



ISSN: 2686-9039 Online

PEER-REVIEWED SCIENTIFIC AND PRACTICAL
**South Russian Journal
of Cancer**

РЕЦЕНЗИРУЕМЫЙ НАУЧНО-ПРАКТИЧЕСКИЙ
**Южно-Российский
онкологический журнал**

vol.
ТОМ **4** № **4/2023**

www.cancersp.com

PEER-REVIEWED SCIENTIFIC AND PRACTICAL JOURNAL

South Russian Journal of Cancer

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Founder and Publisher:

Autonomous Non-profit Organization "Perspectives of Oncology" (ANO "Perspectives of Oncology")

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Phone: +7 (903) 547-04-62, +7 (863) 295-53-62
www.cancersp.com
For correspondence: 111555, Moscow, PO box 3

The journal is registered at the Roskomnadzor on 28.10.2019, EL No. FS 77-80665 – online.
Frequency: 4 issues per year.

Published 09.12.2023.

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РЕЦЕНЗИРУЕМЫЙ НАУЧНО-ПРАКТИЧЕСКИЙ

Южно-Российский онкологический журнал

Журнал входит в рекомендованный ВАК РФ перечень рецензируемых научных журналов и изданий для опубликования основных научных результатов диссертаций на соискание учёной степени кандидата и доктора наук.

«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.

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Адрес редакции и издателя:

344037, Россия, Ростов-на-Дону, 14-я линия, д. 63,
литер Г, комната 1
E-mail: edition@cancersp.com, info@cancersp.com
Телефон: +7 (903) 547-04-62, +7 (863) 295-53-62
Сайт: www.cancersp.com
Для корреспонденции: 111555, Москва, а/я 3

Журнал зарегистрирован в Роскомнадзоре 28.10.2019 г.,
ЭЛ № ФС 77-80665 – сетевое издание.
Периодичность: 4 номера в год.

Опубликовано 09.12.2023.

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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AREA OF COMBINED SURGICAL INTERVENTIONS, INCLUDING THOSE WITH AN ANGIOSURGICAL COMPONENT, IN TERMS OF MALIGNANT NON-ORGAN RETROPERITONEAL TUMORS' TREATMENT

O. I. Kit¹, A. A. Maslov¹, E. N. Kolesnikov¹, O. V. Katelnitskaya¹, M. A. Kozhushko¹, A. V. Snezhko^{1,2}, T. B. Katsieva¹, R. E. Myagkov¹, S. V. Sanamyants¹, A. E. Anisimov¹, K. V. Kolomiets^{1✉}

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ABSTRACT

Purpose of the study. To analyze the immediate outcomes following surgical treatment of locally advanced malignant non-organ retroperitoneal tumors (NRT).

Materials and methods. Surgical interventions for malignant NRTs were performed in 114 patients at Abdominal Oncology Department No. 1, National Medical Research Centre for Oncology. There were 48 males and 66 females among them. According to the histological structure, liposarcoma was commonly detected in 64 (56.1 %) cases.

Results. Intraoperative revision revealed the spread of the tumor to adjacent anatomical structures in 67 (58.8 %) cases. Resection surgical interventions were performed in 109 patients, of which 106 (97.2 %) patients had operations performed in an amount of R0. Combined surgical interventions for NRTs were performed in 62 (54.4 %) patients, and multiorgan happened to be in 45 (72.6 %) patients out of those. Resection of the inferior vena cava was performed in 12 patients. Resection of the superior mesenteric vein was performed in 2 patients and the iliac-colonic vein in 2 patients. In 1 observation the left renal vein was resected with suturing of the lateral defect of the vessel wall. Complications during surgery and in the early postoperative period were noted in 14 (12.3 %) patients. In total, 2 patients died after operations, the mortality rate was 1.8 %.

Conclusions. Tumor invasion of big main blood vessels is not a contraindication for surgical treatment of locally spread malignant non-organ retroperitoneal tumors.

Keywords: locally spread tumors, retroperitoneal tumors, non-organ tumors, liposarcomas, retroperitoneal space, multiorgan resections, combined operations, prosthetics, angiosurgical interventions, resection, marginal resection, circular resection, complications, mortality, survival rates, vascular invasion, bleeding

For citation: Kit O. I., Maslov A. A., Kolesnikov E. N., Katelnitskaya O. V., Kozhushko M. A., Snezhko A. V., Katsieva T. B., Myagkov R. E., Sanamyants S. V., Anisimov A. E., Kolomiets K. V. Area of combined surgical interventions, including those with an angiosurgical component, in terms of malignant non-organ retroperitoneal tumors' treatment. South Russian Journal of Cancer. 2023; 4(4): 6-12. <https://doi.org/10.37748/2686-9039-2023-4-4-1>, <https://elibrary.ru/apgihl>

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Compliance with ethical standards: the research was carried out according to the ethical principles, set forth by World Medical Association Declaration of Helsinki, 1964, ed. 2013. The study was approved by the Biomedical Ethics Committee at the National Medical Research Center for Oncology (extract from the protocol of the meeting No. 22 dated 07/05/2023. Informed consent was obtained from all participants of the study.

