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«Южно-Российский онкологический журнал»: профессиональное медицинское издание. В нем публикуются новости медицинского и фармацевтического сообществ, научно-практические статьи для целевой аудитории – врачей-онкологов. Редакция журнала ставит своей задачей популяризацию научно-исследовательских работ и достижений онкологов Южного федерального округа, анализ процесса глубокой реорганизации здравоохранения в России. Редакция приглашает в качестве авторов всех, кто ищет и находит интересные решения многогранных задач, стоящих перед современной медициной, и хочет поделиться своими мыслями и наблюдениями с коллегами.

Цель: способствовать развитию онкологической медицины Юга России и внедрению её достижений в практику.

Задачи: освещать современные достижения онкологической службы Юга России; содействовать обмену опытом и передовыми знаниями между специалистами; информировать читателей об итогах крупных медицинских форумов.

В журнале размещаются публикации различных рубрик: обзоры литературы, мета-анализы, клинические исследования, наблюдения клинических случаев, обсуждения, анонсы и описания новых методов лечения.

Журнал принимает к публикации: оригинальные статьи, организации здравоохранения, лучевой диагностики, обмен опытом, обзоры, клинические наблюдения.

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Purpose: to promote the development of cancer medicine in the South of Russia and the introduction of its achievements into practice.
Tasks: to highlight the current achievements of the oncology service in the South of Russia; to promote the exchange of experience and advanced knowledge between specialists; to inform readers about the results of major medical forums.
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ORIGINAL ARTICLE

BLOOD LEVELS OF GROWTH AND PROGRESSION FACTORS IN PATIENTS WITH LOCALLY ADVANCED BREAST CANCER DURING NEOADJUVANT CHEMOTHERAPY

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ABSTRACT

Purpose of the study. An analysis of blood levels of TGF-β, TGFR2, TNF-α, TNF-αR1, TNF-αR2, CD44 and MMP9 in patients with various biological subtypes of breast cancer receiving neoadjuvant chemotherapy.
Materials and methods. This article presents an analysis of levels of growth and progression factors (TGF-β, TGFR2, TNF-α, TNF-αR1, TNF-αR2, CD44 and MMP9) in the blood of 162 patients with various biological subtypes of locally advanced breast cancer receiving 8 cycles of neoadjuvant chemotherapy.
Results. Levels of TGF-β, TGFR2, TNF, TNF-α, TNFR1, TNFR2, CD44, MMP9 in patients with all BC subtypes were high before the treatment. After chemotherapy cycles, the values decreased statistically significantly in all BC subtypes: CD44 decreased by 25.2 %, 30 % and 54.7 % in luminal A, luminal B and TNBC, respectively; TNFα– by 26.2 %, 48.3 % and 50.8 %, respectively; TNFα-R1 – by 52.1 %, 39.2 % and 50.3 % respectively; TNFα-R2 – by 31.7 %, 32.8 % and 41.9 % respectively; MMP9 – 35.3 %, 32.6 % and 43.3 % respectively.
Conclusions. We identified a combination of growth and progression factors which determines the chemotherapy sensitivity and resistance in all subtypes of breast cancer; so, a decline in the levels of TGF-β, TNFα, MMP9 and CD44 after neoadjuvant chemotherapy predicts further remission for at least 3 years. On the contrary, stabilization or an increase of these indicators leads to the early tumor progression.

Keywords:
breast cancer, biological subtypes, neoadjuvant polychemotherapy, growth and progression factors, IHC, remission, progression, chemotherapy resistance.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

СОДЕРЖАНИЕ ФАКТОРОВ РОСТА И ПРОГРЕССИРОВАНИЯ В КРОВИ БОЛЬНЫХ МЕСТНОРАСПРОСТРАНЕННЫМ РАКОМ МОЛОЧНОЙ ЖЕЛЕЗЫ В ПРОЦЕССЕ НЕОАДЪЮВАНТНОЙ ХИМИОТЕРАПИИ

Е.М.Франциянц, Н.Ю.Саманева*, Л.Ю.Владимирова, А.Э.Сторожакова, Е.А.Калабанова, С.Н.Кабанов, А.В.Тишина

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РЕЗЮМЕ

Цель исследования. Изучение уровня TGF-β, TGFR2, TNF-α, TNF-αR1, TNF-αR2, CD44 и MMP9 в крови больных раком молочной железы различных биологических подтипов, получивших неоадъювантную химиотерапию.
Материалы и методы. В работе представлены результаты исследования изучения содержания факторов роста и прогрессирования (TGF-β, TGFR2, TNF-α, TNF-αR1, TNF-αR2, CD44 и MMP9) в крови у 162 больных местнораспространенным раком молочной железы различных биологических подтипов, которым было проведено 8 курсов неоадъювантной химиотерапии.
Результаты. Уровни TGF-β, TGFR2, TNF, TNF-α, TNFR1, TNFR2, CD44, MMP9 у пациентов со всеми подтипами РМЖ были высокими до лечения. После циклов химиотерапии значения статистически значимо снизились во всех подтипах РМЖ: CD44 уменьшился на 25,2 %, 30 % и 54,7 % в люминальном А, В и TNBC соответственно; TNFα – на 26,2 %, 48,3 % и 50,8 % соответственно; TNF-αR1 – на 52,1 %, 39,2 % и 50,3 % соответственно; TNF-αR2 – на 31,7 %, 32,8 % и 41,9 % соответственно; MMP9 – 35.3 %, 32,6 % и 43,3 % соответственно.
Заключение. Выявлен комплекс факторов роста и прогрессии, определяющий чувствительность и резистентность к химиотерапии при всех подтипах РМЖ, а именно снижение уровня TGF-β, TNF-α, MMP9 и CD44 после неоадъювантной химиотерапии определяет в дальнейшем ремиссию в течение минимум 3 лет. Напротив, стабилизация или увеличение этих показателей приводит в дальнейшем к раннему прогрессированию злокачественного процесса.

Ключевые слова:
рак молочной железы, биологические подтипы, неоадъювантная полихимиотерапия, факторы роста и прогрессирования, ИГХ, ремиссия, прогрессирование, резистентность к химиотерапии.

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Despite the improvement in the 10-year overall survival rate of breast cancer patients, this disease remains the leading cause of cancer death in women worldwide. One of the main reasons is the occurrence of tumor recurrence and resistance to therapy [1]. There is increasing evidence that the aggressive nature of TNBC tumors may be due to the presence of a higher frequency of cancer stem cells (CD44 high CD24 low/-) compared to other subtypes of breast cancer [2, 3]. These observations suggest that the subgroup of cancer stem cells in tumors is heterogeneous in nature with respect to the phenotype and may function among different subtypes of breast cancer. Single-cell transcriptomic analysis of primary and metastatic tumors of various subtypes of breast cancer can certainly provide very interesting information about the heterogeneity of cancer stem cells. Such information could then provide the basis for a hypothesis about how heterogeneity in the cancer stem cell compartment in different subtypes of breast cancer can be a predictor of response to therapy and resistance to therapy.

Currently, the clinical problem in the treatment of breast cancer is the development of resistance to therapy, the progression of the disease due to the occurrence of relapses and distant metastasis. The regulation of cancer stem cell function and the induction of chemoresistance under the influence of external factors, such as cytokines, chemokines and hypoxia, become obvious as potential strategies. They can be aimed at the interaction of cancer stem cells with cellular and non-cellular components of the extracellular matrix as more effective therapeutic approaches.

The role of transforming growth factor β (TGF-β) in the regulation of tumor cell proliferation, metastasis and remodeling of the extracellular matrix is also well documented [4]. According to the literature, prolonged exposure to TGF-β on human breast epithelial cells enhances the phenotype of the epithelial-mesenchymal transition (EMT) and increases the number of CD44 cells – a known marker of stem cancer cells [5, 6]. In response to the effect of TGF-β, epithelial and carcinoma cells undergo a partial or complete epithelial-mesenchymal transition, which contributes to the progression of cancer. This process is considered reversible, because the cells return to the epithelial phenotype after removal of TGF-β. However, the authors found that long-term exposure to TGF-β contributes to stable EMT in breast epithelial cells and carcinoma, in contrast to reversible EMT caused by shorter exposure. The stabilized EMF was accompanied by a steadily increased production of stem cells and resistance to antitumor drugs.

Other cytokines, such as tumor necrosis factor alpha (TNF-α) and endothelial growth factor (EGF), regulate the activity of cancer stem cells. When tumor cells from the luminal A subtype of breast cancer were exposed to TNF-α, the population of breast cancer cells became enriched for the CD44+ CD29+ CSC phenotype with increased metastatic properties [7]. Further studies should be conducted to assess the content of TNF-α and their receptors in the serum of various subtypes of breast cancer before and after standard treatment [8].

Cancer biomarker research can play an important role in areas such as cancer diagnosis and prediction, monitoring of disease progression, predicting disease recurrence, monitoring and predicting treatment effectiveness, and cancer screening. According to some studies, the level of expression of p53 protein receptors in luminal subtypes is different. In hormone-positive tumors, a high level of p53 expression is associated with overexpression or mutation of Her2neu [9]. It was found that MMR9 is a potential biomarker for several types of cancer [10, 11]. It can be used as a marker in areas such as diagnosis, monitoring the effectiveness of treatment and monitoring the progression of the disease. Some biomarkers may not have sufficient specificity for clinical applicability when they are used as a single marker. The use of a combination of biomarkers is one of the strategies for increasing their specificity. To achieve this goal, MMR9 can also be used in combination with other cancer biomarkers [9].

The purpose of the study: the study of the level of TGF-β, TGFR2, TNF-α, TNF-αR1, TNF-αR2, CD44 and MMR9 in the blood of breast cancer patients of various biological subtypes who received neoadjuvant chemotherapy.

MATERIALS AND METHODS

The study included data on 162 patients with locally advanced primary inoperable Her2 negative stage III breast cancer aged 30 to 65 years, having a somatic status on the ECOG-WHO scale from 0 to 1 points (on the Karnovsky scale from 100 % to 80 %). After the diagnosis and comprehensive treatment, the patients were monitored, according to the results of which they were divided into 2 groups. The first group consisted of data on 58 patients who had previously experienced disease progression (local relapse or distant metastasis) in the period from 6 to 12 months. The second group included 104 patients who had remission after treatment for at least 3 years (36 months).

According to the biological subtype, the patients were distributed as follows: in group 1 there were 58.62 % (34) patients who were diagnosed with a thrice-negative variant of the tumor, in 41.38 % (24) patients-a luminal negative subtype in Her2. Luminal A subtype was not observed in any patients in group 1; hormone-dependent subtypes of the tumor were most common in group 2 (82 patients – 78.85 %). Luminal B, Her2 negative subtype was diagnosed in 40 patients (38.46 %), luminal A subtype – in 42 patients (40.39 % of cases). Also, 21.15 % (22 patients) were diagnosed with a triple-negative variant of the tumor.

The following levels were determined in the blood serum of patients using standard ELISA test systems: TGF-β1, TNF-α, CD44, MMR9 (BenderMedSystem, Austria); TGF-βRII (RayBiotech, USA); TNF-αRI and TNF-αRI (R&D systems, USA&Canada).

Statistical data processing was performed using the STATISTICA 10 statistical package (StatSoft Inc., USA). Descriptive statistics of quantitative features are presented in the form of the arithmetic mean and the standard error of the arithmetic mean (M±s). The reliability of the differences between the samples was evaluated using the nonparametric Mann-Whitney test (the differences were considered reliable at $p<0.05$).

RESEARCH RESULTS AND DISCUSSION

Interesting are the results of studying the blood parameters before treatment, depending on the biological subtype of the tumor in patients with subsequent progression for 6-12 months (Table 1).

First of all, it should be noted that this group included only breast cancer patients with luminal B and TNBC. It was found that in the blood of patients with luminal B before the start of chemotherapy, all the studied indicators had significant differences from the standard values of healthy donors. Thus, the level of TGF-β and its receptor TGF-βR2 was on average 1.9 times higher than the values in the blood of donors. The content of TNF-α and its TNF-αR1 and TNF-αR2 receptors before the start of treatment was increased by 4.3 times, 1.2 times and 1.8 times, respectively. The level of CD44 and MMR9 in the blood of patients with luminal BC during this period of the

Table 1. Growth and progression factors in the blood of breast cancer patients with subsequent progression for 6-12 months

Indicators	Donor	Luminal B		TNBC	
		Before therapy	After chemo	Before therapy	After chemo
TGFβ pg/ml	210.1±19.6	392.9±34.3[1]	331.7±31.5[1]	194.1±18.3	178.9±16.9
TGFβ-R2 pg/ml	99.7±8.2	194.3±17.5[1]	316±28.2[1,2]	255.1±26.1[1]	479.2±43.8[1,2]
TNF-α pg/ml	1.1±0.2	4.7±0.5[1]	5.9±0.5[1,2]	8.5±0.8[1]	8.5±0.9[1]
TNF-α-R1 pg/ml	405.2±35.4	486.6±42.3[1]	747.3±59.4[1,2]	755.4±63.1[1]	773.3±72.5[1]
TNF-α-R2 pg/ml	829.2±74.6	1471±112.8[1]	2309.6±256.7[1,2]	2757.7±242.1[1]	2442.3±252.3[1]
CD44 ng/ml	25.1±2.6	69.6±6.4[1]	94.7±8.3[1,2]	182.8±17.6[1]	181.5±19.4[1]
MMR9 ng/ml	48.3±4.6	181.6±17.9[1]	243.2±21.5[1,2]	162.4±14.2[1]	237.5±24.2[1,2]

Note: [1] – reliable in relation to the indicators of donors; [2] – reliable in relation to the "before treatment" stage ($p\leq0.05$).

study exceeded the normative indicators by 2.8 times and 3.8 times, respectively. In the blood of TNBC patients with subsequent progression, the content of TGFβ unexpectedly turned out to be at the level of values in donors, and the level of its TGF-βR2 receptor was increased by 2.6 times. The content of TNF-α and its TNF-αR1 and TNF-αR2 receptors before the start of treatment was increased by 7.7 times, 1.9 times and 3.3 times, respectively. The level of CD44 and MMR9 in the blood of patients was increased by 7.3 times and 3.4 times, respectively.

Analyzing the dynamics of changes in indicators after chemotherapy in the blood of patients with subsequent progression, it was revealed (Table. 1), the absence of significant changes in the level of TGF-β relative to the indicator before treatment. The TGF-βR2 receptor, on the contrary, increased in luminal BC by 1.6 times relative to the indicator before treatment and became 3.2 times higher than the norm; in TNBC, the increase compared to the indicators before treatment was 1.9 times and, accordingly, the indicator was 4.8 times higher than the normative values.

After neoadjuvant courses of chemotherapy, the level of CD44 in luminal BC increased by 1.4 times and became 3.8 times higher than normal, with TNBC-remained unchanged. The level of TNF-α during this period of the study in luminal BC increased by 25.5 % relative to the values before treatment and 5.4 times exceeded the normative indicators, while in TNBC it remained unchanged. TNF-αR1 and TNF-αR2 in the luminal subtype increased by 1.5 times and 1.6 times and became 1.8 times and 2.8 times higher than normal, respectively. In both studied types of breast cancer, the content of MMR9 in the blood of this contingent of patients increased: in luminal B – by 1.3 times, in TNBC – by 1.5 times, and in both cases, the indicators became higher than normal on average up to 5 times.

