initial diagnosis in most patients, the primary tumor focus was placed in the categories T1 – 90 (30.20%) patients (p=0.0008) and T2 – 88 (29, 53%) patients (p=0.0016). In 55 (18.46%) patients, the primary tumor focus was regarded as T3, and in 40 (13.42%) patients as T4. In 25 (8.39%) patients, the determination of the T descriptor was impossible due to the primary surgical intervention in non-oncological hospitals and the lack of data in the primary medical documentation of patients.

In the majority of patients – 151 (50.67%) – no lesions of regional lymph nodes were detected at the initial diagnosis (p<0.0001). In 93 (31.21%) patients, the lesion of the regional lymphatic apparatus corresponded to criterion N1. A regional lesion in the volume of N2 was registered only in 43 (14.43%) patients (p<0.0001). Only in 1 (0.34%) patient, the lesion of regional lymph nodes was regarded as T3.

And in 10 (3.36%) patients, the lesion of the regional lymphatic apparatus at the primary stage was not evaluated.

The comprehensive examination revealed the presence of distant metastases in 111 (37.25%) patients; 187 (62.75%) patients had no signs of dissemination of the process ($p<0.0001$).

## 2.2 Research methods

All patients included in the clinical study, at the initial stage of diagnosis and final clinical diagnosis, were comprehensively examined in the following volume:

General methods of examination of patients included in the study:

1. Assessment of the patient's compliance with the criteria for inclusion in the study.
2. Collection of anamnestic data.
3. Examination of whole blood to determine hemoglobin, the number of erythrocytes, the number of platelets, the number of leukocytes, the leukocyte formula with the calculation of neutrophils, lymphocytes, monocytes, basophils, eosinophils, the determination of the erythrocyte sedimentation rate – a clinical blood test. The study was carried out on a multidisciplinary analyzer of the company "Abbott Diagnostic", USA: Cell-Dyn 3700 SL.
4. Examination of blood serum to determine the level of alanine aminotransferase, aspartate aminotransferase, total protein, total bilirubin, creatinine, glucose – a biochemical blood test. The study was conducted on a multidisciplinary analyzer of the company "Abbott Diagnostic", USA: Architect c 8000.
5. Physical examination (by organs and systems) and detection of concomitant diseases and conditions.

Specialized methods of examination of patients included in the study:

1. Ultrasound examination (ultrasound) of peripheral lymph nodes, abdominal cavity and pelvis.
2. X-ray examination of the chest organs on the device.
3. Computed tomography of the chest, abdominal cavity and pelvis (with intravenous amplification) on the device.
4. Morphological examination of tumor tissue in order to verify the tumor process and determine the degree of its differentiation.
5. Immunohistochemical examination of a tumor tissue sample to assess the proliferation index – Ki-67.
6. Blood test to determine the level of:

– serum serotonin;
– blood serum chromogranin.

7. Urinalysis to determine the level of 5-hydroxyinolacetic acid.
8. Sequencing of a new generation (NGS) sample of tumor tissue.

### *2.2.1 Morphological research methods*

Histological examination of the surgical material was used to verify the tumor process.

Histological examination was carried out in the pathology department of the St. Petersburg State Medical Institution "City Clinical Oncological Dispensary".

The postoperative material in the operating room was fixed in a 3% formalin solution and delivered to the pathology department, where it was registered in accordance with the established procedure. The duration of the fixation stage averaged 24 hours. After a day, the material was removed from the formalin solution, washed in running water, dried on filter paper and filled with paraffin (paraffin filling method). The paraffin-filled material was placed in a thermostat and kept for 24 hours at a temperature of 37 °C for the purpose of uniform and complete

impregnation of the tissue sample with paraffin. After completion of this stage – the stage of wiring the material – histological sections, no more than 10-15 microns thick, were prepared from the finished paraffin block using a microtome. The slices should be well straightened, without the formation of folds and tears. The resulting sections were applied to slides and stained with hematoxylin and eosin. At this stage, it is necessary to ensure that the color of the slices is uniform, with a clear differentiation of different structures. The resulting slices should be well enlightened.

Ready–made histological preparations were subjected to microscopic examination: the survey was carried out under magnification, a multiple of 5-10, and the sighting – under magnification, a multiple of 25-40.

After verification of the diagnosis of "neuroendocrine tumor of the gastrointestinal tract" with the help of morphological examination, the degree of malignancy of the tumor process was determined, which has an important prognostic value for the nosology under consideration. The distribution of patients depending on the degree of differentiation of the tumor process is presented in Table 3.

Table 3 – Distribution of patients with neuroendocrine gastrointestinal tumors by the degree of malignancy of the tumor process

| G | Quantity, abs. (%) |
|---|---|
| 1 | 144 (48,32%) |
| 2 | 115 (38,59%) |
| 3 | 39 (13,09%) |

The tumor of most patients had a grade of malignancy G1 – 144 (48.32%) of the sample, or G2 – 115 (38.59%). Only in 39 (13.09%) patients with morphological examination, the degree of malignancy was determined as G3 ($p<0.00001$).

### *2.2.2 Immunohistochemical examination of a sample of tumor tissue to determine the level of proliferation*

In order to determine the level of proliferative activity of the tumor, assessed by analyzing the expression of Ki-67, patients of the cohort under consideration, an immunohistochemical study of a sample of tumor tissue was performed.

The material was delivered to the laboratory for immunohistochemical examination in the form of paraffin blocks.

Microscopic examination selected the most suitable block containing tumor tissue. Slices with a thickness of 4 microns were cut from this block, which were placed on glasses with a poly-L-lysine coating. The sections were dried, dewaxed and exposed to antigen unmasking using a citrate buffer in a water bath, t=95 °C, 30 minutes. After that, they were cooled at room temperature and washed with a tris buffer with twin. Each section was outlined with a paraffin pencil, after which the endogenous peroxidase was inhibited with 3% hydrogen peroxide for 20 minutes. Then an antibody was applied to each slice (Clone SP6, rabbit antibodies, monoclonal, 1:200 dilution, manufacturer LabVision), the exposure lasted 1 hour on a thermostick in a "water bath" at a temperature of 30 °C.

DAKO's EnVision polymer detection system was used to visualize the antigen-antibody reaction, diaminobenzidine was used as a chromogen. The control coloring of the nuclei was carried out using Mayer's hematoxylin. After each of the stages, before staining with diaminobenzidine, the cut glasses were washed in a tris buffer with a pH 7.1 twin from BioOptica. The glasses were enclosed in the BioMaunt environment of Bio Optica.

The evaluation was carried out in the percentage (%) of positively colored cells in the presented sample.

### *2.2.3 New generation sequencing (NGS) of a tumor tissue sample*

In order to determine the following genes in the tumor material – ATM, ATR, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CDK12, CHEK1, CHEK2, EPCAM, FANCL,MLH1, MSH2, NBN, NF1, PALB2, PMS2, RAD51B, RAD51C, RAD51D, RAD54L, STK11, TP53, POLE, KRAS, NRAS,BRAF, EGFR, ERBB2, PIK3CA, MET ex14, BAT25, BAT26, NR21, NR24, MONO27, KIT, PDGFRA, Pi3Ca a new generation sequencing method was used (NGS).

Preparation of libraries for sequencing was carried out using NimblGen SepCapEZ Choice ("Roche") and reagents recommended by the manufacturer. Sequencing was carried out on the Illumina MiSeq device ("Illumina").

Bioinformatic analysis was carried out as follows:

1. Removing adapters and sequences with poor reading quality.
2. Mapping readings to the reference sequence of the human genome (hg19) using the BWAMEM algorithm.
3. Quality control of source data, alignment, enrichment and coverage of target regions using FastQC, BAMQC and NGSrich.
4. Search for nucleotide variations for germinal mutations using GATK HaplotypeCaller + UnifiedGenotyper (with getting a merged VCF file).
5. Search for nucleotide variations for somatic mutations using Mutect2 + Strelka (to obtain a combined VCF file).
6. Search for structural variations using Lumpy and Manta (obtaining a combined VCF file and generating visual data to validate the analysis results).
7. Processing of VCF files using the SnpSift program (the filtering criterion is a reading depth of more than 10).
8. Annotation using SnpEff (analysis of all transcripts), ANNOSAR (analysis of allele frequencies in ExAC/gnomAD, 1000G and ESP6500, algorithms for checking the functional significance of SIFT, PolyPhen2, MutationTaster, FATMM, CADD, DANN, Eigen), Alamut Batch (influence on splicing, dbSNP, ClinVar, HGMD Professional databases), BIC databases.

## 2.3 Patients treatment methods

According to the recommendations in force at the time of inclusion of patients in this clinical study, the optimal initial treatment was chosen for all patients (with mandatory consideration of the stage of the disease). The distribution of patients by treatment method is presented in Table 4.

Table 4 – Distribution of patients with neuroendocrine gastrointestinal tumors by the method of initial treatment

| Type of initial treatment | Quantity, abs. (%) |
|---|---|
| Surgical treatment | 239 (80,20%) |
| Drug therapy | 53 (17,79%) |
| Symptomatic therapy | 3 (1,01%) |
| Observation | 3 (1,01%) |

The majority of patients with gastrointestinal NET received surgical treatment as the main stage after the verification of the tumor process and the establishment of a clinical diagnosis: the proportion of patients who were operated on at the first stage of treatment was 80.20% (239/298; $p<0.00001$). 53/298 patients (17.79%) received drug therapy as an option of initial treatment. Patients who received only symptomatic therapy or dynamic follow-up were significantly less: their proportion was 1.01% (3/298) and 1.01% (3/298), respectively ($p<0.00001$).

Despite the fact that the surgical method was the main method of initial treatment, the nature of the surgical intervention was not always radical (Table 5).

Analyzing the volume of surgical intervention at the initial stage, the following patterns can be identified: radical surgical treatment was performed in 74.06% (177/239) patients, cytoreductive surgical treatment was performed in 23.01% (55/239) patients. And 2.93% (7/239) of patients underwent only exploratory laparotomy.

Table 5 – Distribution of patients with neuroendocrine gastrointestinal tumors who received surgical treatment by the volume of the intervention

| The scope of surgical intervention | Quantity, abs. (%) |
|---|---|
| Radical surgery | 177 (74,06%) |
| Cytoreductive surgical treatment | 55 (23,01%) |
| Exploratory laparotomy | 7 (2,93%) |

Those patients who failed to perform surgical treatment received drug therapy with somatostatin analogues, antitumor cytostatics or a combination thereof within the 1st line. The distribution of patients who received drug therapy as an option of initial treatment, by type of treatment, is presented in Table 6.

Table 6 – Distribution of patients with neuroendocrine gastrointestinal tumors who received drug treatment by type of therapy.

| Type of drug therapy | Quantity, abs. (%) |
|---|---|
| Somatostatin analogues | 15 (28,3%) |
| Oral fluoropyrimidines | 6 (11,32%) |
| Interferon alpha + somatostatin analogues | 6 (11,32%) |
| Interferon Alpha | 6 (11,32%) |
| EP | 4 (7,55%) |
| Somatostatin analogues + oral fluoropyrimidines | 3 (5,66%) |
| FOLFOX | 3 (5,66%) |
| Platinum preparations | 1 (1,89%) |
| Analogues of somatostatin + etoposide | 1 (1,89%) |
| GP | 1 (1,89%) |
| Other | 7 (13,21%) |

Somatostatin analogues at the initial stage of treatment were used in the majority of patients – 28.30% (15/53) (p=0.0554). Oral fluoropipimidines (tegafur, capecitabine), interferon alpha in combination with somatostatin analogues and

interferon alpha in a single mode were used for six patients each, receiving a share of 11.32% (6/53) for each of the presented methods of drug treatment. 4 patients (7.55% (4/53)) as the first stage of treatment, we received the EP regimen (etoposide + a platinum-series drug). Three patients received somatostatin analogues in combination with oral fluoropyrimidines (5.66% (3/53)). The remaining three types of drug therapy were applied to one patient each, namely: platinum preparations, somatostatin analogues in combination with etoposide, GP regimen (gemcitabine + cisplatin), accounting for 1.89% (1/53). In 13.21% (7/53) of patients, there is no information about the drug therapy regimen in the primary documentation.

# Chapter 3
# CLINICAL AND EPIDEMIOLOGICAL FEATURES OF NEUROENDOCRINE TUMORS OF THE GASTROINTESTINAL TRACT IN PATIENTS IN THE RUSSIAN FEDERATION ACCORDING TO THE REGISTER OF THE ST. PETERSBURG STATE HEALTHCARE INSTITUTION "CITY CLINICAL ONCOLOGICAL DISPENSARY"

## 3.1 General characteristics of patients

We observed 298 patients with a diagnosis of "Neuroendocrine tumor of the gastrointestinal tract (gastrointestinal tract)" who were treated at the St. Petersburg State Medical Institution "City Clinical Oncology Dispensary" in the period from January 2015 to December 2020.

When analyzing the database, the following features are observed – in the studied cohort of patients, there were significantly more women – 182 (61.08%) than men – 116 (38.93%), $p<0.0001$. Among all age groups, the proportion of elderly patients (60-74 years according to the WHO classification, 2016) prevailed – 136 (45.64%), $p<0.0001$.

The distribution of patients by gender and age (according to the WHO classification of age groups, 2016) is presented in Table 7.

In all patients, the neuroendocrine nature of malignant lesions of the gastrointestinal tract was verified at the stage of primary diagnosis (Table 8).

Table 7 – Distribution of patients with neuroendocrine gastrointestinal tumors by gender and age (n=298)

| Age | Gender | |
|---|---|---|
| | men, abs. (%) | women, abs. (%) |
| 18-44 years old (young age) | 18 (6,04%) | 25 (8,39%) |
| 45-59 years old (middle age) | 25 (8,39%) | 50 (16,78%) |
| 60-74 years old (elderly age) | 54 (18,12%) | 82 (27,52%) |
| 75-90 years old (senile age) | 16 (5,36%) | 25 (8,39%) |
| over 90 years old (centenarians) | 3 (1,01%) | 0 |
| Total: | 116 (38,93%) | 182 (61,08%) |

Table 8 – Distribution according to the localization of the primary tumor (n=298)

| Localization of the primary tumor | Quantity, abs. (%) |
|---|---|
| Pancreas | 98 (32,89%) |
| Stomach | 69 (23,15%) |
| Small intestine | 68 (22,82%) |
| Rectum | 16 (5,37%) |
| Duodenum | 13 (4,36%) |
| The vermiform process | 12 (4,03%) |
| Large intestine | 11 (3,69%) |
| WPL | 11 (3,69%) |

In the majority of patients – 98 (32.89%) – the primary tumor focus was localized in the pancreas (p=0.0081), in 69 (23.15%) – in the stomach, in 68 (22.82%) – in the small intestine. The lesion of other parts of the gastrointestinal tract was much less common: the rectum was primarily affected in 16 (5.37%) patients, the duodenum – in 13 (4.36%), the appendix – in 12 (4.03%), the colon – in 11 (3.69%) patients. It should be noted that in 11 (3.69%) patients, the localization of the primary tumor focus could not be determined, however, the histological and IHC portrait of the tumor indicates that the tumor originates from the gastrointestinal tract.

All patients, after verification of the disease and instrumental examination of the degree of prevalence of the disease, were staged according to the TNM classification (7/8 edition). Taking into account the existing clinical recommendations, TNM staging was carried out depending on the localization of the primary tumor focus.

The distribution of patients for each descriptor is presented in Table 9.

Table 9 – Distribution of neuroendocrine gastrointestinal tumors according to the TNM system (7th/8th edition) (n=298)

| T | Quantity, abs. (%) |
|---|---|
| 1 | 90 (30,2%) |
| 2 | 88 (29,53%) |
| 3 | 55 (18,46%) |
| 4 | 40 (13,42%) |
| x (not defined) | 25 (8,39%) |
| N | Quantity, abs. (%) |
| 0 | 151 (50,67%) |
| 1 | 93 (31,21%) |
| 2 | 43 (14,43%) |
| 3 | 1 (0,34%) |
| x (not defined) | 10 (3,36%) |
| M | Quantity, abs. (%) |
| 0 | 187 (62,75%) |
| 1 | 111 (37,25%) |

At diagnosis in most patients, the primary tumor focus was placed in the categories T1 – 90 (30.20%) patients (p=0.0008) and T2 – 88 (29, 53%) patients (p=0.0016). In 55 (18.46%) patients, the primary tumor focus was regarded as T3, and in 40 (13.42%) patients as T4. In 25 (8.39%) patients, the determination of the T

descriptor was impossible due to the primary surgical intervention in non-specialized hospitals and the lack of data in the primary medical documentation of patients.

In the majority of patients – 151 (50.67%) lesions of regional lymph nodes were not detected ($p<0.0001$). In 93 (31.21%) patients, the lesion of the regional lymphatic apparatus corresponded to criterion N1. Regional lesion in the volume of N2 was registered only in 43 (14.43%) patients ($p<0.0001$), and in 1 (0.34%) patient – N3. In 10 (3.36%) patients, the lesion of regional lymph nodes at the primary stage was not evaluated.

A comprehensive examination revealed the presence of distant metastases in 111 (37.25%) patients; no signs of dissemination of the process were detected in 187 (62.75%) patients ($p<0.0001$).

After morphological verification of the diagnosis, the degree of malignancy of the tumor process was determined in all patients, which has an important prognostic and predictive value for the nosology under consideration. The distribution of patients depending on the degree of malignancy of the tumor process is presented in Table 10.

Table 10 – Distribution of patients with gastrointestinal NET according to the degree of malignancy of the tumor (n=298)

| G | Quantity, abs. (%) |
|---|---|
| 1 | 144 (48,32%) |
| 2 | 115 (38,59%) |
| 3 | 39 (13,09%) |

Most of the tumor samples of most patients had a grade of malignancy G1 – 144 (48.32%) of the sample or G2 – 115 (38.59%). Only in 39 (13.09%) patients with morphological examination the degree of malignancy G3 ($p <0.00001$).

During the examination of patients in the framework of the initial admission under the conditions of the GCD, all patients were assessed for the presence of pain syndrome associated with the underlying disease (Table 11).

Table 11 – The presence of pain syndrome in patients with gastrointestinal NET (at the time of initial diagnosis) (n=298)

| Pain syndrome | Quantity, abs. (%) |
|---|---|
| Absent | 140 (46,98%) |
| The presence of pain syndrome | 158 (53,02%) |
| periodic pain | 99 (62,66%) |
| constant pain | 59 (37,34%) |

Pain syndrome was registered in 158 (53.02%) patients at the time of diagnosis of gastrointestinal NET, and in the majority of patients – 99 (62.66%) pain was periodic, and 59 (37.34%) patients felt pain constantly ($p<0.0001$).

But the presence of signs of carcinoid syndrome at the time of the initial diagnosis was recorded less frequently (Table 12).

Table 12 – The presence of carcinoid syndrome in patients with gastrointestinal NET (at the time of initial diagnosis) (n=298)

| Carcinoid syndrome | Quantity, abs. (%) |
|---|---|
| Absent | 257 (86,24%) |
| The presence of carcinoid syndrome | 41 (13,76%) |
| hot flashes | 21 (51,22%) |
| abdominal pain | 15 (36,59%) |
| diarrhea | 35 (85,37%) |
| The number of symptoms | |
| one | 18 (43,90%) |
| two | 18 (43,90%) |
| three | 5 (12,20%) |

The frequency and severity of carcinoid syndrome were evaluated in the study group. In a significant majority of patients – 257 (86.24%) – at the time of the onset of the disease, no manifestations of carcinoid syndrome were registered ($p<0.0001$).

Among 41 (13.76%), 21 (51.22%) patients felt hot flashes, 15 (36.59%) patients had abdominal pain, 35 (85.37%) patients had diarrhea. In 18 (43.90%) patients, the carcinoid syndrome was manifested by only one symptom – hot flashes or diarrhea. A combination of two symptoms was registered in 18 (43.90%): hot flashes and abdominal pain were registered in 2 (4.88%) patients, hot flashes in combination with diarrhea and abdominal pain in combination with diarrhea – in 8 (19.51%), respectively. In 5 (12.20%) patients, at the time of the initial diagnosis of gastrointestinal NET, the carcinoid syndrome was manifested by a combination of all three symptoms. Our analysis showed that in patients with NET gastrointestinal tract at the time of the appearance of the first symptoms of the disease or the detection of a tumor process during a routine dispensary examination, the presence of carcinoid syndrome is rare.

## 3.2 Characteristics of the treatment performed

The distribution of patients by treatment method is presented in Table 13.

Table 13 – Distribution of patients with neuroendocrine gastrointestinal tumors by the method of initial treatment (n=298)

| Type of initial treatment | Quantity, abs. (%) |
|---|---|
| Surgical treatment | 239 (80,20%) |
| Drug therapy | 53 (17,78%) |
| Symptomatic therapy | 3 (1,01%) |
| Observation | 3 (1,01%) |
| Total | 298 (100%) |

The majority of patients with gastrointestinal NET underwent surgical treatment as the main one after the verification of the tumor process and the establishment of a clinical diagnosis: the proportion of patients who were operated on at the first stage of treatment was 80.20% ($p<0.00001$). These data correlate with Table 9, which suggests that the detection of tumors at an early stage allows performing surgical treatment at the first stage. 53 (17.78%) patients received drug therapy with the first stage of treatment. Those who received only symptomatic therapy or dynamic follow-up were significantly less: their proportion was 1.01% (3/298) and 1.01% (3/298), respectively ($p<0.00001$).

Despite the fact that the surgical method was the main method of initial treatment, the nature of the surgical intervention was not always radical (Table 14).

Table 14 – Distribution of patients with gastrointestinal NET who received surgical treatment by type of intervention (n=239)

| The scope of surgical intervention | Quantity, abs. (%) |
|---|---|
| Radical surgery | 177 (74,06%) |
| Cytoreductive surgical treatment | 55 (23,01%) |
| Exploratory laparotomy | 7 (2,93%) |
| Total | 239 (100%) |

According to the analyzed data, radical surgical treatment was performed in 177 (74.06%) of 239 patients, cytoreductive surgery – in 55 (23.01%). Only exploratory laparotomy was performed in 7 (2.93%) patients.

Those patients who failed to perform surgical treatment received first-line drug therapy with somatostatin analogues, antitumor cytostatics or their combinations. The distribution of patients according to the first-line drug therapy schemes as primary treatment is presented in Table 15.

Table 15 – Distribution of patients with gastrointestinal NET according to the schemes of the 1st line of therapy and the degree of differentiation

| Drug therapy regimens | Quantity, abs. (%) | G1 (12) | G2 (27) | G3 (14) |
|---|---|---|---|---|
| Somatostatin analogues | 15 (28,3%) | 8 (66,7%) | 14 (51,9%) | 2 (14,3%) |
| Oral fluoropyrimidines | 6 (11,32%) | – | 4 (14,8%) | 2 (14,3%) |
| Interferon alpha + somatostatin analogues | 6 (11,32%) | 2 (16,6%) | 2 (7,4%) | 3 (21,5%) |
| Interferon Alpha | 6 (11,32%) | – | – | – |
| EP | 4 (7,55%) | – | 1 (3,7%) | 3 (21,5%) |
| Somatostatin analogues + oral fluoropyrimidines | 3 (5,66%) | – | – | – |
| FOLFOX | 3 (5,66%) | – | 2 (7,4%) | 1 (7,1%) |
| Platinum preparations (monotherapy) | 1 (1,89%) | – | – | 1 (7,1%) |
| Analogues of somatostatin + etoposide | 1 (1,89%) | – | – | – |
| GP | 1 (1,89%) | – | – | 1 (7,1%) |
| Other | 7 (13,21%) | 2 (16,7%) | 4 (14,8%) | 1 (7,1%) |

Somatostatin analogues at the first stage of specialized treatment were prescribed in the majority of patients – 15 (28.30%), p=0.0554. Oral fluoropipimidines (tegafur, capecitabine), interferon alpha in combination with somatostatin analogues and interferon alpha in a single mode were prescribed in six patients, respectively, each scheme, accounting for a proportion of 11.32% (6/53). Four patients (7.55% (4/53)) as the first stage of treatment, we received the EP regimen (etoposide + a platinum-series drug). Three patients received somatostatin analogues in combination with oral fluoropyrimidines – 5.66% (3/53). The remaining three types of drug therapy were prescribed each in one patient, namely platinum preparations, somatostatin analogues in combination with etoposide, GP regimen (gemcitabine + cisplatin), accounting for a share of 1.89% (1/53) for therapy regimens, respectively. In 7/53 (13.21%) patients, there was no information about the prescribed systemic therapy regimen in the primary documentation.

The median OS of patients with gastrointestinal NET at the time of the data cut was not reached (Figure 1).

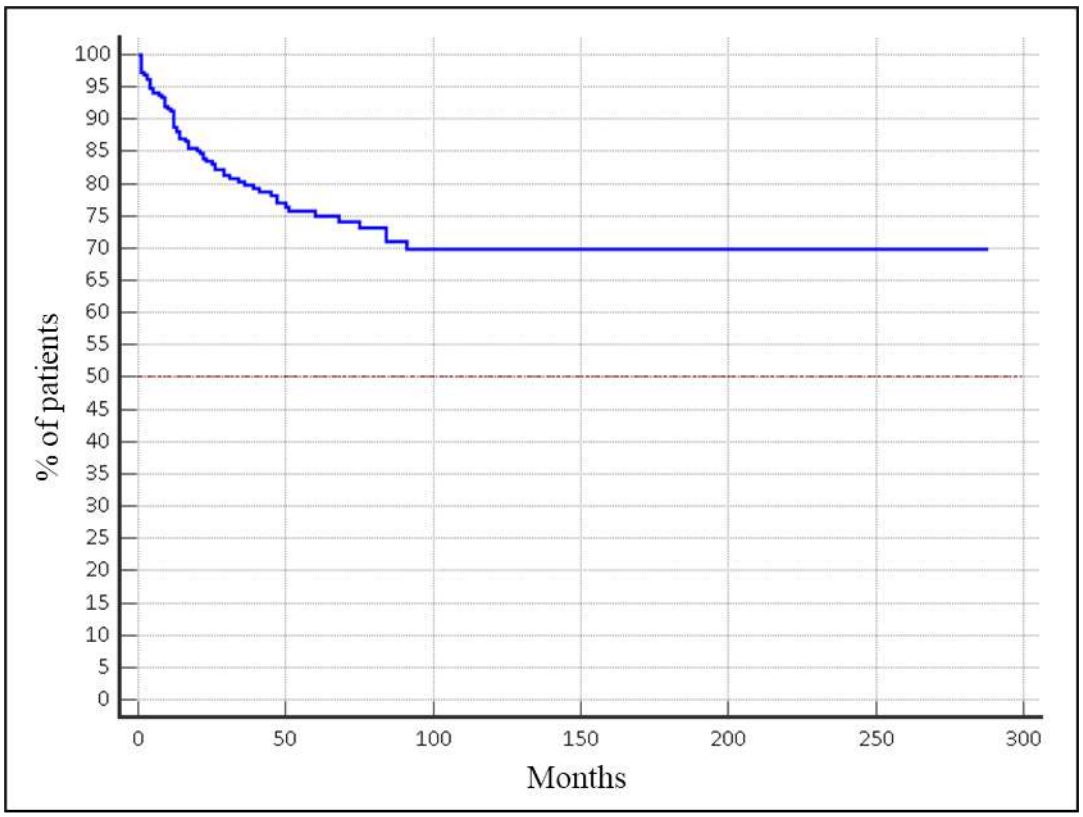


Figure 1 – Overall survival of patients with gastrointestinal NET

The average life expectancy of patients in the cohort under consideration was 210.40±8.51 months (95% CI 193.72-227.08).

The median PFS of patients with gastrointestinal NET at the time of the data cut is shown in Figure 2.

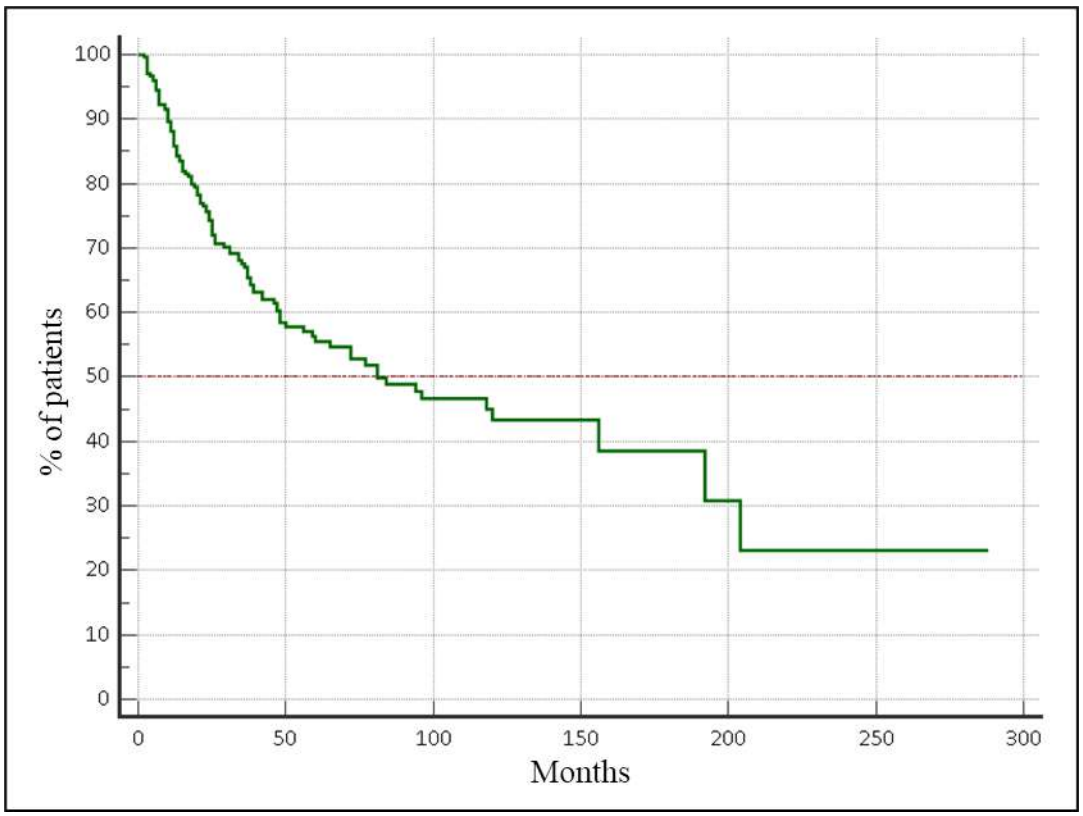


Figure 2 – Progression-free survival in patients with gastrointestinal NET

The median PFS in the cohort of patients under consideration was 81.00 months (95% CI 59.00-156.00).

When analyzing the influence of clinical and morphological factors of the disease on the survival rates of patients, the following results were obtained.

The analysis of the influence of the degree of malignancy of the tumor on the overall survival rate of patients with gastrointestinal NET is presented in Figure 3.