Funding: this work was not funded.

Conflict of interest: the authors declare that there are no obvious and potential conflicts of interest associated with the publication of this article.

The article was submitted 25.10.2022; approved after reviewing 11.10.2023; accepted for publication 09.12.2023.

© Kit O. I., Maslov A. A., Kolesnikov E. N., Katelnitskaya O. V., Kozhushko M. A., Snezhko A. V., Katsieva T. B., Myagkov R. E., Sanamyants S. V., 2023

МЕСТО КОМБИНИРОВАННЫХ ХИРУРГИЧЕСКИХ ВМЕШАТЕЛЬСТВ, В ТОМ ЧИСЛЕ С АНГИОХИРУРГИЧЕСКИМ КОМПОНЕНТОМ, В ЛЕЧЕНИИ ЗЛОКАЧЕСТВЕННЫХ НЕОРГАНЫХ ЗАБРЮШИННЫХ ОПУХОЛЕЙ

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РЕЗЮМЕ

Цель исследования. Анализ непосредственных результатов хирургического лечения злокачественных неорганных забрюшинных опухолей (НЗО).

Материалы и методы. В отделении абдоминальной онкологии № 1 ФГБУ «НМИЦ онкологии» Минздрава России хирургические операции по поводу злокачественных НЗО выполнены у 114 больных. Среди них мужчин было 48, женщин – 66. По гистологической структуре наиболее часто в 64 (56,1 %) наблюдениях выявлена липосаркома.

Результаты. Интраоперационная ревизия выявила распространение опухоли на смежные анатомические структуры в 67 (58,8 %) наблюдениях. Резекционные хирургические вмешательства удалось выполнить 109 пациентам, из них у 106 (97,2 %) больных операции были выполнены в объёме R0. Комбинированные хирургические вмешательства по поводу НЗО выполнены у 62 (54,4 %) больных, из них у 45 (72,6 %) пациентов они были мультивисцеральными. Резекция нижней полой вены выполнена у 12 пациентов. У 2 пациентов выполнена резекция верхней брыжеечной вены и у 2 больных – подвздошно-толстокишечной вены. В 1 наблюдении резецирована левая почечная вена с уширением бокового дефекта стенки сосуда. Осложнения во время операции и в раннем послеоперационном периоде отмечены у 14 (12,3 %) больных. Всего после операций умерли 2 больных, летальность составила 1,8 %.

Заключение. Опухолевая инвазия крупных магистральных кровеносных сосудов не является противопоказанием для хирургического лечения местно-распространенных злокачественных неорганных забрюшинных опухолей.

Ключевые слова: местно-распространенные опухоли, забрюшинные опухоли, неорганные опухоли, липосаркомы, забрюшинное пространство, мультивисцеральные резекции, комбинированные операции, протезирование, ангиохирургические вмешательства, резекция, краевая резекция, циркулярная резекция, осложнения, летальность, выживаемость, сосудистая инвазия, кровотечение

Для цитирования: Кит О. И., Маслов А. А., Колесников Е. Н., Кательнищкая О. В., Кожушко М. А., Снежко А. В., Кацьева Т. Б., Мягков Р. Е., Санамянц С. В., Анисимов А. Е., Коломиец К. В. Место комбинированных хирургических вмешательств, в том числе с ангиохирургическим компонентом, в лечении злокачественных неорганных забрюшинных опухолей. Южно-Российский онкологический журнал. 2023; 4(4): 6-12. <https://doi.org/10.37748/2686-9039-2023-4-4-1>, <https://elibrary.ru/apgihl>

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Соблюдение этических стандартов: в работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). Исследование одобрено Комитетом по биомедицинской этике при ФГБУ «НМИЦ онкологии» Минздрава России (выписка из протокола заседания № 22 от 05.07.2023 г.). Информированное согласие получено от всех участников исследования.

Финансирование: финансирование данной работы не проводилось.

Конфликт интересов: все авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Статья поступила в редакцию 25.10.2022; одобрена после рецензирования 11.10.2023; принята к публикации 09.12.2023.