It was also of interest to consider the studied indicators before treatment, depending on the biological subtype of the tumor in patients with subsequent remission for at least 3 years. The results are presented in Table 2. It was found that in the blood of patients, almost all the studied indicators had significant differences from the standard values in the direction of increase. The exception was the level of TGF-β in patients with luminal A breast cancer, which did not significantly differ from healthy donors. With luminal B and TNBC, this indicator exceeded the standard values by 34.8 % and 73.4 %, respectively. The level of the TGF-βR2 receptor was also higher than normal in all biological subtypes of breast cancer – on average 4.4 times in luminal A and B, 6.3 times in TNBC. This was accompanied by an increase in the CD44 index by 1.8 times, 3.3 times and 7.3 times, respectively, with luminal A, luminal B

Table 2. Growth and progression factors in the blood of breast cancer patients with subsequent remission for 3 years

Indicators	Donors	Luminal A		Luminal B		TNBC	
		Before therapy	After chemo	Before therapy	After chemo	Before therapy	After chemo
TGFβ pg/ml	210.1±19.6	234.4±21.8	201.7±10.3[2]	283.3±29.2[1]	210.3±18.5[2]	364.3±33.1[1]	209.2±19.7[2]
TGFβ-R2 pg/ml	99.7±8.2	441.5±32.9[1]	191.1±17.5[1,2]	435.6±38.3[1]	200.8±19.4[1,2]	628.5±64.6[1]	207.1±22.4[1,2]
TNF-α pg/ml	1.1±0.2	4.2±0.4[1]	3.1±0.3[1,2]	8.7±0.9[1]	4.5±0.5[1,2]	6.3±0.6[1]	3.1±0.3[1,2]
TNF-α-R1 pg/ml	405.2±35.4	930.6±87.2[1]	445.2±46.9[2]	979.4±95.3[1]	595.2±51.6[1,2]	1067.8±89.1[1]	530.3±57.3[1,2]
TNF-α-R2 pg/ml	829.2±74.6	1557.7±132.9[1]	1064.2±96.4[1,2]	2404.4±156.3[1]	1616.1±142.7[1,2]	3508.8±253.1[1]	2036.95±189.2[1,2]
CD44 ng/ml	25.1±2.6	45.6±4.3[1]	34.1±3.2[1,2]	83.9±7.5[1]	58.7±6.0[1,2]	182.5±16.9[1]	82.7±7.5[1,2]
MMR9 ng/ml	48.3±4.6	136.9±11.3[1]	88.6±7.9[1,2]	181.9±14.2[1]	122.5±11.5[1,2]	196.5±17.3[1]	111.5±9.6[1,2]

Note: [1] – reliable in relation to the indicators of donors; [2] – reliable in relation to the "before treatment" stage ($p \leq 0.05$).

and TNBC. Assessing the levels of TNF-α and its TNF-αR1, TNF-αR2 receptors in each subtype of cancer, the following was obtained: with luminal A, an increase of 3.8 times, 2.3 times and 1.9 times, respectively; with luminal B – by 7.9 times, 2.4 times and 2.9 times, respectively; with TNBC – by 5.7 times, 2.6 times and 4.2 times. Also, a significant increase in the level of MMR9 relative to donors was observed in various biological subtypes of breast cancer – 2.8 times in luminal A, 3.8 times in luminal B, 4.1 times in TNBC.

The dynamics of changes in indicators after chemotherapy in the blood of patients with subsequent remission for at least 3 years is clearly shown in Table 2. During this period, the level of TGF-β and its receptor TGF-βR2 decreased relative to the previous period by 14 %, 25.8 %, 42.6 % and 56.7 %, 53.9 %, 67 %, respectively, in luminal A, luminal B and TNBC, respectively.

The dynamics of a decrease in the blood content of breast cancer patients was also noted for other studied indicators after chemotherapy. Thus, the level of CD44 decreased by 25.2 %, 30 % and 54.7 %, respectively, with luminal A, luminal B and TNBC; the level of TNF-α – by 26.2 %, 48.3 % and 50.8 %, respectively; TNF-αR1– by 52.1 %, 39.2 % and 50.3 %, respectively; TNF-αR2 – by 31.7 %, 32.8 % and 41.9 %, respectively; MMR9 – 35.3 %, 32.6 % and 43.3 %, respectively.

Our results are confirmed by the data of literary sources. CD44 is known to serve as a docking molecule for matrix metalloproteases (MMPs), which are matrix-modifying enzymes that destroy the basement membrane and promote cell migration [12]. MMR9, in turn, cleaves TGFβ for activation, which promotes angiogenesis and invasion [13]. According to the results of the study, Kuo Y. C. showed that TGFβ induces the expression of membrane-type MMP in breast cancer cells, which causes CD44 cleavage [14]. The cleaved CD44 then promoted the migration of tumor cells, which indicates a significant role of the CD44-MMP-TGF-β axis in cancer invasion and metastasis. CD44 also contributes to the emergence of multidrug resistance [15].

CONCLUSION

Thus, a complex of growth and progression factors has been identified that determines sensitivity and resistance to chemotherapy. A decrease in the level of TGF-β, TNF-α, MMR9 and CD44 after neoadjuvant chemotherapy determines further remission. On the contrary, the stabilization or increase in these indicators leads to an early progression of the malignant process in the period from 6 to 12 months. The assessment of indicators of growth factors and progression can play an important prognostic role with the help of which it is possible to distinguish a group of patients with the development of resistance to chemotherapy.

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ORIGINAL ARTICLE

FUNCTIONAL STATE OF CARDIOMYOCYTE MITOCHONDRIA IN MALIGNANT PROCESS IN PRESENCE OF COMORBID PATHOLOGY IN EXPERIMENT

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ABSTRACT

Purpose of the study. An analysis of indices of free radical oxidation and respiration of mitochondria of heart cells in a malignant process in presence of diabetes mellitus and chronic neurogenic pain in experimental animals.
Materials and methods. The study included outbred female rats (*n*=32) and C57BL/6 female mice (*n*=84). Experimental groups of rats were: intact group 1 (*n*=8), control group 1 (*n*=8) with diabetes mellitus (DM), comparison group 1 (*n*=8) with standard subcutaneous transplantation of Guerin's carcinoma, main group 1 (*n*=8) with Guerin's carcinoma transplanted after 1 week of persistent hyperglycemia. Experimental groups of mice were: intact group 2 (*n*=21), control group 2 (*n*=21) with a model of chronic neurogenic pain (CNP), comparison group 2 (*n*=21) with standard subcutaneous transplantation of melanoma (B16/F10), main group 2 (*n*=21) (CNP+B16/F10) with melanoma transplanted 3 weeks after the CNP model creation. Heart mitochondria were isolated by differential centrifugation. Levels of cytochrome C (ng/mg of protein), 8-hydroxy-2'-deoxyguanosine (8-OHdG) (ng/mg of protein), and malondialdehyde (MDA) (μmol/g of protein) were measured in mitochondrial samples by ELISA. Statistical analysis was performed using the Statistica 10.0 program.
Results. DM in rats upregulated 8-OHdG by 6.3 times and MDA by 1.9 times (p=0.0000) and downregulated cytochrome C by 1.5 times (p=0.0053) in heart cell mitochondria, compared to intact values. DM+Guerin's carcinoma in rats increased 8-OHdG by 14.0 times and MDA by 1.7 times (p=0.0000) and decreased cytochrome C by 1.5 times (p=0.0000), compared to intact values. CNP in mice did not affect the studied parameters in mitochondria of the heart. CNP+B16/F10 in mice increased 8-OHdG by 7.1 times and MDA by 1.6 times (p=0.0000) and decreased cytochrome C by 1.6 times (p=0.0008).
Conclusions. Comorbidity (diabetes mellitus, chronic neurogenic pain) together with malignant pathology aggravates mitochondrial dysfunction of heart cells with destabilization of the respiratory chain mediated by free radical oxidation processes.

Keywords:
mitochondria, heart, experimental animals, Guerin's carcinoma, B16/F10 melanoma, cytochrome C, 8-hydroxy-2'-deoxyguanosine, malondialdehyde.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

ФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ МИТОХОНДРИЙ КАРДИОМИОЦИТОВ ПРИ ЗЛОКАЧЕСТВЕННОМ ПРОЦЕССЕ НА ФОНЕ КОМОРБИДНОЙ ПАТОЛОГИИ В ЭКСПЕРИМЕНТЕ

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РЕЗЮМЕ

Цель исследования. Изучение показателей свободнорадикального окисления и дыхания митохондрий клеток сердца при злокачественном процессе на фоне сахарного диабета и хронической нейрогенной боли у экспериментальных животных.

Материалы и методы.Работа выполнена на нелинейных крысах-самках (*n*=32) и мышах-самках линии C57BL/6 (*n*=84). Экспериментальные группы крыс: интактная 1 (*n*=8), контрольная группа 1 (*n*=8) с сахарным диабетом (СД), группа сравнения 1 (*n*=8) – стандартная подкожная перевивка карциномы Герена, основная группа 1 (*n*=8) – через 1 неделю стойкой гипергликемии перевивали карциному Герена. Экспериментальные группы мышей: интактная 2 (*n*=21), контрольная 2 (*n*=21) – воспроизведение модели хронической нейрогенной боли (ХНБ), группа сравнения 2 (*n*=21) – стандартная подкожная перевивка меланомы (B16/F10), основная группа 2 (*n*=21) (ХНБ+B16/F10) – меланому перевивали через 3 недели после создания модели ХНБ. Митохондрии сердца получали методом дифференциального центрифугирования. В образцах митохондрий методом ИФА определяли концентрацию: цитохрома С (нг/мг белка), 8-гидрокси-2'-дезоксигуанозина (8-OHdG) (нг/мг белка), малонового диальдегида (МДА) (мкмоль/г белка). Статистический анализ – Statistica 10.0.

Результаты. Наличие СД у крыс способствовало повышению 8-OHdG в 6,3 раза, МДА в 1,9 раза (*p*=0,0000) и снижению цитохрома С в 1,5 раза (*p*=0,0053) в митохондриях клеток сердца по сравнению с интактными значениями. СД+карцинома Герена у крыс вызывало повышение уровня 8-OHdG в 14,0 раз, МДА в 1,7 раза (*p*=0,0000) и снижение цитохрома С в 1,5 раза (*p*=0,0093) по сравнению с интактными значениями. Присутствие ХНБ у мышей не повлияло на уровень изучаемых показателей в митохондриях сердца. ХНБ+меланома B16/F10 у мышей приводило к повышению уровня 8-OHdG в 7,1 раза, МДА в 1,6 раза (*p*=0,0000) и снижению уровня цитохрома С в 1,6 раза (*p*=0,0008).

Заключение. Коморбидная патология (сахарный диабет, хроническая нейрогенная боль), сопряженная со злокачественным процессом, усугубляет дисфункцию митохондрий клеток сердца с дестабилизацией дыхательной цепи, опосредованной процессами свободнорадикального окисления.

Ключевые слова:
митохондрии, сердце, экспериментальные животные, карцинома Герена, меланома B16/F10, цитохром С, 8-гидрокси-2'-дезоксигуанозин, малоновый диальдегид.

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RELEVANCE

Currently, a new multidisciplinary direction is actively developing – cardiac oncology, based on a comprehensive personalized approach to the prevention and treatment of cardiovascular diseases (CVD) in cancer patients. Advances in oncology have led to an increase in their survival rate, which in turn has increased the number of patients who live to develop long-term cardiac complications [1]. In general, such complications are not uncommon and can occur in almost every cancer patient [2]. The reasons are not only the cardiotoxic effects of antitumor therapy, but also the fact that the oncological disease itself provokes the appearance of cardiovascular problems, especially in patients with prerequisites for this, as well as CVD can be associated with the decompensation of chronic comorbid pathology existing in an oncological patient. The addition of a cardiological disease reduces the quality of life, and sometimes makes it impossible to continue special treatment.

In addition to cardiological pathology, the malignant process is often accompanied by other comorbid diseases. The connection of diabetes mellitus with a malignant process is known, often diabetes is present in patients with malignant tumors [3]. Pain is quite often an accompanying component of the tumor process and is present in 30-50 % of cancer patients after antitumor therapy, in 65-90 % of patients due to the progression of the disease, 33 % of patients suffer from pain after the end of antitumor treatment [4]. The origin of pain in cancer patients is usually multifactorial: concomitant diseases, direct and indirect effects of tumor growth, side effects of antitumor therapy [4].

The heart is an organ that consumes a lot of energy, and its function largely depends on ATP (adenosine triphosphate) produced by mitochondria. It is well known that mitochondria ensure the functioning of various organs under normal and pathological conditions [5-7]. Mitochondrial dysfunction is one of the main causes of various heart diseases, which requires a close study and understanding of the mechanisms of mitochondrial remodeling in the heart [5, 8, 9]. The most well-known role of mitochondria is energy production, which is of paramount importance for organs with a high level of metabolism, such as the heart. Moreover, mitochondria mediate the fate of cells, including proliferation, differentiation and viability, and can also change the normal development of the body [10]. It is proved that the mitochondria of cardiomyocytes are the largest producers of reactive oxygen species (ROS), and hydroxyl radicals damage mitochondrial proteins, mitochondrial DNA (mtDNA) and membrane lipids. The latter, called lipid peroxidation, can disrupt the functions of mitochondria, including the oxidation of fatty acids (FA) [11] and the production of ATP, which in turn can potentially cause systolic dysfunction [12, 13]. Significant oxidative damage detected in mitochondria can thus contribute to their functional deficiency in the heart [5].

Diabetes mellitus occurs when glucose tolerance is impaired due to insulin resistance. Various factors interact in the pathogenesis and progression of diabetes mellitus [14]. Diabetic cardiomyopathy is the main cause of heart failure and death in patients with diabetes [15].

Chronic neuropathic pain is defined as "pain resulting from a direct lesion or disease of peripheral or central neurons that affects the somatosensory system" [16] and is associated with a violation of the structural and functional connections of the brain [17, 18]. At the moment, the question of the effect of pain syndrome on the cardiovascular system has not been fully studied, therefore it is an area of science that requires more careful research.

In connection with the above, it is very relevant to study the influence of comorbid diseases accompanying the malignant process and to identify dysfunctional parameters at the subcellular level in organs not affected by the malignant process.

The purpose of the study was to study the indicators of free radical oxidation and respiration of mitochondria of heart cells in a malignant process against the background of diabetes mellitus and chronic neurogenic pain in experimental animals.

MATERIALS AND METHODS

The work was performed on non-linear female rats (*n*=32) 180-220 g and female mice of the C57BL/6 line (*n*=84) 8 weeks of age with an initial weight of 21-22 g. The animals were obtained from FSBI NMRC Scientific Center for Biomedical Technolo-

gies "Andreevka" FMBA (Moscow region). The study used a cell line of mouse melanoma B16/F10 and a strain of Guerin's carcinoma. The tumor strains were obtained from the N. N. Blokhin National Research Center of Oncology. The work with animals was carried out in accordance with the rules of the "European Convention for the Protection of Animals Used in Experiments" (Directive 86/609/EEC) and the Helsinki Declaration, as well as in accordance with the "International Recommendations for conducting Biomedical research using Animals" and Order No. 267 of the Ministry of Health of the Russian Federation dated June 19, 2003 "On approval of the rules of laboratory practice". The animals were kept under natural lighting conditions with free access to water and food. The Commission on Bioethics of the National Medical Research Centre for Oncology of the Ministry of Health of Russia of 31.05.2018 approved the protocol of the study (protocol of the ethical committee No. 2) on working with mice of the C57BL/6 line and on working with nonlinear rats of 01.09.2020, protocol of the ethical committee No. 21/99. Manipulations with animals were performed in the box in compliance with the generally accepted rules of asepsis and antiseptics.