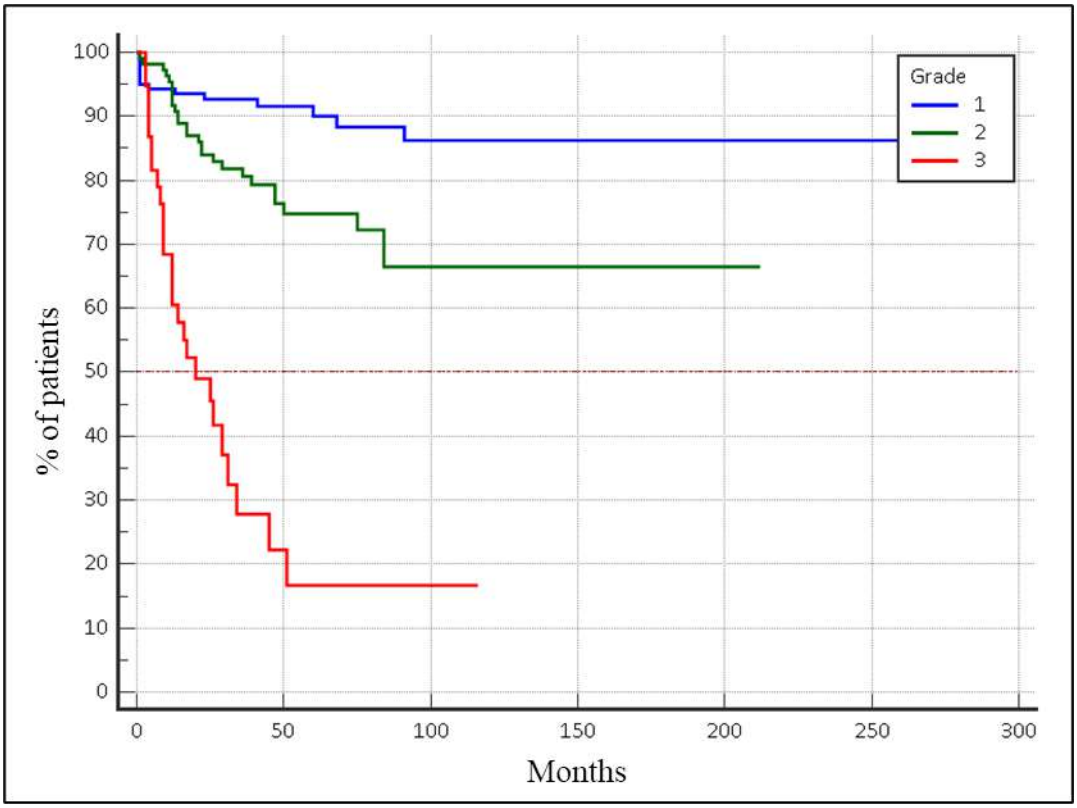


Figure 3 – Overall survival of patients with gastrointestinal NET depending on the degree of malignancy of the tumor

The median S of patients whose tumor corresponds to the grade of malignancy G1 was not reached at the time of data collection and significantly ($p<0.0001$) exceeds the median S of patients with G2 (HR=0.39, 95% CI 0.24-0.65), which was also not reached at the time of data cut) and G3, which was 20.0 months (95% CI 12.00-31.00) (HR=0.09, 95% CI 0.04-0.21). The most unfavorable group are patients whose tumor has a grade of G3 malignancy, since their OS index is significant ($p<0.0001$) worse than both the index of G1 patients and G2 patients with gastrointestinal NET (HR=4.48, 95% CI 1.81-11.07).

Regarding the assessment of the influence of the degree of malignancy of the tumor process on the survival rate without progression of patients with gastrointestinal NET, a similar trend is observed (Figure 4).

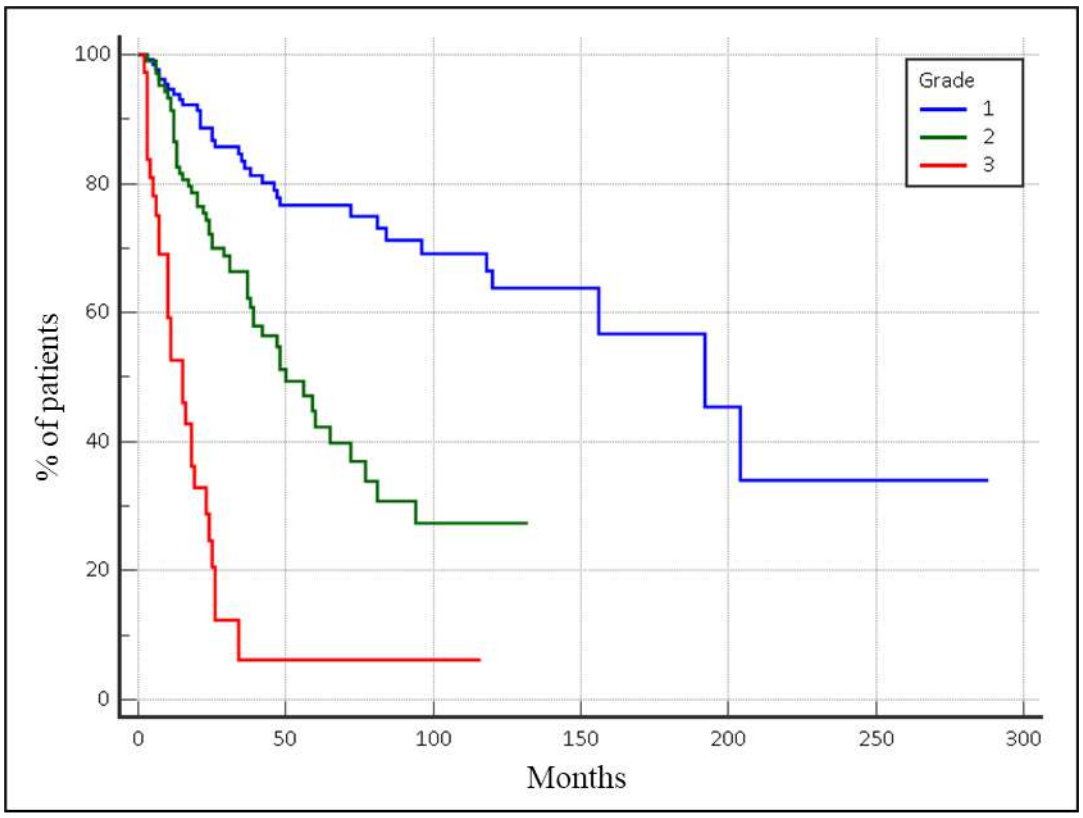


Figure 4 – Progression-free survival in patients with gastrointestinal NET depending on the degree of malignancy of the tumor

The median PFS of patients whose tumor has a grade of G1 malignancy was 192.0 months (95% CI 156.0-204.0) and significantly (by 142.0 months) exceeded the median PFS of patients with G2, where it was only 50.0 months (95% CI 38.0-72.0) (HR=0.40, 95% CI 0.27-0.59) ($p<0.0001$). In patients whose tumor had a grade of G3 malignancy, the median PFS was only 15.0 months (95% CI 10.0-19.0), and the risk of disease progression was the highest, even when compared with patients whose tumor corresponded to G 2 (HR=3.59, 95% CI 1.53-8.41) ($p<0.0001$).

The effect of the localization of the primary tumor focus on the indicator of OS in patients with gastrointestinal NET is shown in Figure 5.

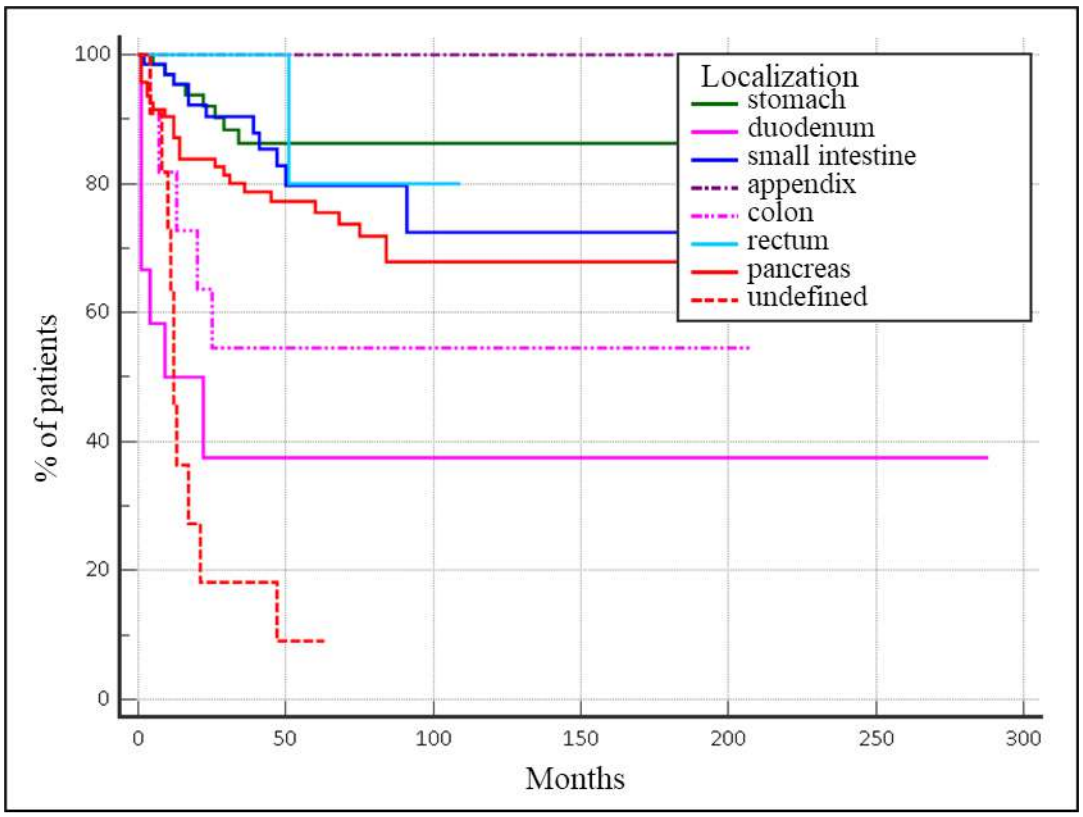


Figure 5 – Overall survival of patients with gastrointestinal NET depending on the location of the primary tumor focus

Localization of the primary tumor in the area of the duodenum (duodenum) and NET without an identified primary focus turned out to be factors that negatively affect the indicator of patients. The median S of patients whose primary tumor focus could not be determined (WPL) was 12.0 months and was significantly ($p<0.0001$) less than all other groups (with the exception of patients whose tumor was localized in the area of the duodenum) (HR=6.16, 95% CI 1.20-31.68). The median of patients with primary tumor foci localized in the area of the duodenum was equal to only 9.0 months (95% CI 1.0-22.0) and significantly differed from the median of patients with primary tumor foci of other primary localizations (HR=7.03, 95% CI 1.28-38.76). In the remaining subgroups of patients with gastrointestinal NET, the median S at the time of the data cut was not reached.

The effect of the localization of the primary tumor focus on the index of PFS in patients with gastrointestinal NET is shown in Figure 6.

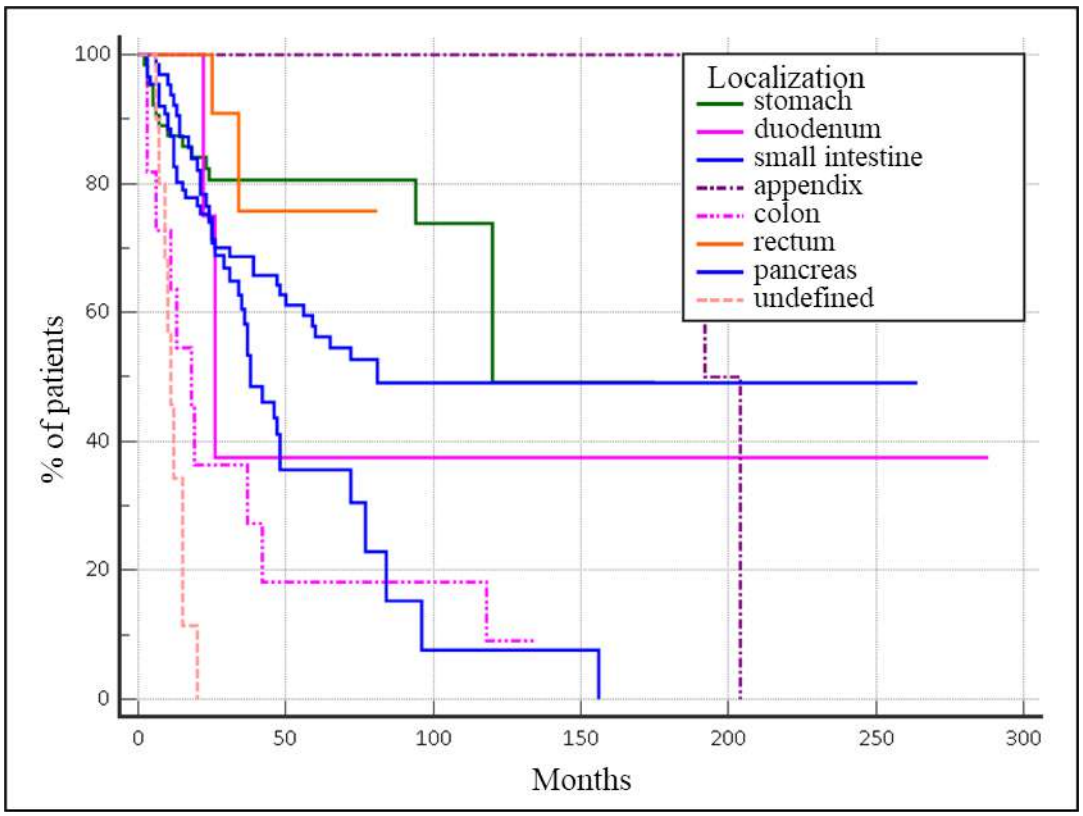


Figure 6 – Progression-free survival in patients with gastrointestinal NET depending on the location of the primary tumor focus

At the time of the data cut, the median of IBP was not reached only in the group of patients, the primary tumor focus was localized in the rectum. The greatest median of PFS was determined in patients whose primary tumor focus was localized in the area of the appendix, where it was 192.0 months (95% CI 192.0-204.0) and significantly ($p<0.0001$) exceeded the median of PFS in the group of patients with a primary focus in the small intestine (median PFS 38.0 months (95% CI 34.0-156.0); HR=0.25, 95% CI 0.10-0.64), colon (median PFS 18.0 months (95% CI 3.0-42.0); HR=0.13, 95% CI 0.03-0.51) and without identified primary focus (median PFS 11.0 (95% CI 6.0-20.0); HR=0.06, 95% CI 0.01-0.38). Median PFS in patients with gastric NET was 120.0 months (95% CI 120.0-120.0), pancreatic NET - 81.0 months (50.0-81.0), NET duodenum – 26.0 months (95% CI 22.0-26.0).

The treatment, of course, had a significant impact on the survival rates of patients with gastrointestinal NET. The surgical component of the treatment was important.

The effect of surgical treatment on the indicator of OS in patients with gastrointestinal NET is shown in Figure 7.

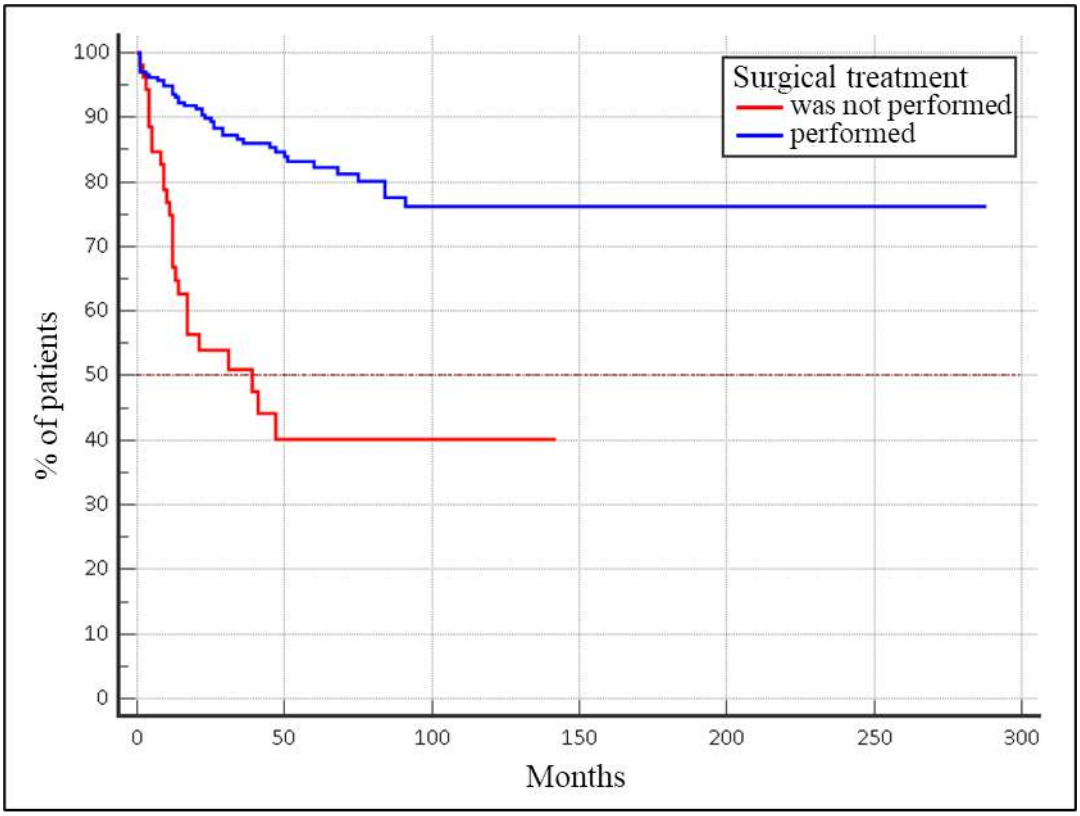


Figure 7 – Overall survival of patients with gastrointestinal NET depending on the surgical stage of treatment

The median OS of patients who underwent surgical treatment was not reached at the time of the data cut (the average OS was 228.49±8.80 months (95% CI 211.25-245.74), and the risk of death of patients during the surgical aid significantly decreased: HR=0.09, 95% CI 0.04-0.19; $p<0.0001$, according to compared with a group of patients whose complex of therapeutic measures did not include surgical treatment: the median S in this subgroup was 39.0 months (95% CI 13.0-47.0).

The effect of surgical treatment on the GDP of patients with gastrointestinal NET is shown in Figure 8.

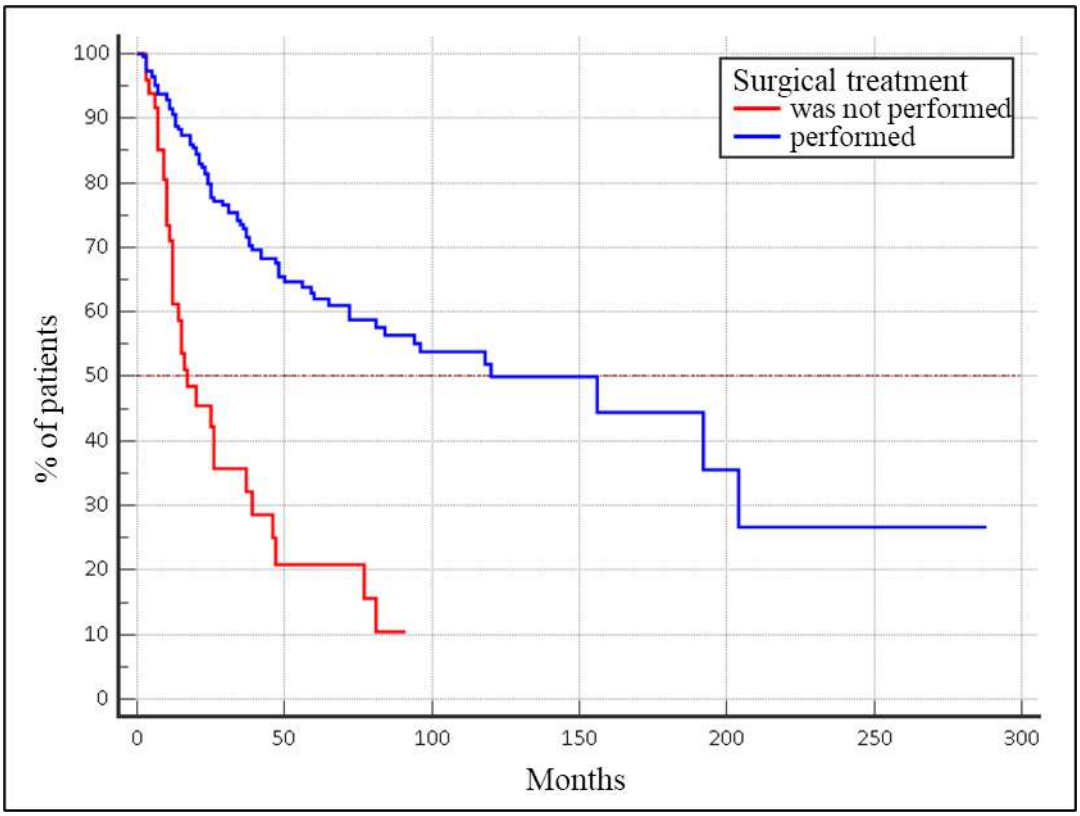


Figure 8 – Progression-free survival in patients with gastrointestinal NET depending on the surgical stage of treatment

The median PFS of patients whose complex of therapeutic measures included the surgical stage was 120.0 months (95% CI 81.0-204.0), which was 103.0 months higher than the median PFS of patients who did not undergo surgical treatment: median PFS 17.0 months (95% CI 12.0-37.0). In other words, treatment significantly reduces the risk of progression of gastrointestinal NET: HR=0.12, 95% CI 0.07-0.23, $p<0.0001$ [2].

# Chapter 4
# INVESTIGATION OF SYSTEMIC INFLAMMATION FACTORS IN NEUROENDOCRINE TUMORS OF THE GASTROINTESTINAL TRACT

## 4.1 General characteristics of patients

To determine the prognostic role of systemic inflammation factors on the course of gastrointestinal NET, we conducted a prospective study, which included 71 patients with gastrointestinal NET treated and observed at the St. Petersburg State Medical Institution "City Clinical Oncological Dispensary" in the period from 2015 to 2021. All patients were treated according to standard protocols from 2015 to 2021.

The main criteria for the inclusion of patients in the planned study:

1. The opportunity and consent to sign a form of voluntary informed consent to participate in the study.
2. The age of patients is over 18 years.
3. Morphologically verified diagnosis of "neuroendocrine tumor of the gastrointestinal tract".

The main criteria for non-inclusion of patients in the study:

1. The registered presence of any inflammatory process in the patient's body (both associated with the tumor process and unrelated to it) within 14 days prior to the patient's inclusion in the study.
2. Taking antibacterial drugs for 14 days before the patient is included in the study.

There were 25 men (35.21%) and 46 women (64.79%) in the study cohort. The age of the patients ranged from 20 to 82 years, the average age of the patient was 54.97±13.88 years (95% CI 51.69-58.26). The general characteristics of the patients included in the study are presented in Table 16.

Table 16 – General characteristics of patients included in the study

| Sign | Abs., (%) |
|---|---|
| Total number of patients | n=71 |
| Gender:<br>men<br>women | <br>25 men (35,21%)<br>46 women (64,79%) |
| Age, average (years) | 54,97 years [20-82] |
| Stage of the tumor process<br>I<br>II<br>III<br>IV | <br>22 (31,88%)<br>6 (8,7%)<br>11 (15,94%)<br>30 (43,48%) |
| Localization of the primary focus:<br>stomach<br>pancreas<br>colon<br>small intestine<br>undefined | <br>11 (15,94%)<br>21 (30,43%)<br>15 (21,74%)<br>15 (21,74%)<br>7 (10,14%) |
| The degree of malignancy<br>G1<br>G2<br>G3 | <br>28 (40,58%)<br>29 (42,03%)<br>12 (17,39%) |
| Ki-67,%: median [Q25-Q75],<br>(min.-max.) | 5,00 [2,00-14,50]<br>(1,00-95,00) |

The majority of 30 patients (43.48%) were diagnosed with stage IV of the disease at the initial treatment, 22 (31.88%) patients had stage I. Stage III was typical for 11 (15.94%) cases. The most rare – 6 (8.7%) were diagnosed with stage II of the disease.

In the majority of patients – 21 (30.43%) the primary tumor focus was localized in the pancreas, in 15 (21.74%) in the small intestine, in 15 (21.74%) in the colon. Tumors localized in the stomach were found in 11 (15.94%) cases. In 7 (10.14%) – without a primary focus.

Immunohistochemical examination revealed the degree of G1 malignancy in the majority of patients – 28 (40.58%). The degree of G2 malignancy was found in 29 (42.03%) cases. The most rare – 12 (17.39%) - was the degree of malignancy of G3.

The level of proliferative activity measured by the level of Ki-67 expression varied from 1.00% to 95.00%. The median expression level of Ki-67 was 5.00 [2.00-14.50].

## 4.2 Assessment of systemic inflammation factors

To assess the effect on the median progression-free survival before the start of treatment of patients with gastrointestinal NET, the following indicators were evaluated:

1. Peripheral blood leukocytes.
2. Neutrophils of peripheral blood.
3. Peripheral blood lymphocytes.
4. Peripheral blood monocytes.
5. Peripheral blood eosinophils.
6. Peripheral blood platelets.

In order to assess the level of endogenous intoxication of the patient's body (based on the data obtained during the clinical analysis of peripheral blood at the initial assessment stage before the start of treatment), the following indices were calculated:

1. Neutrophil-lymphocyte index (NLI): the ratio of the absolute number of neutrophils to the absolute number of lymphocytes.

2. Platelet-lymphocyte index (TLI): the ratio of the absolute number of platelets to the absolute number of lymphocytes.

3. Lymphocyte-monocyte index (LMI): the ratio of the absolute number of lymphocytes to the absolute number of monocytes.

Derivative of NLI (dNLR): the calculation formula is as follows: dNLR=Absolute number of neutrophils / (leukocytes – the absolute number of neutrophils).

At the next stage of the study, using ROC analysis, the threshold values of each of the analyzed indicators were determined, as well as an assessment of the survival rates of patients of the cohort under study, depending on the indicators of general body reactions and indicators of the level of the studied calculated blood parameters (systemic inflammation factors).

The results of the analysis of peripheral blood parameters of patients are presented in Table 17.

Table 17 – Peripheral blood parameters of patients with gastrointestinal NET

| Indicator | All patients (n=71) | | |
|---|---|---|---|
| | median (Me)/ Mean (M±SD) | quartiles [Q25-Q75]/ 95% CI | min-max |
| Leukocytes, $\times 10^9$/l | 6,15 | [4,50-7,60] | 2,2-20,75 |
| Neutrophils, $\times 10^9$/l | 3,53 | [2,39-4,64] | 1,01-17,51 |
| Lymphocytes, $\times 10^9$/l | 1,86±0,71 | 1,70-2,04 | 0,61-3,74 |
| Lymphocytes, % | 31,08±12,12 | 28,12-34,04 | 6,40-63,60 |
| Monocytes, $\times 10^9$/l | 0,57 | [0,40-0,7574] | 0,06-1,70 |
| Monocytes, % | 8,65 | [7,40-10,95] | 1,60-18,00 |
| Eosinophils, $\times 10^9$/l | 0,12 | [0,06-0,20] | 0,00-0,36 |
| Eosinophils,% | 2,11 | [1,00-2,90] | 0,00-6,50 |
| Platelets, $\times 10^9$/l | 241,50 | [189,00-327,00] | 120,00-565,00 |

The absolute number of peripheral blood leukocytes in the study cohort of patients ranged from $2.2\times10^9$/l to $20.75\times10^9$/l, the median was $6.15\times10^9$/l [4.50-7.60]. The absolute number of neutrophils varied from $1.01\times10^9$/l to $17.51\times10^9$/l, the median was $3.53\times10^9$/l [2.39-4.64]. Analysis of the lymphocyte level showed that the median absolute number of lymphocytes was $1.86\pm0.71\times10^9$/l [1.70-2.04], with a

minimum value of $0.61\times10^9$/l, and a maximum of $3.74\times10^9$/l. The relative number of lymphocytes varied in the range of 6.40-63.60%, the average value was 31.08±12.12% (95% CI 28.12-34.04%). The median absolute number of peripheral blood monocytes was $0.57\times10^9$/l [0.40-0.7574], the minimum value was $0.06\times10^9$/l, the maximum value was $1.70\times10^9$/l. The relative number of monocytes ranged from 1.60% to 18.00%, the average was 8.65% (95% CI 7.40-10.95). Analysis of the level of peripheral blood eosinophils revealed that the median absolute number of eosinophils was $0.12\times10^9$/l [0.06-0.20], with its minimum value equal to $0.00\times10^9$/l, the maximum – $0.36\times10^9$/l; the median relative number of eosinophils was 2.11% [1.00-2.90], with its minimum value equal to 0.00%, the maximum – 6.50%. The median level of peripheral blood platelets in patients was $241.00\times10^9$/l [189.00-327.00], the minimum platelet level was $120.00\times10^9$/l, the maximum was $565.00\times10^9$/l.

Taking into account the data obtained, relative indices were calculated in order to assess the level of endogenous intoxication. The results obtained are presented in Table 18.

Table 18 – Relative indexes

| Index | All patients (n=71) | | |
|---|---|---|---|
| | median (Me) | quartiles (Q25-Q75) | min-max |
| Neutrophil-lymphocytic | 1,91 | 1,20-3,05 | 0,51–12,43 |
| Platelet-lymphocytic | 134,88 | 101,92-211,28 | 61,50-445,90 |
| Lymphocytic-monocytic | 3,18 | 2,24-4,92 | 0,89-41,33 |

The median neutrophil-lymphocytic index was 1.91 [1.20-3.05], with a minimum index of 0.51 and a maximum of 12.43. The platelet-lymphocyte index ranged from 61.50-445.90; the median aphid was 134.88 [101.92-211.28]. The median lymphocytic-monocytic index was 3.18 [2.24-4.92]; its minimum value was 0.89, the maximum was 41.33.

## 4.3 Research results

### *4.3.1 The results of the evaluation of the diagnostic significance of the proliferative activity of the tumor and factors of systemic inflammation in patients with neuroendocrine tumors of the gastrointestinal tract*

At this stage of the study, a ROC analysis was performed to identify the prognostic significance and optimal threshold values (cut-off) of the Ki-67 proliferative activity index and systemic inflammation factors and indices characterizing the level of endogenous intoxication of patients with gastrointestinal NET.

The assessment of the influence of the index of proliferative activity and the considered factors of systemic inflammation on the time without progression of patients with gastrointestinal NET is presented in Table 19.