INTRODUCTION

Non-organ retroperitoneal tumors (NRTs) are a combined group of neoplasms without organ affiliation, localized in the retroperitoneal space proper, between the peritoneal leaves of the intestinal mesentery or peritoneal in the pelvic cavity. The histogenetic source of NRTs can be fatty, connective, vascular or nervous tissue, as well as embryonic elements located in the retroperitoneal space.

NRTs are extremely rare and account for 0.03 to 1 % of all tumor diseases. They are more common in young and middle-aged women. According to various researchers, up to 85 % of NRTs are malignant [1; 2]. There are no epidemiological data on the incidence of retroperitoneal non-organ tumors in Russia. The treatment of malignant non-organ retroperitoneal tumors remains one of the difficult and complex problems of modern clinical oncology, while due to the relatively low sensitivity of NRTs to radiation therapy and treatment with cytostatics [3], the surgical method is the main one.

Important characteristics of almost all morphological forms of NRTs are frequent recurrence and relatively rare metastasis. The frequency of recurrence is variable and depends on many factors, the most important of which are the morphological characteristics of the tumor and the degree of radicality of the surgical treatment. According to a number of authors, relapses after radical operations amount to 20–90 % [2; 4]. Operations for NRTs are technically complex, due to the prevalence of the tumor process, the deep location of neoplasms and the relatively frequent need for resection of neighboring organs [5]. A special place among malignant NRTs is occupied by locally advanced tumors with invasion into the main vessels due to the fact that performing a combined radical operation in the volume of R0 is technically possible only if angiosurgical intervention is performed [6–9].

MATERIALS AND METHODS

During the period from 2016 to 2021, surgical operations for malignant NRTs were performed in 114 patients in the Abdominal Oncology Department No. 1 of the thoracoabdominal Department of the Rostov National Medical Research Centre for Oncology.

Among them, there were 48 men and 66 women, the ratio of men and women was 1 to 1.4. The operated women were younger than men. The average age of men was 57.4 years (in the range from 21 to 82 years) and women 49.4 years (in the range from 18 to 79 years).

According to the histological structure of the studied postoperative material, there was a significant variety. As a result of morphological examination, liposarcoma was detected most often in 64 (56.1 %) cases, leiomyosarcoma was the second most common malignant NRTs in 13 (11.4 %) cases. Malignant schwannomas and gastrointestinal stromal tumors were somewhat less common (in 7 (6.1 %) and 6 (5.3 %)) accordingly (Table 1). Such tumors as rhabdomyosarcoma, angiosarcoma, fibrosarcoma, malignant paraganglioma, malignant lymphoma were relatively rare. Extremely rare cases such as extraosseous localization of osteogenic sarcoma and bladder cancer with a predominantly extraperitoneal solid component have also been identified. In 3 cases, the source of NRTs was metastases of malignant tumors of other localizations.

In the studied group of patients, a primary tumor was detected in 78 (68.4 %) cases, and in 36 (31.6 %) cases, a recurrent one. Among patients with newly diagnosed NRTs, stage I was in 17 (21.8 %) patients, stage II and III in 28 (35.9 %) and 30 (38.5 %), respectively, and stage IV in 3 (3.8 %) patients (Fig. 1).

Table 1. Histological identity of NRTs

Histological identity	Percentage (%)
Liposarcoma	64 (56.1 %)
Leyomyosarcoma	13 (11.4 %)
Schwannoma	7 (6.1 %)
Gastrointestinal stromal tumors	6 (5.3 %)
Others	24 (21.1 %)

STUDY RESULTS AND DISCUSSION

Intraoperative revision revealed the spread of the tumor to adjacent anatomical structures in 67 (58.8 %) cases. Most commonly affected organs were kidneys, colon and its mesentery, pancreas, spleen, small intestine, as well as various musculo-skeletal structures (lumbar muscles, abdominal wall muscles, sacrum, diaphragm). Liver germination was observed in 2 cases.

Resection surgical interventions were performed in 109 patients, out of which 106 (97.2 %) patients had operations performed in the amount of R0. In 5 patients, the amount of the operation was diagnostic.

Combined surgical interventions were performed in locally advanced tumors of malignant NRTs, out of which 45 (72.6 %) patients had multiorgan. The most common components of combined surgical interventions were right-sided or left-sided nephrenalectomies, right-sided or left-sided hemicolectomy, diaphragm resection, lumbar muscle resection, splenic and pancreatic resection. Combined interventions were performed in 26 (82.3 %) cases out of 32 operations carried out for recurrent tumors.