Female rats (*n*=32) were randomly assigned to the following experimental groups: intact group 1 (*n*=8), control group 1 with diabetes (*n*=8), comparison group 1 (*n*=8) – rats with standard subcutaneous transplantation of Guerin's carcinoma, main group 1 (*n*=8) – rats who were first reproduced diabetes mellitus (DM) (once, intraperitoneal alloxan was administered at a dose of 150 mg/kg of weight) and after 1 week Guerin's carcinoma was transplanted to persistent hyperglycemia with 0.5 ml of a suspension of tumor cells in saline solution at a dilution of 1:5. At the time of transplantation of Guerin's carcinoma in animals of the main group 1 (*n*=8), the average blood glucose values were 25.4±1.2 mmol/l, whereas in the group of intact animals 1 (*n*=8) 5.2±0.3 mmol/l. Decapitation of animals was performed on a guillotine 14 days after transplantation of Guerin's carcinoma and 21 days after reproduction of experimental DM.

Female mice of the C57BL/6 line (*n*=84) were randomly assigned to the following experimental groups: intact group 2 (*n*=21), control group 2 (*n*=21) – reproduction of a model of chronic neurogenic pain (CNP) [19]. Comparison group 2 (*n*=21) – mice with standard subcutaneous melanoma grafting (B16/F10), main group 2 (*n*=21) (CNP+B16/F10) – mice that had melanoma grafted 3 weeks after the creation of the CNP model.

Female mice of the main group 2 (CNP+B16/F10) underwent ligation of the sciatic nerves from 2 sides under xyl-zoletil anesthesia: xylazine (Xyl preparation) intramuscularly, at a dose of 0.05 ml/kg (according to the instructions), after 10 minutes Zoletil-50 was administered at a dose of 10 mg/100g. 3 weeks after the healing of the surgical wound, 0.5 ml of a suspension of melanoma B16/F10 tumor cells was injected subcutaneously under the right shoulder blade in a 1:10 saline solution. Animals from the comparison group were transplanted with melanoma B16/F10 subcutaneously in the same dose and volume as in the main group, but without reproducing the model of chronic pain. Decapitated mice on the guillotine. Animals from the main group 2 and the comparison group 2 were decapitated 14 days after transplantation of experimental melanoma B16/F10.

After decapitation, the heart was quickly extracted from the animals using refrigerants and mitochondria were isolated according to the method of Egorova MV, Afanasiev SA (2011) [20] using differential centrifugation on a high-speed refrigerated centrifuge Avanti J-E, BECMAN COULTER, USA. The tissues were washed with an icy 0.9 % KCl solution. To destroy the intercellular connections, the cell wall and plasma membranes, mechanical processing of tissues with grinding with scissors and homogenization in a glass homogenizer with a Teflon pestle (Potter-Elvegame homogenizer) was used. For each gram of tissue, 10 ml of the isolation medium was added (0.22 M mannitol, 0.3 M sucrose, 1 mM EDTA, 2 mM TRIS-HCL, 10 mM HEPES, pH 7.4). The tissues were homogenized and centrifuged for the first time for 10 min at a speed of 1000 g, temperature 0-2 °C, the second and third centrifugation is carried out at 20,000 g, 20 min, temperature 0-2 °C. Between centrifugation, the procedure of resuspending the mitochondrial sediment in the isolation medium was performed. The mitochondrial fraction was additionally purified from lysosomes, peroxisomes, melanosomes, etc., by centrifuging in a 23 % Percoll

gradient. The suspension of subcellular structures was layered on a Percoll gradient, centrifuged for 15 minutes at 21000 g, after which separation into 3 phases was observed, the lower layer of mitochondria was left and resuspended with an isolation medium. The next washing of the mitochondria was carried out by centrifugation for 10 minutes at 15,000 g, temperature 0-2 °C. The obtained mitochondrial samples (protein concentration 4-6 g/l) were stored at-80 °C in the isolation medium before analysis. The concentration of cytochrome C (ng/mg protein) (Bioscience, Austria), 8-hydroxy-2'-deoxyguanosine (8-OHdG) (ng/mg protein) (Enzo Life Sciences, Switzerland), malondialdehyde (MDA) (μmol/g protein) was determined in mitochondrial samples of all groups using test systems on an ELISA analyzer (Infinite F50 Tecan, Austria) (BlueGene Biotech, China); by the biochemical method-the amount of protein (mg/ml) – the biuret method (Olvex Diagnosticum, Russia) on the ChemWell automatic analyzer (Awareness Technology INC, USA).

Statistical analysis of the results was carried out using the Statistica 10.0 software package. The obtained data were analyzed for compliance of the distribution of features with the normal distribution law using the Shapiro-Wilk criterion (for small samples). Comparison of quantitative data in groups (independent samples) was carried out using the Kraskel-Wallis criterion (multiple comparisons). The data of the tables are presented in the form M±m, where M is the arithmetic mean, m is the standard error of the mean, $p<0.05$ was taken as the level of statistical significance. The obtained results were statistically processed in compliance with the general recommendations for medical research.

RESEARCH RESULTS AND DISCUSSION

When analyzing experimental data, obtained using the model of diabetes mellitus and the growth of Guerin's carcinoma, it was necessary to establish the effect of diabetes mellitus (control group 1) on the mitochondria of female rat heart cells. Thus, compared with the values in intact group 1, an increase in 8-OHdG by 6.3 times, MDA by 1.9 times ($p<0.05$) and a decrease in cytochrome C by 1.5 times ($p<0.05$) were found (Table 1).

No significant changes were found in comparison group 1. The combination of two pathological processes in the animal's body – diabetes mellitus and a malignant tumor caused the following changes in the studied parameters: the level of 8-OHdG increased by 14.0 times, MDA by 1.7 times ($p<0.05$), and cytochrome C decreased by 1.5 times ($p<0.05$) compared to the values in intact group 1. Statistically significant differences between animal groups were determined only in the level of 8-OHdG, this indicator was 2.2 times higher in the main group when compared between the control 1 and the main 1 groups, and when compared with the values in the comparison group it was 17.4 times higher in the main 1. The level of MDA in the main group 1 exceeded the values in the comparison group 1 by 1.5 times ($p<0.05$).

Table 1. Changes in indicators of free radical oxidation and respiration of mitochondria of female rat heart cells in diabetes mellitus and Guerin's carcinoma

	8-OHdG ng/mg protein	MDA μmol/g protein	Cytochrome C ng/mg protein
Intact group 1 (n=8)	0.927±0.048	14.912±1.110	1.382±0.137
Control group 1 – diabetes mellitus (n=8)	5.890±0.529[1] p^1=0.0000	27.866±0.813[1] p^1=0.0000	0.900±0.048[1] p^1=0.0053
Comparison group 1-Guerin's carcinoma (n=8)	0.748±0.058	16.992±0.599	1.215±0.101
Main group 1-diabetes mellitus + Guerin's carcinoma (n=8)	13.050±0.942[1,2,3] p^1=0.0000 p^2=0.0000 p^3=0.0000	26.092±0.642[1,3] p^1=0.0000 p^3=0.0000	0.949±0.041[1] p^1=0.0093

Note: p^1 – statistically significant differences compared to the values in the intact group; p^2 – statistically significant differences compared to the values in the control group; p^3 – statistically significant differences in relation to the values in the comparison group.

Thus, the most pronounced changes in the level of the studied parameters (8-OHdG, MDA, cytochrome C) in the mitochondria of the heart cells of female rats were determined in the group of animals where the malignant process developed against the background of comorbid pathology-diabetes mellitus.

The next section of the work was devoted to the study of the influence of another comorbid pathology-chronic neurogenic pain accompanying the growth of melanoma B16/F10 in female mice in the mitochondria of heart cells. First of all, it was necessary to assess the state of free radical processes and respiration of the mitochondria of the heart cells of female mice, under the influence of CNP, as a result, it was determined that there were no significant differences between all the studied parameters (8-OHdG, MDA, cytochrome C) with the values in intact group 2 (Table 2).

With the independent growth of melanoma B16/F10 in female mice, as in the case of the independent growth of Guerin's carcinoma in rats, no significant changes were found compared to the level in intact group 2.

The combination of two pathological processes (CNP+melanoma B16/F10) in the animal's body led to a change in all the studied biochemical parameters in the mitochondria of the heart of female mice. Thus, with the growth of melanoma against the background of chronic neurogenic pain, compared with the values in intact group 2, an increase in the level of 8-OHdG was recorded by 7.1 times, MDA by 1.6 times ($p<0.05$) and a decrease in the level of cytochrome C by 1.6 times ($p<0.05$). Compared with the values in control group 2, an increase in the level of 8-OHdG was determined by 6.6 times, the level of MDA – by 2.0 times, and the level of cytochrome C, on the contrary, decreased by 2.1 times. When comparing the results obtained between the main group 2 (CNP+melanoma B16/F10) and the comparison group 2 (melanoma B16/F10), it was determined that the level of peroxidation products – 8-OHdG and MDA exceeded the corresponding indicators for comparison group 2 by 7.7 times and 1.8 times ($p<0.05$), and cytochrome C was reduced by 1.3 times ($p<0.05$).

The totality of the presented changes in the indicators of 8-OHdG, MDA and cytochrome C in the case of standard melanoma growth and concomitant CNP in female mice characterizes different mitochondrial dysfunction depending on the presence of comorbid pathology – CNP. At the same time, more pronounced dysfunctional changes in the mitochondria of the heart cells of female mice were noted when a malignant process was superimposed on the comorbid pathology – CNP.

Among the most important biological markers of oxidative stress, purines can be distinguished: 8-OHdG or its oxidized form-8-oxo-7,8-dihydro-2' – deoxyguanosine (8-oxodG). Although all living cells develop a wide range of DNA repair mechanisms, their enzymatic repair system does not always lead to the complete removal of all DNA modifications. Therefore, incorrectly reconstructed DNA is a serious problem for cells, mainly due to changes in genetic information, as well as related mutagenesis and apoptosis of cells [21]. Modifications of 8-OHdG and 8-oxodG occur as a result of the interaction of hydroxyl or superoxide radicals and guanine G in the

Table 2. Indicators of free radical processes and respiration of mitochondria of heart cells in female mice with chronic neurogenic pain and the growth of melanoma B16/F10

	8-OHdG ng/mg protein	MDA μmol/g protein	Cytochrome C ng/mg protein
Intact group 2 (n=21)	1.525±0.078	3.728±0.189	4.611±0.57
Control group 2 –CNP (n=21)	1.63±0.082	2.909±0.254	6.18±0.59
Comparison group 2 – melanoma B16/F10 (n=21)	1.399±0.101	3.254±0.227	3.81±0.47
Main group 2 – CNP+melanoma B16/F10 (n=21)	10.785±0.387^{1,2,3} p^1=0.0000 p^2=0.0000 p^3=0.0000	6.003±0.216^{1,2,3} p^1=0.0000 p^2=0.0000 p^3=0.0000	2.934±0.47^{1,2,3} p^1=0.0008 p^2=0.0010 p^3=0.0024

Note: $p1$ – statistically significant differences compared to the values in the intact group; p^2 – statistically significant differences compared to the values in the control group; p^3 – statistically significant differences in relation to the values in the comparison group.

DNA chain. Free radicals attack the G chains of DNA or free 2' – deoxyguanosine, resulting in the formation of radical adducts. The separation of electrons forms 8-OHdG, which, as a result of a reaction known as keto-enol tautomerism, turns into the main oxidized product of 8-oxodG [21].

Numerous experimental data emphasize the direct connection between oxidative stress and diabetes by measuring biomarkers of oxidative stress both in patients with diabetes and in experimental animals with reproduced diabetes mellitus [15, 22, 23]. A hyperglycemic condition can lead to increased levels of markers of DNA damage caused by oxidative stress, such as 8-hydroxy-2' – deoxyguanosine (8-OHdG) and 8-oxo-7,8-dihydro-2' – deoxyguanosine; lipid peroxidation products, measured as substances reacting with thiobarbituric acid (TBARS); protein oxidation products, such as the level of nitrothyrosine and carbonyl, and also reduce the activity of antioxidant enzymes [15].

In the presented study on experimental animals, an increase in the processes of lipid peroxidation was confirmed by an increase in the oxidation products of 8-OHdG, MDA and a decrease in the component of the respiratory chain – cytochrome C in diabetes mellitus in the mitochondria of heart cells.

One of the end products of lipid peroxidation (LP) is MDA. The formation of MDA occurs as a result of free radical oxidation of polyunsaturated fatty acids of phospholipids of cell membranes by active oxygen forms [24]. At the sites of the addition of peroxide radicals, fatty acids are torn into fragments, on the edges of which there are aldehyde groups with high reactivity. If the gap occurred on both sides, an MDA is formed. Reacting with the SH-and CH3-groups of proteins, MDA suppresses the activity of enzymes: cytochrome oxidase, hydroxylase, etc. MDA is a highly toxic compound that causes polymerization of proteins, destruction of DNA, sulfhydryl antioxidants, modification of the lipid layer of cell membranes. As a result, there is a suppression of the generation of high-energy compounds by mitochondria, in particular, adenosine triphosphate, which is necessary to ensure the vital activity of cells, growth rates, and development of the whole organism. MDA is considered to be the most mutagenic product of lipid peroxidation [24].

We believe that the detected changes in MDA and 8-OHdG in a rodent experiment using a model of diabetes mellitus and chronic neurogenic pain combined with two strains of a malignant tumor (Guerin's carcinoma, melanoma B16/F10) may indicate the ability of MDA to damage DNA, through an increase in the biomarker of oxidative stress – 8-OHdG.

Cytochrome C is an "extremely multifunctional" protein [25]. It mediates the transfer of electrons in the respiratory chain and acts as a detoxifying agent to get rid of ROS. In addition, cytochrome C participates in cell apoptosis as a precursor of internal apoptosis mediated by mitochondria [25, 26]. As a protein of the mitochondrial peripheral membrane, cytochrome C acts between the inner and outer mitochondrial membrane of healthy cells, where it mediates electron transfer between complexes III and IV of the respiratory chain [25].

Within the framework of this experiment, it was shown that the level of cytochrome C in the mitochondria of the heart with the standard growth of Guerin's carcinoma and melanoma B16/F10 had no statistically significant changes, whereas with Guerin's carcinoma and the growth of melanoma against the background of comorbid pathology – diabetes mellitus and CNP, its decrease was noted.

In this work, using two models of comorbid pathology (diabetes mellitus, chronic neurogenic pain) and two strains of malignant tumor (Guerin's carcinoma, melanoma B16/F10) on different experimental animals (rats, mice), both general dysfunctional changes in the mitochondria of heart cells and fundamental differences were demonstrated. As a result, differences between the two comorbid pathologies were revealed, so in diabetes mellitus, an increase in free radical oxidation with suppression of mitochondrial respiration was revealed, and in chronic neurogenic pain, the mitochondria of heart cells were stable. It is worth noting that the independent development of the tumor process-Guerin's carcinoma and melanoma B16/F10 in the body of female rats and female mice did not affect the functional state of the mitochondria of heart cells. General dysfunctional disorders in the mitochondria of heart cells were recorded with a combination of comorbid pathologies and a malignant process, which resulted in an increase in all products of lipid peroxidation and suppression of cytochrome C.