Table 19 – Evaluation of the effect of the Ki-67 proliferative activity index and peripheral blood parameters on the time without progression of patients with gastrointestinal NET (ROC analysis results)

| Indicator | Area under the curve (AUC) (95% CI) | p-value | Cut-off threshold (cut-off) | Sensitivity | Specificity |
|---|---|---|---|---|---|
| Ki-67, % | 0,698±0,062 (0,577-0,802) | 0,0015 | >5 | 61,76 | 69,44 |
| White blood cells, $\times 10^9$/l | 0,532±0,071 (0,408-0,654) | 0,646 | >6,5 | 48,48 | 63,89 |
| Neutrophils, $\times 10^9$/l | 0,594±0,070 (0,469-0,711) | 0,179 | >3,05 | 72,73 | 55,56 |
| Neutrophils, % | 0,629±0,072 (0,500-0,746) | 0,074 | >58,3 | 58,06 | 76,47 |

Continuation of table 19

| Indicator | Area under the curve (AUC) (95% CI) | p-value | Cut-off threshold (cut-off) | Sensitivity | Specificity |
|---|---|---|---|---|---|
| Lymphocytes, $\times 10^9$/l | 0,633±0,067 (0,508-0,746) | 0,053 | ≤2,26 | 87,88 | 41,67 |
| Lymphocytes, % | 0,651±0,070 (0,523-0,764) | 0,030 | ≤30,0 | 68,75 | 67,65 |
| Eosinophils, $\times 10^9$/l | 0,554±0,071 (0,429-0,675) | 0,442 | >0,04 | 87,50 | 27,78 |
| Eosinophils, % | 0,515±0,073 (0,388-0,641) | 0,835 | >0,6 | 37,17 | 73,47 |
| Monocytes, $\times 10^9$/l | 0,539±0,073 (0,412-0,663) | 0,589 | >0,37 | 87,10 | 28,57 |
| Monocytes, % | 0,539±0,073 (0,403-0,660) | 0,658 | >8,5 | 60,00 | 54,55 |
| Platelets, $\times 10^9$/l | 0,512±0,071 (0,388-0,634) | 0,868 | >342 | 24,24 | 88,89 |

Most of the analyzed peripheral blood parameters did not have a significant effect on the time without progression of patients: the models obtained were statistically insignificant ($p>0.05$), and the area under the ROC curve varied from 0.512±0.071 to 0.651±0.070, which indicated the unsatisfactory quality of the models.

During the analysis, threshold values were determined for all indicators (cut-off).

Significant models were obtained only by analyzing the effect of the Ki-67 proliferative activity index, the relative number of neutrophils, and the absolute and relative number of lymphocytes on the time without progression.

The area under the ROC curve characterizing the effect of Ki-67 expression level on the time without progression of patients with gastrointestinal NET was 0.698±0.062 (95% CI 0.577-0.802). This model was statistically significant (p=0.0015), the quality of the model was average (Figure 9).

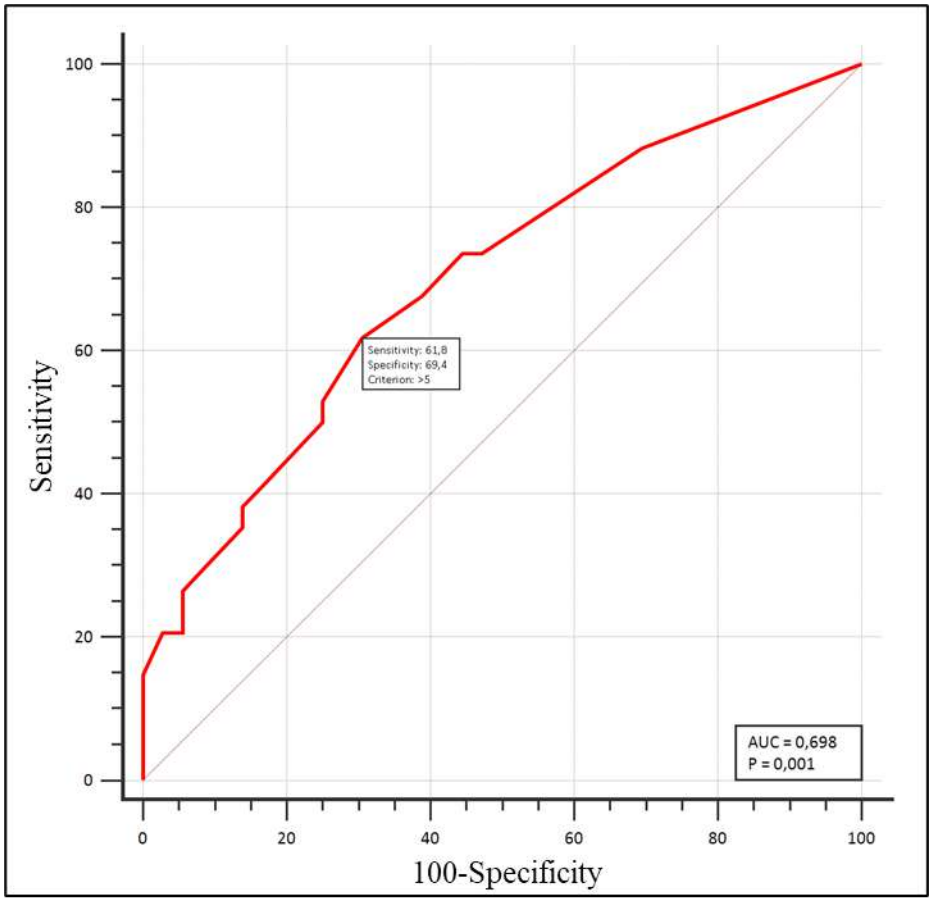


Figure 9 – Dependence of
progression-free time on Ki-67 expression level

The optimal threshold level of Ki-67 expression at the cut-off point was 5.00%: the level of Ki-67 expression, not reaching or equal to 5.00%, indicated a possible favorable course of the disease and a lower risk of progression. The level of Ki-67 expression exceeding the threshold value of 5.00% had a negative effect on the indicator of time without progression in patients with gastrointestinal NET. The sensitivity of this test was only 61.76%, and the specificity was 69.44%, which

indicates that this test can be considered both screening and confirmation. However, the predictive value of this test will be average.

The area under the ROC curve, indicating the effect of the relative number of neutrophils on the time without progression of patients with gastrointestinal NET, was 0.629± 0.072 (95% CI 0.500-0.746). This model was statistically significant (p=0.074), although the quality of the model was average (Figure 10).

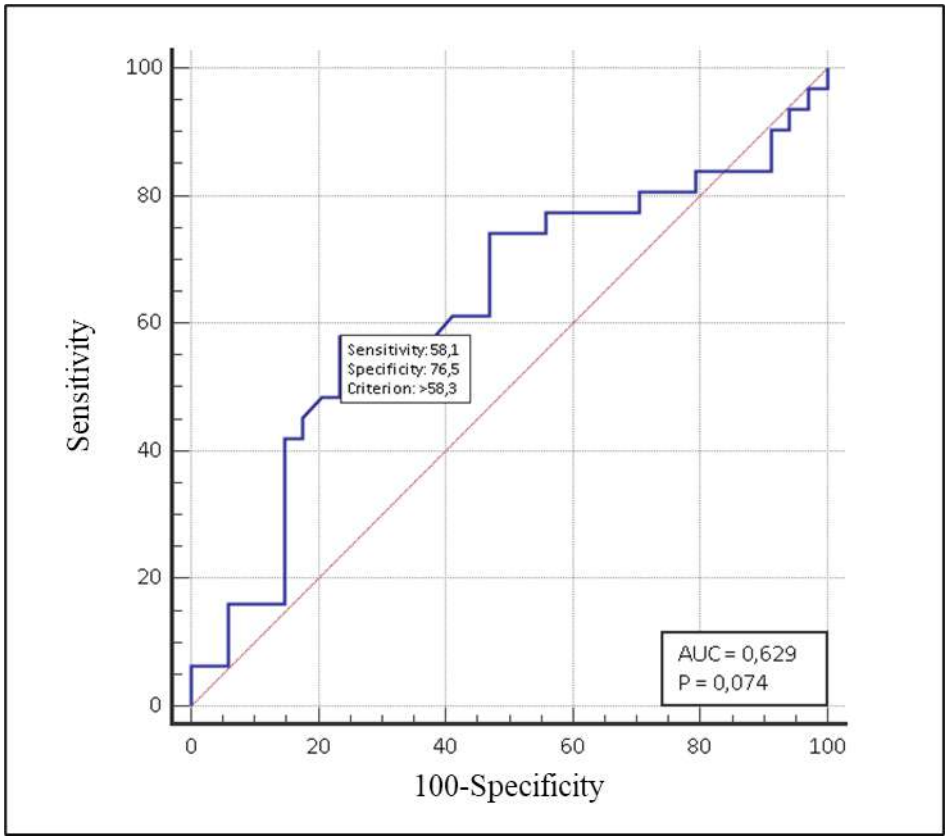


Figure 10 – Dependence of progression-free time
on the relative number of peripheral blood neutrophils

The optimal threshold value of the absolute number of lymphocytes at the cut-off point was $2.26\times10^9$/l: the absolute number of lymphocytes equal to or exceeding the value "$2.26\times10^9$/l" indicated a possible favorable course of the disease and a longer life expectancy. The absolute number of lymphocytes, which did not reach the threshold value of $2.26\times10^9$/l, had a negative impact on the duration of patients with gastrointestinal NET. The sensitivity of this test was only 87.6%, and the specificity

was 41.7%, which indicates that this test can be considered both screening and confirmation. However, the predictive value of this test will be average.

Another potential prognostic indicator is the absolute number of peripheral blood lymphocytes. The area under the ROC curve characterizing the effect of the absolute number of lymphocytes on the life expectancy of patients with gastrointestinal NET was 0.633±0.067 (95% CI 0.508-0.746). This model was statistically insignificant (p=0.053), but with this value of p-value it is possible to talk about the presence of a tendency to the influence of the indicator. Unfortunately, the quality of the model was unsatisfactory (Figure 11).

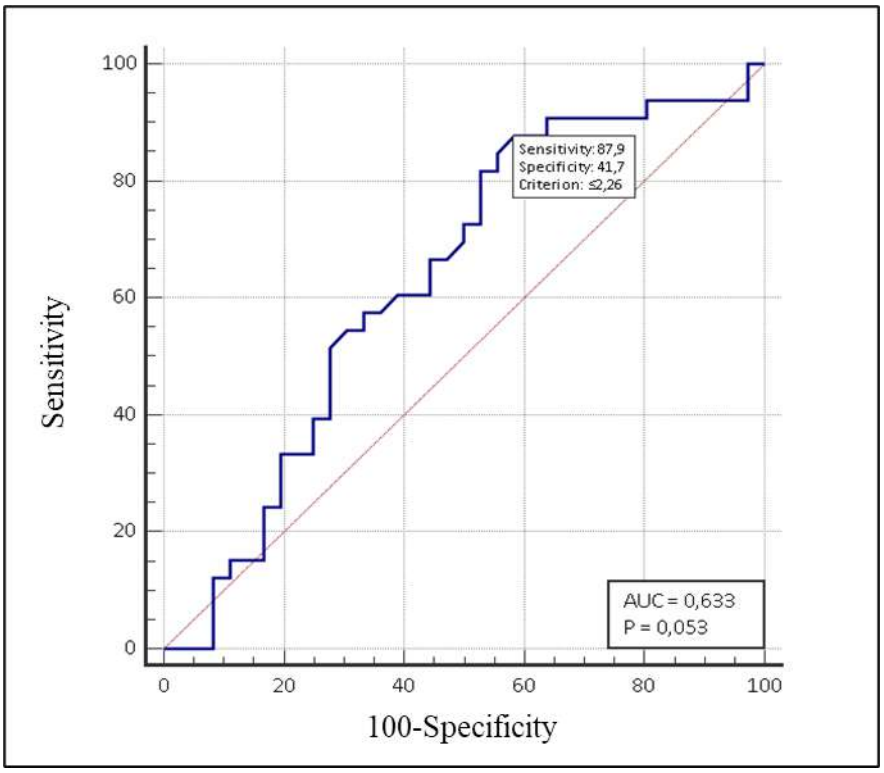


Figure 11 – Time dependence without progression
from the absolute number of peripheral blood lymphocytes

The optimal threshold value of the relative number of lymphocytes at the cut-off point was 30.0%: the relative number of lymphocytes equal to or exceeding the value of "30.0%" indicated a possible favorable course of the disease and a longer life expectancy. The absolute number of lymphocytes, which did not reach the threshold

value of 30.0%, had a negative impact on the life expectancy of patients with gastrointestinal NET. The sensitivity of this test was only 68.7%, and the specificity was 67.6%, which indicates that this test can be considered both screening and confirmation. However, the predictive value of this test will be average (Figure 12).

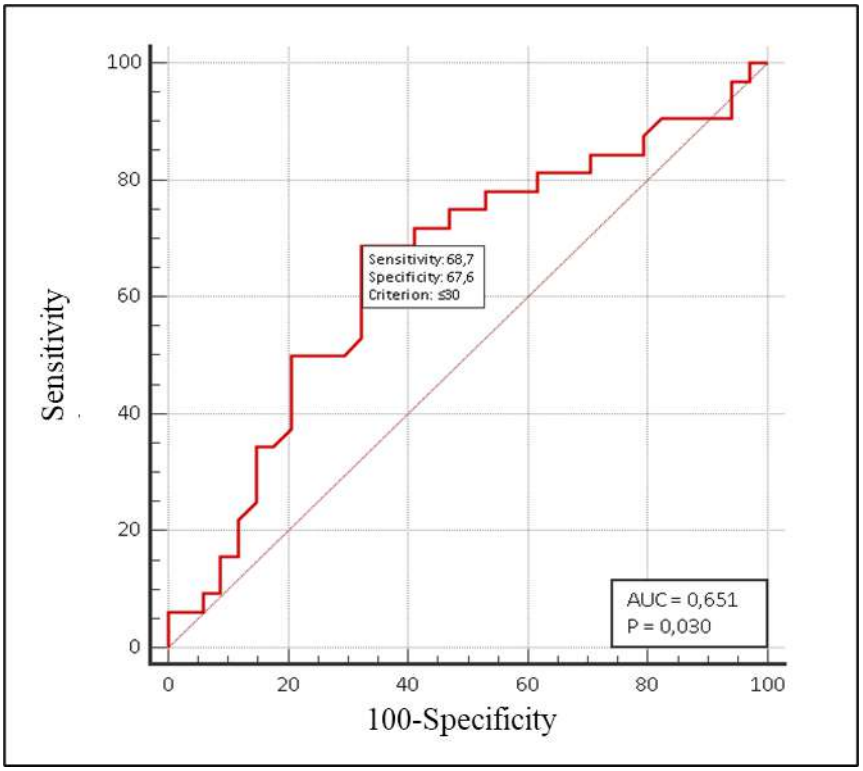


Figure 12 – Dependence of progression-free time
on the relative number of peripheral blood lymphocytes

The optimal threshold value of the relative number of neutrophils at the cut-off point was 58.3%: the relative number of neutrophils exceeding 58.3% was a factor in the negative prognosis of disease progression. On the contrary, the relative number of neutrophils equal to or below the threshold value of 58.3% had a positive effect on the indicator of time without progression of patients with gastrointestinal NET. However, the sensitivity of this test was 58.06%, and the specificity was 76.47%, which does not allow us to consider this test as a screening test, but it can definitely be considered as a confirmatory (confirmatory) prognostic indicator [10].

### *4.3.2 Analysis of the prognostic significance of relative indices characterizing the level of endogenous intoxication for a time without progression in patients with neuroendocrine tumors of the gastrointestinal tract*

When assessing the prognostic significance of relative indices characterizing the level of endogenous intoxication of patients, all the relationships under consideration had a significant impact on the indicator of time without progression of patients. A threshold value (cut-off) was determined for all indicators. The results of the analysis are presented in Table 20.

Table 20 – Evaluation of the effect of relative indices characterizing the level of endogenous intoxication on the time without progression of patients with gastrointestinal NET (results of ROC analysis)

| Index | Area under the curve (AUC) | p-value | Cut-off threshold (cut-off) | Sensitivity | Specificity |
|---|---|---|---|---|---|
| Neutrophil-lymphocytic | 0,641±0,069 (0,516-0,753) | 0,0415 | >1,85 | 72,73 | 61,11 |
| dNLR | 0,638±0,069 (0,513-0,750) | 0,0446 | >1,40 | 54,55 | 77,78 |
| Platelet-lymphocytic | 0,590±0,070 (0,465-0,707) | 0,1967 | >170,19 | 45,45 | 75,00 |
| Lymphocytic-monocytic | 0,616±0,071 (0,488-0,733) | 0,1023 | ≤2,90 | 54,84 | 71,43 |

### *4.3.3 Analysis of the prognostic significance of relative indices characterizing the level of endogenous intoxication for a time without progression in patients with neuroendocrine tumors of the gastrointestinal tract*

When assessing the prognostic value of relative indices characterizing the level of endogenous intoxication of patients with gastrointestinal NET, the neutrophil-lymphocytic index, dNLR had a significant effect on the indicator of time without progression of patients. A threshold value (cut-off) was determined for all indicators.

When analyzing the effect of NLI on the time without progression of patients with gastrointestinal NET, the following results were obtained: the area under the ROC curve was 0.641±0.069 (95% CI 0.516-0.753) and, despite the fact that the prognostic model was reliable in general - p=0.0415, the quality of this model was average (Figure 13).

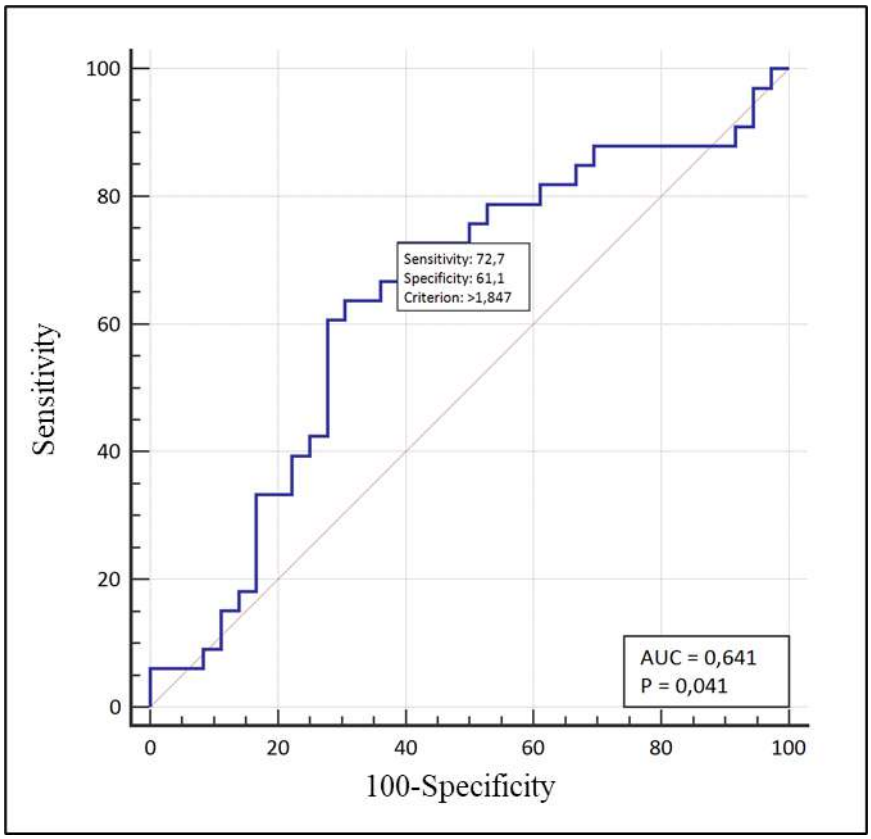


Figure 13 – Results of the ROC analysis of the dependence of the progression-free time index on the neutrophil-lymphocytic index

The optimal threshold value of the NLI at the cut-off point was 1.85: the value of the NLI, greater than the level of 1.85, was a factor of unfavorable prognosis with respect to the time without progression of patients. The value of NLI equal to or less than 1.85, on the contrary, had a positive effect on the indicator of time without progression of patients with gastrointestinal NET. The sensitivity of this test was 72.73%, the specificity was 61.11%, which allows us to consider this test both as a screening and as a confirming (confirmatory) prognostic indicator.

When analyzing the effect of dNLR on the time without progression of patients with gastrointestinal NET, the following results were obtained: the area under the ROC curve was 0.638±0.069 (95% CI 0.513-0.750) and, despite the fact that in general the prognostic model was reliable – p=0.0446, the quality of this model was average (Figure 14).

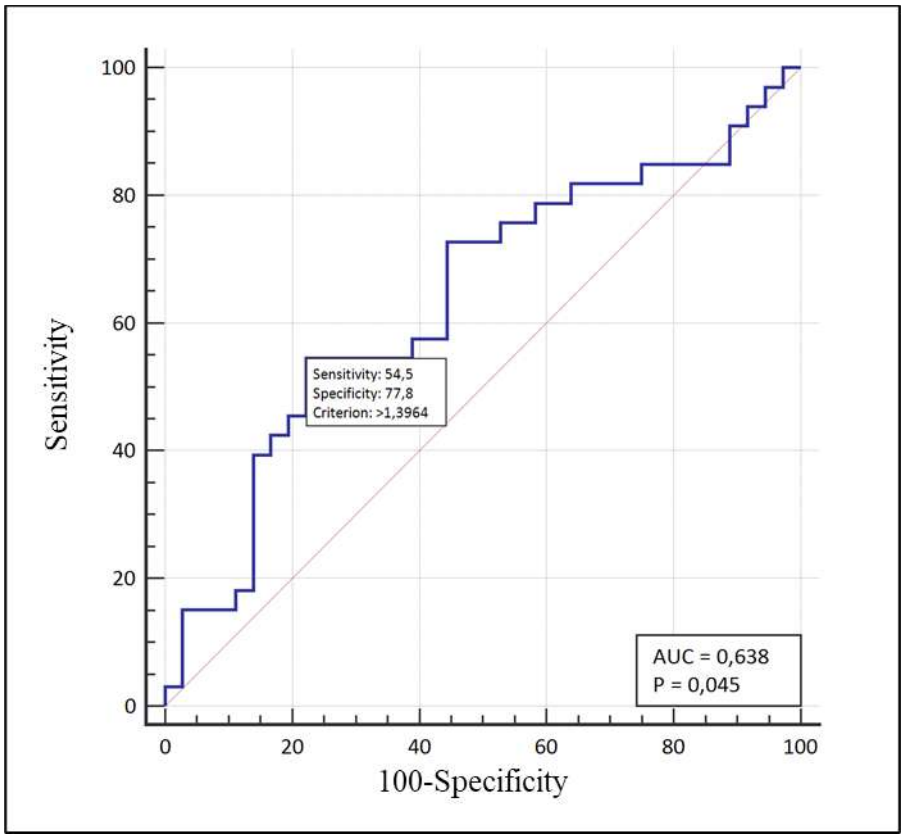


Figure 14 – Results of the ROC analysis of the dependence of the progression-free time indicator on dNLR

The optimal threshold value of dNLR at the cut-off point was 1.40: the value of NLI, greater than the level of 1.40, was a factor of unfavorable prognosis with respect to the time without progression of patients. A dNLR value equal to or less than 1.40, on the contrary, had a positive effect on the indicator of time without progression in patients with gastrointestinal NET. The sensitivity of this test was 54.55%, the specificity was 77.78%, which allows us to consider this test both as a screening and as a confirming (confirmatory) prognostic indicator.

Analysis of the effect of aphids on the time without progression of patients with gastrointestinal NET showed that the area under the ROC curve was 0.590±0.070 (95% CI (0.465-0.707)) and, despite the fact that the prognostic model was not reliable in general – p=0.1967.

The optimal threshold value of aphids at the cut-off point was 170.19: the value of aphids, above the level of 170.19, was a factor of unfavorable prognosis and negatively affected the time without progression of patients. An APHID value equal to or less than 170.19, on the contrary, had a positive effect on the indicator of time without progression of patients with gastrointestinal NET. The sensitivity of this test was 45.45%, the specificity was 75.00%, which allows us to consider this test as a screening test, and its value as a confirmatory (confirmatory) prognostic indicator is low.

When assessing the effect of the LMI level on the time without progression of patients with gastrointestinal NET, the area under the ROC curve was 0.616±0.071 (95% CI 0.488-0.733), the model was reliable - p=0.1023, the quality of the model was average.

The optimal threshold value of LMI at the cut-off point was 2.90: the value of LMI, lower than or equal to the level of 2.90, was a factor of unfavorable prognosis and negatively affected the time without progression of patients. The LMI value greater than 2.90, on the contrary, had a positive effect on the indicator of time without progression of patients with gastrointestinal NET. The sensitivity of this test was 54.84%, the specificity was 71.43%, which allows us to consider this test both as a screening and as a confirming (confirmatory) prognostic indicator.

### *4.3.4 Long-term results of treatment of patients with neuroendocrine tumors of the gastrointestinal tract*

Based on the threshold values of each of the considered indicators of the level of proliferative activity and systemic inflammation of peripheral blood identified by ROC analysis, a one-factor analysis of the time indicator without progression of patients with gastrointestinal NET was carried out.

### *4.3.5 The effect of proliferative tumor activity and systemic inflammation factors on the time without progression of patients with neuroendocrine tumors of the gastrointestinal tract (results of a single-factor analysis)*

The results of a one-factor analysis of the effect of the level of proliferative activity of the tumor and factors of systemic inflammation in peripheral blood on the time without progression of patients with gastrointestinal NET, taking into account the threshold values, are presented in Table 21.

The univariate analysis did not reveal significant differences in time without progression of patients depending on the absolute and relative number of monocytes, absolute and relative number of eosinophils, absolute number of peripheral blood platelets at the initial assessment stage. Moreover, in all cases, the median time without progression did not exceed 39 months.

Table 21 – Influence of the level of proliferative activity of peripheral blood indicators on the time without progression of patients with gastrointestinal NET

| Indicator | Median (month) (95% CI) | Risk ratio (HR) (95% CI) | Log-rank test p-value |
|---|---|---|---|
| Ki-67, % | | | |
| >5 | 15,0 (95% CI 10,0-38,0) | | |
| ≤5 | 84,0 (95% CI 36,0-96,0) | 0,26 (95% CI 0,13-0,55) | 0,0004 |
| White blood cells, $\times10^9$/l: | | | |
| >6,50 | 25,0 (95% CI 11,0-96,0) | | |
| ≤6,50 | 39,0 (95% CI 31,0-59,0) | 0,54 (95% CI 0,26-1,12) | 0,0992 |
| Neutrophils, $\times10^9$/l: | | | |
| >3,05 | 25,0 (95% CI 12,0-96,0) | | |
| ≤3,05 | Not reached | 0,39 (95% CI 0,20-0,79) | 0,0085 |
| Neutrophils, %: | | | |
| >58,30 | 25,0 (95% CI 11,0-96,0) | | |
| ≤58,30 | Not reached | 0,39 (95% CI 0,19-0,79) | 0,0090 |
| Lymphocytes, $\times10^9$/l: | | | |
| ≤2,26 | 31,0 (95% CI 15,0-96,0) | | |
| >2,26 | Not reached | 0,42 (95% CI 0,20-0,89) | 0,0241 |
| Lymphocytes, %: | | | |
| ≤30 | 25,0 (95% CI 12,0-96,0) | | |
| >30 | Not reached | 0,40 (95% CI 0,20-0,81) | 0,0106 |
| Monocytes, $\times10^9$/l: | 36,0 (95% CI 24,0-59,0) | | |
| >0,37 | Not reached | 0,57 (95% CI 0,24-1,34) | 0,1966 |
| ≤0,37 | | | |

Continuation of table 21

| Indicator | Median (month) (95% CI) | Risk ratio (HR) (95% CI) | Log-rank test p-value |
|---|---|---|---|
| Monocytes, %: | | | |
| >8,50 | 31,0 (95% CI 13,0-96,0) | | |
| ≤8,50 | 59,0 (95% CI 20,0-59,0) | 0,75 (95% CI 0,36-1,56) | 0,4386 |
| Eosinophils, $\times 10^9$/l: | | | |
| >0,04 | 34,0 (95% CI 15,0-96,0) | | |
| ≤0,04 | 39,0 (95% CI 25,0-39,0) | 0,60 (95% CI 0,27-1,37) | 0,2265 |
| Eosinophils, %: | | | |
| >0,6 | 34,0 (95% CI 15,0-96,0) | | |
| ≤0,6 | 39,0 (95% CI 9,0-39,0) | 0,73 (95% CI 0,28-1,88) | 0,5137 |
| Platelets, $\times 10^9$/l: | | | |
| >342 | 31,0 (95% CI 6,0-96,0) | | |
| ≤342 | 39,0 (95% CI 25,0-59,0) | 0,55 (95% CI 0,21-1,41) | 0,2141 |

With respect to the indicator of the absolute number of leukocytes, it is possible to talk about the presence of a trend in the increase in PFS in those patients whose initial absolute number of leukocytes was less than or equal to 6.5: 39 months (95% CI 31.0-59.0) versus 25 months (95% CI 11.0-96.0) (p=0.0992; HR 0.54: 95% DI 0.26-1.12).

The other analyzed factors: the level of proliferative activity and peripheral blood parameters had a statistically significant effect on the indicator of time without progression.

The index of proliferative activity – Ki-67, the threshold value of which was determined by the expression level of 5%, became an indicator that has a significant impact on the indicator of time without progression of patients with gastrointestinal NET (Figure 15).