Recently, mitochondria have attracted considerable attention from both academic circles and pharmacological concerns, since mitochondrial dysfunction is a sign of many diseases, including heart failure. In general, many studies confirm the leading role of impaired activity of the mitochondrial electron transport chain, oxidative stress, etc.in cardiomyocyte dysfunction and, as a result, in the establishment or progression of heart failure [23, 27]. Why do mitochondria play such a central role in heart failure? First of all, the mitochondria are the powerhouse of the cell. Mitochondria make up ~ 35 % of the volume of cardiomyocytes and form a long, dynamic and well-organized network that facilitates both physical and chemical interactions between mitochondria and other intracellular structures. Recent studies on cellular and molecular mechanisms involved in the pathophysiology of heart failure and in the field of cardioncology point to mitochondria as strategic and dynamic nodes that actually affect every biochemical process in heart cells [23]. A dysfunctional mitochondrial network of cardiomyocytes can quickly spread damage inside cardiomyocytes in heart failure [23]. Therefore, the identification of points or markers of the dysfunctional state of mitochondria can be considered as a promising scientific direction, the results of which can be used to develop various new therapeutic strategies, including small molecules and peptides aimed at various mitochondrial anomalies that can improve heart function.

CONCLUSION

Summarizing the results obtained for all the used models of comorbid pathology (diabetes mellitus, chronic neurogenic pain) and malignant process (Guerin's carcinoma, melanoma B16/F10) in rodents, it can be argued that comorbid pathology associated with a malignant process exacerbates the dysfunction of the mitochondria of heart cells with the destabilization of the respiratory chain mediated by the processes of free radical oxidation.

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ORIGINAL ARTICLE

INFLUENCE OF ONCOLYTIC STRAINS OF A NEW UNCLASSIFIED GROUP OF HUMAN ROTAVIRUSES ON PERIPHERAL BLOOD LYMPHOCYTES

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ABSTRACT

Purpose of the study. Evaluation of the cytotoxic effect of strains RVK100 and RVK228 of a new unclassified group of human rotaviruses on human peripheral blood mononuclear cells *in vitro*.
Materials and methods. As a material for the study, we used peripheral blood mononuclear cells of a healthy donor. The cells were exposed to two strains of rotaviruses RVK100 and RVK228 for 24 and 48 hours. The cytotoxicity of the tested viruses was assessed using the Colorimetric Cell Viability Kit I (WST-8) (PromoCell, Germany). Analysis of lymphocytes subpopulation composition was assessed on a FACSCantoII flow cytometer (BD, USA) using monoclonal antibodies to human antigens: CD3, CD4, CD8, CD16/56, CD19, CD45, CD38, HLA-DR.
Results. According to the cell viability test, there was no significant decrease in the number of living cells in the samples with the addition of viruses in comparison with the control. On the contrary, after 48 hours of cultivation in the samples with the addition of RVK228, the number of living cells was significantly higher than in the control. The study of lymphocytes subpopulation composition showed a relative increase in the number of early activation markers on T cells in samples with viruses, which was also more pronounced in samples with the addition of RVK228.
Conclusion. The investigated strains of rotaviruses have no cytotoxic effect on human peripheral blood mononuclear cells. Moreover, the RVK228 strain is likely to have the ability to activate lymphocytes.

Keywords:
oncolytic viruses, rotaviruses, peripheral blood mononuclear cells, cytotoxicity assay, flow cytometry.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

ВЛИЯНИЕ ОНКОЛИТИЧЕСКИХ ШТАММОВ НОВОЙ НЕКЛАССИФИЦИРОВАННОЙ ГРУППЫ РОТАВИРУСОВ ЧЕЛОВЕКА НА ЛИМФОЦИТЫ ПЕРИФЕРИЧЕСКОЙ КРОВИ

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РЕЗЮМЕ

Цель исследования. Оценка цитотоксического действия штаммов RVK100 и RVK228 новой неклассифицированной группы ротавирусов человека на мононуклеарные клетки периферической крови человека *in vitro*.
Материалы и методы. В качестве материала для исследования использовали мононуклеарные клетки периферической крови здорового донора. На клетки воздействовали двумя штаммами RVK100 и RVK228 в течение 24 и 48 часов. Цитотоксичность тестируемых вирусов оценивали с помощью теста Colorimetric Cell Viability Kit I (WST-8) (PromoCell, Германия). Анализ субпопуляционного состава лимфоцитов оценивали на проточном цитофлуориметре FACSCantoII (BD, США) с использованием панели моноклональных антител к человеческим антигенам: CD3, CD4, CD8, CD16/56, CD19, CD45, CD38, HLA-DR.
Результаты. По данным теста на жизнеспособность не было обнаружено достоверно значимого снижения количества живых клеток в образцах с добавлением вирусов по сравнению с контролем. Напротив, после 48 часов культивирования в образцах с добавлением RVK228 количества живых клеток было достоверно больше, чем в контроле. Исследование субпопуляционного состава лимфоцитов показало относительное увеличение количества маркеров ранней активации на Т-клетках в образцах с вирусами, которое также было более выражено в образцах с добавлением RVK228.
Заключение. Исследуемые штаммы ротавирусов не оказывают цитотоксического действия на мононуклеарные клетки периферической крови человека. При этом штамм RVK228, вероятно, обладает способностью к активации лимфоцитов.

Ключевые слова:
онколитические вирусы, ротавирусы, мононуклеарные клетки периферической крови, цитотоксический тест, проточная цитофлуориметрия.

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INTRODUCTION

Despite the success of modern oncology, malignant tumors are the second most common cause of death worldwide [1]. Oncolytic virotherapy is one of the antitumor strategies, the development of which remains relevant for a number of decades. The potential of oncolytic viruses (OV) was finally realized when the first drug for therapy based on OV – Oncorin (H101) – was approved by the State Food and Drug Administration of China (SFDA) for clinical use in the treatment of nasopharyngeal carcinoma in 2005 [2]. Later, in 2015, the US Food and Drug Administration (FDA) approved the drug T-Vec based on a modified herpes simplex virus (HSV) for the treatment of metastatic melanoma [3]. Both natural attenuated and engineered viruses that are nonpathogenic to humans are being studied all over the world. Identifying the most active and safe of them would allow us to offer new methods of treating malignant tumors.

The safety of the use of OV depends on their ability to infect and replicate only in tumor cells. It is known, in particular, that the selective replication of viruses in cancer cells is mainly due to impaired mechanisms of the antiviral response in transformed cells (for example, the type I interferon signaling pathway) [4]. Disorders of cellular metabolism [5] and signaling pathways regulating cell division [6] are also considered as possible reasons for the OV tropism to tumor cells. Since it is impossible to draw a conclusion about its safety only on the basis of data on the interaction of OV with tumor cells, a necessary stage in research to find new OV is testing their cytotoxic activity in relation to normal cells of the body.

In the Rostov Institute of Microbiology and Parasitology (the Rostov Research Institute of Microbiology and Parasitology of Rospotrebnadzor), during the work on the adaptation of group A human rotaviruses to growth on transplanted cell cultures for their use as a vaccine for children, strains that did not belong to any of the known groups of human rotaviruses were detected and isolated [7]. The new group is called group K rotaviruses (RVK), its representatives have common features both with group A rotaviruses, which are the most common pathogens of gastroenteritis in children, and with reoviruses. Further joint studies of the National Medical Research Centre for Oncology of the Ministry of Health of Russia and the Rostov Research Institute of Microbiology and Parasitology showed the oncolytic activity and immunomodulatory properties of this group of viruses in vivo and *in vitro* [8-10]. The future prospects of using new rotavirus strains in oncolytic virotherapy will be determined by their safety in relation to normal human cells.

The purpose of the study was to evaluate the cytotoxic effect of RVK100 and RVK228 viruses of a new unclassified group of human rotaviruses of the family Reoviridae on peripheral blood mononuclear cells of healthy donors.

MATERIALS AND METHODS

Oncolytic strains of rotaviruses

The study used two live attenuated, nonpathogenic rotavirus strains previously isolated at the Rostov Institute of Microbiology and Parasitology, with the working name rotaviruses of group K – RVK100 and RVK228 [7].

Cultivation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (MNCs) were obtained from a healthy male donor of 25 years. The donor signed an informed consent to participate in the study. Mononuclear cells were isolated by centrifugation using Vacutainer® CPT ™ tubes (BD, USA) according to the manufacturer's instructions. The resulting cells were counted with the addition of 0.4 % trypan blue on an automatic Eve counter (NanoEnTek Inc, Korea).

Determination of the cytotoxic activity of viruses

MNCs were introduced into a 96-well tablet (Eppendorf, Germany) in an equal seeding dose of 10^4 cells per well in 100 ml of RPMI 1640 nutrient medium (Gibco, USA) without the addition of serum and antibiotics. Then the cells were cultured for 24 hours at 37 °C in a humid atmosphere containing 5 % CO_2. After 24 hours, viruses were introduced into the wells with MNCs to a final concentration of 10^7 particles/ml, for which 1 ml of a suspension of viruses with a concentration of 10^9 particles/ml in the RPMI 1640 nutrient medium without serum was added to each well of the 96-well tablet. An equal amount of virus-free medium was introduced into

the control wells. Next, the MNCs were cultured for 24 or 48 hours at 37 °C in an atmosphere of 5 % CO_2. WST-8-the test used to determine cytotoxicity is an analog of the popular MTT test. The number of living cells was evaluated by colorimetric measurement of the concentration of formazan obtained during the reduction of WST-8 by cellular reductases. The study used the Colorimetric Cell Viability Kit I (WST-8) (PromoCell, Germany) according to the manufacturer's instructions. The optical density (OD) was measured using an ELISA reader Stat Fax2100 (Awareness Technology, USA) at a wavelength of 492 nm. Each variant of the experiment was put in 16 repeats, including 2 incubations and 2 strains.

Determination of the population composition of lymphocytes

For cytofluorimetric analysis, cells were introduced 5×10^5 cells per well of a 6-well tablet (Eppendorf, Germany) in 1.5 ml of RPMI 1640 nutrient medium without the addition of serum and antibiotics. The cultivation of cells with viruses was carried out as described above. The study of the population and subpopulation composition of lymphocytes was carried out on a 6-color flow cytometer BD FACSCanto II (Becton Dickinson, USA) using a panel of monoclonal antibodies to human antigens: CD3, CD4, CD8, CD16/56, CD19, CD45, CD38, HLA-DR (Becton Dickinson, USA).

Statistical analysis

The colorimetric test data is presented as an average value of ± 95 % confidence interval. The reliability of the difference between the average values was determined using the Student's t-test. The significance level adopted in the study, taking into account the Bonferroni correction for multiple comparison, was $\alpha' = \alpha/6 = 0.005/6 = 0.008$. The critical value of the Student's t-test for the adjusted $\alpha' = 0.008$ and $df = 2n-2 = 30$ was $t_{crit} = 2.84$.

RESEARCH RESULTS AND DISCUSSION

The addition of viruses to the culture medium did not have a cytotoxic effect on the MNC. According to the results of the colorimetric test, the level of living cells in both samples with the addition of viruses and in control samples did not differ significantly after 24 hours of cultivation (OD_{contr}=0.21±0.007, OD_{RVK100}=0.22±0.007, OD_{RVK228}=0.21±0.01). After 48 hours of cultivation, there was a significant decrease in the proportion of living cells in all samples, but in the sample with the addition of the RVK228 strain, the decrease was less pronounced (OD_{contr}=0.14±0.003, OD_{RVK100}=0.14±0.01, OD_{RVK228}=0.15±0.005) (Fig. 1). Testing the hypothesis of equality of mean values using the Student's t-test showed that the difference in the proportion of living cells between the control and

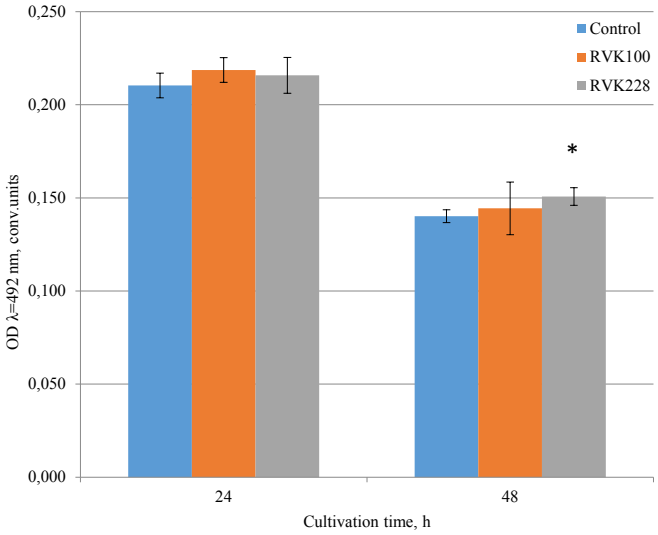

Fig. 1. The results of the WST-8 test under the action of oncolytic rotavirus strains RVK100 and RVK228 on MNCs under in vitro conditions (mean value ± 95% confidence interval). * – the difference in the average values compared to the control is statistically significant at the significance level α'≤0.008.

the variant with the addition of RVK228 is significant at the significance level α'≤0.008 (t_{exp}=3.53, df=30), while the differences between the other variants did not reach the accepted significance level.

A decrease in the proportion of living MNCs after a short period of time after passage indicates the death of some cells, probably caused by the absence of specific signals in the serum-free environment that support the activation and proliferation of lymphocytes [11]. The fact that the decrease in the level of living cells after 48 hours of cultivation in the sample with RVK228 was not as pronounced as in the control sample and in the sample with RVK100, may be due to the fact that this strain has a greater ability to activate cells of the immune system compared to RVK100. This assumption is supported by the results cytofluorimetric studies are shown in tables 1, 2.

It should be noted that incubation of donor samples MNC for 24 hours under these conditions without viruses has led to significant changes in their

Table 1. Effect of RVK100 and RVK228 strains on the population composition of lymphocytes under *in vitro* conditions

Samples	Lymphocyte populations, % of CD45+ (mean value ± standard deviation)			
	$CD3^+$	$CD19^+$	$CD16/56^+$	$CD3/16/56^+$
Before incubation	80.4±7.4	1.7±0.2	14.3±2.1	2.6±0.9
		24 hours		
Control	67.4±6.2	0.7±0.05	25.6±3.0	5.3±1.1
RVK100	72.1±8.0	0.9±0.1	24.4±2.8	4.1±1.0
RVK228	67.2±7.8	0.7±0.08	27.3±3.1	4.1±0.7
		48 hours		
Control	68.8±5.8	0.9±0.15	23.9±2.3	3.6±0.5
RVK100	68.3±7.1	0.4±0.03	25.6±3.05	2.5±0.5
RVK228	69.1±6.5	0.5±0.03	25.6±2.8	3.1±0.8

Table 2. Effect of RVK100 and RVK228 strains on the subpopulation composition of T-lymphocytes under *in vitro* conditions

Samples	T-lymphocyte subpopulations (mean value ± standard deviation)					
	$CD4^+$, % of $CD3^+$	$CD8^+$, % of $CD3^+$	$CD4^+HLA\text{-}DR^+$, % of $CD4^+$	$CD4^+CD38^+$, % of $CD4^+$	$CD8^+HLA\text{-}DR^+$, % of $CD8^+$	$CD8^+CD38^+$, % of $CD8^+$
Before incubation	38.1±4.8	38.0±5.1	6.4±0.9	33.8±3.8	10.4±1.8	10.7±2.1
			24 hours			
Control	15.8±2.1	39.6±3.5	17.0±1.3	12.8±1.7	4.3±3.1	3.8±0.45
RVK100	13.8±1.05	48.0±5.6	23.5±1.9	7.8±0.9	2.7±1.9	6.0±0.8
RVK228	13.2±1.4	39.5±4.2	19.6±2.1	13.7±1.2	6.0±0.7	8.8±0.74
			48 hours			
Control	14.0±1.7	40.8±4.02	20.5±2.5	9.5±1.05	2.3±0.4	4.5±0.6
RVK100	12.9±1.07	39.0±4.5	18.9±2.1	8.7±0.9	2.7±0.3	4.6±0.8
RVK228	11.2±1.5	39.9±3.8	21.3±2.8	14.7±1.9	3.5±0.7	3.5±0.4

composition: there was a decrease in the percentage of cells expressing $CD3^+$ and $CD4^+$, with preservation of the percentage of $CD8^+$, the marked increase in the percentage of NK-cells, which, apparently, was more resistant to such cultivation; the changes were made in subpopulations of activated T-cells.

After 24 hours, in both samples with the addition of viruses, compared with the cultivation control, there was a relative increase in the proportion of T-killers expressing an early activation marker ($CD8^+CD38^+$), while the reaction to RVK228 was more pronounced (RVK228 – 8.8 % ± 0.74 %, RVK100 – 6.0 % ± 0.8 %, control – 3.8 %±0.45 %). The 24-hour incubation of MNCs with RVK was also accompanied by an increase in the percentage of $CD4^+$ cells and a decrease in $CD8^+$ cells expressing $HLA\text{-}DR^+$ under the action of RVK100. After 48 hours of cultivation, the expression of the early activation marker on T-helpers ($CD4^+CD38^+$) exceeded the control values only in samples with the addition of RVK228 (RVK228 – 14.7 ± 1.9 %, RVK100 – 8.7 ± 0.9 %, control – 9.5 %±1.05 %).

There were no significant changes in the relative number of T-lymphocytes ($CD3^+$), T-killers ($CD8^+$), T-helpers ($CD4^+$), B-lymphocytes ($CD19^+$), as well as natural killers (NK) and natural killer T-cells (NKT) in the experimental samples compared with the control ones (Tables 1, 2).

Activation of lymphocytes in response to interaction with a foreign agent, including a virus, is a well-known fact. However, at this stage, without additional studies, it is impossible to accurately determine the mechanisms underlying the ability of the RVK228 strain to maintain lymphocyte proliferation. However, there are a number of works that provide results similar to ours and investigate possible intermediary cells of the mitogenic activity of rotaviruses *in vitro*. So, in the work of Yasukawa et al. [12], it was suggested that the source of the mitogenic effect on the lymphocytes of the Wa strain of human rotavirus and the NCDC strain of cow rotavirus are memory T cells that previously came into contact with this group of viruses, since this effect was not observed in experiments with umbilical cord blood, whose lymphocytes were naive to pathogens. The repeated encounter with the virus, according to the authors, provoked the active proliferation of memory T cells and the production of such factors as interleukin-2 (IL-2) and interferon-γ (IFN-γ), which stimulate the proliferation of lymphocytes [12]. However, in more recent studies by other authors, it was shown that the primary source of activation of lymphocytes, including T cells, by rotaviruses are plasmacytoid dendritic cells (PDC) [13, 14], which are present in a small amount in all MNC preparations. Thus, in the work of Mesa et al. [13], it was shown that macs, when infected with the Wa strain, secrete proinflammatory cytokines IL-6, tumor necrosis factor-α (TNF-α) and IFN-α into the medium, which, in turn, leads to the activation of T-helpers and their production of regulatory cytokines IFN-γ, IL-2 and IL-10. In the same study, it was shown that in addition to PDC, monocytes and B cells are susceptible to rotavirus infection, with almost complete resistance of T cells to it, which is associated with α4-integrin and other unexplored factors [13]. It is emphasized that the infection is of a short-term nature and does not affect the viability of these cells, which is consistent with the results of our experiment.

Despite the fact that our study did not observe a relative increase in the number of T-helpers, which is mentioned in the study of Yasukawa et al. [12], we can assume that this subpopulation is activated, as evidenced by an increase in the proportion of $CD4^+CD38^+$ cells. Whether the activation of T-helpers is direct or mediated by antigen-presenting cells, as well as how much T-helpers are involved in maintaining the proliferative activity of MNCs, has yet to be established. Given that in our study we are studying the properties of a previously unexplored group of rotaviruses, the mechanism of their interaction with blood cells may differ from mechanisms given in the works cited above.

CONCLUSION

The studied strains of human rotaviruses belonging to group K, first described at the Rostov Institute of Microbiology and Parasitology, do not have cytotoxic activity against human lymphocytes *in vitro*. Moreover, the RVK228 strain probably has the ability to activate T cells, but further research is required to test this hypothesis.

Authors contribution:

Kit O.I. – scientific editing.

Filippova S.Yu. – analysis and interpretation of data, preparation of illustrations, writing the text of the article.

Timofeeva S.V. – conducting experiments with the isolation and cultivation of lymphocytes, setting up a colorimetric study, primary processing of the results.

Sitkovskaya A.O. – research design, technical editing.

Zlatnik E.Yu. – the concept of research and scientific editing.

Kolpakov S.A. – isolation and growth of RVK strains.

Kolpakova E.P. – isolation and growth of RVK strains.

Bondarenko E.S. – cytofluorimetric analysis.

Novikova I.A. – scientific editing.

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REVIEW

BIOMARKERS FOR NON-SMALL CELL LUNG CANCER IMMUNOTHERAPY

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ABSTRACT

The discovery of immune checkpoint inhibition has revolutionized the treatment of many solid malignancies, including non-small cell lung cancer (NSCLC). Immune checkpoint inhibitors (ICI) can restore the antitumor immune response by blocking the inhibition of T-cell activation. Anti-programmed death-ligand 1 (PD-L1) is currently the main biomarker of the effectiveness of anti-PD-1 / PD-L1 blockade in the treatment of NSCLC without driver mutations. High tumor mutational burden suggests an increased neoantigens load and has been associated with the effectiveness of ICI therapy. Microsatellite instability, a biomarker approved for immunotherapy across solid tumors, but it is uncommon in NSCLC. Primary resistance to ICIsis characteristic of NSCLC with driver mutations, acquired is associated with immunoediting resulting in the depletion of potentially immunogenic neoantigens. The review discusses recent advances and future directions for predicting the results of immunotherapy in patients with NSCLC.

Keywords:
non-small cell lung cancer, immunotherapy, biomarkers, checkpoint inhibitor, anti-programmed death-ligand 1, tumor mutation burden.

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ОБЗОР

БИОМАРКЕРЫ ДЛЯ ИММУНОТЕРАПИИ НЕМЕЛКОКЛЕТОЧНОГО РАКА ЛЕГКОГО

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РЕЗЮМЕ

Открытие ингибирования иммунных контрольных точек произвело революцию в лечении многих солидных злокачественных новообразований, включая немелкоклеточный рак легкого (НМРЛ). Ингибиторы иммунных контрольных точек (ИИКТ) обладают способностью восстанавливать противоопухолевый иммунный ответ, блокируя торможение активации Т-лимфоцитов. Anti-programmed death-ligand 1, трансмембранный белок, лиганд к рецептору PD-1(PD-L1) в настоящее время является основным биомаркером эффективности анти-PD-1/ PD-L1 препаратов лечении НМРЛ без драйверных мутаций. Высокая мутационная нагрузка опухоли, предполагающая повышенную продукцию неоантигенов, также ассоциируется с эффективностью иммунотерапии. Микросателлитная нестабильность – другой биомаркер, одобренный для иммунотерапии при солидных опухолях, – редко наблюдается при НМРЛ. Первичная резистентность к ИИКТ характерна для онкодрайверного НМРЛ, приобретенная связана с иммунноредактированием в результате истощения потенциально иммуногенных неоантигенов. В обзоре обсуждаются последние достижения и будущие направления прогнозирования результатов иммунотерапии у больных НМРЛ.

Ключевые слова:
немелкоклеточный рак легкого, иммунотерапия, биомаркеры, ингибиторы иммунных контрольных точек, anti-programmed death-ligand, опухолевая мутационная нагрузка.

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INTRODUCTION

The discovery of immune checkpoint inhibition has revolutionized the treatment of many solid malignancies, including non-small cell lung cancer (NSCLC). Anti-programmed death-ligand 1, transmembrane protein, ligand to the PD-1 receptor (PD-L1) is currently the main biomarker of the effectiveness of anti-PD-1/ PD-L1 drug blockade in the treatment of NSCLC without driver mutations.

To date, the biomarkers approved by the Food and Drug Administration (FDA) for determining indications for immunotherapy of progressive NSCLC are: the proportion of a tumor proportion score (TPS) expressing PD-L1 on tumor cells and microsatellite instability. Other promising markers studied for immunotherapy are: tumor mutation burden (TMB), tumor-infiltrating lymphocytes (TILs), and the density of CD8+T cells in the tumor microenvironment. The genomic landscape of a tumor affects its immunogenicity and response to immunotherapy. The review discusses the latest achievements and future directions for predicting the results of immunotherapy in NSCLC patients.

PD-L1 tumor expression

PD-L1 is a transmembrane protein encoded by the PD-L1 gene found on chromosome 9 in humans. Constitutive expression of low-level PD-L1, characteristic of resting lymphocytes, antigen-presenting cells of other tissues, is necessary for maintaining homeostasis in anti-inflammatory conditions [1]. The inhibitory PD-1 molecule present on B-lymphocytes, activated T-lymphocytes and natural killer cells binds to the ligands PD-L1 (B7-H1 or CD271+) and PD-L2 (B7-DC or CD273+) [2]. The interaction of the PD-1 molecule with the PD-L1 sligand inhibits the proliferation, survival and activity of cytotoxic T-lymphocytes, induces apoptosis of tumor-infiltrating lymphocytes (TILs) and the accumulation of immunosuppressive regulatory T cells (T-reg.) in the tumor microenvironment [3]. With advanced NSCLC, approximately 40 to 58 % of patients have PD-L1-negative tumors, 28 to 31 % have tumors with low (1-49 %) expression of PD-L1, and only 10 to 32 % have tumors with high (50 % or more) expression of PD-L1 [4, 5]. Antibody blockade of immune control points of the PD-1/PD-L1 axis revolutionized the treatment of advanced and metastatic NSCLC, becoming the standard of first-line treatment of patients both in isolation and in combination with chemotherapy [6].

The expression of PD-L1 is determined by the immunohistochemical method. 5 different anti-PD-L1 immunoglobulins of the IgG1 class are used for testing in clinical trials: 22C3, 28-8, SP142, SP263 and 73-10. The percentage of expression is most often measured using the TPS indicator, which is estimated by quantifying viable tumor cells with partial or complete staining of cell membranes [7].

Numerous clinical studies [8] of the use of anti-PD-1 and anti-PD-L1 antibodies have shown the value of studying the expression of PD-L1 as a predictive biomarker. A randomized clinical trial of KEYNOTE-010, which compared the effectiveness of pembrolizumab at two different doses of 2 or 10 mg/kg every 3 weeks with docetaxel chemotherapy in previously treated patients with progressive NSCLC with a TPSPD-L1 index of ≥1 %. The main endpoints of the study were determined by the overall survival (s) and progression – free survival (PFS-progression-free survival). Patients treated with pembrolizumab had a significantly longer median S: 10.4 months. when prescribing pembrolizumab at a dose of 2 mg/kg (HR0, 71, p=0.008) and 12.7 months at a dose of 10 mg/kg (HR 0.61 p<0.00001) compared with patients receiving only docetaxel-8.5 months. After 1 year, most of the patients receiving pembrolizumab were alive: in the group of pembrolizumab at a dose of 10 mg/kg, OV was 52.3 %, and at a dose of 2 mg/kg – 43.2 %, compared with those receiving docetaxel – 34.6 %. A subgroup analysis revealed that a higher PD-L1 TPS is a predictor of longer survival. The median OV of patients with TPS PD-L1≥50 % was 14.9 months in the group of patients receiving pemrolizumab at a dose of 2 mg/kg versus 8.2 months. In the docetaxel group (HR 0.54; 95 % [CI] 0.38-0.77; p=0.0002) and 17.3 months. In the group of patients receiving pemrolizumab at a dose of 10 mg/kg versus 8.2 months in the docetaxel group (HR 0.50; 95 % [CI] 0.36-0.70; p<0.0001) [8].

In a study by Reck M, Rodriguez-Abreu D, Robinson AG, et al. PHASE 3 KEYNOTE-024 The efficacy of pembrolizumab immunotherapy compared to standard two – component platinum – containing chemo-

therapy in the first line for EGFR-and ALK-negative advanced NSCLC with PD-L1 TPS expression ≥ 50 % was studied. As a result, the study demonstrated clear advantages in patients receiving immunotherapy in terms of median PFS, S and the frequency of objective responses to treatment. The median response duration in the pembrolizumab group was not reached [5].

Recently, the results of the follow-up study of the KEYNOTE-024 study were published [9]. The median OS in the group of patients receiving pembrolizumab in the first line was 30.0 months versus 14.2 months in the chemotherapy group [9]. The presented results ultimately led to the approval of pembrolizumab monotherapy in patients with metastatic NSCLC without activating mutations with high PD-L1 expression (Table 1).

A phase 3 clinical trial of KEYNOTE-042 led to the approval of pembrolizumab for PD-L1 – positive progressive NSCLC with any level of PD-L1 expression. The KEYNOTE-042 protocol is a randomized open-label international double-blind study of pembrolizumab immunotherapy compared to standard chemotherapy in patients with untreated metastatic PD-L1-positive (TPS≥1 %) NSCLC. Patients who started treatment showed significantly longer GS in the group receiving pembrolizumab compared to first-line chemotherapy in all PD-L1 positive groups: PD-L1 TPS≥50 % – HR 0.69, 95 % [CI] 0.56-0.85, p=0.0003; PD-L1 TPS≥20 % – HR 0.77, 95 % [CI] 0.64-0.92, p=0.002 and PD-L1 TPS≥1 %-HR 0.81, 95 % [CI] 0.71-0.93, p=0.0018 [10]. The median GS was 17.7 months in the pembrolizumab group versus 12.2 months in the chemotherapy group; among patients with PD-L1 TPS≥50 %, the median GS reached 17.7 months versus 16.7 months in the group with PD-L1 TPS≥20 % and against 12.1 months. in the group with PD-L1 TPS ≥1 %, respectively [10].

It was shown that smoking or quit smoking patients with progressive non-squamous NSCLC who received nivolumab had better GS indicators compared to non-smoking patients [12]. Two studies have linked the history of smoking with an increase in TPS PD-L1 [11, 12]. The group of patients with the nicotine addiction gene had a higher level of objective response – 56 % compared to the group of patients without it – 17 % (p=0.03) [12]. In addition, the clinical study of KEYNOTE-024 demonstrated an increase in the survival rate when quitting smoking during immunotherapy [5].