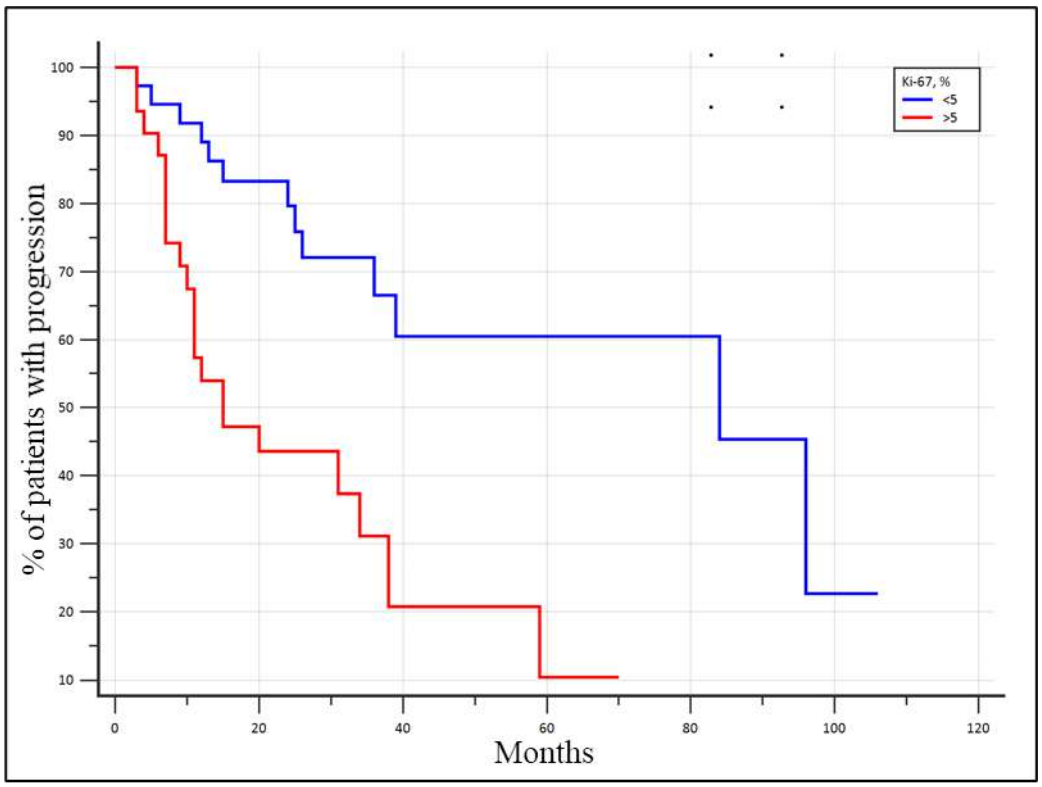


Figure 15 – Time without progression of patients with gastrointestinal NET depending on the expression level of Ki-67 (cut-off threshold =5%)

The median progression-free time of patients with an initial Ki-67 expression level exceeding 5% was 15.0 months (95% CI 10.0-38.0) and was significantly less than the median PFS of patients with an initial Ki-67 level equal to or below 5%, where it was 84.0 months (95% CI 36.00-96.00) – p=0.0004; HR 0.26: 95% CI 0.13-0.55.

The next indicator of peripheral blood of patients with gastrointestinal NET, which had a significant effect on the indicator of time without progression, was the absolute number of neutrophils with a threshold value defined as the level of $3.05\times10^{9}$/l (Figure 16).

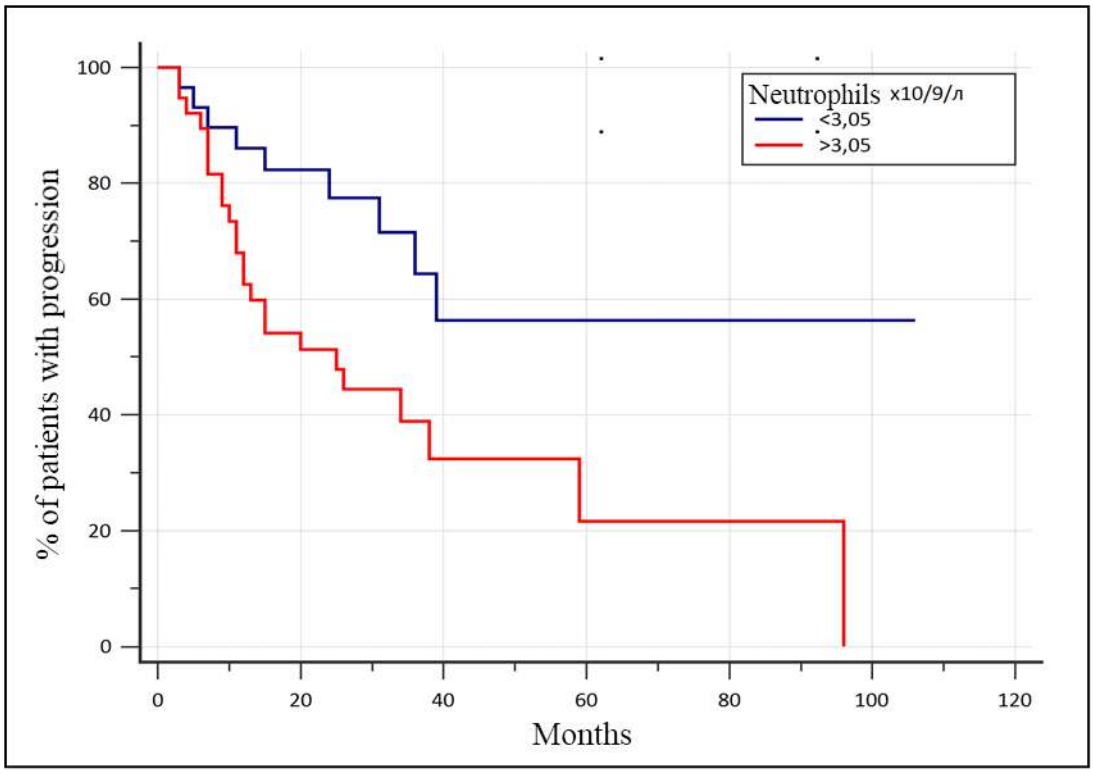


Figure 16 – Time without progression of patients with gastrointestinal NET depending on the absolute number of neutrophils (cut-off threshold =$3.05\times10^9$/l)

The median progression-free time of patients with baseline neutrophil levels exceeding 3.05 was 25.0 months (95% CI 12.0-96.0) and was significantly less than the median PFS of patients with baseline absolute neutrophil count equal to or below $3.05\times10^9$/l, where it was not reached (p=0.0085; HR 0.39 95% CI 0.20-0.79).

The relative number of neutrophils with a threshold value of 58.30% also had a significant effect on the indicator of time without progression in patients with gastrointestinal NET (Figure 17).

The median progression-free time of patients with baseline relative neutrophil count exceeding 58.30% was 25.0 months (95% CI 12.0-96.0) and was significantly less than the median PFS of patients with baseline relative neutrophil count equal to or below $58.30\times10^9$/l, where it was not reached (p=0.0090; HR 0.39: 95% CI 0.19-0.79).

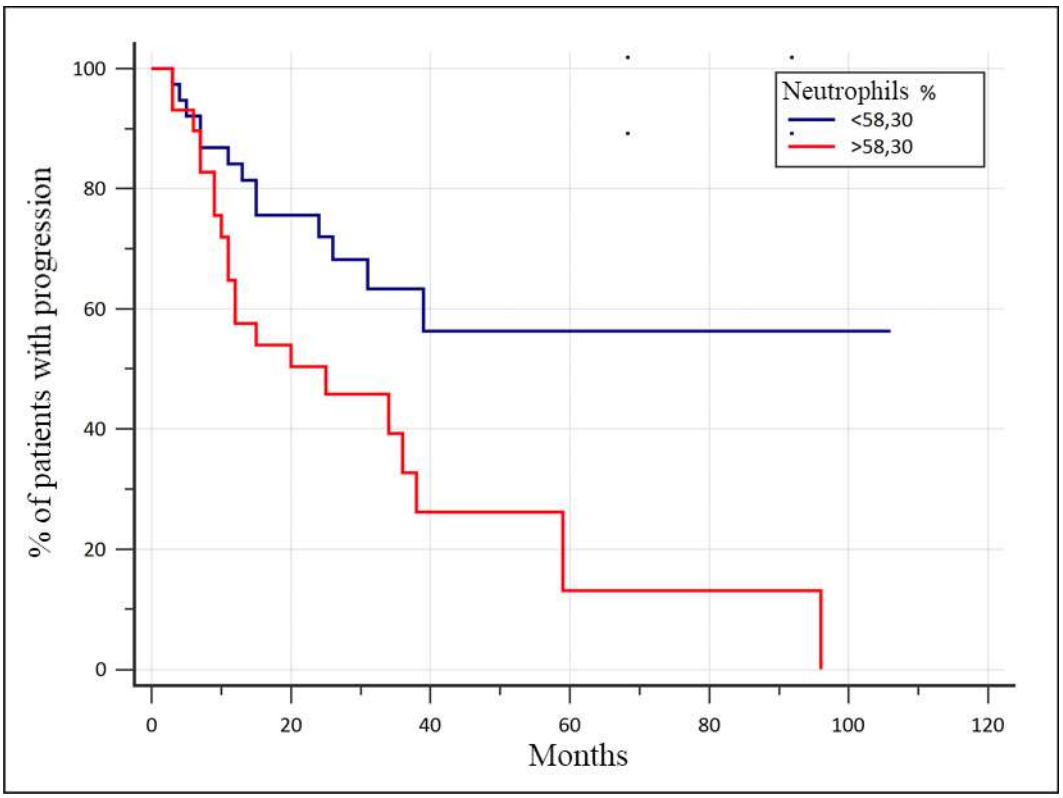


Figure 17 – Time without progression of patients with gastrointestinal NET depending on the relative number of neutrophils (cut-off threshold =58.30%)

The next indicator of peripheral blood of patients with gastrointestinal NET, which had a significant effect on the indicator of time without progression, was the absolute number of lymphocytes with a threshold value defined as the level of $\leq 2.26 \times 10^9$/l (Figure 18).

The median time without progression of patients with an initial level of absolute number of lymphocytes less than or equal to the value of $2.26 \times 10^9$/l, was 31.0 months (95% CI 15.0-96.0) and was significantly less than the median PFS of patients with baseline absolute lymphocyte count of more than $2.26 \times 10^9$/l, where it was not achieved (p=0.0241; HR 0.42: 95% CI 0.20-0.89).

Also, the relative number of lymphocytes with threshold values, defined as the level of 30%, had a significant effect on the indicator of time without progression (Figure 19).

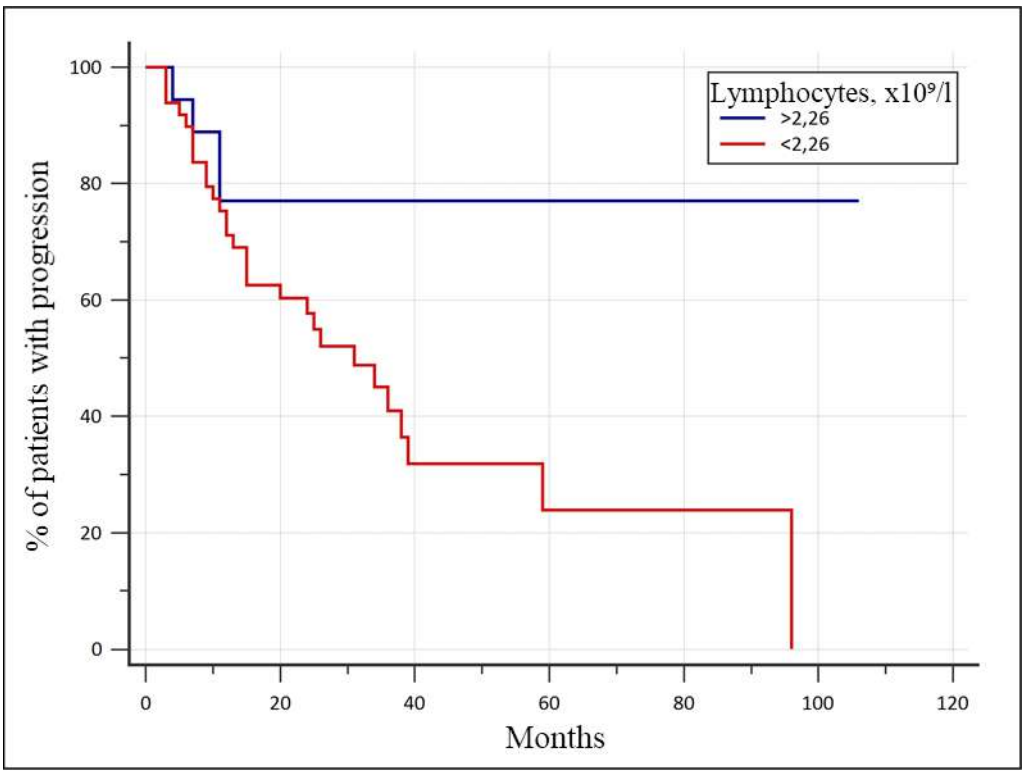


Figure 18 – Time without progression of patients with gastrointestinal NET depending on the absolute number of lymphocytes (cut-off threshold =$2.26\times10^9$/l)

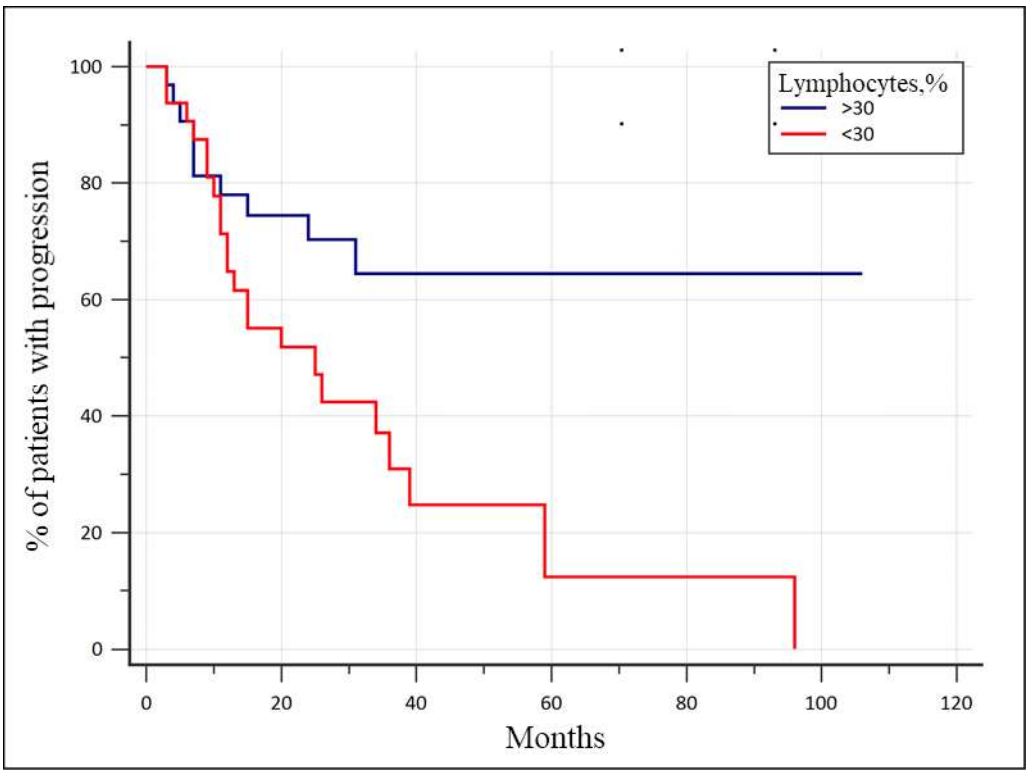


Figure 19 – Time without progression of patients with gastrointestinal NET depending on the relative number of lymphocytes (cut-off threshold =30%)

The median progression-free time of patients with baseline relative lymphocyte count less than or equal to 30% was 25.0 months (95% CI 12.0-96.0) and was significantly less than the median PFS of patients with baseline relative lymphocyte count more than 30%, where it was not achieved (p=0.0106; HR 0.40: 95% CI 0.20-0.81).

The indicator with a potential effect on the indicator of time without progression of the disease was the level of peripheral blood leukocytes (Figure 20).

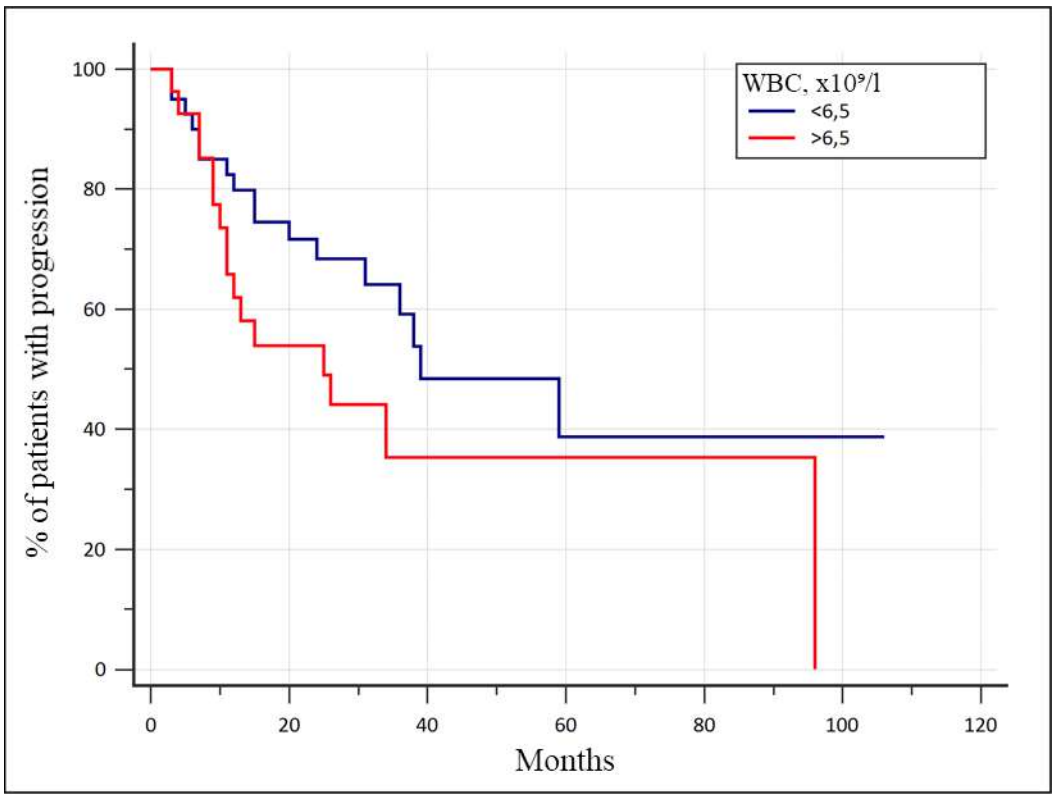


Figure 20 – Time without progression of patients with gastrointestinal NET depending on the absolute number of leukocytes (cut-off threshold =$6.50\times10^9$/l)

The threshold value of the level of the absolute number of peripheral blood leukocytes was a value exceeding the level of $6.50\times10^9$/L. The median time without progression in patients with gastrointestinal NET with an initial level of absolute leukocyte count exceeding $6.50\times10^9$/L was 25.0 months (95% CI 12.0-96.0) and was significantly less than the median in patients with an initial level of absolute leukocyte count equal to or below $6.50\times10^9$/l, where it was 39 months (95% CI 31.0-59.0) (p=0.0992; HR 0.54: 95% CI 0.26-1.12).

### *4.3.6 The effect of relative indices characterizing endogenous inflammation on the survival rates of patients with neuroendocrine tumors of the gastrointestinal tract (results of a single-factor analysis)*

The results of a one-factor analysis of the effect of the calculated relative indices on the time without progression of patients with gastrointestinal NET, taking into account the threshold values, are presented in Table 22.

Table 22 – Effect of the level of endogenous intoxication on the time without progression of patients with gastrointestinal NET

| Indicator | Median (month) (95% CI) | Risk ratio (HR) (95% CI) | Log-rank test p-value |
|---|---|---|---|
| Neutrophil-lymphocytic index: | | | |
| >1,85 | 25,0 (95% CI 12,0-96,0) | 0,44 (95% CI 0,22-0,89) | 0,0213 |
| ≤1,85 | Not reached | | |
| dNLR: | | | |
| >1,40 | 20,0 (95% CI 10,0-96,0) | 0,37 (95% CI 0,17-0,74) | 0,0055 |
| ≤1,40 | Not reached | | |
| Platelet-lymphocyte index: | | | |
| >170,19 | 36,0 (95% CI 15,0-96,0) | 0,67 (95% CI 0,33-1,37) | 0,2707 |
| ≤170,19 | Not reached | | |
| Lymphocytic-monocytic index: | | | |
| ≤2,90 | 26,0 (95% CI 11,0-96,0) | 0,53 (95% CI 0,25-1,11) | 0,0921 |
| >2,90 | Not reached | | |

The univariate analysis made it possible to establish the presence of an effect on the time without progression of patients with gastrointestinal NET NLI, dNLI. The effect of aphids was negligible (p=0.2707).

The threshold value of aphids, with its effect on the time without progression, was 1.85, and a comparison of the survival curves is shown in Figure 21.

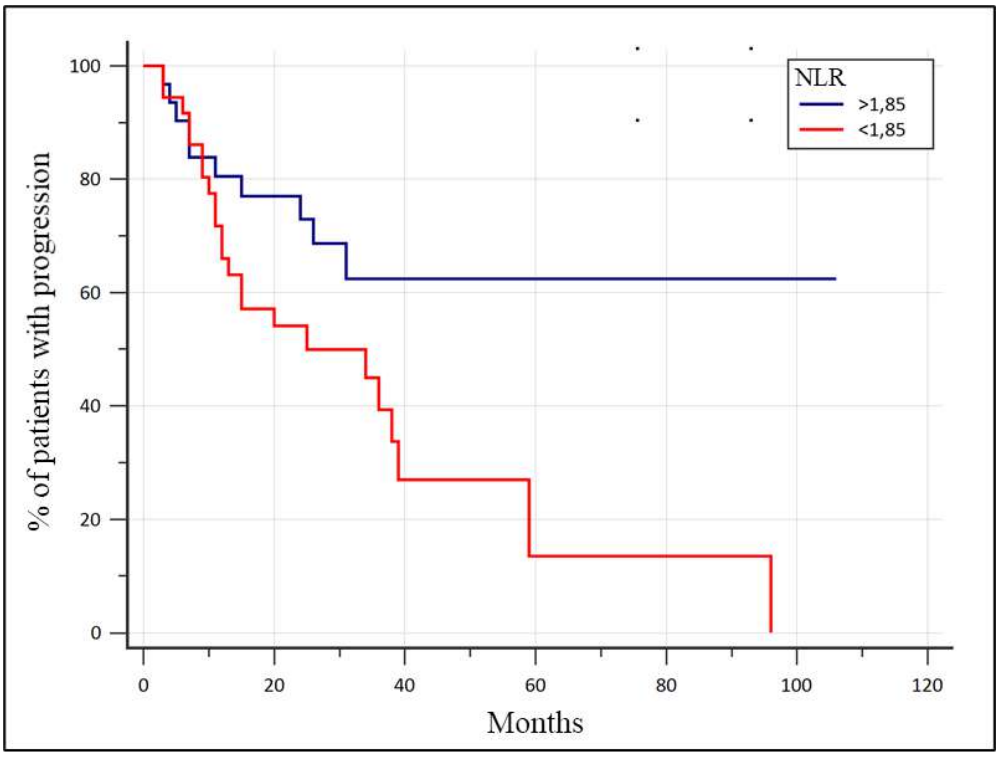


Figure 21 – Time without progression of patients with gastrointestinal NET depending on the neutrophil-lymphocytic index (cut-off threshold = 1.85)

The median time to progression of patients whose NLI was more than the threshold value of 1.85 was 25.0 months (95% CI 12.0-96.0). Median progression-free time in patients with NLR, less than and equal to 1.85, was not achieved (p=0.0213; HR 0.44: 95% CI 0.22-0.89).

The threshold value of dNLR was determined to be 1.40 (Figure 22).

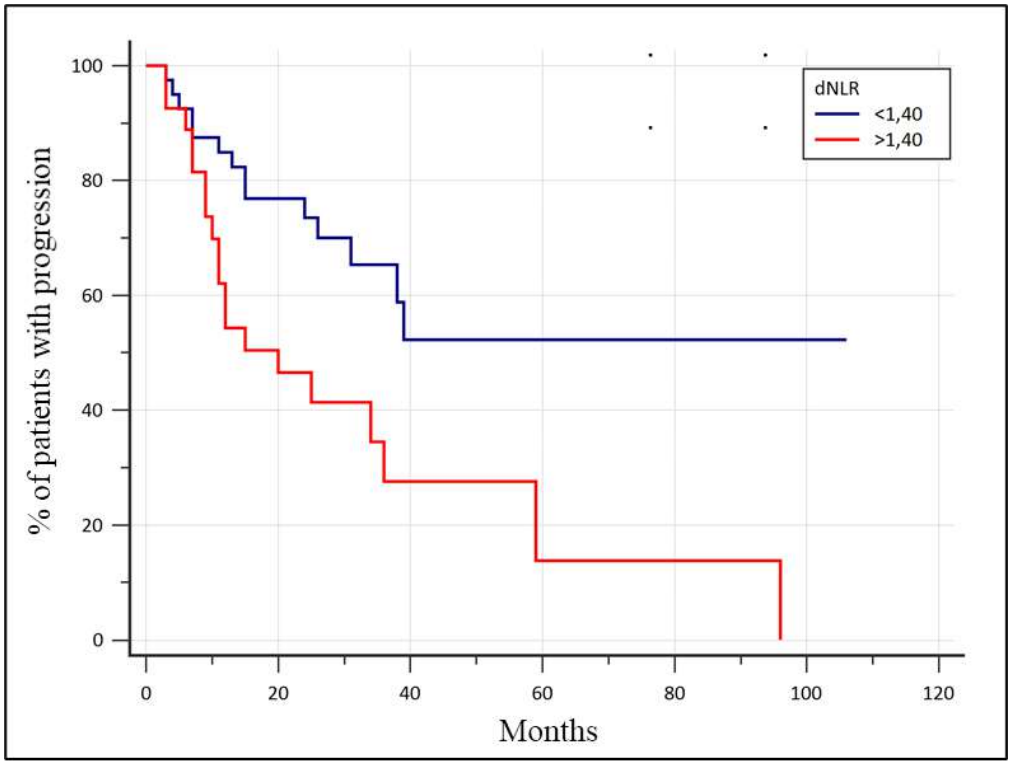


Figure 22 – Time without progression of patients with gastrointestinal NET depending on dNLR (cut-off threshold =1.40)

The median time to progression of patients whose dNLR corresponded to the threshold value of 1.40 or was less than it was not reached. The time without progression in patients with dNLR exceeding 1.40 was 20.0 months (95% CI 10.0-96.0) (p=0.0055; HR 0.37: 95% CI 0.17-0.74).

The effect of LMI tended to have a significant effect on the time without progression of patients. The threshold value of the LMI was 2.90 (Figure 23).

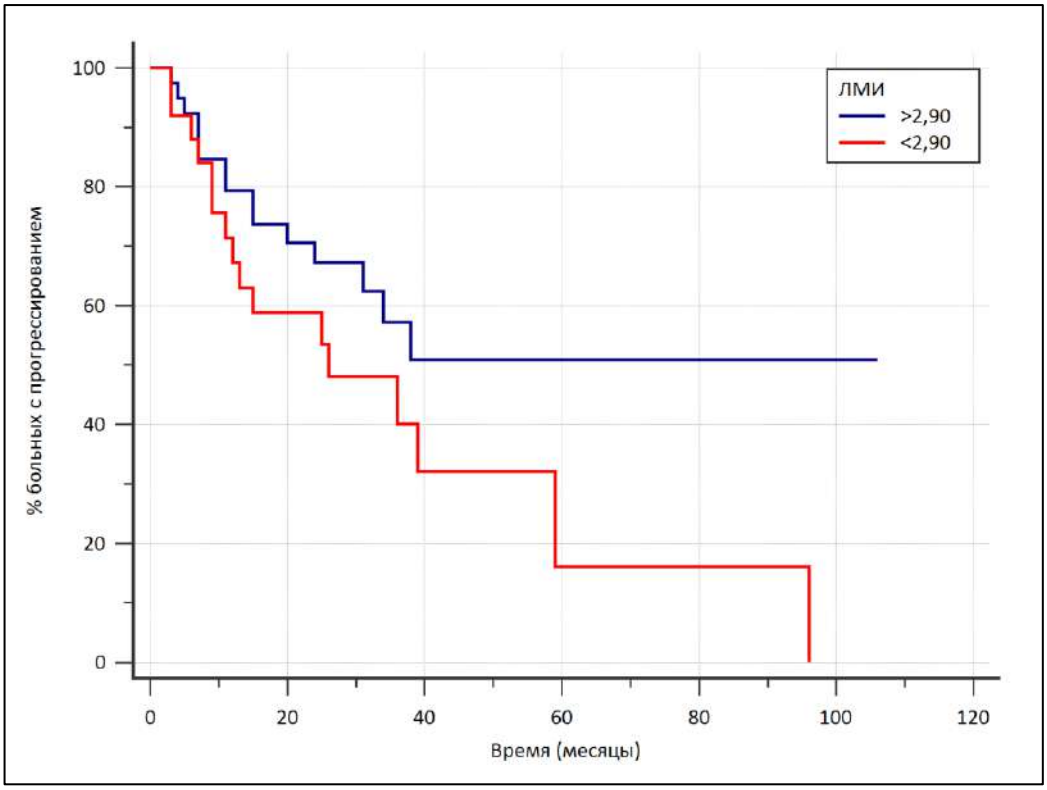


Figure 23 – Time without progression of patients with gastrointestinal NET depending on the lymphocyte-monocyte index (cut-off threshold =2.90)

The median time without progression of patients whose LMI was greater than 2.90 was not achieved. Patients in whom the LMI value was equal to or did not reach the level of 2.90, the median time without progression was 26.0 months (95% CI 11.0-96.0) (p=0.0921; HR 0.53: 95% CI 0.25-1.11).

### *4.3.7 Long-term results of treatment of patients with neuroendocrine tumors of the gastrointestinal tract depending on factors of systemic inflammation (results of multivariate analysis)*

In order to assess the true prognostic value of the considered factors of systemic inflammation and indicators characterizing the level of endogenous intoxication of patients with gastrointestinal NET on indicators of life expectancy and

time without progression, all significant parameters identified as a result of the univariate analysis were included in the multifactorial model of proportional risks of Coke.

The model assessing the influence of the factors under consideration on the life expectancy of patients included the following indicators: the absolute number of neutrophils (threshold value: $>3.05\times10^9$/l), the relative number of neutrophils (threshold value: >58.30%), the absolute number of lymphocytes (threshold value: $\leq2.26\times10^9$/l), NLI (threshold value: >1.85), dNLR (threshold value: >1.40).

The results of a multifactorial analysis of the influence of systemic inflammation factors on the life expectancy of patients conducted by constructing a model of proportional Cox risks are presented in Table 23.

Table 23 – Results of multivariate regression analysis of Coke to assess the risk of death in patients with gastrointestinal NET

| Indicator | Risk ratio (HR) | 95% CI | p-value (Cox) |
|---|---|---|---|
| Neutrophils, %<br>>58,30 | 1,05 | 1,01-1,09 | 0,0336 |
| Lymphocytes, %<br>≤30 | 1,03 | 1,01-1,06 | 0,0443 |
| NLR<br>>1,85 | 1,17 | 1,02-1,34 | 0,0228 |

The multivariate analysis made it possible to establish that the indicators of peripheral blood having an independent statistically significant effect on the time without progression of patients with gastrointestinal NET were the relative number of neutrophils and the relative number of peripheral blood lymphocytes. In addition, the neutrophil-lymphocyte ratio (NLR) had an independent statistically significant effect on the patients' PFS.

The effect of the initial relative number of peripheral blood neutrophils on the risk of progression of patients is shown in Figure 24.