In most clinical studies of IT immunotherapy in EGFR – and ALK-negative progressive NSCLC, high levels of PD-L1 expression correlated with better GS, PFS indicators and the frequency of objective responses to treatment compared to first-line chemotherapy [9, 13]. However, for patients with metastatic NSCLC, whose disease progressed on platinum-containing two-component chemotherapy, both nivolumab and atezolizumab are approved in the second line regardless of PD-L1 expression [14-17].

Microsatellite instability and MMR-deficient malignant tumors

The defective DNA repair process is known to lead to hypermutation genomic status, otherwise called high microsatellite instability (MSI-H). Mismatch repair (MMR) DNA repair proteins are represented by: MutL homolog 1 (MLH1), MutS homolog 2 (MSH2), MutS homolog 6 (MSH6) and PMS1 Homolog 2 (PMS2). Inactivation of any of the genes encoding

Table 1. Approved biomarkers for IT immunotherapy in NSCLC

Biomarkers approved by FDA	Drug	Therapy outcomes	Evidence-based clinical studies
PD-L1 ≥ 50 %	Pemrolizumab in the first line against chemotherapy	The best indicators of GS and PFS in the pemrolizumab group	KEYNOTE-024 [9]
PD-L1 ≥ 50 %	Pemrolizumab in the first line	The best indicators of GS and PFS in the pemrolizumab group	KEYNOTE-042 [10]
MSI-H	Pemrolizumab for any morphological subtype	The best indicators of GS and PFS	D.T.Le et al, 2015 [22]

Note: MSI-H – microsatellite instability high.

these proteins occurs in 80 % of cases as a result of somatic mutations, and in 20 % is secondary to germinal mutations, followed by a second inactivating somatic damage in the remaining wild-type allele [18]. MMR-deficient colorectal cancer carries 100 times more somatic mutations than MMR-deficient adenocarcinomas. MMR-deficient cancers and among them NSCLC have pronounced lymphocytic infiltrates that correlate with the immune response [19].

MSI-H (microsatellite instability high) tumors or tumors with high microsatellite instability show increased regulation of control points in the tumor microenvironment, including PD1, PD-L1, LAG3 (lymphocyte activation gene 3) and IDO (indolamine 2,3-dioxygenase). These control points that suppress the activity of CD8+cytotoxic T-lymphocytes that infiltrate the tumor microenvironment are also found in MMR-deficient malignant neoplasms [20]. In a phase 2 clinical study, the results of therapy of patients with MMR-deficient and MMR-surplus solid tumors, including NSCLC, treated with pembrolizumab were compared. WES-whole-exome sequencing revealed approximately 1,782 somatic mutations per tumor in patients with MMR-deficient cancer and an average of 73 mutations per tumor in patients with MMR-surplus cancer (p=0.007). The observed objective response rate was 39.6 % in a cohort of 149 patients with 15 different solid tumors, including NSCLC, of which 7 % had a complete response. Four out of 10 patients with MMR-deficient colorectal cancer responded to imunotherapy with pembrolizumab (Table. 1) [21].

Based on the study under discussion, pembrolizumab is approved by the FDA for the treatment of adults and children with unresectable or metastatic, MSI-H – positive or MMR-deficient solid tumors that do not have alternative treatment options after progression [19].

Mutational load of the tumor

The mutational burden of a tumor (TNB) is a set of somatic non-synonymous mutations: insertions, deletions and substitutions of protein-coding bases in the coding region of the tumor genome. The increased mutational load is the result of exposure to smoking, radiation, ultraviolet rays and other environmental and nutritional factors that lead to inflammation. It is suggested that high TMB enhances immunogenicity by increasing the number of neoantigens expressed by cancer cells, which are recognized by T-lymphocytes as foreign, causing a stronger immune response in the presence of IT (Table. 2) [22, 23].

TMB is measured by various methods, including full-exome sequencing (WES-whole-exome sequencing) and targeted next-generation sequencing (NGS) panels. The use of WES for the determination of TMV in NSCLC patients revealed an association between a higher load of somatic non-synonymous mutations and the clinical efficacy of pembrolizumab in 2 different groups of patients [24]. In the group with high TMB, consisting of 16 patients with a predominant clinical response duration of more than 6 months, the average number of non-synonymous mutations was 302 versus 148 for the group with a short response (p=0.02). In patients with a high load of tumors with non-synonymous mutations, an increase in the level of objective response was observed to 63 % versus its complete absence (p=0.03) and survival rates to progression with a median of 14.5 versus 3.7 months (HR 0.19, 95 % CI 0.05-0.70; p=0.01) [24]. An inde-

Table 2. Potential biomarkers for IT immunotherapy in NSCLC

Studied biomarkers	Medication	Therapy outcomes	Evidence-based clinical studies
TMB high	Nivolumab, Ipilimumab	ORR, PFS indicators improvement	CheckMate-227 [25] CheckMate-026 [23]
STK11/LKB1 Mutation	Anti-PD-1 or anti-PD-L1 antibodies or a combination of anti-PD-L1 with anti-CTLA-4 antibodies	Shorter PFS	SU2C and CheckMate-057 [28]
HLA class I allele C03:04	IT	Shorter PFS	M.V.Negrao et al, 2019 [32]
Acquired loss of beta-2-microglobulin	Combination of anti-PD-L1 with anti-CTLA-4 antibodies	IT resistance	S.Gettinger et al, 2017 [31]

pendent set of 18 NSCLC samples from patients receiving pembrolizumab formed a validation group. The average load of non-synonymous mutations was 244 in the tumors of patients with a long-term clinical response, compared with 125 in tumors without one (p=0.04). Significantly longer PFS was observed in patients with a non-synonymous mutational load above 200: their median PFS was not reached compared to 3.4 months in the group with low TMV (HR 0.15, 95 % CI0, 04-0.59; p=0.006) [24].

Subsequently, as part of the CheckMate-026 study, TMV was calculated using tumor WES and compared with blood DNA in 312 patients. The patients were divided into trigroups in accordance with the values of TMV. TMV from 0 to less than 100 mutations was considered a low load, from 100 to 242 mutations were considered an average load, and from 243 or more mutations were considered a high load. Patients with high TMV treated with nivolumab had higher objective response rates – 47 % vs. 28 % and longer PFS with a median of 9.7 months vs. 5.8 months. compared with patients who received chemotherapy [23].

CheckMate-227-an open phase 3 clinical trial compared the results of immunotherapy with nivolumab, nivolumab in combination with ipilimumab – an CTLA-4-anti–cytotoxic Tlymphocyte–associated protein (4anti-CTLA-4 antibody) and nivolumab in combination with platinum-containing two-component chemotherapy in patients with stage IV NSCLC. TMV was calculated using the NGS target panel after applying various filters and, as a result, was divided into a calculated area (0.8 Mb) to calculate the number of mutations per megabase. Among the patients selected for TMV, a predetermined TMV reference point of 10 mutations per mega base was selected for a preliminary study of the effectiveness of combined immunotherapy with nivolumab and ipilimumab in comparison with chemotherapy in the group. Patients who received combined immunotherapy with anti-PD-1 and anti-CTLA-4 antibodies had a higher level of objective response – 45.3 % versus 26.9 % in those who were treated with chemotherapy. The median PFS was 7.2 months. when using nivolumab and ipilimumab against 5.5 months. with chemotherapy (HR-0.58; 97.5 % [CI] 0.41-0.81; p<0.001), a1-year PFS: 42.6 % vs. 13.2 %, respectively [25].

It was found that TMV calculated using MSK-IMPACT (Memorial Sloan Kettering-Integrated Mutation Profilingof Actionable Cancer Targets), a clinical diagnostic platform for molecular oncology of solid tumors based on NGS, predicts survival after immunotherapy for several types of cancer. The study also included 350 NSCLC patients who received IT therapy. The reference point determined by the 30 % normalized MSK-IMPACT mutation load for NSCLC was 10.8 mutations per megabase. The patients of this group had the best OV (HR 0.75; p<0.032) [26].

Thus, TMV is a new biomarker that, as shown in several clinical studies, predicts the response to IT immunotherapy. A higher TMV of the tumor, apparently, increases the probability that immunogenic neoantigens cause a pronounced antitumor response. Currently, it is necessary to harmonize the methods of measuring TMV for the use of a biomarker in clinical practice in order to optimally select patients who will benefit from the appointment of immunotherapy.

Tumor infiltrating lymphocytes

The infiltration of the tumor by lymphocytes correlates with the best survival of patients who underwent surgical treatment. In the Lung Adjuvant Cisplatin Evaluation Biomarker (LACE) Bio study, which included patients with localized NSCLC, the degree of tumor infiltration by lymphocytes was studied based on the criterion-intensive or non-intensive, with the primary endpoint chosen by the S, and the secondary endpoint-relapse-free survival. Intensive lymphocytic infiltration was defined as more than 50 % of lymphocytes in the volume of tumor tissue compared to epithelial tumor cells. The study established the lymphocytic infiltration of the tumor as an independent prognostic factor predicting a longer life of patients whose removed tumors had intense lymphocytic infiltration [27].

The above-mentioned effectiveness of IT in MMR-deficient and MSI-H tumors is not least associated with their high lymphocytic infiltration. However, it is important not only the total number of lymphocytes, but also their subpopulation composition and functional activity. Thus, CD8+ infiltration by lymphocytes is associated with a positive effect of immuno-

therapy, and the accumulation of Tregs cells in the tumor is an unfavorable predictor sign. As shown in experimental models, their removal with CD25 antibodies before the use of anti-PD-1 antibodies leads to an increased antitumor response and, in the future, may be one of the therapeutic strategies [28].

STK11/LKB1 gene

The CTK11/LKB1(Serine/threonine-proteinkinase/liverkinaseB1) gene is a tumor suppressor gene inactivated in approximately one-third of KRAS-mutant NSCLC, playing a key role in the primary resistance of NSCLC to IT. The STK11/LKB1 gene encodes serine-threonine kinase, which, when inactivated by mutational or non-mutational mechanisms, affects the immune microenvironment of the tumor, leading to a decrease in the number of tumor-infiltrating cytotoxic CD8+ T lymphocytes both in human tumors and in genetically engineered mouse models [29]. Increased accumulation of T-cell depletion markers and increased production of interleukin-6 by tumor cells, leading to the recruitment of myeloid cells and neutrophils with suppressive properties against T-lymphocytes, was shown in mice with the knockout CTK11/LKB1 gene. Neutrophil depletion and neutralization of interleukin-6 in mouse models with loss of STK11/LKB1 enhanced the function and increased the number of T-lymphocytes in the tumor [30]. STK11/LKB1 is an ascending activator of the AMPK (adenosinemonophosphate-activated proteinkinase) signaling pathway, which, being inactive, cannot block the mTOR (mammalian target of rapamycin) signaling pathway or induce mitochondrial autophagy. The activated mTOR signaling pathway ultimately leads to the growth of tumor cells. Repeated induction of LKB1 restored the level of PD-L1 expression on the surface of tumor cells, stimulating the chemotaxis of T-lymphocytes [31].

Retrospective clinical studies of SU2C and CheckMate-057 of two different groups of patients showed that the alteration of the STK11/LKB1 gene in lung adenocarcinomas makes them less sensitive to IT immunotherapy with a significant decrease in the level of objective response, PFS (p<0.001) and OV (p=0.0015) compared with lung adenocarcinomas with REDSTK11/LKB1 mutations of the wild type [29].

HLA class I

HLA (human leukocyte antigen) of class I plays an important role in the antitumor immune response, and a wider range of molecules is believed to lead to an increase in the chances of presentation of an immunogenic antigen and the probability of a response to IT. The main histocompatibility complex I (MHC-I) in humans is represented by the classical molecules HLA-A, HLA-B and HLA-C. A decrease in the expression of beta-2-microglobulin, which is a component of MHC-I, is described as a mechanism of acquired resistance to IT immunotherapy [32]. However, a recent study conducted in MD Anderson Cancer Center, which compared 3 different groups of patients with progressive NSCLC who received anti-PD-1/PD-L1 immunotherapy, did not reveal differences in results depending on HLA status. The study evaluated the expression of PD-L1, TMB, HLA genotype, mutational status and the presence of STK11 mutations; all these biomarkers were correlated with the results of treatment. After HLA typing, 2 groups were identified: HLA-heterozygous, with heterozygosity of patients in all classes of HLA, and homozygous, with homozygosity of at least 1 locus of the HLAI class. HLA-A and HLA-B were grouped into supertypes. There was no statistically significant difference in PFS between heterozygous and homozygous HLA patients [33].

In recent years, the role of non-classical molecules of the main histocompatibility complex has been considered, among which HLA-G attracts special attention. It is expressed on a number of cells, including tumor cells, and exhibits an immunosuppressive effect by interacting with inhibitory ILT2 and ILT4 receptors expressed on many cells of the immune system (NK, T, B, DC). These receptors bind to HLA-G 3-4 times more strongly than with classical MHCI, which indicates the leading role of this interaction in regulating the activity of T cells and antigen-presenting cells. In addition, ILT2 and ILT4 receptors compete with CD8+ lymphocytes for binding to MHCI, which results in inhibition of their cytotoxicity [34]. Such features of the tumor microenvironment can affect the IT effect.

Predictive multi-ohmic model

Given the complexity of the interaction between the immune system and the tumor, it is likely that one biomarker will not be enough to determine the

treatment tactics, so it may be necessary to use a combination of biomarkers. It was found that a trivariant multiomic model consisting of TMV, estimatedCD8+T-cell abundance (eCD8T) and fraction ofhigh PD-1 messenger RNA (fPD1) improves the ability to predict the response to IT immunotherapy in various types of malignant tumors [35]. TMB and the level of objective response to anti-PD-1/PD-L1 therapy have a high and statistically significant correlation, eCD8T is also characterized by a strong positive correlation with the level of objective response. Most types of malignant tumors with a higher objective response rate than predicted by the TMB regression model have higher levels of ECD8T and vice versa. The integration of TMB and ECD8T models significantly improved the prediction of the response, demonstrating a significantly improved likelihood function compared to one-dimensional models (p<0.001) [35]. The addition of FPD1 to the two-dimensional TMB – eCD8T model showed that the resulting three-dimensional regression model has significantly better prediction accuracy (p<0.02). Subtypes of malignant tumors with a higher level of responses than predicted by the bivariant prognostic model have higher levels of fPD1, and those with low levels of response are characterized by lower levels of fPD1 [35].

NSCLC Mutation Status

Tumors of patients with driver NSCLC give different responses to IT immunotherapy. For example, it is known that tumors carrying the EGFR gene mutation are characterized by feedback with PD-L1 expression, low TMV, absence of T-lymphocytic infiltration and a reduced ratio of PD-L1+ / CD8+tumor-infiltrating lymphocytes (p=0.034) [36]. In a promising phase 2 study of the effectiveness of pembrolizumab in patients with EGFR-mutant NSCLC, objective responses to IT therapy with activating EGFR mutations were not observed [37].

An international retrospective study of IMMUNOTARGET examined data from 551 patients with driver mutations, including KRAS, EGFR, ALK, ROS1, BRAF, RET, MET amplification or MET mutation in the 14exone and activating mutationher2. Anti–PD-1 antibodies were received by 94 % of patients and anti-PD-L1 antibodies by 6 %. Only 5 % of patients received IT in the first line of therapy and 40 % in the second line; the rest received immunotherapy as the third line and subsequent lines. The percentage expression of PD-L1 in driver mutations is as follows: HER2-0, EGFR – 3.5 %, ALK – 7.5 %, KRAS – 12.5 %, RET – 26 %, MET – 30 %, BRAF – 50 % and ROS1-90 %. The overall objective response, depending on the driver alteration, was: KRAS – 26 %, BRAF – 24 %, ROS1 – 17 %, MET – 16 %, EGFR – 12 %, HER2 – 7 %, RET – 6 % and ALK – 0 %. For patients with KRAS-mutant NSCLC, no difference in PFS between the subtypes of the KRAS mutation was established. However, PD-L1 positivity was statistically significantly correlated with a longer median PFS: 7.2 months versus 3.9 months (p=0.01). Patients with BRAF mutant and HER2-mutant NSCLC smokers had a longer PFS compared to those who had never smoked: 4.1 months versus 1.9 months (p=0.03) and 3.4 months versus 2.0 months (p=0.04), respectively. PD-L1-positive driver NSCLC with fusion and rearrangements: ALK, ROS1 and RET did not give any response to IT immunotherapy, amedian PFS in never-smokers was equal to 2.6 months. it turned out to be slightly longer compared to smokers-1.8 months (p=0.03) [38].

CONCLUSION

Currently, the considered biomarkers are being studied to determine the relationship of immunotherapy with long-term results. Thus, high PD-L1 expression, high TMB and intensive infiltration of the tumor by CD8+T-lymphocytes are associated with the clinical effectiveness of blocking immune control points. The expression of PD-L1, in turn, correlates with the severity of infiltration by T-lymphocytes and S. The study of the composition of 3 biomarkers suggests a high potential of the multicomic model for predicting the long-term results of treatment of patients receiving immunotherapy. Soluble PD-L1 and TMB in the blood are tested as biomarkers for the selection of candidates who are indicated for immunotherapy. It is even more necessary to identify biomarkers of acquired NSCLC resistance to IT blockade in order to identify patients who need treatment correction to achieve the best results.

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Lazutin Yu.N. – writing the text, processing the material.
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REVIEW

THE IMPORTANCE OF DEVELOPING NEW MANNAN TESTS IN THE DIAGNOSIS OF INVASIVE CANDIDIASIS IN ONCOLOGY PATIENTS

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ABSTRACT

The regimens of anticancer therapy have been intensified and methods of high-dose chemotherapy (HDCT) have been introduced for recent years which made it possible to achieve significant progress in the results of tumor treatments. Intensification of chemotherapy regimens in cancer patients leads to the emergence of risk factors of invasive candidiasis (IC) development: agranulocytosis, disruption of the integrity of the mucous membranes, prolonged use of CVC, repeated antibiotic therapy, long-term parenteral nutrition. Thus, intensification of anticancer therapy may be accompanied by an increase in infection-mediated mortality.

IC is the most common invasive mycosis in Russia. More than 11 thousand cases of IC occur in our country every year. The frequency IC in Russia is 8.29 per 100 thousand of the population, which corresponds to the results of the LIFE study in European countries where this indicator varies from 2.2 to 11 per 100 thousand of the population. There are no clinical signs or symptoms specific for IC. It develops in patients with concomitant diseases, which significantly complicates the diagnosis. In this regard, an urgent issue is to improve the diagnosis of candidal infectious complications in cancer patients in order to optimize treatment by studying serological markers that have the greatest value in the diagnosis of infectious complications in cancer patients.

Keywords:
invasive, candidiasis, candidemia, oncology, *Candida spp*, mannan.

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ОБЗОР

ЗНАЧЕНИЕ РАЗРАБОТКИ НОВЫХ МАННАНОВЫХ ТЕСТОВ В ДИАГНОСТИКЕ ИНВАЗИВНОГО КАНДИДОЗА У ОНКОЛОГИЧЕСКИХ БОЛЬНЫХ

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РЕЗЮМЕ

За последние годы были интенсифицированы режимы противоопухолевой терапии и внедрены методы высокодозной химиотерапии (ВДХТ), что позволило достичь значимого прогресса в результатах лечения опухолевых процессов. Интенсификация режимов химиотерапии у онкологических больных приводит к возникновению факторов рисков развития инвазивного кандидоза (ИК): агранулоцитозу, нарушению целостности слизистых оболочек, длительному применению центральных венозных катетеров (ЦВК), повторной антибактериальной терапии, длительному парентеральному питанию. Таким образом, усиление противоопухолевой терапии может сопровождаться повышением инфекционно-опосредованной летальности.
Инвазивный кандидоз – самый распространенный микоз в России. Ежегодно в нашей стране возникает более 11 тысяч случаев ИК. Согласно данным многоцентровых исследований, частота ИК в России составляет 8,29 на 100 тысяч населения. В странах Европы, данный показатель варьируется от 2,2 до 11 на 100 тысяч населения. Не существует клинических признаков или симптомов, специфичных для инвазивного кандидоза который, как правило, развивается у пациентов на фоне сопутствующих заболеваний, что существенно затрудняет диагностику. В связи с этим актуальной задачей является улучшить диагностику кандидозных инфекционных осложнений у больных онкологического профиля для оптимизации лечения за счет исследования серологических маркеров, имеющих наибольшую диагностическую значимость в возникновении инфекционных осложнений у онкологических больных.

Ключевые слова:
инвазивный, кандидоз, кандидемия, онкология, *Candida spp*., маннан.

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RELEVANCE

In recent years, the regimens of antitumor therapy have been intensified and methods of high-dose chemotherapy (HDCT) have been introduced, which has made it possible to achieve significant progress in the results of treatment of tumor processes [1]. The intensification of chemotherapy regimens in cancer patients leads to the emergence of risk factors for the development of invasive candidiasis: agranulocytosis, violation of the integrity of the mucous membranes, prolonged use of central venous catheters (CVC), repeated antibacterial therapy, long-term parenteral nutrition. Thus, intensification of anticancer therapy may be accompanied by an increase in infection-mediated mortality [1-2].

Invasive candidiasis is the most common mycosis in Russia. Every year, more than 11 thousand cases of IC occur in our country [3]. According to the data of multicenter studies, the frequency of IR in Russia is 8.29 per 100 thousand of the population. In European countries, this indicator varies from 2.2 to 11 per 100 thousand population [4]. The most common variants of invasive candidiasis are candidemia, acute disseminated candidiasis and candidal peritonitis, other forms are somewhat less common [5].

According to the expert group of the Russian Association of Perinatal Medicine Specialists (RAPMS), the frequency of IC in newborns in the structure of infectious and inflammatory diseases is from 15 to 30 %. The incidence of IC in newborns is inversely proportional to the gestation period and body weight at birth and ranges from 2.6 % to 3.1 % in newborns with very low body weight and from 10 % to 16 % in newborns with extremely low body weight [6].

Candida spp. they are pathogens in 9-22 % of cases of all nosocomial infections. The frequency of invasive candidiasis in patients in intensive care units (ICU) varies from 0.3 to 10 %, depending on the profile of the departments. The mortality rate for invasive candidiasis in patients in the ICU is 10-47 % [7-9].

In 2020, due to the spread of the new coronavirus infection SARS-CoV-2, reports of cases of COVID-19-associated invasive candidiasis (COVID – 19 associated candidiasis-CAC) began to appear and systematize. To date, the development of acute respiratory distress syndrome with a concomitant risk of developing superinfection and stay in the ICU are identified as the main risk factors for the development of CAC [10].

IC usually develops in patients with concomitant diseases, which significantly complicates the diagnosis. The main risk factors for the development of IC are:

- surgical abdominal interventions, in particular, accompanied by the failure of anastamoses and repeated laparotomies;
- perforation of the gastrointestinal tract;
- staying in the ICU;
- chemo-and radiotherapy in cancer patients;
- oncohematological diseases;
- multiple and long-term colonization of *Candida spp*. (colonization index >0.5 or adjusted colonization index >0.4);
- the presence of a central venous catheter;
- complete parenteral nutrition;
- the use of broad-spectrum antibacterial agents;
- artificial ventilation of the lungs;
- infected pancreatic necrosis;
- hemo – and peritoneal dialysis;
- organ and tissue transplantation;
- the state of prematurity of children with very low and extremely low body weight;
- stay in burn units;
- diabetes mellitus;
- HIV infection;
- immunodeficiency conditions, including those caused by immunosuppressive therapy [6, 7, 11].

There are no clinical signs or symptoms specific to invasive candidiasis. Invasive candidiasis should be suspected in patients with known risk factors with fever of unknown origin that cannot be treated with antibacterial agents [12].

Thus, a large contingent of patients at risk of IC, a change in the structure of pathogens of nosocomial infections, accompanied by an increasing role of fungal pathogens, an increase in resistance to antimycotic drugs, bring the problem of diagnosing invasive mycoses to a new level. Timely diagnosis of IR is the key to ensuring a favorable outcome. In fact, 1-2 days of delay in starting effective antifungal therapy doubles the risk of death from IC [13, 14].

Microbiological methods and blood culture testing remain the gold standard for the diagnosis of inva-

sive candidiasis, but the difficulties of cultivating *Candida spp*. in the study of hemoculture, as well as a long growth time, this method is not sufficiently reliable [12]. Thus, a positive result of hemoculture is observed only in 21-71 % of patients with invasive candidiasis confirmed at autopsy, depending on the frequency of sampling and the volume of blood taken [15].

The development of additional molecular and serological methods for timely and accurate diagnosis of IR is becoming increasingly relevant.

One of the first commercial ELISA test systems were developed that allow detecting the mannan antigen – the main component of the *Candida spp*. cell wall and antibodies to mannan, PLATELIA™ Candida Ag PLUS and PLATELIA™ Candida Ab PLUS (Bio-Rad Laboratories, Marnes-la-Coquette, France). Under the auspices of the Third European Conference on Infectious Diseases in Leukemia, the characteristics of these test systems were analyzed based on the results of published studies [16, 17]. The diagnosis of IC in this study was established in accordance with the 2008 recommendations of the European Organization for Research and Treatment of Cancer / Mycoses Study Group. Sensitivity and specificity were calculated for the separate determination of mannan antigen, mannan antibodies and for combined testing. Data from 14 studies were analyzed (all studies, with the exception of one, were retrospective). The total number was 453 patients and 767 control cases. In the studies, the sample was represented by patients of oncological and oncohematological departments, departments of surgery and intensive care. The sensitivity of the determination of the mannan antigen was 58 % (95 % confidence interval [CI], 53-62); the specificity was 93 % (95 % CI, 91-94). When determining mannan antibodies, the sensitivity was 59 % (95 % CI, 54-65); the specificity was 83 % (95 % CI, 79-97). In the combined determination of mannan and antibodies to it, the sensitivity was 83 % (95 % CI, 79-87), the specificity was 86 % (95 % CI, 82-90). A significant heterogeneity of sensitivity was noted in the determination of both mannan and mannan antibodies for different *Candida* species. The highest was found for *C. albicans*, followed by *C. glabrata* and *C. tropicalis* [18].

However, in a 2016 study, including among patients in the ICU with severe abdominal pathology, a combined determination of mannan antigen and antibodies to the mannan antigen *Candida spp*. it turned out to be ineffective (sensitivity 55 % and specificity 60 %). Antibodies are often present in immunocompromised patients with previous candidemia or colonization [19].

Thus, the prognostic value of detecting antibodies remains low with a single test and the absence of subsequent detection of their increasing concentration. This observation and the unexplained variability of tests in various studies are an important warning for doctors, since the unreliability of the results of laboratory diagnostics can lead to unjustified prescribing of antifungal drugs to patients whose candidiasis is unlikely [20, 21].

According to the updated 2016 Guidelines for the Clinical Practice of IC Management of the American Society of Infectious Diseases (Infectious Diseases Society of America), the role of existing tests for the determination of *Candida spp*. mannan and mannan antibodies remains unclear. These tests are not approved by the US FDA and are available mainly in Europe, where their use is allowed [22]. A number of domestic clinical recommendations suggest the possibility of using tests to determine mannan and antibodies to mannan [6, 7, 23].

CONCLUSION

The development of additional molecular and serological methods for timely and accurate diagnosis is becoming increasingly relevant. In this situation, it is necessary to search for biomarkers that would be an objective and reliable opportunity for a clinician to quickly respond to the possible development of a severe infectious complication [24-25]. The study of the structure of carbohydrate antigens of fungal pathogens is a key link in the successful development of more valid diagnostic tests. The development of additional molecular and serological methods for timely and accurate diagnosis is becoming increasingly relevant. The use of tests for the determination of *Candida spp*. mannan and antibodies to mannan will help to improve diagnosis and clarify the etiological factor of life-threatening infectious complications.

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CLINICAL CASE REPORTS

CHANGES IN THE LEVEL OF CARDIOMARKERS IN THE DEVELOPMENT OF ACUTE MYOCARDIAL INFARCTION ON THE BACKGROUND OF CHEMOTHERAPY OF A PATIENT WITH TONGUE CANCER

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ABSTRACT

Cancer is one of the leading causes of death and disability worldwide. Timely diagnosis and the introduction of new effective treatments, including intensive radiation and chemotherapy regimens, have significantly improved survival forecasts in recent years. At the same time, the use of these types of treatment increases the risk of complications, one of which includes chemotoxic cardiopathies. In this regard, timely detection and treatment of complications from the cardiovascular system in patients receiving chemotherapy courses in combination with surgical methods of treatment is important. This paper presents an assessment of the significance of the use of cardiomarkers in the early diagnosis of acute myocardial infarction that developed during chemotherapy in a patient with tongue cancer with a complicated cardiac history. Patient M., 45 years old, was admitted for surgical treatment for cancer of the tongue St. IVA, T4aN1M0, cl. gr. 2. Planned laboratory and instrumental studies were performed. Contraindications for surgical treatment were not identified. A preoperative course of chemotherapy was performed, against the background of which the patient's condition worsened with symptoms of acute cardiopathy. A second ECG was urgently performed, as a result of which an increase in the ST segment in III, aVF was established, as well as a study of the concentration of cardiomarkers: highly sensitive troponin I, N-terminal propeptide of natriuretic hormone, creatine phosphokinase MB, myoglobin, the dynamics of changes in the level of which indicated the development of acute coronary syndrome. The complex application of diagnostic procedures, including the determination of the level of cardiomarkers, made it possible to timely diagnose the development of acute type 1 myocardial infarction in a patient with tongue cancer on the background of chemotherapy. When analyzing the entire array of clinical and laboratory data, the leading initiating factor that played a decisive role in the development of myocardial infarction in this case was, in our opinion, a preoperative course of polychemotherapy with paclitaxel and carboplatin, which have cardiotoxicity. Thus, the category of patients with an initial unfavorable background, due to a common malignant process and the presence of a history of cardiodisfunction, requires more careful preparation for preoperative courses of polychemotherapy, including cardiotropic therapy with mandatory monitoring of the level of the main cardiomarkers. The most significant changes were in the levels of creatine phosphokinase MB, troponin I, and myoglobin, which were recorded in the first hours of myocardial infarction. An association was found between an increase in troponin I concentration and an increase in the ST segment of the electrocardiogram.

Keywords:
myocardial infarction, cardiomarkers, creatine phosphokinase MB, myoglobin, troponin I, natriuritic propeptide.