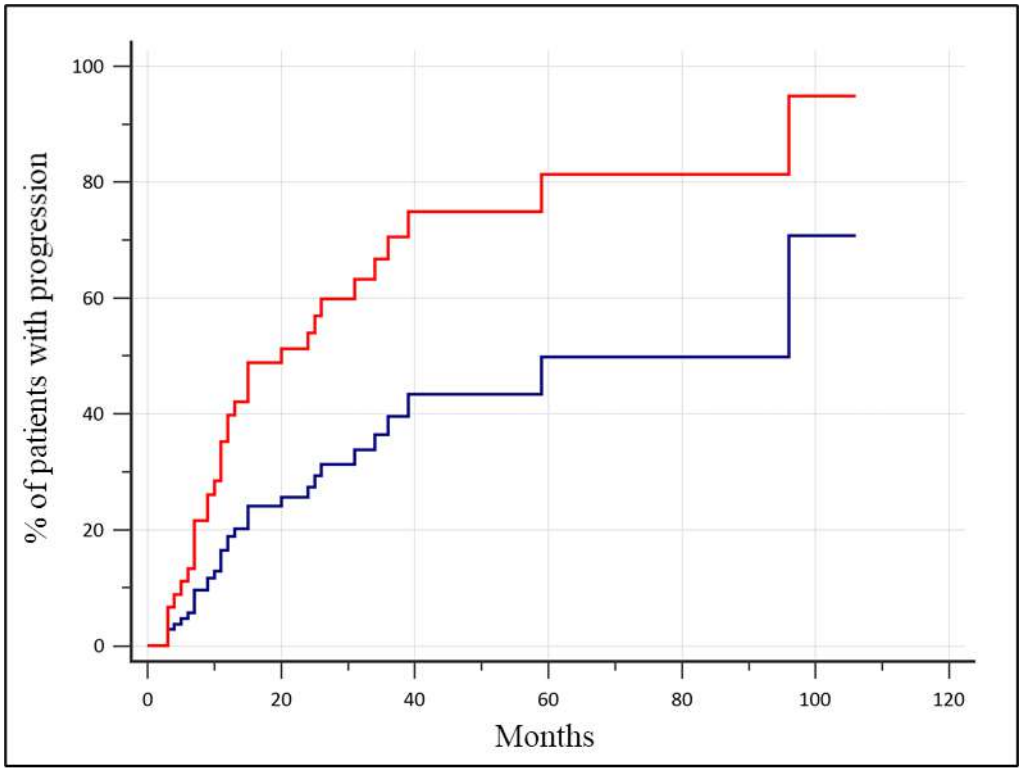


Figure 24 – A model of the relative risk of progression of patients Gastrointestinal NET depending on the initial relative number of peripheral blood neutrophils (cut-off threshold =58.30%)

An increase in the relative number of peripheral blood monocytes at the initial assessment stage above 58.30% increased the risk of disease progression by 1.05 times (p=0.0336; HR 1.05: 95% CI 1.01-1.09).

The effect of the initial relative number of peripheral blood lymphocytes on the risk of progression of patients is shown in Figure 25.

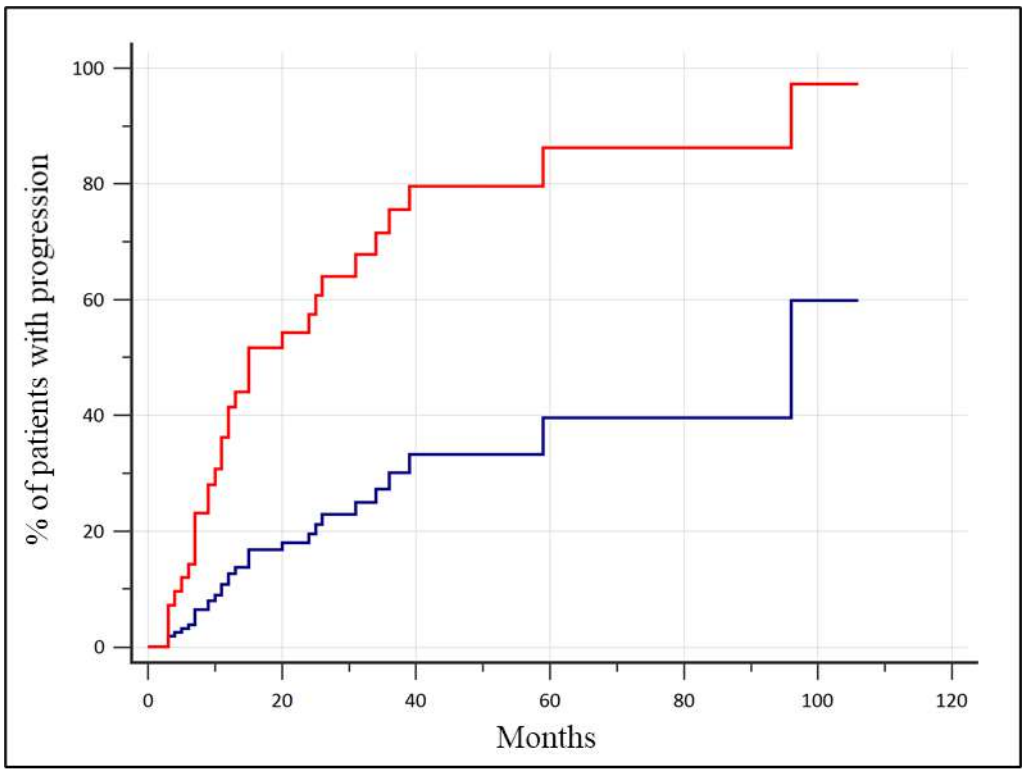


Figure 25 – A model of the relative risk of progression of patients Gastrointestinal NET depending on the initial relative number of peripheral blood lymphocytes

Compliance with the level of the relative number of peripheral blood lymphocytes at the initial assessment stage of 30% or not exceeding it increased the risk of death of the patient by 1.03 times (p=0.0443; HR 1.03: 95% CI 1.01-1.06).

The effect of the neutrophil-lymphocyte ratio (NLI) on the risk of disease progression in patients with gastrointestinal NET is presented on Figure 26.

An increase in the level of neutrophil-lymphocyte ratio (NLI) at the initial assessment stage above the level of 2.30 increased the risk of death of the patient by 1.17 times (p=0.0228; HR 1.17: 95% CI 1.02-1.34).

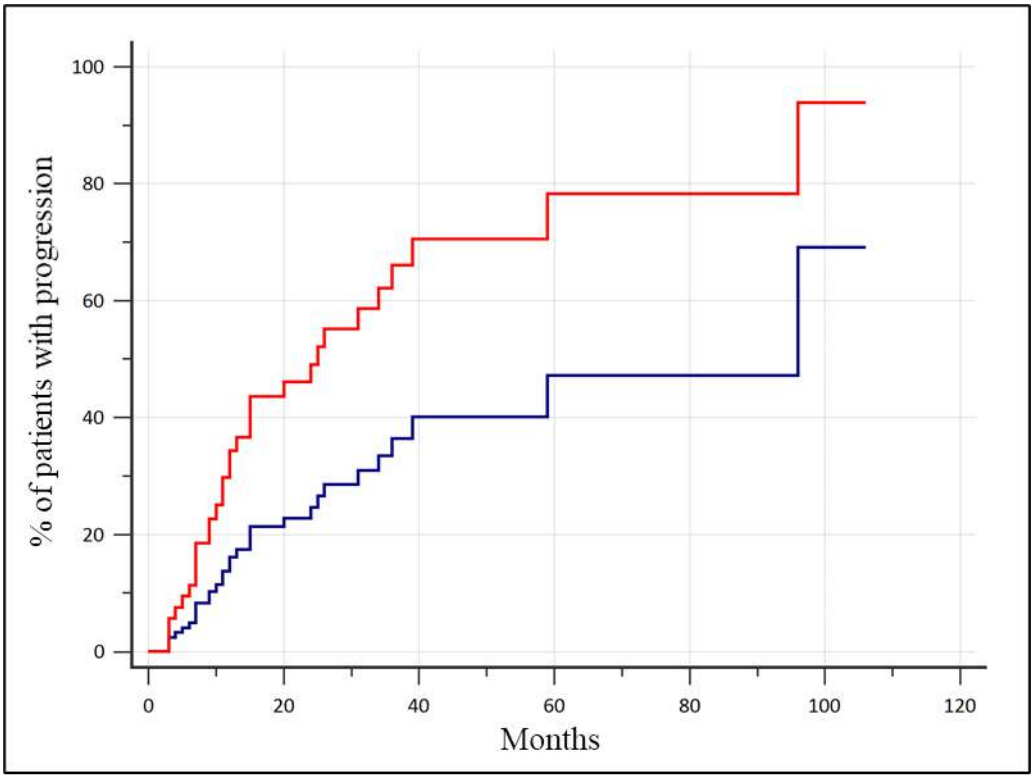


Figure 26 – A model of the relative risk of progression of patients Gastrointestinal NET depending on the neutrophil-lymphocytic index

Thus, the results obtained indicate that independent adverse factors increasing the risk of disease progression were: the initial level of the relative number of peripheral blood neutrophils >58.30% (p=0.0336, HR 1.05: 95% CI 1.01-1.09), the initial level of the relative number of peripheral blood lymphocytes <30% (p=0.0443, HR 1.03: 95% CI 1.01-1.06) and NLI >1.85 (p=0.0228; HR 1.17: 95% CI 1.02-1.34).

# Chapter 5
# STUDY OF METABOLIC SYNDROME IN NEUROENDOCRINE TUMORS OF THE GASTROINTESTINAL TRACT

To determine the prognostic role of MS factors on the course of gastrointestinal NET, we conducted a prospective study that included 34 patients with gastrointestinal NET with type 2 diabetes who received treatment and follow-up at the St. Petersburg State Medical Institution "City Clinical Oncology Dispensary" in the period from 2015 to 2021. As a control group, 30 patients without DM. All patients were treated according to standard protocols from 2015 to 2021.

The main criteria for the inclusion of patients in the planned study:

1. The opportunity and consent to sign a form of voluntary informed consent to participate in the study.
2. The age of patients is over 18 years.
3. Morphologically verified "neuroendocrine tumor of the gastrointestinal tract".

The main criteria for non-inclusion of patients in the study:

1. The presence of type 1 diabetes mellitus.

The majority of 13 patients (38.24%) were diagnosed with stage IV of the disease at the initial treatment, 11 (32.35%) patients had stage II. Stage I was typical for 5 (14.71%) cases. Also, 5 (14.71%) were diagnosed with stage III of the disease.

In the majority of patients – 12 (35.29%) the primary tumor focus was localized in the pancreas, in 10 (29.41%) in the stomach, in 9 (26.47%) in the small intestine. Tumors localized in the colon were found in 1 (2.94%) cases. In 2 (5.88%) – without a primary focus.

Immunohistochemical examination revealed an intermediate degree of malignancy in the majority of patients – 20 (58.82%). A low degree of malignancy was found in 10 (29.41%) cases. The most rare – 4 (11.76%) - was the grade of G3 malignancy.

In the presented cohort of patients, carcinoid syndrome occurred only in 6 (17.65%) patients. For 28 (82.35%) cases, carcinoid syndrome was not characteristic.

## 5.1 General characteristics of patients (control group)

The general characteristics of the patients included in the study are presented in Table 24.

Table 24 – General characteristics of patients included in the study

| Sign | Patients with DM (abs., (%)) | Control Group (abs., (%)) | p-value |
|---|---|---|---|
| Total number of patients | n=34 (100%) | n=30 (100%) | |
| Gender: | | | |
| men | 13 (38,24%) | 9 (30,00%) | 0,4886 |
| women | 21 (61,76%) | 21 (70,00%) | 0,4886 |
| Age: | | | |
| Median /average±SD | 68,00 years | 61,43±17,67 | |
| [Q25-Q75]/95% CI | [65,00-72,00] | [54,84-68,03] | 0,0777 |
| min. – max. | 44,00-86,00 | 27,00-86,00 | |
| Stage of the tumor process | | | |
| I | 5 (14,71%) | 7 (23,33%) | 0,3780 |
| II | 11 (32,35%) | 3 (10,00%) | 0,0309 |
| III | 5 (14,71%) | 5 (16,67%) | 0,8294 |
| IV | 13 (38,24%) | 15 (50,00%) | 0,3440 |
| Localization of the primary focus: | | | |
| stomach | 10 (29,41%) | 7 (23,33%) | 0,5826 |
| pancreas | 12 (35,29%) | 9 (30,00%) | 0,6529 |
| colon | 1 (2,94%) | 4 (13,33%) | 0,1222 |
| small intestine | 9 (26,47%) | 8 (26,67%) | 0,9856 |
| undefined | 2 (5,88%) | 2 (6,67%) | 0,8963 |

Continuation of table 24

| Sign | Patients with DM (abs., (%)) | Control Group (abs., (%)) | p-value |
|---|---|---|---|
| The degree of malignancy | | | |
| G1 | 10 (29,41%) | 13 (43,33%) | 0,0920 |
| G2 | 20 (58,82%) | 11 (36,67%) | 0,0768 |
| G3 | 4 (11,76%) | 6 (20,00%) | 0,3649 |
| Carcinoid syndrome | | | |
| Yes | 6 (17,65%) | 4 (13,33%) | 0,6348 |
| No | 28 (82,35%) | 26 (86,67%) | 0,6348 |

The comparative (Mann-Whitney criterion) analysis allowed us to establish that both groups of patients were comparable in age (U=379; p=0.0777). Also, when comparing data by gender (Mann-Whitney criterion), the groups were comparable – U=468; p=0.4886. Women were comparably more common in both groups (p=0.4886). The group of patients with diabetes mellitus consisted of 61.76% of women and 38.24% of men aged 44 to 86 years, the median age was 68 years [65.00-72.00]. In the control group there were 70.00% of women and 30.00% of men, aged 27 to 86 years, the median age was 61.43 years [54.84-68.03].

In the group of patients with diabetes mellitus, both early – I and II stages of the disease (14.71% and 32.35%), and III (14.71%), IV (38.24%) stages of the tumor process were registered with the same frequency. In the control group, stage IV of the disease was registered more often (50.00%) at the initial treatment of patients. At the same time, a comparative analysis of the indicator of stage II of the disease revealed a significant (p=0.0309) difference. 11 (32.35%) patients with stage II disease were registered in the group with diabetes mellitus, while 3 (10.00%) patients were registered in the control group.

A comparative analysis of the groups of patients under consideration revealed no significant differences between them in the localization of the primary tumor focus.

Analysis of the degree of malignancy of the tumor process in the group with diabetes mellitus allowed us to determine that in this group of patients an intermediate (58.82%) degree of malignancy was more often registered.

In both groups, carcinoid syndrome was significantly more often absent – 82.35% in the group with diabetes mellitus, 86.67% in the group without diabetes mellitus. Comparative analysis of the differences between the groups did not show.

## 5.2 Assessment of metabolic syndrome factors

To assess the impact on overall survival and progression-free survival before the start of treatment of patients with gastrointestinal NET, the following indicators were studied:

- presence/absence of type 2 SD;
- blood glucose level before treatment;
- patient's body mass index;
- the presence of carcinoid syndrome.

In general, the median progression-free survival in all patients with gastrointestinal NET was 45 (±12.3) months (median overall survival was not achieved).

Table 25 presents the results of assessing the impact on survival of the presence or absence of type 2 diabetes mellitus.

The five–year survival rate in the group without type 2 diabetes is 76.4% (±7.8), in the group with type 2 diabetes - 73.6% (± 8.5). The median overall survival in the group without type 2 diabetes is not achieved, in the group with type 2 diabetes is 101 (±5.7) months, the differences between the groups are statistically insignificant ($p>0.05$).

Table 25 – Effect of the presence of type 2 diabetes mellitus on overall survival and progression-free survival in patients with gastrointestinal NET

| Survival rate | DM | n (%) | 5-year survival rate,% (±SE) | Median (±SE) | 95% confidence interval | | HR (95% CI) | Chi-square (p) |
|---|---|---|---|---|---|---|---|---|
| | | | | | lower limit | upper limit | | |
| OS, months | no | 30 (46,9) | 76,4 (±7,8) | not reached | not reached | not reached | C | 0,734 |
| | yes | 34 (53,1) | 73,6 (±8,5) | 101,0 (±5,7) | 89,7 | 112,3 | 1,18 (0,45-3,11) | |
| PFS, months | no | 30 (46,9) | 41,6 (±11,0) | 47,0 (±11,2) | 25,0 | 69,0 | C | 0,535 |
| | yes | 34 (53,1) | 36,1 (±8,6) | 19,0 (±19,7) | 0,00 | 57,6 | 1,22 (0,65-2,29) | |
| C – the level of the trait is taken as the baseline when evaluating the Cox regression. | | | | | | | | |

Not reaching the median means that all patients dropped out of follow-up before 50% of patients died. Five–year progression-free survival in the group without DM is 41.6% (±11.0), in the group with DM - 36.1% (±8.6). The median progression–free survival in the group without DM is 47 (±11.2) months in the group with DM - 19 (±19.7) months, however, despite the significant difference, these differences are also statistically insignificant ($p>0.05$). Graphs of survival functions for overall survival and progression-free survival in the DM groups are shown in the figures 27, 28 respectively.

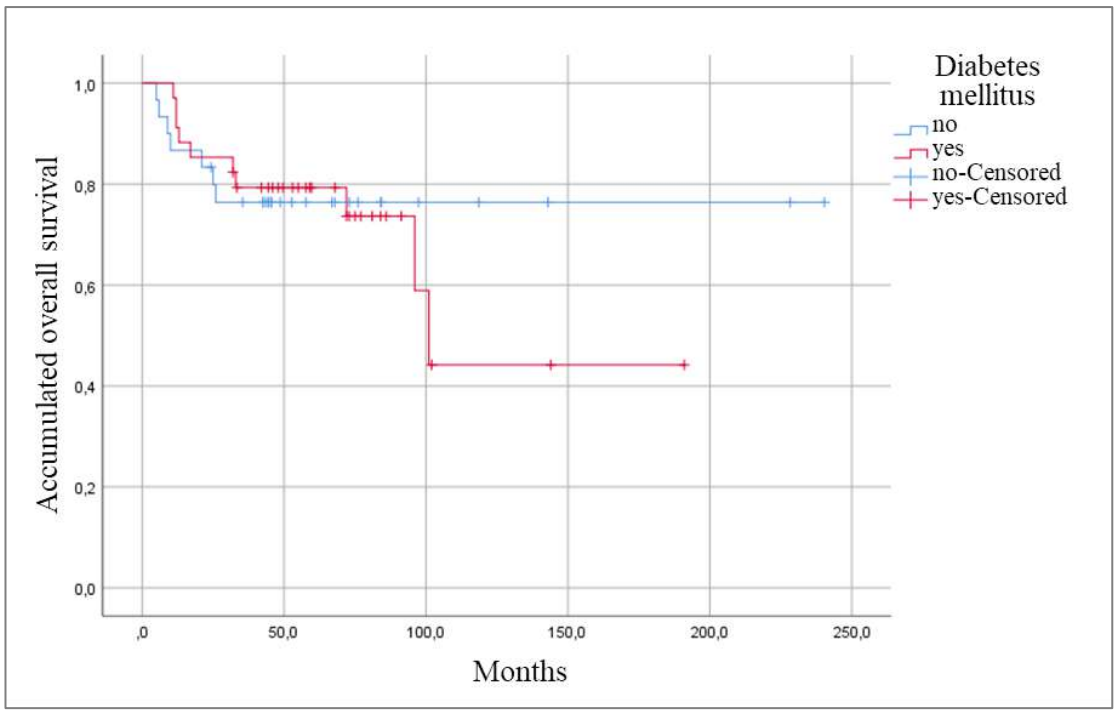


Figure 27 – Survival function (overall survival) of patients with gastrointestinal NET according to groups of presence/absence of type 2 diabetes mellitus

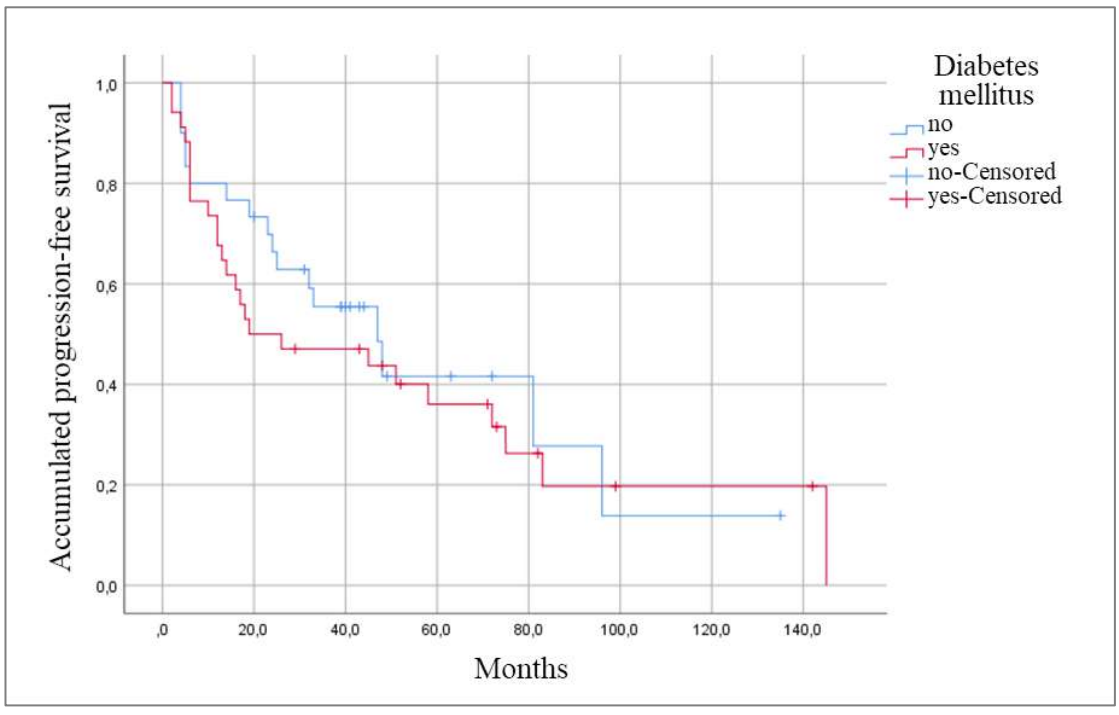


Figure 28 – Survival function (progression-free survival) of patients with gastrointestinal NET according to groups of presence/absence of type 2 diabetes mellitus

Table 26 presents estimates of overall survival and progression-free survival in groups with elevated or normal glucose levels. The five–year overall survival rate is 83.7% (±6.7) in the group of patients with normal, and 68.2% (±9.5) - with elevated blood glucose levels. The median overall survival in the group with normal glucose levels is not achieved, in the group with elevated is 101 (±15.1) months. The differences are statistically insignificant (p>0.05).

Table 26 – Effect of elevated glucose levels on overall survival and progression-free survival in patients with gastrointestinal NET

| Survival rate | Glucose level | n (%) | 5-year survival rate,% (±SE) | Median (±SE) | 95% confidence interval | | HR (95% CI) | Chi-square (p) |
|---|---|---|---|---|---|---|---|---|
| | | | | | lower limit | upper limit | | |
| OS, months | increased | 32 (50,7) | 68,2 (±9,5) | 101,0 (±15,1) | 71,49 | 130,51 | 2,27 (0,79-6,55) | 0,130 |
| | standard | 31 (49,2) | 83,7 (±6,7) | not reached | not reached | not reached | C | |
| PFS, months | increased | 32 (50,7) | 26,8 (±9,2) | 25,0 (±16,9) | 0,00 | 58,26 | 1,65 (0,88-3,12) | 0,121 |
| | standard | 31 (49,2) | 41,9 (±12,0) | 81,0 (±37,9) | 6,59 | 155,42 | C | |
| C – the level of the trait is taken as the baseline when evaluating the Cox regression. | | | | | | | | |

The five-year progression-free survival rate is 41.6% (±12) in the group with normal, and 26.8% (±9.2) in the group with elevated glucose levels. Median progression-free survival is 81 (±37.9) in the normal group, and 25 (±16.9) in the group with elevated glucose levels. However, these differences are also statistically insignificant (p>0.05).

The survival functions are shown in Figures 29, 30, respectively.

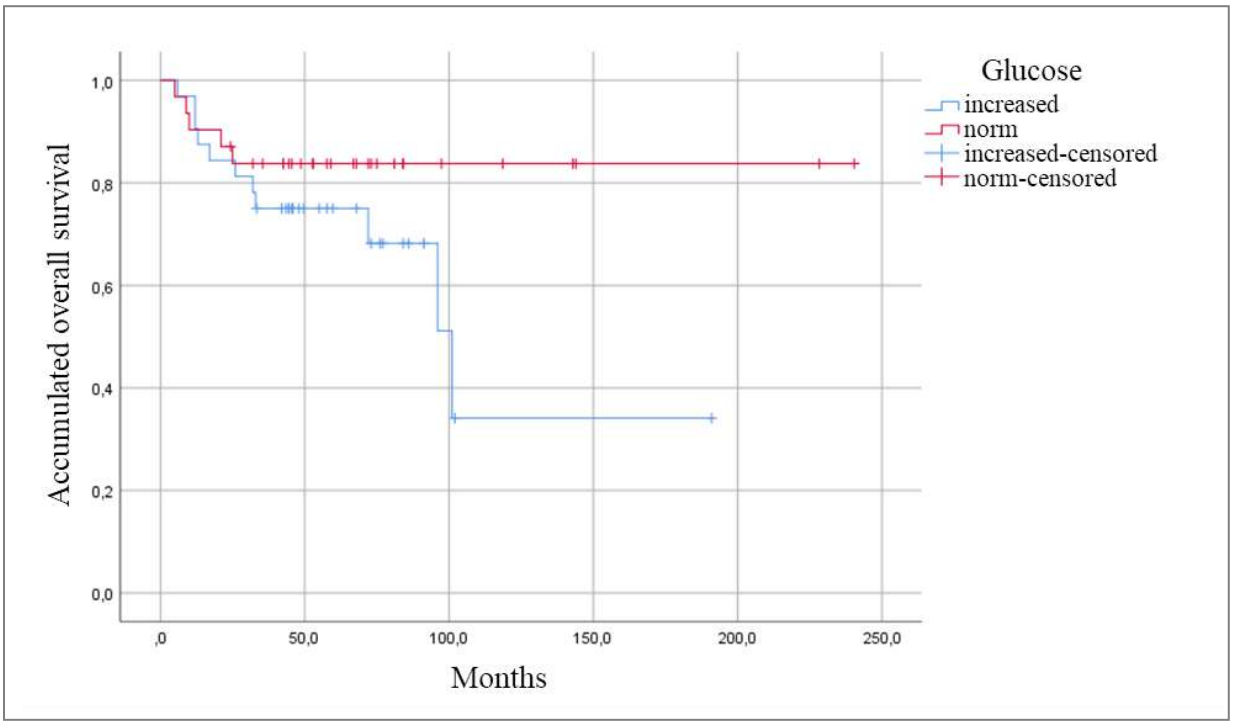


Figure 29 – Survival function (overall survival) of patients with gastrointestinal NET by groups of elevated/normal glucose levels

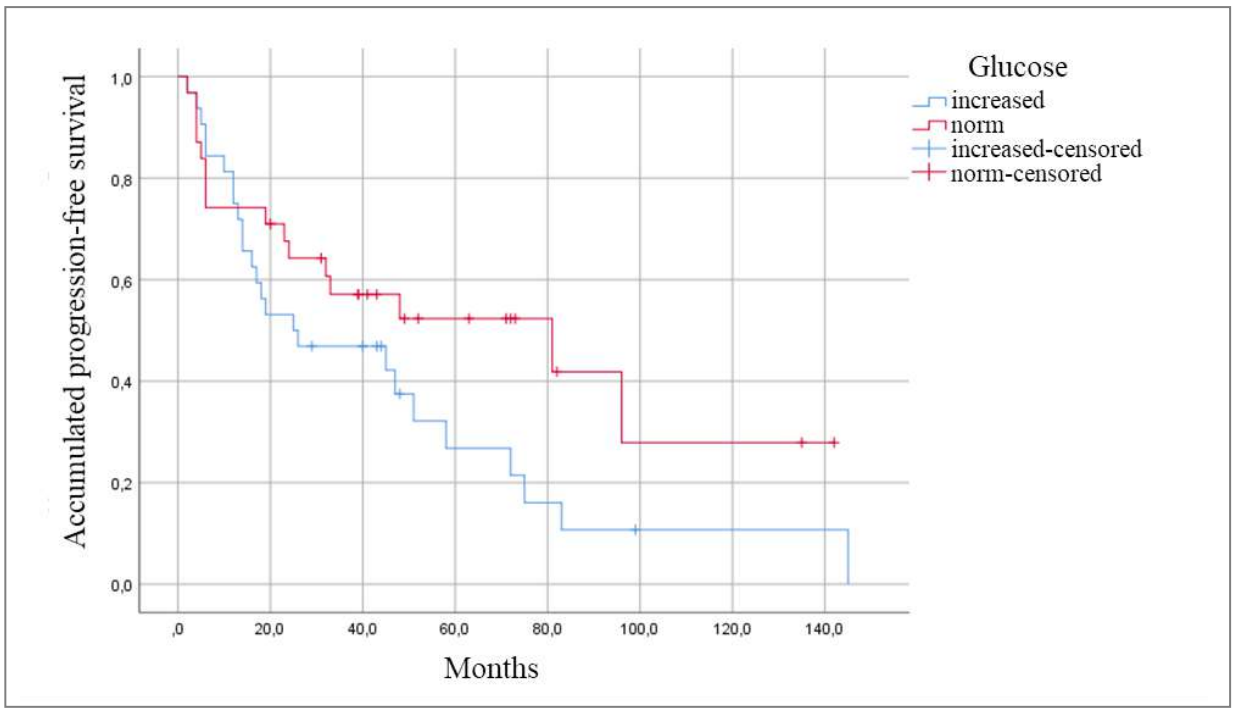


Figure 30 – Survival function (progression-free survival) of patients with gastrointestinal NET according to groups of elevated/normal glucose levels

Table 27 presents the results of assessing the impact of weight on overall survival and progression-free survival. Depending on the BMI value, patients are divided into the following groups: those with normal weight, overweight, type 1 and type 2 obesity. The five-year survival rate was 75.7% (±7.1) for patients with normal, 70% (±20.8) for overweight patients, 80% (±12.6) for patients with type 1 obesity (only 1 patient had type 2 obesity, which does not allow calculating five-year survival).

Table 27 – Effect of weight on overall survival and progression-free survival in patients with gastrointestinal NET

| Survival rate | Weight | n (%) | 5-year survival rate,% (±SE) | Median (±SE) | 95% confidence interval | | HR (95% CI) | Chi-square (p) |
|---|---|---|---|---|---|---|---|---|
| | | | | | lower limit | upper limit | | |
| OS, months | normal | 37 (58,7) | 75,7 (±7,1) | not reached | not reached | not reached | C | 0,451 |
| | redundant | 15 (23,8) | 70,0 (±20,8) | 96,0 (±18,4) | 59,93 | 132,07 | 0,86 (0,23-3,19) | 0,818 |
| | 1 degree of obesity | 10 (15,9) | 80,0 (±12,6) | 101,0 (±0,00) | – | – | 1,26 (0,34-4,69) | 0,728 |
| | 2 degree of obesity | 1 (1,6) | 0,00 (±0,00) | 32,0 (-) | – | – | 5,14 (0,63-41,78) | 0,126 |
| PFS, months | normal | 37 (58,7) | 38,8 (±9,6) | 47,0 (±23,4) | 1,14 | 92,86 | C | 0,643 |
| | redundant | 15 (23,8) | 18,8 (±14,8) | 24,0 (±4,4) | 15,37 | 32,63 | 1,53 (0,72-3,25) | 0,271 |
| | 1 degree of obesity | 10 (15,9) | 25,0 (±19,4) | 16,0 (±26,2) | 0,00 | 67,36 | 1,51 (0,64-3,59) | 0,346 |
| | 2 degree of obesity | 1 (1,6) | 0,00 (±0,00) | 51,0 (-) | – | – | 1,41 (0,19-10,58) | 0,738 |

(-) – standard errors cannot be calculated due to the small number of uncensored observations;
C – the level of the trait is taken as the baseline when evaluating the Cox regression.

The median overall survival was 96 (±18.4) months for overweight patients, 101 (±0.00) months for patients with grade 1 obesity, 32 months for a patient with type 2 obesity and is not achieved for patients with normal weight. Differences between the groups are statistically insignificant (p>0.05).

The five-year progression-free survival rate was 38.8% (±9.6) for patients with normal, 18.8% (±14.8) for overweight patients, 25% (±19.4) for patients with type 1 obesity. Median progression-free survival is 47 (±23.4) months for normal patients, 24 (±4.4) months for overweight patients, 16 (±26.2) months for type 1 obese patients and 51 months for type 2 obese patients. The differences between the groups are statistically insignificant (p>0.05).

The corresponding survival functions are shown in Figures 31, 32.

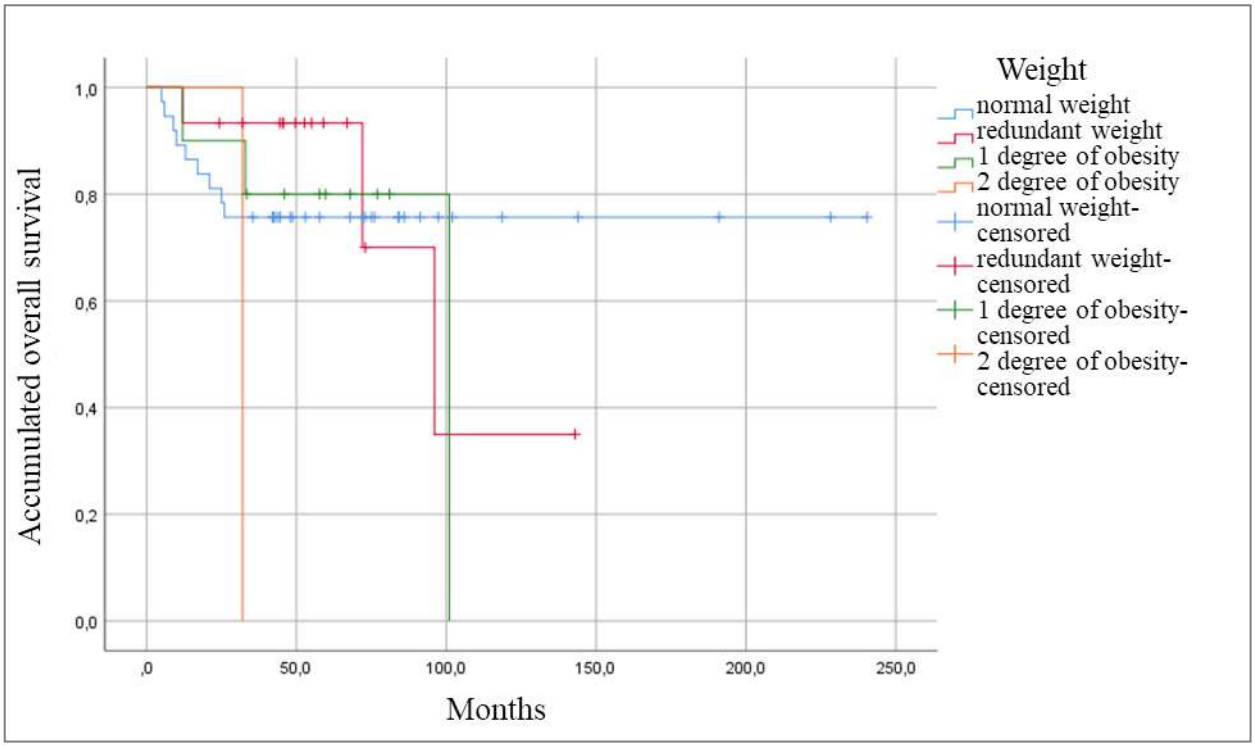


Figure 31 – Survival function (overall survival) of patients with gastrointestinal NET by weight groups

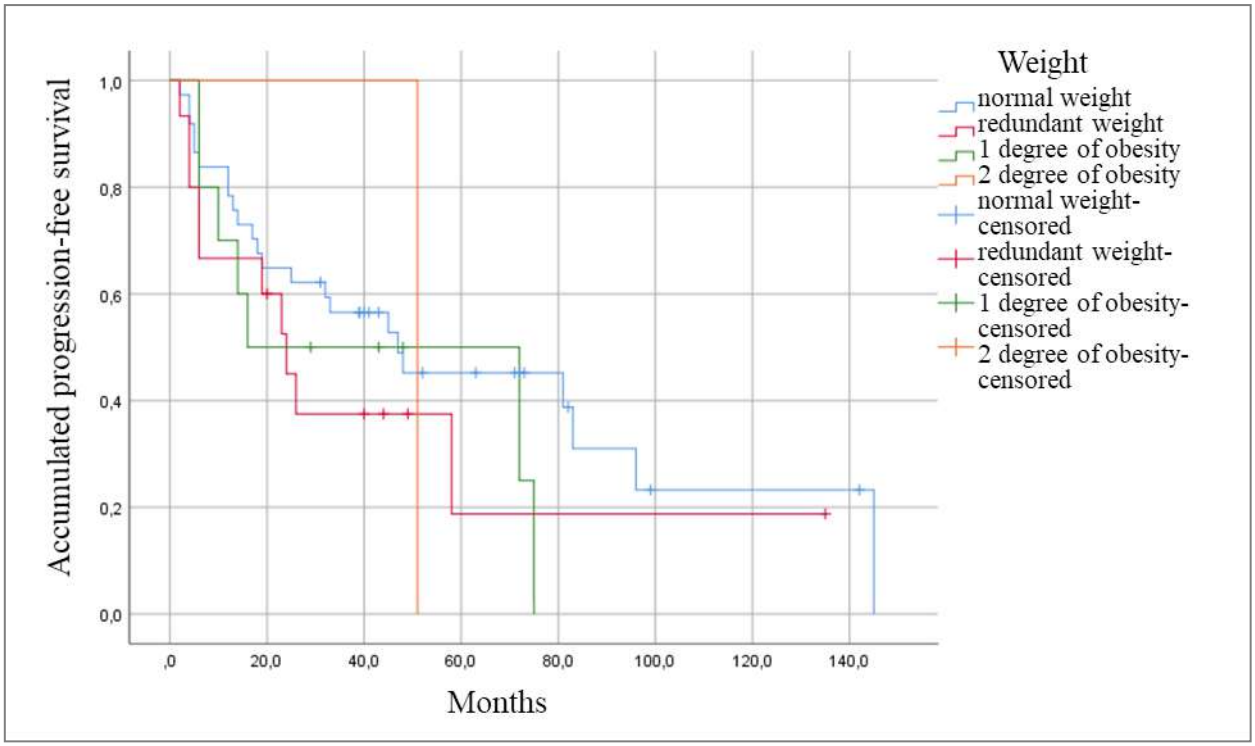


Figure 32 – Survival function (progression-free survival) of patients with gastrointestinal NET by weight groups

Table 28 presents an assessment of overall survival and progression-free survival for groups of patients with or without carcinoid syndrome. The five–year survival rate of patients with carcinoid syndrome is 70% (±14.5), without - 75% (±6.8), median survival is not achieved, differences between groups are statistically insignificant ($p>0.05$).

The five–year progression–free survival of patients with carcinoid syndrome is 10% (±9.5), without - 45.4% (±7.26), the median progression-free survival is 17 (±10.3) months and 51 (±20.8) months, respectively. The differences are statistically significant ($p<0.05$). The presence of carcinoid syndrome increases the risk of progression by 2.63 times (95% CI 1.27-5.43).

Table 28 – The effect of the presence of carcinoid syndrome on the overall survival and progression-free survival of patients with gastrointestinal NET

| Survival rate | Carcinoid syndrome | n (%) | 5-year survival rate,% (±SE) | Median (±SE) | 95% confidence interval | | HR (95% CI) | Chi-square (p) |
|---|---|---|---|---|---|---|---|---|
| | | | | | lower limit | upper limit | | |
| OS, months | No | 54 (84,4) | 75,0 (±6,8) | not reached | not reached | not reached | C | 0,711 |
| | Yes | 10 (15,6) | 70,0 (±14,5) | not reached | not reached | not reached | 1,27 (0,36-4,42) | |
| PFS, months | No | 54 (84,4) | 45,4 (±7,26) | 51,0 (±20,8) | 10,27 | 91,73 | C | 0,006 |
| | Yes | 10 (15,6) | 10,0 (±9,5) | 17,0 (±10,3) | 0,00 | 37,14 | 2,63 (1,27-5,43) | |
| C – the level of the trait is taken as the baseline when evaluating the Cox regression. | | | | | | | | |

The corresponding survival functions are presented in Figures 33, 34.

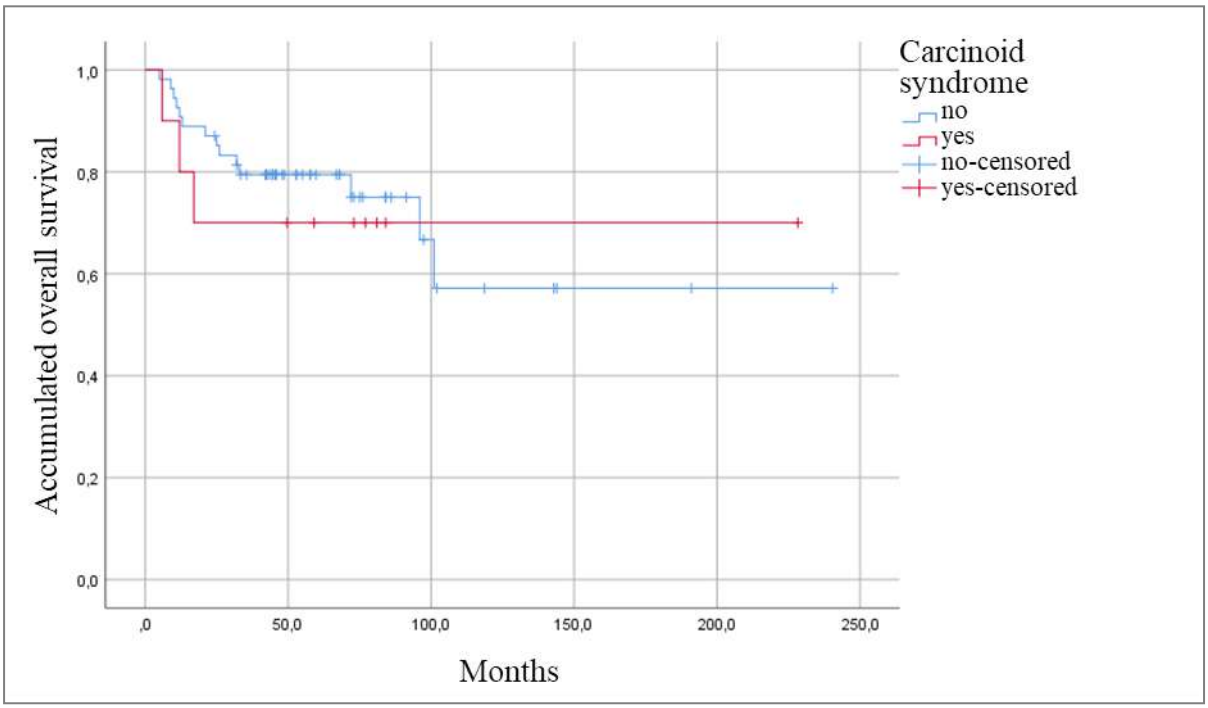


Figure 33 – Survival function (overall survival) of patients with gastrointestinal NET by groups of presence/absence of carcinoid syndrome

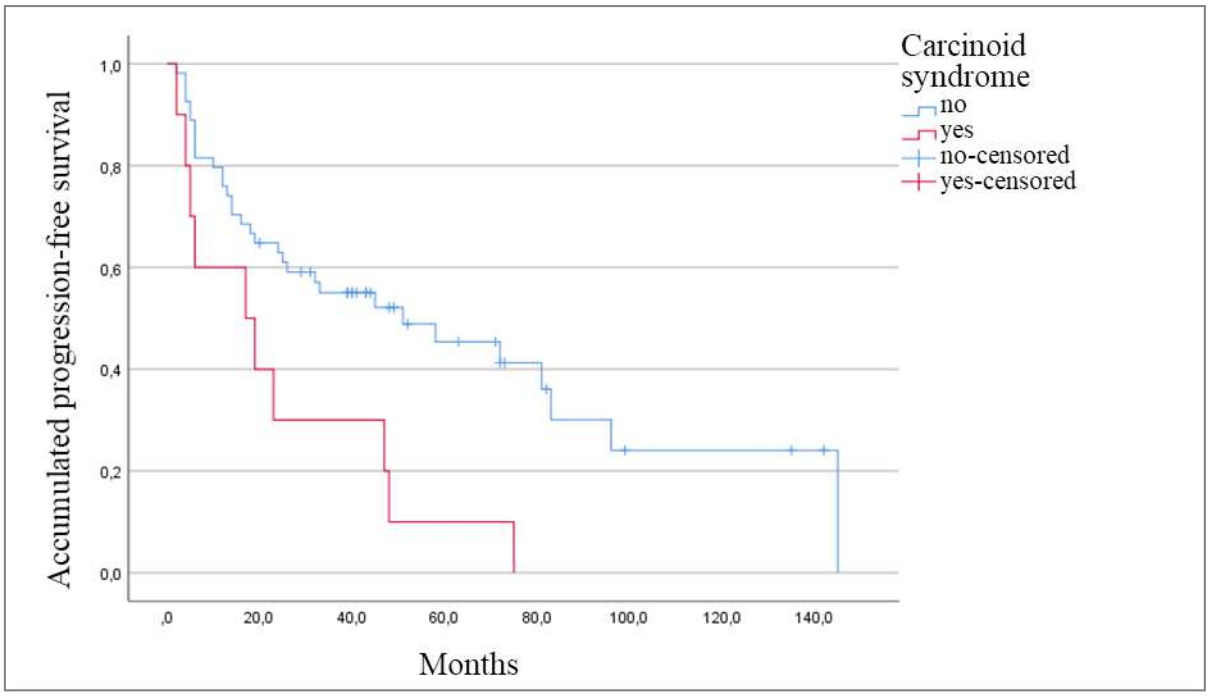


Figure 34 – Survival function (progression-free survival) of patients with gastrointestinal NET according to groups of presence/absence of carcinoid syndrome

Table 29 presents the results of assessing the effect of blood glucose and body mass index on overall survival and progression-free survival in patients with gastrointestinal NET. As can be seen, BMI does not have a statistically significant effect on survival ($p>0.05$). Glucose level affects statistically significantly ($p<0.05$). With an increase in glucose level by 1 mmol/l, the risks of death increase by 1.43 times (95% CI 1.12-1.82), the risks of progression – by 1.2 times (95% CI 1.02-1.42).

Table 29 – Effect of glucose level and BMI on overall survival and progression-free survival in patients with gastrointestinal NET

| Survival rate | Factor | HR (95% CI) | Chi-square (p) |
|---|---|---|---|
| OS, month | Glucose | 1,43 (1,12-1,82) | 0,004 |
| | BMI | 1,03 (0,93-1,13) | 0,626 |
| PFS, month | Glucose | 1,20 (1,02-1,42) | 0,029 |
| | BMI | 1,05 (0,98-1,11) | 0,154 |

Thus, it can be concluded that the overall survival is affected by the level of glucose in the blood, the progression–free survival is affected by the presence of carcinoid syndrome and glucose levels.

Further, a study was conducted on the influence of factors on survival in groups with type 2 diabetes and in its absence. The results for overall survival are presented in Table 30. To the factors discussed above, the localization of the tumor, its stage and prevalence were added.

Elevated glucose levels, BMI, and carcinoid syndrome have no statistically significant effect on overall survival in both groups ($p>0.05$).

Table 30 – The influence of factors on the overall survival of patients with gastrointestinal NET according to the groups of presence/absence of type 2 diabetes mellitus

| DM | Factor | Meaning | n (%) | Median (±SE) | HR (95% CI) | Chi-square (p) |
|---|---|---|---|---|---|---|
| No | Glucose | increased | 7 (23,3) | not reached | 1,29 (0,25-6,68) | 0,758 |
| | | normal | 23 (76,7) | not reached | C | |
| Yes | | increased | 25 (73,5) | 101,0 (±15,3) | 0,03 (0,00-17,08) | 0,280 |
| | | normal | 8 (23,5) | not reached | C | |
| No | Glucose, value | | | | 0,81 (0,31-2,12) | 0,666 |
| Yes | | | | | 2,15 (1,4-3,3) | <0,001 |
| No | BMI | | | | 0,87 (0,70-1,09) | 0,222 |
| Yes | | | | | 1,09 (0,97-1,24) | 0,154 |
| No | Weight | normal | 22 (73,3) | 25,0 (±11,14) | C | 0,314 |
| | | redundant | 8 (26,7) | not reached | 0,03 (0,00-28,57) | |
| | | 1 degree of obesity | – | – | – | – |
| | | 2 degree of obesity | – | – | – | – |

Continuation of table 30

| DM | Factor | Meaning | n (%) | Median (±SE) | HR (95% CI) | Chi-square (p) |
|---|---|---|---|---|---|---|
| Yes | Weight | normal | 15 (44,1) | not reached | C | 0,126 |
| | | redundant | 7 (20,6) | 72,0 (±43,4) | 5,36 (0,82-35,03) | 0,08 |
| | | 1 degree of obesity | 10 (29,4) | 101,0 (±0,00) | 3,09 (0,51-18,80) | 0,222 |
| | | 2 degree of obesity | 1 (2,9) | 32,0 (-) | 19,37 (1,47-253,7) | 0,024 |
| No | Carcinoid syndrome | No | 26 (86,7) | not reached | C | 0,872 |
| | | Yes | 4 (13,3) | not reached | 1,19 (0,14-9,90) | |
| Yes | | No | 28 (82,4) | 101,0 (-) | C | 0,505 |
| | | Yes | 6 (17,6) | not reached | 1,73 (0,35-8,57) | |
| No | Localization | stomach | 7 (23,3) | not reached | C | 0,930 |
| | | duodenum | 1 (3,3) | not reached | 1,10 (0,00) | 1,00 |
| | | small intestine | 7 (23,3) | not reached | 1,10 (0,00-1,16*$10^{207}$) | 1,00 |
| | | colon | 1 (3,3) | 25,0 (-) | 287470 (0,00-5,56*$10^{157}$) | 0,944 |
| | | rectum | 3 (10,0) | not reached | 1,10 (0,00-7,62*$10^{262}$) | 1,00 |
| | | pancreas | 9 (30,0) | not reached | 127491 (0,00-2,45*$10^{157}$) | 0,948 |
| | | without a primary lesion | 2 (6,7) | 10,0 (-) | 455212 (0,00-8,78*$10^{157}$) | 0,942 |
| Yes | | stomach | 10 (29,4) | not reached | C | 0,013 |
| | | duodenum | – | – | – | – |
| | | small intestine | 9 (26,5) | 101,0 (±0,00) | 1,49 (0,21-10,67) | 0,692 |
| | | colon | – | – | – | – |
| | | rectum | 1 (2,9) | 32,0 (-) | 13,45 (1,01-177,7) | 0,049 |
| | | pancreas | 12 (35,3) | 96,0 (±56,8) | 1,62 (0,27-9,78) | 0,602 |
| | | without a primary lesion | 2 (5,9) | 11,0 (-) | 46,7 (4,21-518,8) | 0,002 |

Continuation of table 30

| DM | Factor | Meaning | n (%) | Median (±SE) | HR (95% CI) | Chi-square (p) |
|---|---|---|---|---|---|---|
| No | Stage | I | 13 (43,3) | not reached | C | 0,172 |
| | | II | 11 (36,7) | not reached | 3,51 (0,37-33,74) | 0,277 |
| | | III | 6 (20,0) | 25,0 (-) | 8,28 (0,86-79,84) | 0,068 |
| Yes | | I | 10 (29,4) | not reached | C | 0,074 |
| | | II | 20 (58,8) | 96,0 (±56,8) | 5,62 (0,62-50,6) | 0,124 |
| | | III | 4 (11,8) | 33,0 (±20,0) | 16,54 (1,41-194,24) | 0,026 |
| No | Prevalence | localized | 12 (40,0) | not reached | C | 0,608 |
| | | locally distributed | 5 (16,7) | not reached | 84140,6 (0,00-6,1*$10^{163}$) | 0,952 |
| | | generalized | 13 (43,3) | not reached | 246987,8 (0,00-1,8*$10^{164}$) | 0,947 |
| Yes | | localized | 16 (47,1) | not reached | C | 0,101 |
| | | locally distributed | 5 (14,7) | 101,0 (±0,00) | 3,16 (0,45-22,45) | 0,250 |
| | | generalized | 13 (38,2) | 96,0 (±0,00) | 6,42 (1,17-35,18) | 0,032 |

(-) – standard errors cannot be calculated due to the small number of uncensored observations;
C – the level of the trait is taken as the baseline when evaluating the Cox regression.

The glucose level has no significant effect on the overall survival of patients without type 2 diabetes, and in patients with diabetes significantly ($p<0.05$) increases the risk of death by 2.15 times (95% CI 1.4-3.3) with an increase in glucose levels by 1 mmol/L.

The presence of grade 2 obesity in type 2 diabetes increases the risk of death by 19.37 times (95% CI 1.47-253.7, $p <0.05$) compared with normal weight. In the absence of DM, weight has no significant effect on overall survival.

In the absence of type 2 diabetes, tumor localization has no significant effect on overall survival ($p>0.05$). In the presence of DM and localization of the tumor in

the rectum, the risk of death increases by 13.45 times (95% CI 1.01-177.7), without a primary lesion – by 46.7 times (95% CI 4.21-518.8) compared with localization in the stomach ($p<0.05$).

In the absence of DM, the stage does not have a statistically significant effect on overall survival ($p>0.05$). In the presence of DM and stage III, the risk of death increases by 16.54 times (95% CI 1.41-194.24, $p<0.05$) compared with stage I.

In the absence of type 2 diabetes, the prevalence of the process does not affect overall survival ($p>0.05$). In the presence of type 2 diabetes and a generalized process, the risks of death increase by 6.42 times (95% CI 1.17-35.18, $p<0.05$).

Table 31 presents the results of a multifactorial analysis of the impact on overall survival.

Table 31 – Multivariate analysis of the effect on overall survival in a group of patients with gastrointestinal NET with type 2 diabetes

| Factor | Meaning | HR (95% CI) | Chi-square (p) |
|---|---|---|---|
| Glucose | | 3,03 (1,60-5,74) | 0,001 |
| Localization | stomach | C | 0,043 |
| | small intestine | 2,25 (0,25-20,15) | 0,467 |
| | rectum | 1,18 (0,07-19,27) | 0,906 |
| | pancreas | 1,17 (0,14-9,70) | 0,881 |
| | without a primary lesion | 608,7 (8,96-41370,8) | 0,003 |
| C – the level of the trait is taken as the baseline when evaluating the Cox regression. | | | |

Various combinations of factors were considered, the selection of factors in the model was carried out according to the chi-square criterion, taking into account the mutual correlation of factors. The model includes two factors – glucose level and tumor localization. In the presence of type 2 diabetes and the same tumor localization, an increase in blood glucose by 1 mmol/l increases the risk of death by 3 times (95% CI 1.6-5.7, $p<0.01$). In the presence of type 2 diabetes and a fixed level of glucose in the blood, localization of the tumor without a primary identified focus

increases the risk of death by 608.7 times (95% CI 8.96-41370.8, p<0.01) compared with localization in the stomach. The resulting model is statistically significant (chi-squared =19.38, p=0.004).

Table 32 presents the results of assessing the influence of factors on progression-free survival in groups with and without type 2 diabetes.

Table 32 – Influence of factors on progression-free survival in patients with gastrointestinal NET by type 2 diabetes mellitus presence/absence groups

| DM | Factor | Meaning | n (%) | Median (±SE) | HR (95% CI) | Chi-square (p) |
|---|---|---|---|---|---|---|
| No | Glucose | increased | 7 (23,3) | 47,0 (±0,00) | 1,31 (0,41-4,18) | 0,653 |
| | | normal | 23 (76,7) | 48,0 (±20,14) | C | |
| Yes | | increased | 25 (73,5) | 19,0 (±7,49) | 2,46 (±0,73-8,36) | 0,141 |
| | | normal | 8 (23,5) | not reached | C | |
| No | Glucose, value | | | | 1,18 (0,61-2,28) | 0,628 |
| Yes | | | | | 1,22 (0,99-1,51) | 0,062 |
| No | BMI | | | | 0,96 (0,83-1,11) | 0,577 |
| Yes | | | | | 1,06 (0,99-1,14) | 0,102 |
| No | Weight | normal | 22 (73,3) | 47,0 (±13,1) | C | 0,336 |
| | | redundant | 8 (26,7) | not reached | 0,54 (0,15-1,89) | |
| | | 1 degree of obesity | – | – | – | – |
| | | 2 degree of obesity | – | – | – | – |
| Yes | | normal | 15 (44,1) | 83,0 (±48,93) | C | 0,058 |
| | | redundant | 7 (20,6) | 6,0 (±1,3) | 4,63 (1,54-13,87) | 0,006 |
| | | 1 degree of obesity | 10 (29,4) | 16,0 (±26,2) | 2,09 (0,72-6,05) | 0,174 |
| | | 2 degree of obesity | 1 (2,9) | 51,0 (-) | 2,17 (0,26-18,09) | 0,475 |

Continuation of table 32

| DM | Factor | Meaning | n (%) | Median (±SE) | HR (95% CI) | Chi-square (p) |
|---|---|---|---|---|---|---|
| No | Carcinoid syndrome | No | 26 (86,7) | 81,0 (±42,7) | C | 0,154 |
| | | Yes | 4 (13,3) | 23,0 (±21,0) | 2,31 (0,73-7,32) | |
| Yes | | No | 28 (82,4) | 45,0 (±25,88) | C | 0,060 |
| | | Yes | 6 (17,6) | 6,0 (±7,96) | 2,46 (0,96-6,29) | |
| No | Localization | stomach | 7 (23,3) | not reached | C | 0,201 |
| | | duodenum | 1 (3,3) | not reached | 0,00 (0,00) | 0,991 |
| | | small intestine | 7 (23,3) | 47,0 (±11,4) | 2,25 (0,44-11,70) | 0,332 |
| | | colon | 1 (3,3) | 19,0 (-) | 10,35 (0,83-128,56) | 0,069 |
| | | rectum | 3 (10,0) | not reached | 0,00 (0,00) | 0,982 |
| | | pancreas | 9 (30,0) | 14,0 (±13,4) | 4,74 (0,94-23,84) | 0,059 |
| | | without a primary lesion | 2 (6,7) | 5,0 (-) | 18,79 (2,10-167,9) | 0,009 |
| Yes | | stomach | 10 (29,4) | 26,0 (±22,93) | C | 0,036 |
| | | duodenum | – | – | – | – |
| | | small intestine | 9 (26,5) | 6,0 (±0,99) | 2,99 (1,04-8,61) | 0,043 |
| | | colon | – | – | – | – |
| | | rectum | 1 (2,9) | 51,0 (-) | 1,56 (0,18-13,28) | 0,683 |
| | | pancreas | 12 (35,3) | 58,0 (±27,58) | 0,81 (0,26-2,52) | 0,714 |
| | | without a primary lesion | 2 (5,9) | 5,0 (-) | 6,66 (1,21-36,74) | 0,030 |
| No | Stage | I | 13 (43,3) | 48,0 (±1,0) | C | 0,638 |
| | | II | 11 (36,7) | 33,0 (±7,7) | 1,68 (0,56-5,05) | 0,359 |
| | | III | 6 (20,0) | 19,0 (±17,1) | 1,50 (0,41-5,44) | 0,536 |
| Yes | | I | 10 (29,4) | 18,0 (±34,39) | C | 0,389 |
| | | II | 20 (58,8) | 19,0 (±29,66) | 1,30 (0,50-3,38) | 0,597 |
| | | III | 4 (11,8) | 16,0 (±12,0) | 2,44 (0,67-8,87) | 0,175 |

Continuation of table 32

| DM | Factor | Meaning | n (%) | Median (±SE) | HR (95% CI) | Chi-square (p) |
|---|---|---|---|---|---|---|
| No | Prevalence | localized | 12 (40,0) | 81,0 (±0,00) | C | 0,003 |
| | | locally distributed | 5 (16,7) | 96,0 (±57,1) | 0,42 (0,04-4,44) | 0,471 |
| | | generalized | 13 (43,3) | 23,0 (±10,8) | 7,46 (1,93-28,84) | 0,004 |
| Yes | | localized | 16 (47,1) | 145,0 (±0,00) | C | 0,028 |
| | | locally distributed | 5 (14,7) | 51,0 (±41,63) | 2,46 (0,77-7,82) | 0,128 |
| | | generalized | 13 (38,2) | 16,0 (±6,59) | 3,58 (1,40-9,21) | 0,008 |
| (-) – standard errors cannot be calculated due to the small number of uncensored observations; C – the level of the trait is taken as the baseline when evaluating the Cox regression. | | | | | | |

Elevated glucose levels, blood glucose levels, BMI, the presence of carcinoid syndrome, stage do not have a statistically significant effect on progression-free survival in both the group with type 2 diabetes and in the group of patients without type 2 diabetes ($p>0.05$).

In the group of patients without type 2 diabetes, survival without progression, overweight or obesity have no statistically significant effect ($p>0.05$). In the group with DM, being overweight increases the risk of progression by 4.63 times (95% CI 1.54-13.87, $p <0.01$) compared to normal weight.