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КЛИНИЧЕСКОЕ НАБЛЮДЕНИЕ

ИЗМЕНЕНИЕ УРОВНЯ КАРДИОМАРКЕРОВ ПРИ РАЗВИТИИ ОСТРОГО ИНФАРКТА МИОКАРДА НА ФОНЕ ХИМИОТЕРАПИИ БОЛЬНОГО РАКОМ ЯЗЫКА

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РЕЗЮМЕ

Онкологические заболевания являются одной из основных причин смертности и инвалидизации во всем мире. Своевременная диагностика и внедрение новых эффективных методов лечения, включающих интенсивные схемы химиотерапии, значительно улучшили прогнозы выживаемости. Вместе с тем, применение химиотерапии увеличивает риск осложнений, к одним из которых относят химиотоксические кардиопатии. В этой связи актуально своевременное выявление и лечение осложнений со стороны сердечно-сосудистой системы у пациентов, получающих курсы химиотерапии в комплексе с хирургическими методами лечения. В данной работе представлена оценка информативности изменений уровня отдельных кардиомаркеров при развитии острого инфаркта миокарда на фоне химиотерапии больного раком языка с осложненным кардиологическим анамнезом. Пациент М., 45 лет поступил на оперативное лечение по поводу рака языка St. IVA, T4aN1M0, кл. гр.2. Выполнены плановые лабораторные и инструментальные исследования. Противопоказаний для проведения хирургического лечения не выявлено. Проведен предоперационный курс химиотерапии, на фоне которого у пациента отмечено ухудшение состояния с симптомами развития острой кардиопатии. В срочном порядке выполнены повторная ЭКГ, в результате которой установлен подъем сегмента ST в III, aVF, а также исследование концентрации кардиомаркеров: высокочувствительного тропонина I, N-концевого пропептида натрийуретического гормона, креатинфосфокиназы МВ, миоглобина, динамика изменения уровня которых указывала на развитие острого коронарного синдрома. Комплексное применение диагностических процедур, в числе которых немаловажное значение имело определение уровня кардиомаркеров, позволило своевременно диагностировать развитие острого инфаркта миокарда 1 типа на фоне проведения предоперационного курса химиотерапии у больного раком языка. При анализе всего массива клинико-лабораторных данных ведущим инициирующим фактором, сыгравшим решающую роль в развитии инфаркта миокарда в данном случае, явился, на наш взгляд, предоперационный курс полихимиотерапии паклитакселом и карбоплатином, обладающими кардиотоксичностью. Таким образом, категория больных с исходным неблагоприятным фоном, обусловленным распространенным злокачественным процессом и наличием в анамнезе кардиодисфункции, требует более тщательной подготовки к проведению предоперационных курсов полихимиотерапии, включающей кардиотропную терапию с обязательным мониторированием уровня основных кардиомаркеров. Наиболее показательными были изменения уровня тропонина I, креатинфосфокиназы МВ, и миоглобина, которые регистрировались в первые часы развития инфаркта миокарда.

Ключевые слова:
инфаркт миокарда, кардиомаркеры, тропонин I, креатинфосфокиназа МВ, миоглобин, натрийуретический пропептид.

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RELEVANCE

Oncological diseases (OD) are one of the main causes of mortality and disability worldwide [1, 2]. However, in recent years, mortality statistics have begun to improve due to timely diagnosis and the introduction of new effective treatment methods that have significantly improved survival forecasts. This includes intensive radiation and chemotherapy regimens. However, the use of these types of treatment increases the risk of complications, one of which includes chemotoxic cardiopathies, including myocardial dysfunction, heart failure, hypertension, vasospastic and thromboembolic ischemia, arrhythmias of various types and sudden cardiac death [3, 4]. In this regard, timely detection and treatment of complications from the cardiovascular system (CCC) in patients receiving chemotherapy courses in combination with surgical methods of treatment is important. Currently, the diagnosis of CCC disorders includes a number of studies: instrumental-electrocardiographic (ECG), ultrasound (EchoCG), cardiometric (CM), X-ray, magnetic resonance imaging, positron emission tomography, cardiac catheterization and laboratory-hematological, biochemical, coagulological, immunochemical methods [5]. A special place for early detection of cardiovascular disorders in oncological hospitals and intensive care units is occupied by determining the level of cardiac biomarkers, such as highly sensitive troponin I, N-terminal propeptide of natriuretic hormone, creatine phosphokinase MB, myoglobin [6].

The aim of the study was to assess the informative value of changes in the level of individual cardiomarkers in the development of acute myocardial infarction against the background of chemotherapy in a patient with tongue cancer with a complicated cardiological history.

Clinical Case Report

Patient M., at the age of 45 years, was admitted to the Department of Head and neck tumors of the Ministry of Health of the Russian Federation at the Department of Head and Neck tumors on 17.02.2021 for surgical treatment for tongue cancer, St. IVA, T4aN1M0, cl. gr. 2. From the anamnesis: suffers from coronary heart disease: angina pectoris, II FC; hypertension II art. I did not receive treatment. Planned laboratory and instrumental studies were carried out. There are no contraindications for surgical treatment and chemotherapy.

On 18.02.2021, the patient was consulted by a chemotherapist: a preoperative course of polychemotherapy (PCT) was recommended according to the scheme: carboplatin AUC5 655 mg, paclitaxel 350 mg intravenously against the background of pre-and postmedication with dexamethasone-8 mg intravenously, which was performed from 18.02.2021 to 24.02.2021. The administration of the drugs was carried out satisfactorily, he did not complain.

On 24.02.2021, at 9:15, a planned ECG was performed in preparation for surgical treatment: sinus rhythm, normasystole with a heart rate of 61 beats/min, there are no rhythm disturbances, left ventricular myocardial hypertrophy with signs of systolic overload.

At 16:00, a deterioration of the condition was noted, the patient complained of pressing pain behind

Table 1. Results of cardiomarkers level measurement in patient M. in dynamics

Studied indicators/units of measurement	Research stages		Referent values
	I	II	
Troponin I, ng/ml	0.0	0.01	<0.03
N-terminal propeptide of natriuretic hormone, pg/ml	42.3	43.1	15–128.3
Myoglobin, ng/ml	30.5	47.4	8.95–48.8
Creatine Phosphokinase MB, ng/ml	9.77	11.2	2.0–4.99

the sternum, weakness, sticky sweat. The patient underwent a repeated ECG. The ST segment lift is set to III, aVF. Due to the deterioration of the patient's condition, he was urgently transferred to the department of anesthesiology and intensive care. Studies of the level of cardiomarkers were urgently performed: highly sensitive troponin I (Abbott i-STAT critical condition analyzer, USA), N-terminal propeptide of natriuretic hormone, creatine phosphokinase MB, myoglobin (PATHFAST immunochemiluminescent analyzer, Japan). The studies were conducted twice with an interval of 40 minutes. The results of the study of the level of cardiomarkers are presented in Table 1.

In the first study, 2 hours after the patient's complaints, an increase in the level of creatine phosphokinase MB by 2 times in comparison with the upper limit of the reference interval was noticed. However, there were no changes in the level of other cardiomarkers – troponin I, N-terminal propeptide of natriuretic hormone and myoglobin at this stage.

The results of the second study showed a tendency to increase the values of the analyzed group of indicators, with the exception of the N-terminal propeptide of natriuretic hormone. Thus, there was an increase in the level of troponin I within the reference interval, the concentration of creatine phosphokinase MB by 2.2 times in comparison with the upper limit of the reference interval and by 1.2 times with the result of the I-th study. The level of myoglobin increased within the reference values, similar to troponin I, and was 1.6 times higher than the data of the first study. An increase in the concentration of myoglobin at this stage of observation of the patient may be due to the expansion of the area of ischemic myocardial lesions. In general, the dynamics of changes in the levels of the studied markers indicated the development of acute coronary syndrome.

The obtained laboratory data corresponded to the patient's clinical condition, the indications of an ECG performed after the patient complained, and the results of cardiological monitoring conducted in the intensive care unit.

The ECG revealed negative dynamics in comparison with the initial study: sinus bradycardia with a heart rate of 55 beats / min. ST segment elevation in leads II, III st and aVF, pronounced depression of ST segment I, aVL, V1 – V6 – (discordant myocardial changes). Conclusion: acute myocardial injury of the posterior-lower parts of the left ventricle (Fig. 1).

According to the results of cardiological monitoring carried out in the department of anesthesiology

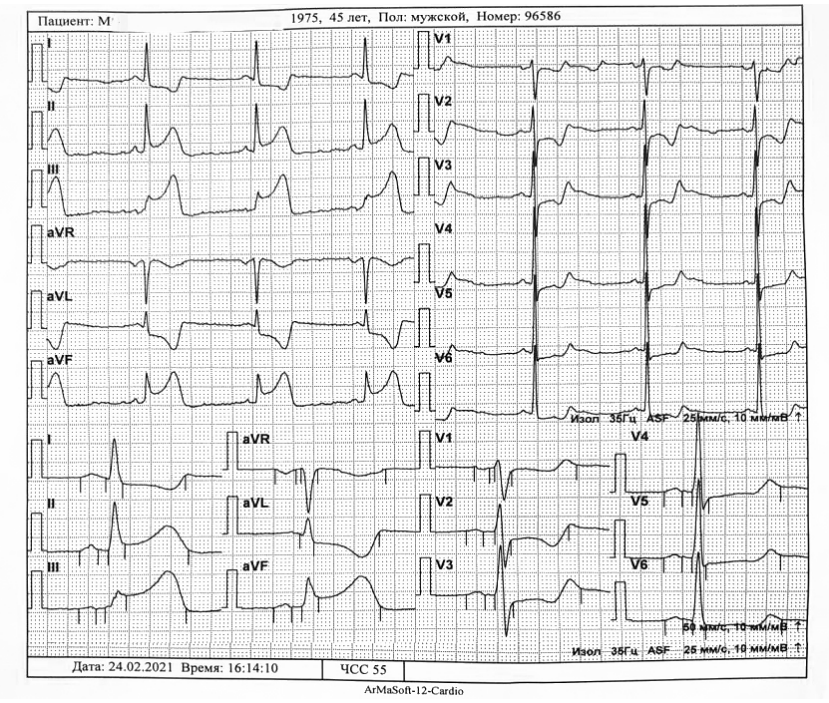

Fig. 1. ECG of patient M. with a significant ST segment elevation on leads II, III and aVF.

and intensive care, there was a preservation of ST wave rises in leads II, III st and aVF, sinus bradycardia with a heart rate of 54 beats/min, which indicated an increase in ischemic injuries. Increases in the ST segment of the teeth were associated with changes in the level of the analyzed cardiomarkers, mainly creatine phosphokinase MB, troponin I and myoglobin, which made it possible to establish the diagnosis of acute myocardial infarction type 1 in the shortest possible time. A complex of therapeutic measures was performed that led to an improvement in the patient's condition: relief of pain syndrome, stabilization of hemodynamic parameters, which made it possible to urgently transfer the patient for further treatment and percutaneous surgery to the cardiology department of the Emergency Hospital. After the treatment and rehabilitation, the patient continued treatment of the underlying disease at the FSBI "NMRC Oncology" of the Ministry of Health of the Russian Federation.

DISCUSSION

According to the Russian clinical guidelines for the diagnosis and treatment of acute myocardial infarction with ST-segment elevation of the electrocardiogram (MOH of the Russian Federation 2020), the primary treatment strategy is based on the clinical picture and ECG. For the final confirmation of the diagnosis, it is necessary to determine cardiomarkers. There are cases of a connection between changes in the ST segment of the electrocardiogram and an increase in the concentration of troponin I in patients with acute myocardial infarction and the possibility of assessing the volume of myocardial necrosis by the degree of changes in the level of troponin I [7]. Nevertheless, despite the high specificity, troponins are "late" markers of myocardial necrosis, the peak of growth is recorded after 6-8 hours from the onset of the disease, reaching a maximum by 24 hours [8]. It is also known that it is extremely difficult to measure the concentration of troponin in the blood, since it is normally extremely low [9]. Therefore, the recorded increase in the patient's troponin I level, which does not go beyond the reference boundaries, is significant, in our opinion, since according to the available data, even the smallest increase in their level in the blood is dangerous and may indicate a myocardial infarction [10].

According to International recommendations, studies of an early cardiomarker, creatine phosphokinase MB, are performed in addition to troponin I for patients admitted within 6 hours from the onset of pain syndrome. An increase in the level of creatine phosphokinase MB in the blood is specific for myocardial damage [11]. In our observation, an increase in the level of the indicator in the patient's blood was noted almost from the moment of filing complaints.

The informative value of the use of myoglobin is shown to exclude the diagnosis of acute myocardial infarction [12]. An increase in myoglobin is noted during the first 3.5 hours from the onset of the attack with a maximum of values by 6-12 hours and a return to the initial level within 24-36 hours [13], which explains the absence of changes in the patient's myoglobin level in the first study. However, after 40 minutes, an increase in the indicator was noted in comparison with the initial value, which confirms the expediency of using myoglobin in this case.

Thus, the ECG data, along with the study of the level of creatine kinase MB, myoglobin, troponin I, made it possible to establish the development of acute type 1 myocardial infarction in the patient in the shortest possible time. At the same time, there is evidence that the N-terminal propeptide of natriuretic hormone, not being a direct indicator of necrosis, characterizes the functional capabilities of the myocardium [14], and is also effective in assessing the development of chronic heart failure in cancer patients on the background of chemotherapy [15] and as a prognostic factor for adverse outcomes [16]. The absence of changes in the indicator in the presented case does not contradict these data, but requires additional observations.

When analyzing the entire array of clinical and laboratory data, the question was also raised about the factors that played a decisive role in the development of myocardial infarction. One of the reasons can be attributed to the initial unfavorable background caused by a widespread malignant process and accompanied by cancer intoxication. However, this is the case in most cancer patients. In our opinion, the leading initiating factor was the preoperative course of polychemotherapy with paclitaxel and carboplatin, which have cardiotoxicity, the main mechanism of which is acute vasospasm leading to ischemic

complications [17]. At the same time, the presence of the patient's initial cardiodisfunction, namely, coronary heart disease, stable angina pectoris, arterial hypertension, could certainly serve as an aggravating factor.

CONCLUSION

The complex application of diagnostic procedures, including the determination of the level of cardiomarkers, made it possible to timely diagnose the development of acute myocardial infarction of type 1 in a patient with tongue cancer on the background of chemotherapy. Informative, along with troponin I, were changes in the level of creatine phosphokinase MB and myoglobin, which were recorded in the first hours of the development of myocardial infarction. The high sensitivity of the cardiovascular system to drug therapy due to tumor progression requires more thorough preparation of patients for preoperative courses of polychemotherapy, including cardiotropic therapy with mandatory monitoring of the level of the main cardiomarkers, especially in persons with a burdened cardiological history.

Authors contribution:

Guskova N.K. – the concept and design of the study, systematization and analysis of the data obtained, writing the text of the manuscript, scientific editing.

Vladimirova L.Yu. – analysis of the received data, scientific editing.

Sycheva E.A. – analysis of the received data, consultation, writing the text of the manuscript.

Morozova A.A. – collection of clinical material, systematization and analysis of the data obtained, review of publications on the topic of the article, writing the text of the manuscript.

Rosenko D.A. – analysis of the received data, consultation.

Donskaya A.K. – analysis of the received data, writing the text of the manuscript, consultation.

Selyutina O.N. – processing of the material, review of publications on the topic of the article, writing the text, technical editing, design of the bibliography.

Skopintsev A.M. – collection of clinical data, consultation.

Golomeeva N.V. – collection of clinical data.

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