In patients without type 2 diabetes, localization of a tumor without a primary lesion increases the risk of progression by 18.79 times (95% CI 2.1-167.9, $p<0.01$) compared with localization in the stomach. In patients with type 2 diabetes, localization of the tumor in the small intestine increases the risk of progression by 2.99 times (95% CI 1.04-8.61), and with localization without a primary lesion – by 6.66 times (95% CI 1.21-36.74) compared with localization in the stomach ($p<0.05$).

In patients without type 2 diabetes, the generalized process increases the risk of progression by 7.46 times (95% CI 1.93-28.84), and in patients with DM – by 3.58 times (95% CI 1.4-9.21) compared with the localized process ($p<0.05$).

It was not possible to find a multifactorial model that statistically significantly explains progression-free survival in patients with type 2 diabetes.

Thus, there are differences between the groups of patients with the presence and absence of type 2 diabetes in terms of the effect on the overall survival of obesity, blood glucose levels, tumor localization, stage and prevalence of the process. According to the effect on progression-free survival between groups of patients with type 2 diabetes and without type 2 diabetes, there are differences in the effect of overweight, tumor localization, and the prevalence of the process. Despite the fact that the presence or absence of DM does not directly affect overall survival and progression-free survival, in combination with these factors, it statistically significantly increases the risks of death and progression in patients with gastrointestinal NET.

# Chapter 6
# GENOMIC SEQUENCING OF A NEW GENERATION

## 6.1 General characteristics of patients

Considering that at the moment the main prognostic markers of the course of gastrointestinal NET are only the proliferation index and the number of mitoses, understanding the molecular genetic nature of this nosology will allow us to expand our views on prognostic and predictive factors.

The main objective of this chapter is a detailed description of patients whose tumor material is aimed at genomic sequencing of a new generation.

Criteria for inclusion of patients in the study:

1. The opportunity to sign a form of voluntary informed consent to participate in this study.
2. Age – over 18 years.
3. Verified neuroendocrine tumor of the gastrointestinal tract.

Criteria for non-inclusion of patients in the study:

1. Lack of tumor material suitable for testing.

We observed 40 patients aged from 27 to 86 years (average age 63.23±2.62 (95% CI 57.92-69.53)) who received treatment and follow-up at the St. Petersburg State Medical Institution "City Clinical Oncology Dispensary" in the period from 2015 to 2022. The follow-up period was 84 months. In the study cohort of patients whose tumor material was examined, there were more women – 27 (67.5%) than men - 13 (32.5%) ($p<0.0001$). Neuroendocrine tumor of the gastrointestinal tract was verified in all patients at the stage of primary diagnosis. In the majority of patients – 13 (32.5%) – the primary focus was localized in the pancreas, in 11 (27.5%) – the small intestine, in 8 (20%) - the stomach. Other parts of the gastrointestinal tract were affected much less frequently: rectum – in 3 (7.5%), duodenum – in 1 (2.5%), colon –

in 1 (2.5%) patients. It should be noted that in 3 (7.5%) patients, the localization of the primary tumor focus could not be determined, but the IHC subtype of the tumor indicates that the tumor belongs to the gastrointestinal tract. The distribution of patients by gender and age (according to the WHO classification of age groups, 2016) is presented in Table 33.

Table 33 – Distribution of patients with neuroendocrine gastrointestinal tumors by gender and age

| Age | Gender | |
|---|---|---|
| | men, abs. (%) | women, abs. (%) |
| 18-44 years old (young age) | 1 (2,5%) | 7 (17,5%) |
| 45-59 years old (middle age) | 3 (7,5%) | 3 (7,5%) |
| 60-74 years old (elderly age) | 6 (15%) | 8 (20%) |
| 75-90 years old (senile age) | 3 (7,5%) | 9 (22,5%) |
| over 90 years old (centenarians) | 0 | 0 |
| Total: | 13 (32,5%) | 27 (67,5%) |

When analyzing the age of patients, it should be noted that among all age groups, elderly patients predominated (60-74 years according to the WHO classification, 2016) – 14 (35%) ($p<0.0001$).

All patients, after verification of the process and instrumental examination of all systems and organs, staged the tumor process according to the TNM classification, 7th edition. Taking into account the existing clinical recommendations, TNM staging was carried out depending on the localization of the primary tumor focus.

The distribution of patients for each descriptor is presented in Table 34.

At the initial diagnosis in most patients, the primary tumor focus was placed in the categories T1 – 8 (20%) patients and T2 – 6 (15%) patients. In 8 (20%) patients, the primary tumor focus was regarded as T3, and in 9 (22.5%) patients as T4. In 9 (22.5%) patients, the determination of the T descriptor was impossible due to the

primary surgical intervention in non-oncological hospitals and the lack of data in the primary medical documentation of patients.

Table 34 – Distribution of patients with neuroendocrine gastrointestinal tumors according to the TNM system (7th edition)

| T | Quantity, abs. (%) |
|---|---|
| 1 | 8 (20%) |
| 2 | 6 (15%) |
| 3 | 8 (20%) |
| 4 | 9 (22,5%) |
| Not defined | 9 (22,5%) |
| N | Quantity, abs. (%) |
| 0 | 15 (37,5%) |
| 1 | 15 (37,5%) |
| 2 | 10 (25%) |
| 3 | 0 |
| X | 0 |
| M | Quantity, abs. (%) |
| 0 | 21 (52,5%) |
| 1 | 19 (47,5%) |

No lesions of regional lymph nodes were detected in 15 (37.5%) patients at the initial diagnosis. Also, in 15 (37.5%) patients, the lesion of the regional lymphatic apparatus corresponded to criterion N1. A regional lesion in the volume of N2 was registered only in 10 (25%) patients. Patients whose lesion of regional lymph nodes, which would be regarded as T3 or lesion of the regional lymphatic apparatus was not evaluated at the initial stage, were absent.

The comprehensive examination revealed the presence of distant metastases in 19 (47.5%) patients; 21 (52.5%) patients had no signs of dissemination of the process.

## 6.2 Methods of morphological research

Histological examination of the surgical material was used to verify the tumor process.

Histological examination was carried out in the pathology department of St. Petersburg State Medical Institution "City Clinical Oncological Dispensary".

The postoperative material in the operating room was fixed in a 3% formalin solution and delivered to the pathology department, where it was registered in accordance with the established procedure. The duration of the fixation stage averaged 24 hours. After a day, the material was removed from the formalin solution, washed in running water, dried on filter paper and filled with paraffin (paraffin filling method). The paraffin-filled material was placed in a thermostat and kept for 24 hours at a temperature of 37 ° C in order to evenly and completely impregnate the tissue sample with paraffin. After completion of this stage – the stage of wiring the material – histological sections, no more than 10-15 microns thick, were prepared from the finished paraffin block using a microtome. The slices should be well straightened, without the formation of folds and tears. The resulting sections were applied to slides and stained with hematoxylin and eosin. At this stage, it is necessary to ensure that the color of the slices is uniform, with a clear differentiation of different structures. The resulting slices should be well enlightened.

Ready–made histological preparations were subjected to microscopic examination: the survey was carried out under magnification, a multiple of 5-10, and the sighting - under magnification, a multiple of 25-40.

After verification of the diagnosis of "neuroendocrine cancer of the gastrointestinal tract" with the help of morphological examination, the degree of differentiation of the tumor process was determined, which has an important prognostic value for the nosology under consideration. The distribution of patients depending on the degree of differentiation of the tumor process is presented in Table 35.

Table 35 – Distribution of patients with neuroendocrine gastrointestinal tumors by grade of malignancy

| G | Quantity, abs. (%) |
|---|---|
| 1 | 14 (35%) |
| 2 | 19 (47,5%) |
| 3 | 7 (17,5%) |

The tumor in 14 (35%) patients was with a grade of G1 malignancy, in the majority of 19 (47.5%) – with a grade of G2 malignancy. Only in 7 (17.5%) patients with morphological examination the degree of malignancy G3 ($p<0.00001$).

### *6.2.1 Immunohistochemical examination of a sample of tumor tissue to determine the level of proliferation*

In order to determine the level of proliferative activity of the tumor, assessed by analyzing the expression of Ki-67, in patients of the cohort under consideration, an immunohistochemical study of a sample of tumor tissue was performed.

The material was delivered to the laboratory for immunohistochemical examination in the form of paraffin blocks.

Microscopic examination selected the most suitable block containing tumor tissue. Slices with a thickness of 4 microns were cut from this block, which were placed on glasses with a poly-L-lysine coating. The sections were dried, dewaxed and exposed to antigen unmasking using a citrate buffer in a water bath, t = 95 °C, 30 minutes. After that, they cooled down at room temperature and washed with a tris buffer with twin. Each section was outlined with a paraffin pencil, after which the endogenous peroxidase was inhibited with 3% hydrogen peroxide for 20 minutes. Then an antibody was applied to each slice (Clone SP6, rabbit antibodies,

monoclonal, 1:200 dilution, manufacturer LabVision), the exposure lasted 1 hour on a thermostick in a "water bath" at a temperature of 30 °C.

DAKO's EnVision polymer detection system was used to visualize the antigen-antibody reaction, diaminobenzidine was used as a chromogen. The control coloring of the nuclei was carried out using Mayer's hematoxylin. After each of the stages, before staining with diaminobenzidine, the cut glasses were washed in a tris buffer with a pH 7.1 twin from BioOptica. The glasses were concluded in the BioMaunt environment of the company BioOptica.

The evaluation was carried out in the percentage (%) of positively colored cells in the presented sample.

### *6.2.2 Molecular genetic testing of tumor material by new generation sequencing (NGS)*

In order to determine the following genes in the tumor material-ATM, ATR, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CDK12, CHEK1, CHEK2, EPCAM, FANCL,MLH1, MSH2, NBN, NF1, PALB2, PMS2, RAD51B, RAD51C, RAD51D, RAD54L, STK11, TP53, POLE, KRAS, NRAS,BRAF, EGFR, ERBB2, PIK3CA, MET ex14, BAT25, BAT26, NR21, NR24, MONO27, KIT, PDGFRA, Pi3Ca a new generation sequencing method was used (NGS).

Preparation of libraries for sequencing was carried out using NimblGen SepCapEZ Choice ("Roche") and reagents recommended by the manufacturer. Sequencing was carried out on the Illumina MiSeq device ("Illumina").

Bioinformatic analysis was carried out as follows:

1. Removing adapters and sequences with poor reading quality.
2. Mapping of readings on the reference sequence of the human genome (hg19) using the BWAMEM algorithm.

3. Quality control of source data, alignment, enrichment and coverage of target regions using FastQC, BAMQC and NGSrich.

4. Search for nucleotide variations for germinal mutations using GATK HaplotypeCaller + UnifiedGenotyper (with the receipt of a combined VCF file).

5. Search for nucleotide variations for somatic mutations using Mutect2 + Strelka (to obtain a combined VCF file).

6. Search for structural variations using Lumpy and Manta (obtaining a combined VCF file and generating visual data to validate the analysis results).

7. Processing of VCF files using the SnpSift program (the filtering criterion is a reading depth of more than 10).

8. Annotation using SnpEff (analysis of all transcripts), ANNOSAR (analysis of allele frequencies in ExAC/gnomAD, 1000G and ESP6500, algorithms for checking the functional significance of SIFT, PolyPhen2, MutationTaster, FATMM, CADD, DANN, Eigen), Alamut Batch (influence on splicing, dbSNP, ClinVar, HGMD Professional databases), BIC databases.

## 6.3 Results

Genomic sequencing of 40 tumor samples of patients diagnosed with gastrointestinal NET was performed.

At the first stage, 40 tumor blocks of patients diagnosed with neuroendocrine tumors of the gastrointestinal tract were reviewed. Histological and immunohistochemical examination of the tumor material was used to verify the tumor process.

Morphological examination was carried out in the pathology department of the St. Petersburg State Medical Institution "City Clinical Oncological Dispensary".

After repeated confirmation of the diagnosis of "neuroendocrine tumor of the gastrointestinal tract", the degree of differentiation of the tumor process was

determined using morphological examination, which has an important prognostic value for the nosology under consideration.

According to the division according to the degree of differentiation of the gastrointestinal NET14 tumors had a grade of **malignancy** G1 (35%), 19 – G2 (47.5%), 7 – G3 (17.5%).

The second stage of the work was molecular genetic testing of tumor material by the new generation sequencing (NGS) method. The study was conducted in the conditions of the Medical and Diagnostic Center of the International Institute of Biological Systems named after Sergei Berezin (performer - M.G. Gordiev). The following genes were determined in the tumor material: ATM, ATR, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CDK12, CHEK1, CHEK2, EPCAM, FANCL, MLH1, MSH2, NBN, NF1, PALB2, PMS2, RAD51B, RAD51C, RAD51D, RAD54L, STK11, TP53, POLE, KRAS, NRAS,BRAF, EGFR, ERBB2, PIK3CA, MET ex14, BAT25, BAT26, NR21, NR24, MONO27, KIT, PDGFRA, PIK3Ca.

Preparation of libraries for sequencing was carried out using NimblGen SepCapEZ Choice ("Roche") and reagents recommended by the manufacturer. Sequencing was carried out on the Illumina MiSeq device ("Illumina").

Bioinformatic analysis was carried out as follows:

1. Removing adapters and sequences with poor reading quality.
2. Mapping readings to the reference sequence of the human genome (hg19) using the BWAMEM algorithm.
3. Quality control of source data, alignment, enrichment and coverage of target regions using FastQC, BAMQC and NGSrich.
4. Search for nucleotide variations for germinal mutations using GATK HaplotypeCaller + UnifiedGenotyper (with the receipt of a combined VCF file).
5. Search for nucleotide variations for somatic mutations using Mutect2 + Strelka (to obtain a combined VCF file).
6. Search for structural variations using Lumpy and Manta (obtaining a combined VCF file and generating visual data to validate the analysis results).

7. Processing of VCF files using the SnpSift program (the filtering criterion is a reading depth of more than 10).

8. Annotation using SnpEff (analysis of all transcripts), ANNOSAR (analysis of allele frequencies in ExAC/gnomAD, 1000G and ESP6500, algorithms for checking the functional significance of SIFT, PolyPhen2, MutationTaster, FATMM, CADD, DANN, Eigen), Alamut Batch (influence on splicing, dbSNP, ClinVar, HGMD Professional databases), BIC databases.

Results: pathogenic mutations were detected in 9 samples out of 40 (22.5%): PTEN (2.5%/1) (in combination with BRCA 1), PIK3CA (2.5%/1), RB1 (2.5%/1) (in combination with BRCA 2), CHEK2 (2.5%/1) (in combination with POLE), MLH1 (2.5%/1) (in combination with BRCA 1). The most frequent mutations were BRCA 1 (3/7.5%) and BRCA 2 (3/7.5%).

Analyzing the frequency of mutations, it is worth noting that only one mutation occurred in 4 out of 9 cases (44%), in the remaining 5 cases there were 2 mutations in 1 tumor sample (56%) [8]. The total percentage distribution among mutations is shown in Figure 35.

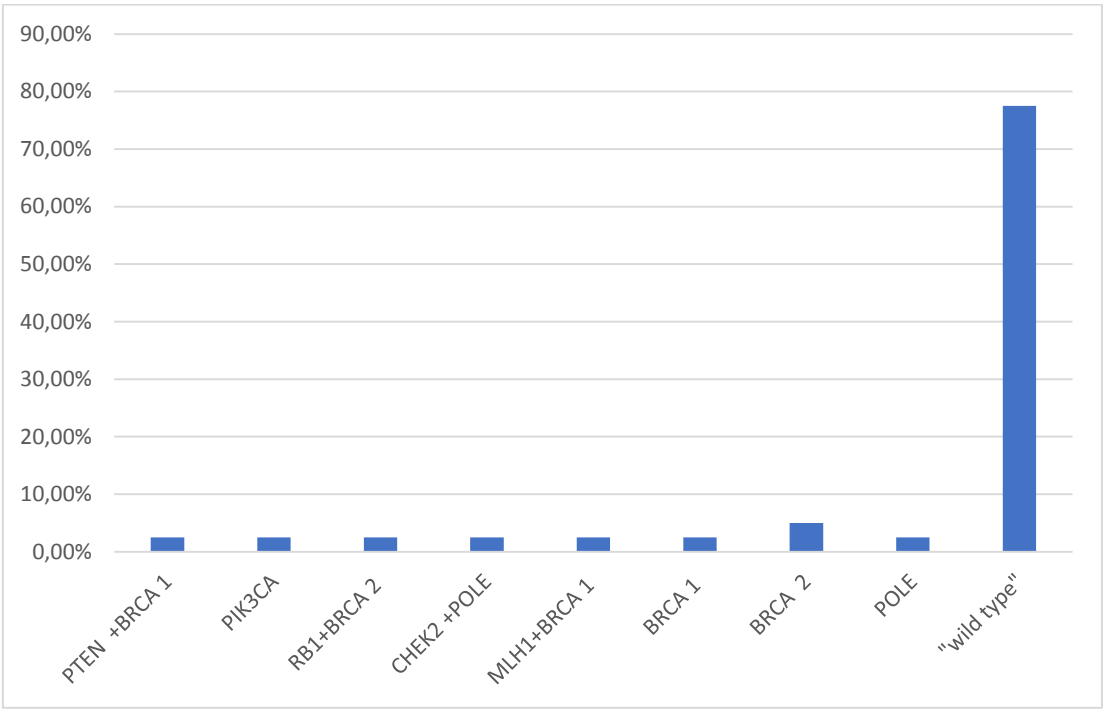


Figure 35 – Percentage distribution among mutations

We have made an attempt to analyze the effect of mutations in the structure of the NET gastrointestinal tract of the analyzed cohort of patients on the survival rates of patients. Despite the fact that the median overall survival was not achieved either in

the group of patients with mutations or in the group of patients with the "wild" type of tumor at the time of the data cut, the average OS in the "wild" type group was 188.81±17.34 (95% CI 155.42-222.19) months, and in a group of patients with the presence of any mutation – 49,35±8,35 (95% 32,98-65,72) ( p=0.527). The median progression-free time in the group of patients with the "wild" type of genes was 33.00 (95% CI 13.00–81.00) months, in the group of patients with mutations – 24.00 (95% CI 5.00-24.00) months (p=0.830).

As shown in Figure 36, the median S in the group of patients with the absence and presence of mutations have not yet been reached.

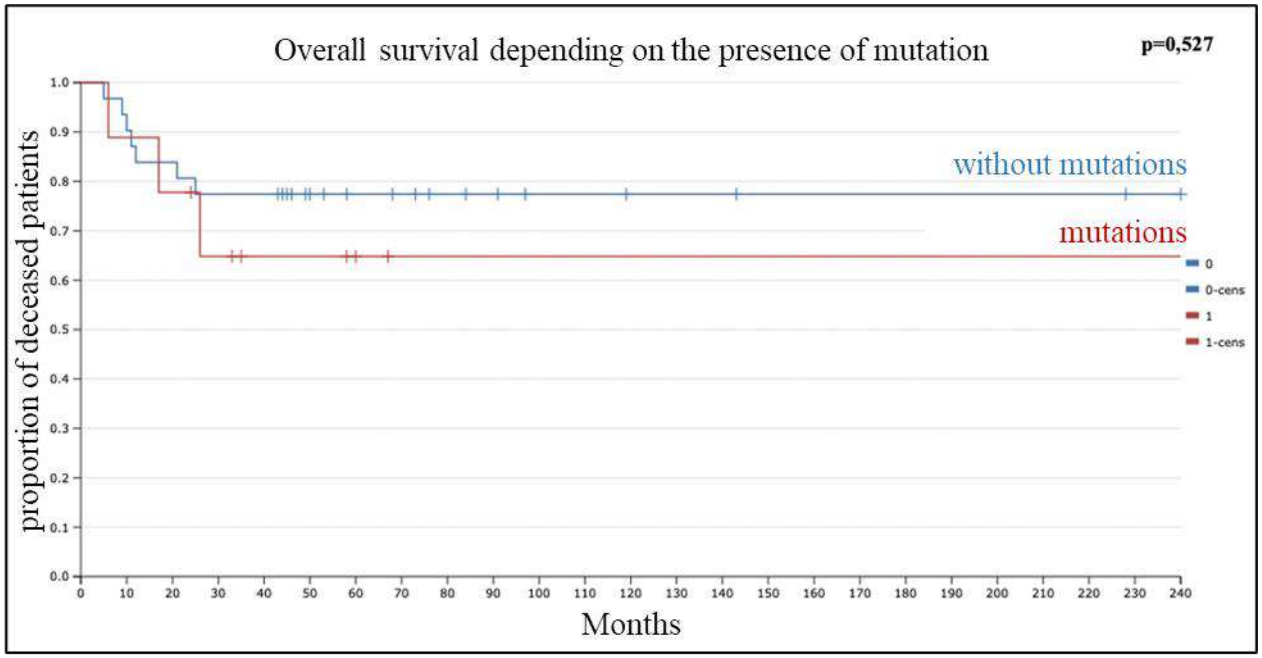


Figure 36 – Overall survival depending on the presence of mutation

The average OS in the group of patients without detected mutations was 188.81±17.03 months (95% CI 155.42-222.19). The average OS in the group with the presence of any mutation was 49.35±8.35 months (95% CI 32.98-65.72).

No significant difference was obtained when comparing the survival curves (p=0.527), however, the divergence of the curves indicates the presence of a possible confident trend.

Figure 37 shows progression-free survival depending on the presence of mutations, the median PFS in the group of patients with the absence of any mutation in the tumor structure was 33.00 months (95% 19.00-81.00).

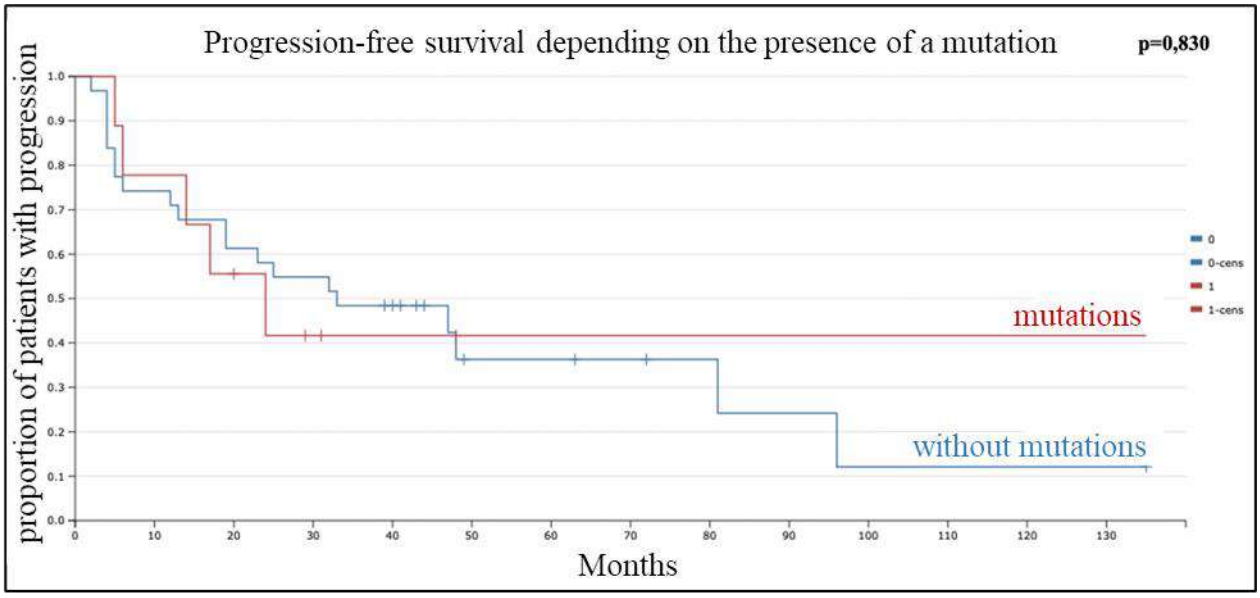


Figure 37 – Progression-free survival
depending on the presence of mutations

The median PFS in the group of patients with the identified mutation was 24.00 months (95% CI 14.00-49.00) and, although insignificant, was inferior to the median PFS of patients with the "wild type" of the tumor – p=0.830.

However, analyzing the progression-free survival curves, it is necessary to note the presence of their intersection, which indicates an increase in the risk of disease progression in the group of patients with a "wild type" tumor at the 48th month of the course of the disease, which requires further study.

It is molecular genetic profiling that can become the basis for building more individualized algorithms for treating patients. The results of this study allow us to count on the fact that when conducting a study on a larger cohort of patients with

gastrointestinal NET, it is possible to obtain results that will expand our knowledge of this nosology and allow us to supplement the already available information about prognosis factors and predictors of response to treatment. To improve the survival rates and individualization of treatment of patients, it is worth paying special attention to the molecular genetic study of the NET gastrointestinal tract. The results of this study can subsequently expand our knowledge about this nosology and will allow us to supplement the already available information about prognostic factors and predictors of response to treatment.

# Chapter 7
# DEVELOPMENT OF THE OPTIMAL SELECTION ALGORITHM TACTICS OF PRIMARY TREATMENT OF PATIENTS

## 7.1 Development of an algorithm for choosing the optimal tactics of primary treatment for patients with neuroendocrine tumors of the gastrointestinal tract

The general characteristics of patients with neuroendocrine tumors of the gastrointestinal tract are presented in Table 36.

Table 36 – General characteristics of patients with gastrointestinal NET included in the final analysis

| Sign | Abs., (%) |
|---|---|
| Total number of patients | 16 |
| Gender:<br>Men<br>women | <br>6 (37,5%)<br>10 (62,5%) |
| Age, average age<br>(years) | 66,13 years<br>[61-78] |
| Stage of the tumor process<br>I<br>II<br>III<br>IV<br>I | <br>2 (12,5%)<br>1 (6,25%)<br>2 (12,5%)<br>11 (68,75%)<br>2 (12,5%) |
| Localization of the primary focus:<br>stomach<br>pancreas | <br>1 (6,25%)<br>8 (50%) |

Continuation of table 36

| Sign | Abs., (%) |
|---|---|
| large intestine | 2 (12,5%) |
| small intestine | 1 (6,25%) |
| WPL | 4 (25%) |
| The degree of malignancy: | |
| G1 | 3 (18,75%) |
| G2 | 9 (56,25%) |
| G3 | 4 (25%) |
| Treatment performed: | |
| Drug therapy | 7(43,75%) |
| Surgery + Drug therapy | 3 (18,75%) |
| Surgery | 5 (31,25%) |
| Symptomatic therapy | 1 (6,25%) |

In order to determine the algorithm for choosing the tactics of treatment of patients with neuroendocrine tumors of the gastrointestinal tract, based on the data we obtained on the influence of the studied factors on the prognosis of the disease and the risk of its progression, we conducted a final analysis, which included 16 patients with a verified diagnosis of "Neuroendocrine tumor of the gastrointestinal tract, treated and monitored in St. Petersburg GBUZ "City Clinical Oncological Dispensary" in the period from June 2015 to 2021., in which all the studied prognostic markers were analyzed.

There were 6 men (37.5%) and 10 women (62.5%) in the study cohort of patients with verified gastrointestinal NET. The age of the patients ranged from 27 to 86 years, the average age was 66.13±10.27 years (95% CI 61.1-71.16). The general characteristics of the patients included in the final analysis are presented in Table 36.

The majority of patients 11 (68.75%) were diagnosed with stage IV of the disease at the initial treatment, 2 (12.5%) patients had stage I. Stage III was typical for 2 (12.5%) cases. The most rare – 1 (6.25%) was diagnosed with stage II of the disease.

In the majority of patients – 8 (50%) the primary tumor focus was localized in the pancreas, in 1 (6.25%) in the small intestine, in 2 (12.5%) in the colon. Tumors localized in the stomach were found in 1 (6.25%) of cases. In 4 (25%) – without a primary focus.

Immunohistochemical examination revealed an intermediate degree of malignancy in the majority of patients – 9 (56.25%). A low degree was found in 4 (25%) cases. The most rare – 3 (18.75%) - was the degree of malignancy G1.

In order to optimize the choice of primary treatment tactics at the first stage, all patients were divided into the following groups: a group of patients who received only drug treatment – 7 patients, a group of patients who received only surgical treatment – 3 patients, a group of patients who received surgical and drug treatment – 5 patients. Also, 1 patient received only symptomatic therapy. All patients were treated according to the current protocols.

### *7.1.2 General characteristics of clinical, laboratory and morphological factors of prognosis of the course neuroendocrine tumors of the gastrointestinal tract*

For the purpose of the final analysis, only patients who had at least one adverse factor (NF) identified by the results of the subgroup analysis were included in the analyzed cohort. The list of adverse factors and the threshold value of each of them, indicating an unfavorable course of the disease or a high risk of progression of the tumor process, is presented in Table 37.

Table 37 – Factors of unfavorable prognosis of the course and early progression of gastrointestinal NET

| No | Factor | Threshold value |
|---|---|---|
| 1 | Ki-67 | >5% |
| Peripheral blood parameters (initial assessment visit) | | |
| 2 | Relative number of neutrophils | >58,30% |
| 3 | Relative number of lymphocytes | ≤30% |
| 4 | Neutrophil-lymphocytic index (NLR) | >1,85 |

As a result of the final analysis, it was found that the number of unfavorable prognostic factors for high risk of progression in the cohort of patients with a diagnosis of "NET gastrointestinal tract" varied from 0 to 5; the average value was 2.63±1.45 (95% CI 1.85-3.40).

## *7.2 The results of the evaluation of the diagnostic significance of unfavorable prognosis factors in patients with neuroendocrine tumors of the gastrointestinal tract*

At the next stage of our study, we conducted a ROC analysis to determine the prognostic significance and threshold values (cut-off) of the number of significant factors of unfavorable prognosis of the course of the disease in patients with gastrointestinal NET.

## *7.3 Analysis of the influence of unfavorable prognosis factors on time without progression of patients with neuroendocrine tumors of the gastrointestinal tract*

The assessment of the influence of the identified factors of unfavorable prognosis on the life expectancy and time without progression of patients is presented in Table 38.

Table 38 – Assessment of the influence of unfavorable prognosis factors on life expectancy and progression-free time in patients with gastrointestinal NET (ROC analysis results)

| Indicator | Area under the curve (AUC) (95% CI) | p-value | Cut-off threshold (cut-off: number of adverse factors) | Sensitivity | Specificity |
|---|---|---|---|---|---|
| Time without | 0,936±0,073 (0,695-0,998) | <0,0001 | >2 | 76,92 | 100,00 |

The area under the ROC curve characterizing the effect of significant adverse factors on the time without progression of patients with gastrointestinal NET was 0.936±0.073 (95% CI 0.695-0.998). This model was statistically significant ($p<0.0001$), and the quality of the model was excellent (Figure 38).

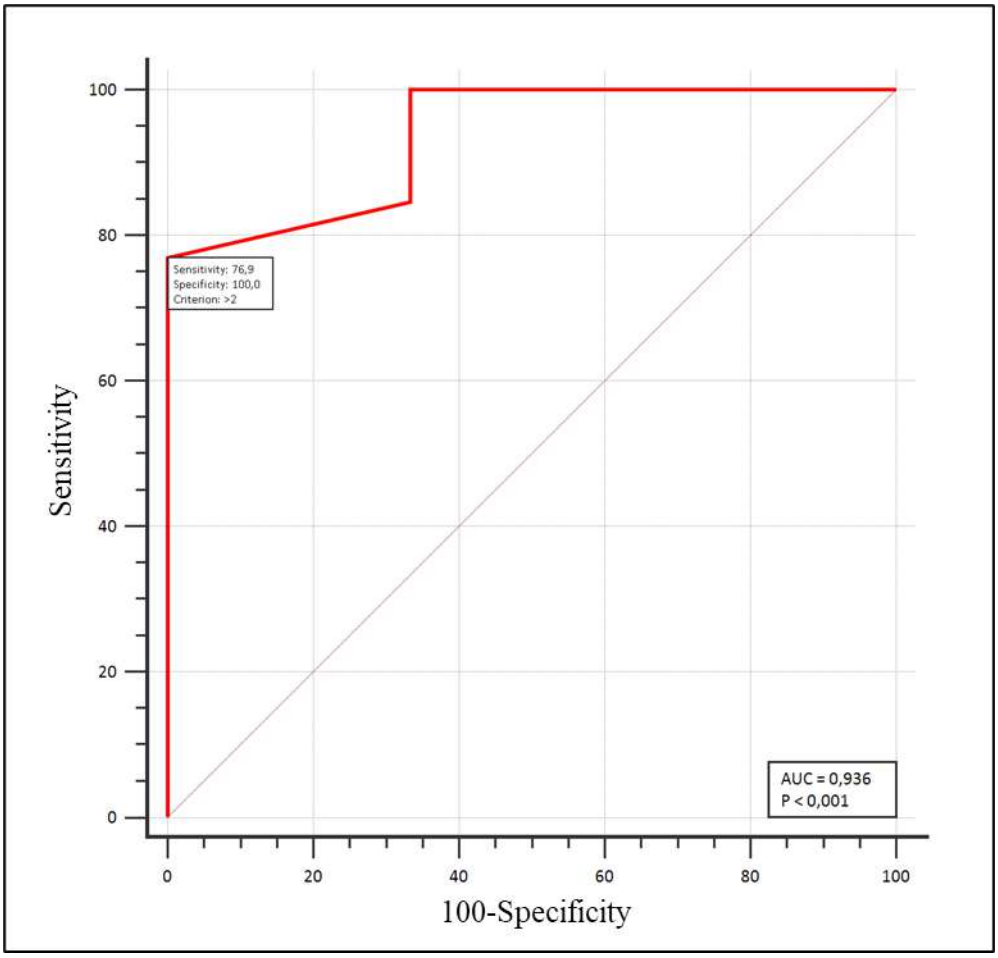


Figure 38 – Results of the ROC analysis of the dependence of the time indicator without progression on the number of factors of an unfavorable prognosis

The optimal threshold value of the number of unfavorable prognosis factors at the cut-off point was 2: the presence of 3 or more unfavorable prognosis factors at the time of the initial assessment negatively affected the time without progression of patients. The sensitivity of this test was 76.92%, which makes it possible to use it as a screening test, and the specificity was 100.00%, which makes it possible to use this test as a confirmatory (confirmatory).

The influence of the number of adverse factors on the course of the disease is shown in Figure 39.

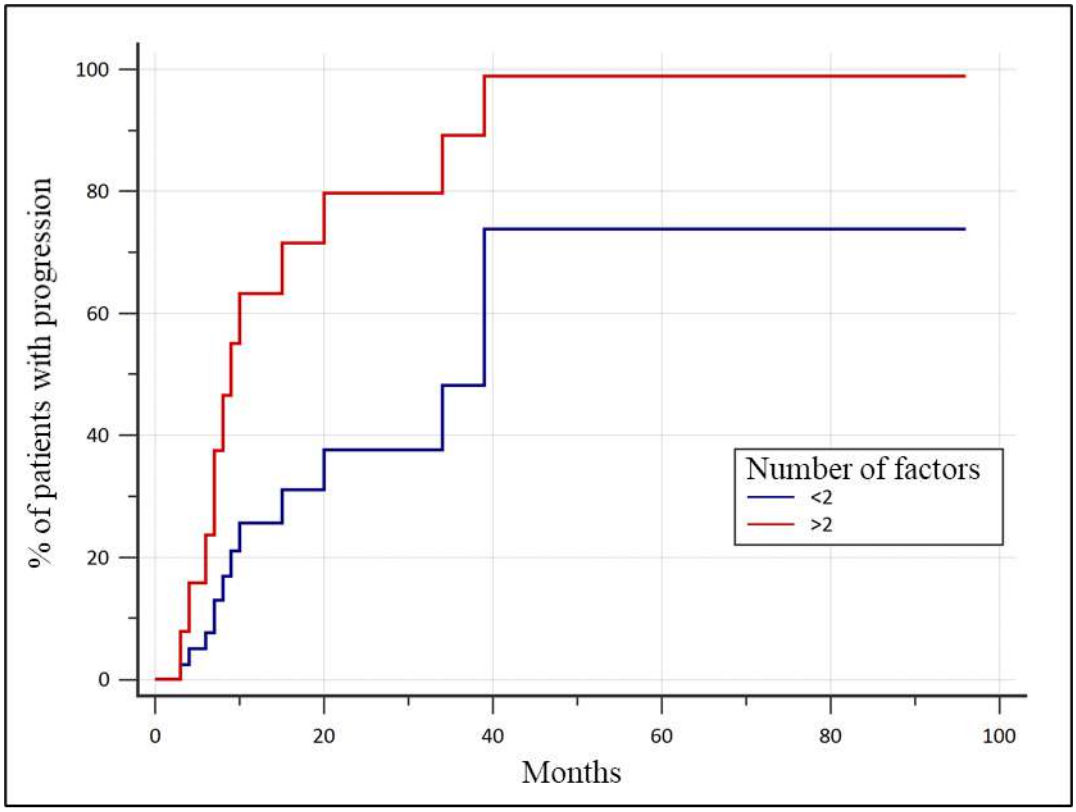


Figure 39 – A model of the relative risk of disease progression in patients with gastrointestinal NET depending on the presence of unfavorable prognostic factors for the course of the disease and their number

The presence of >2 factors of unfavorable prognosis of the course of the disease increased the risk of disease progression by 67%: p=0.0013; HR=1.67, 95% CI 1.05-1.78.

Thus, our study allowed us to identify independent additional prognostic factors that significantly affect the risk of developing gastrointestinal NET progression, which allows us to create a scale for use in clinical practice (Table 39).

Table 39 – Assessment of factors of unfavorable prognosis of NET gastrointestinal tract

| No. | Factor | Threshold value | Scores:<br>0 – no<br>1 – yes |
|---|---|---|---|
| 1 | Expression Ki-67 | >5% | |
| Peripheral blood parameters (initial assessment visit) | | | |
| 2 | Relative number of neutrophils | >58,30% | |
| 3 | Relative number of lymphocytes | ≤30% | |
| 4 | Neutrophil-lymphocytic index | >1,85 | |
| Total number of factors (points) | | | |

# CONCLUSION

Data from foreign and domestic studies over the past 40 years indicate a significant increase in the incidence of neuroendocrine tumors of all localizations, including the gastrointestinal tract.

Over the past decades, algorithms and approaches to the classification of neuroendocrine tumors have been revised, however, among the factors determining the prognosis and course of the disease, the proliferative activity index (Ki-67) and the localization of the primary focus of the neuroendocrine tumor can be distinguished.

However, these generally recognized factors of unfavorable prognosis in some clinical situations do not reflect the actual course of the disease. It is this paradox in the clinical course of neuroedocrine tumors of the gastrointestinal tract that requires additional study.

In Russia, a series of studies devoted to the search for unfavorable prognosis factors among the factors of systemic inflammation and some parameters of the metabolic syndrome in neuroendocrine tumors of the gastrointestinal tract has not been conducted, and therefore the work performed at the St. Petersburg State University (St. Petersburg) is of particular importance. The study included 298 patients treated for neuroendocrine tumors of the gastrointestinal tract in the period from 2015 to 2021 according to standard protocols. The aim of the study was to improve the results of treatment of cancer patients by determining prognostic factors in patients with neuroendocrine tumors of the gastrointestinal tract**.**

When analyzing the database, it is necessary to focus on some points. Namely, the majority of tumor samples of most patients had a grade of malignancy G1 – 144 (48.32%) of the sample or G2 – 115 (38.59%). Only in 39 (13.09%) patients with morphological examination the degree of malignancy G3 ($p < 0.00001$). It is also worth noting that in a significant majority of patients – 257 (86.24%) – at the time of the onset of the disease, no manifestations of carcinoid syndrome were registered

($p<0.0001$). Among 41 (13.76%), 21 (51.22%) patients felt hot flashes, 15 (36.59%) patients had abdominal pain, 35 (85.37%) patients had diarrhea. In 18 (43.90%) patients, the carcinoid syndrome manifested only one symptom – hot flashes or diarrhea. A combination of two symptoms was registered in 18 (43.90%): hot flashes and abdominal pain were registered in 2 (4.88%) patients, hot flashes in combination with diarrhea and abdominal pain in combination with diarrhea – in 8 (19.51%), respectively. In 5 (12.20%) patients, at the time of the initial diagnosis of gastrointestinal NET, the carcinoid syndrome was manifested by a combination of all three symptoms. Our analysis showed a complete correlation with the world data, where the frequency of registration of carcinoid syndrome is no more than 20% and the most frequent symptom of carcinoid syndrome is hot flashes.

When analyzing the data obtained during the evaluation of the treatment, it is worth focusing on a fairly large proportion of patients who underwent surgical treatment at stage 1 (80.20% ($p<0.00001$)). These data suggest that the detection of tumors at an early stage allows performing surgical treatment at the first stage. Despite the fact that the surgical method was the main method of initial treatment, the nature of the surgical intervention was not always radical. According to the analyzed data, radical surgical treatment was performed in 177 (74.06%) of 239 patients, cytoreductive surgery – in 55 (23.01%). Considering that surgical treatment is of key importance in the treatment of neuroendocrine tumors, such a percentage of surgical treatment extremely positively characterizes the institution in which the patients were curated.

Focusing on the survival rates of patients, it is worth noting that the median S of patients with gastrointestinal NET was not reached at the time of the data cut, and the average life expectancy of patients in the cohort under consideration was 210.40±8.51 months (95% CI 193.72-227.08). The median PFS in the cohort of patients under consideration was 81.00 months (95% CI 59.00-156.00). The data obtained characterize neuroendocrine tumors of the gastrointestinal tract as an indolent oncological disease.

The degree of malignancy of highly differentiated neuroendocrine tumors significantly affects the survival rates of patients with neuroendocrine tumors. Thus, the median S of patients with G1, at the time of data collection was not reached and significantly ($p<0.0001$) exceeds the median S of patients with G2 (HR=0.39, 95% CI 0.24-0.65), which at the time of data cut was also not reached) and G3 tumors, which was 20.0 months (95% CI 12.00-31.00) (HR=0.09, 95% CI 0.04-0.21). The figures we have obtained absolutely justify the introduction of a new classification of neuroendocrine tumors where the degree of malignancy G3 is introduced.

The greatest median of PFS was determined in patients whose primary tumor focus was localized in the area of the appendix, where it was 192.0 months (95% CI 192.0-204.0) and significantly ($p<0.0001$) exceeded the median of PFS in the group of patients without a primary focus (median PFS 11.0 (95% CI 6.0-20.0); HR=0.06, 95% CI 0.01-0.38). The obtained data allow us to think about how to optimize the algorithm for the treatment of neuroendocrine tumors without a primary identified focus in the direction of intensification.

When analyzing the influence of systemic inflammation factors, it was revealed that independent adverse factors that increase the risk of disease progression were: the initial level of the relative number of peripheral blood neutrophils >58.30% ($p=0.0336$, HR 1.05: 95% CI 1.01-1.09), the initial level of the relative number of peripheral blood lymphocytes <30% ($p=0.0443$, HR 1.03: 95% CI 1.01-1.06) and NLI >1.85 ($p=0.0228$; HR 1.17: 95% CI 1.02-1.34). The results obtained indicate that the analyses and calculated indices so accessible in routine clinical practice are extremely promising prognostic factors that will optimize existing algorithms for the treatment of patients.

Analyzing the influence of some factors of the metabolic syndrome, we can say that there are differences between groups of patients with the presence and absence of type 2 diabetes in terms of the impact on overall survival of obesity, blood glucose levels, tumor localization, stage and prevalence of the process. According to the effect on progression-free survival between groups of patients with type 2 diabetes and without type 2 diabetes, there are differences in the effect of overweight, tumor

localization, and the prevalence of the process. Despite the fact that the presence or absence of DM does not directly affect overall survival and progression-free survival, in combination with these factors, it statistically significantly increases the risks of death and progression in patients with gastrointestinal NET. The obtained data lead to conclusions that it is necessary to continue to engage in research on the search for prognostic factors in the chosen direction. Considering that neuroendocrine tumors originate from endocrine cells, which in turn regulate metabolic processes.

One of the stages of the work was devoted to testing the tumor blocks of patients with neuroendocrine gastrointestinal tumors using a new generation sequencing. The results obtained: pathogenic mutations were detected in 9 samples out of 40 (22.5%): PTEN (2.5%/1) (in combination with BRCA 1), PIK3CA (2.5%/1), RB1 (2.5%/1) (in combination with BRCA 2), CHEK2 (2.5%/1) (in combination with POLE), MLH1 (2.5%/1) (in combination with BRCA 1). The most frequent mutations were BRCA 1 (3/7.5%) and BRCA 2 (3/7.5%). Of course, the data obtained do not allow us to draw any practical conclusion, given the small sample. However, these results allow us to hope that further research can expand our knowledge about the molecular nature of neuroendocrine gastrointestinal tumors. Germline mutations deserve special attention, given the frequency of their occurrence in our study. In patients who have pathogenic somatic BRCA mutations, it makes sense to study responses to treatment with platinum preparations. It is also noteworthy that such mutations as POLE (which is a favorable sign for endometrial cancer), PIK3CA mutation (as a possible point of application of a targeted drug that is registered in hormone-positive breast cancer with this mutation), MLH1 mutation (a manifestation of microsatellite instability) are found in patients.

In order to determine the algorithm for choosing the tactics of treatment of patients with neuroendocrine tumors of the gastrointestinal tract, based on the data we obtained on the influence of the studied factors on the prognosis of the disease and the risk of its progression, we conducted a final analysis, which included 16 patients with a verified diagnosis of "Neuroendocrine tumor of the gastrointestinal tract, treated and monitored in St. Petersburg GBUZ "City Clinical Oncological

Dispensary" in the period from June 2015 to 2021., in which all the studied prognostic markers were analyzed.

For the purpose of the final analysis, only patients who had at least one adverse factor identified by the results of the subgroup analysis were included in the analyzed cohort. The list of unfavorable factors and the threshold value of each of them, indicating an unfavorable course of the disease or a high risk of progression of the tumor process – Ki-67 >5%, the relative number of lymphocytes < 30%, neutrophil-lymphocyte index > 1.85. As a result of the final analysis, it was found that the number of unfavorable prognostic factors of high risk of progression in the cohort of patients with a diagnosis of "NET gastrointestinal tract" varied from 0 to 5; the average value was 2.63±1.45 (95% CI 1.85-3.40).

At the final stage of the work, a ROC analysis was performed to determine the prognostic significance and threshold values (cut-off) of the number of significant factors of unfavorable prognosis of the course of the disease in patients with gastrointestinal NET.

The optimal threshold value of the number of unfavorable prognosis factors at the cut-off point was 2: the presence of 2 or more unfavorable prognosis factors at the time of the initial assessment negatively affected the time without progression of patients. The sensitivity of this test was 76.92%, which makes it possible to use it as a screening test, and the specificity was 100.00%, which makes it possible to use this test as a confirmatory (confirmatory). The presence of >2 factors of unfavorable prognosis of the course of the disease in the patient increased the risk of disease progression by 67%: p=0.0013; HR=1.67, 95% CI 1.05-1.78.

Thus, our study revealed independent additional prognostic factors that significantly affect the risk of developing gastrointestinal NET progression, which allows us to create a scale for use in clinical practice. The developed scale will subsequently make it possible to rationalize the choice of treatment tactics for patients with neuroendocrine tumors of the gastrointestinal tract.

# SUMMARY

1. Independent adverse factors increasing the risk of disease progression were: baseline relative neutrophil count >58.30% (p=0.0336, HR 1.05: 95% CI 1.01-1.09), baseline relative lymphocyte count <30% (p=0.0443, HR 1.03: 95% CI 1.01-1.06) and NLI >1.85 (p=0.0228; HR 1.17: 95% CI 1.02-1.34).

2. In the presence of type 2 diabetes and the same tumor localization, an increase in blood glucose by 1 mmol/l increases the risk of death by 3 times (95% CI 1.6-5.7, p<0.01). In the presence of type 2 diabetes and a fixed level of glucose in the blood, localization of the tumor without a primary identified focus increases the risk of death by 608.7 times (95% CI 8.96-41370.8, p<0.01) compared with localization in the stomach.

3. pathogenic mutations were detected in 9 samples out of 40 (22.5%): PTEN (2.5%/1) (in combination with BRCA 1), PIK3CA (2.5%/1), RB1 (2.5%/1) (in combination with BRCA 2), CHEK2 (2.5%/1) (in combination with POLE), MLH1 (2.5%/1) (in combination with BRCA 1). The most frequent mutations were BRCA 1 (3/7.5%) and BRCA 2 (3/7.5%).

4. According to the results of multivariate analysis, it was possible to identify factors of unfavorable prognosis of the course and early progression of gastrointestinal NET- Ki-67 >5%, the relative number of neutrophils >58.30%, the relative number of lymphocytes ≤ 30%, neutrophil-lymphocytic index >1.85. The optimal threshold value of the number of unfavorable prognosis factors at the cut-off point was 2: the presence of 2 or more unfavorable prognosis factors at the time of the initial assessment negatively affected the patients' PFS. The presence of >2 factors of unfavorable prognosis of the disease increased the risk of disease progression by 67%: p=0.0013; HR=1.67, 95% CI 1.05-1.78.

# PRACTICAL RECOMMENDATIONS

Analyzing all the obtained clinical, morphological and immunohistochemical data, it became possible to identify the following recommendations for improving treatment algorithms for patients with gastrointestinal NET.

1. It is advisable to determine the following factors in patients with NET gastrointestinal tract at the stage of primary diagnosis in order to determine the prognosis of the course of the disease and the choice of optimal treatment tactics:

- initial level of the relative number of neutrophils);
- initial level of the relative number of lymphocytes;
- NLI (neutrophil-lymphocytic index);
- Ki 67.

2. If the main registered options for the treatment of gastrointestinal NET are exhausted, it is recommended to carry out **next generation sequencing.**

3. According to the result of multifactorial analysis, it was possible to identify factors of unfavorable prognosis of the course and early progression of gastrointestinal NET- Ki-67 >5%, the relative number of neutrophils >58.30%, the relative number of lymphocytes ≤ 30%, neutrophil-lymphocytic index >1.85. The presence of >2 factors of unfavorable prognosis of the disease increased the risk of disease progression by 67%: p=0.0013; HR=1.67, 95% CI 1.05-1.78.

Our study allowed us to identify independent additional prognostic factors that significantly affect the risk of developing gastrointestinal NET progression, which allows us to create a scale for use in clinical practice.

# LIST OF ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| RFS | relapse-free survival |
| HAC | higher attestation commission |
| PFS | progression-free survival |
| WHO | World Health Organization |
| CI | confidence interval |
| IHC | immunohistochemistry |
| BMI | body mass index |
| CT | computed tomography |
| LMI | lymphocytic-monocytic index |
| MRI | magnetic resonance imaging |
| MS | metabolic syndrome |
| MEN | Multiple Endocrine Neoplasia |
| ND | no data |
| NLI | neutrophil-lymphocytic index |
| WPL | without a primary lesion |
| NSE | neuron - specific enolase |
| NET | a group of tumors that develop from neuroendocrine cells |
| NEC | neuroendocrine cancer |
| TAMs | tumor associated macrophages |
| OS | overall survival |
| HR | relative risk |
| HR | odds ratio |
| PET | positron emission tomography |
| PET-CT | positron emission tomography-computed tomography |
| LINE1 | Long INterspersed Element-1 |
| DM | diabetes mellitus |
| PLI | platelet-lymphocyte index |
| USE | ultrasound examination |
| FDG | fluordeoxyglucose |
| 25(OH)D | 25-hydroxyvitamin D |
| AHR- repressor | arylhydroxyantranarate-repressor, protein |

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|---|---|
| ALK-translocations | a genetic change in which the ALK gene (anaplastic lymphoma kinase) moves and combines with another gene |
| Ang2 | a protein that plays a key role in the regulation and development of blood vessels |
| ANNOSAR | ANNOtation of VARiation, a tool for analyzing genetic variants |
| APC | Adenomatous Polyposis Coli, gene |
| APC1 | one of the many exons of gene APC |
| ASCO GI | American Society of Clinical Oncology Gastrointestinal Cancers Symposium, annual scientific conference |
| ATM | Ataxia Telangiectasia Mutated, gene |
| ATR | Ataxia Telangiectasia and Rad3-related, gene |
| ATRX | Alpha-thalassemia/mental retardation syndrome X-linked, gene |
| AUC | Area Under the Curve |
| BAMQC | Bacterial Antimicrobial Resistance Gene-Profile Quality Control, a tool for verifying the correctness and completeness of gene profiles |
| BARD1 | BRCA1 associated RING domain 1, gene |
| BAT25 | Battelle Memorial Institute 25, microsatellite DNA marker |
| BAT26 | Battelle Memorial Institute 26, microsatellite DNA marker |
| BIC | Bayesian Information Criterion, statistical indicator |
| BRAF | B-Rapidly Accelerated Fibrosarcoma, gene |
| BRCA1 | BReast CAncer gene 1, gene |
| BRCA2 | BReast CAncer gene 2, gene |
| BRIP1 | RCA1 interacting protein C-terminal helicase 1, gene |
| BWAMEM | Burrows-Wheeler Aligner MEM, DNA or RNA sequence alignment algorithm |
| CADD | Combined Annotation-Dependent Depletion, methodology for predicting the pathogenicity of depeetic variants |
| CADM1 | Cell Adhesion Molecule 1, or TSLC, cell adhesion molecule |
| CD163 | Cluster of Differentiation 163, molecule |
| CDH1 | Cluster of Differentiation 1, gene |
| CDK12 | Cyclin-Dependent Kinase 12, gene |
| CgA | Chromogranin A, protein |
| CHEK1 | Checkpoint Kinase 1, gene |
| CHEK2 | Checkpoint Kinase 2, gene |
| c-kit | (CD117 or stem cell factor receptor) protein receptor |

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| ClinVar | public and curated database |
| Cyclin D1 | a protein that participates in the regulation of the cell cycle |
| DANN | Domain Adversarial Neural Network, machine learning algorithm |
| DAPK | Death-Associated Protein Kinase, protein |
| DAPK1 | Death-Associated Protein Kinase 1, isoform of the DAPK protein |
| DAXX | Death Domain-Associated Protein, protein |
| DAXX/ATRX | a genetic complex: DAS (Death Domain-Associated Protein) and ATRX (Alpha Thalassemia/Mental Retardation Syndrome X-Linked) |
| dbSNP | Database of Single Nucleotide Polymorphisms, database |
| EC | epirubicin-cyclophosphamide |
| EGFR | Epidermal Growth Factor Receptor, epidermal growth factor receptor |
| Eigen | The Eigen3 algorithm, a linear algebra library |
| ENETs | European Neuroendocrine Tumor Society, uniting specialists engaged in the study and treatment of neuroendocrine tumors |
| EP | Etoposide and Cisplatin |
| EPCAM | Epithelial Cell Adhesion Molecule, glycoprotein |
| ERBB2 | (HER2 or HER2/neu) gene |
| ExAC | Exome Aggregation Consortium, database |
| gnomAD | Genome Aggregation Database, database |
| EZH2 | Enhancer of Zeste Homolog 2, gene |
| FANCL | Fanconi Anemia Complementation Group L, gene |
| FastQC | Fast Quality Control, a tool for assessing the quality of source data |
| FATMM | Fraud Analysis Through Metadata, a method that uses metadata |
| FLI | Fatty Liver Index, index of assessment of the degree of fatty liver dystrophy |
| FOLFIRI | chemotherapy regimen, calcium folinate (folinic acid), fluorouracil and irinotecan |
| FOLFOX | chemotherapy regimen, calcium folinate (folinic acid), fluorouracil and oxaliplatin |
| G1 | a well-differentiated tumor |
| G2 | average tumor differentiation |
| G3 | poorly differentiated tumor |
| Ga68-DOTA-octreotide | radioactive medicinal substance, gallium 68 (Ga68) and DOTA-octreotide |
| GATK | Genome Analysis Toolkit, a set of tools for data analysis |
| GEMOX | gemcitabine (Gemzar) and oxaliplatin (Eloxatin) |

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| GP | gemcitabine (Gemzar) and paclitaxel (Taxol) |
| HER2 | human epidermal growth factor receptor 2 |
| HGMD | Human Gene Mutation Database, database |
| HIC1 | Hypermethylated in Cancer 1, gene |
| HIF | Hypoxia-Inducible Factor, transcription factor |
| hMLH1 | human MutL homolog 1, gene |
| HR | Hazard Ratio |
| IL-8 | Interleukin-8, cytokine, protein |
| JIS | Joint Interim Statement Criteria for Metabolic Syndrome |
| Ki-67 | marker of complex protein |
| KIT | (CD117), protein receptor |
| KRAS | gene, coding protein KRAS |
| LOH | Loss of Heterozygosity, a change in the genome |
| MET | gene, coding receptor for growth hormone |
| MET ex14 | exon 14 of the MET gene, a variant of the MET gene |
| MGMT | O^6-methylguanine-DNA methyltransferase, the enzyme |
| MINEN | Mixed neuroendocrine-non-neuroendocrine neoplasms, type of tumors |
| MLH1 | Mismatch Repair Protein 1, gene |
| MONO27 | refers to the gene designated as MONO27 |
| MSH2 | MutS Homolog 2, gene |
| MSH3 | MutS Homolog 3, gene |
| MSH4 | MutS Homolog 4, gene |
| MSH6 | MutS Homolog 6, gene |
| mTOR | mammalian target of rapamycin, protein kinase |
| MVD | Microvessel Density, density of microvascular structures in the tumor |
| MVI | Microvascular Invasion, microvascular invasion |
| MEN-1 | Multiple Endocrine Neoplasia Type 1, hereditary disease |
| MEN-2A | Multiple Endocrine Neoplasia Type 2A, hereditary disease |
| MEN-2B | Multiple Endocrine Neoplasia Type 2B, hereditary disease |
| NBN | gene, encodes a protein known as Nibrin |
| NF1 | neurofibromine |
| NGS | Next Generation Sequencing |
| NGSrich | software |
| NLR | the ratio of the absolute number of neutrophils to the difference between |

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| | leukocytes and the absolute number of neutrophils |
| NR21 | gene, NR3C1 |
| NR24 | gene, NR2E1 |
| NRAS | gene, which encodes the NRAS protein |
| P16 | or CDKN2A, gene |
| p16 INK4a/P14 ARF | (P16/P14) it is a complex of proteins |
| p18 INK4c | (P18), protein tumor suppressor |
| p27 Kip1 | (P27), protein tumor suppressor |
| P53 | protein tumor suppressor |
| PALB2 | Partner and Localizer of BRCA2, gene |
| PAX5 | B-cell transcription factor 1 (BSAP) |
| PDGFRA | Platelet-Derived Growth Factor Receptor Alpha, gene |
| PDGFRβ | Platelet-Derived Growth Factor Receptor Beta, gene |
| PD-L1 | programmed death 1 ligand |
| P-gp | pi-globulin, glycoprotein carrier |
| Pi3Ca | PI3Kα, phosphoinositide-3-kinase α, an isoform of phosphoinositide-3-kinase |
| PIK3CA | PI3K catalytic subunit alpha, gene |
| PLR | platelet-to-lymphocyte ratio, the ratio of the number of platelets to the number of lymphocytes |
| PMS2 | Postmeiotic Segregation Increased 2, gene |
| PNI | perineural invasion |
| POLE | epidermal growth factor polymerase |
| PolyPhen2 | Polymorphism Phenotyping v2, prediction algorithm |
| PTEN | Phosphatase and Tensin Homolog, gene |
| RAD50 | gene, coding enzyme protein RAD50 |
| RAD51AP2 | gene, coding protein RAD51-associated protein 2 |
| RAD51B | gene, coding protein RAD51B |
| RAD51C | gene, coding protein RAD51C |
| RAD51D | gene, coding protein RAD51D |
| RAD54L | DNA repair and recombination protein RAD54-like, gene |
| RAR-β | Retinoic Acid Receptor Beta, gene |
| Ras/MAPK | Ras/mitogen-activated protein kinase, signal path |
| RASSF1 | Ras association domain family 1, gene |

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| RASSF1A | Ras association domain family 1 isoform A, protein isoform RASSF1 |
| RB1 | retinoblastoma gene 1 |
| ROC | Receiver Operating Characteristic, graphical evaluation tool |
| RUNX3 | Runt-related transcription factor 3, gene |
| RUSSCO | Russian Stabilization and Creation Organization |
| SE | Standard Error, a measure of the spread or statistical parameter |
| SEER | Surveillance, Epidemiology, End Results, US state program |
| SIFT | Scale-Invariant Feature Transform, computer vision algorithm |
| SMAD4 | SMAD family member 4, protein |
| SP6 | antibody clone |
| SSTR-2a | somatostatin receptor 2a |
| STK11 | serine/threonine kinase 11 or LKB1 (lifeobelkinase B1) |
| SYNE1 | ankyrin is repetitive of gene 1 |
| t790m | mutation in the epidermal growth factor receptor (EGFR) gene |
| TemCap | Temodal (temozolomide) and Capecitabine |
| TIMP3 | Tissue Inhibitor of Metalloproteinase 3, gene |
| TNM | cancer staging system |
| TP53 | gene, coding protein p53 |
| TSC1 | Tuberous Sclerosis Complex 1, gene |
| TSC2 | Tuberous Sclerosis Complex 2, gene |
| VAI | visceral adiposity index |
| VCF- file | Variant Call Format, text file |
| VEGF | Vascular Endothelial Growth Factor, protein |
| VEGFR-2 | Vascular Endothelial Growth Factor Receptor 2, the receptor |
| VEGFR-3 | Vascular Endothelial Growth Factor Receptor 3, the receptor |
| VHL | Von Hippel-Lindau, gene |
| XELOX | Capecitabine (Xeloda) and Oxaliplatin (Eloxatin) |

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