Combined surgical operations for locally spread non-organ retroperitoneal tumors with resection of large venous blood vessels were performed in 25 patients. Angiosurgical interventions were generally a component of combined multivisceral resections and were performed in connection with direct tumor invasion of large blood vessels. X-ray endosurgical embolization of arterial blood vessels of the tumor

was applied 24–48 hours before surgery to reduce intraoperative blood loss in 4 patients with a massive tumor and the main type of arterial blood supply of the latter.

Resection of the inferior vena cava was performed in 12 patients, 8 of them were marginal and 4 circular. Resection of the external iliac vein was performed in 8 cases. Resection of the superior mesenteric vein was performed in 2 patients and the iliac-colonic vein in 2 patients. In 1 observation, the left renal vein was resected with suturing of the lateral defect of the vessel wall.

Prosthetics of the inferior vena cava was performed in all cases of its resection, in 8 patients with marginal resection, the defect of the vessel wall was replaced with a synthetic "patch", in 4 patients with circular resection, the defect was replaced with an adequate tubular prosthesis.

Prosthetics of the external iliac vein with a synthetic graft after its resection was required in 3 out of 8 cases, in 3 cases the defect in the vessel wall was sutured, in 2 cases, due to a well-defined network of collaterals, ligation was performed. In all 4 cases of resection of the superior mesenteric and iliac-colonic veins, their prosthetics were performed.

Significant complications (class II–V according to Clavien-Dindo) during surgery and in the early postoperative period were noted in 14 (12.3 %) patients. The most serious and frequent complication was bleeding, observed in 3 patients directly during extensive combined surgical intervention, and in 2 patients in the early postoperative period. The main cause of bleeding was damage to the pathologically

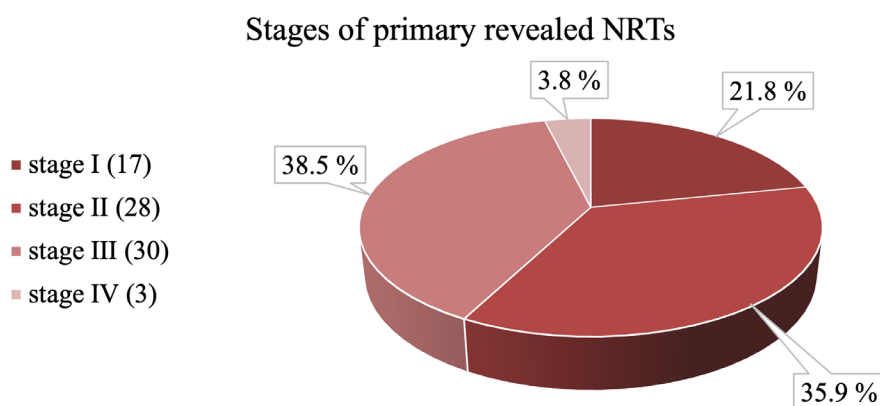


Fig. 1. Stages of primary revealed NRTs.

branched vascular network with insufficient visual control during the technically difficult mobilization of locally widespread NRTs. In 2 cases, intraoperative bleeding was successfully managed during surgery (Clavien-Dindo class IIIB), however, in one patient, massive simultaneous blood loss led to the development of hemorrhagic shock, DIC syndrome, multiple organ failure and death on the first day after surgery, which, according to the Clavien-Dindo classification, belongs to class V. Patients with bleeding in the postoperative period were successfully re-operated. 2 patients had perforation of the small intestine, 2 had adhesive intestinal obstruction. These patients underwent repeated laparotomies (Clavien-Dindo class IIA). In 2 cases, the development of acute postoperative pancreatitis was noted, which was stopped by conservative therapy (Clavien-Dindo class II).

One patient died 13 days after surgery from pulmonary embolism (Clavien-Dindo class V).

In the group of patients with combined surgical operations for locally spread NRTs with resection of large blood vessels, 2 patients had femoral vein thrombosis in the early postoperative period, which required thrombectomy (Clavien-Dindo class IIIB). There were no fatal complications following angiosurgical operations.

Overall, 2 patients died after operations, so the mortality rate was 1.8 %.

The results of treatment of patients with primary non-organ tumors of the retroperitoneal space remain unsatisfactory, and the prognosis is not encouraging. This is due to several circumstances. In particular, the complexity of diagnosis, a pronounced tendency to relapse in the next one and a half to two years, compression or germination into adjacent organs, including spread to vital structures, which leads to serious, sometimes fatal complications, the severity and traumatism of surgical interventions, low sensitivity of the most frequent morphological

variants malignant NRTs tumors to standard drug and/or radiation therapy. The growth of the tumor into large blood vessels significantly complicates surgical intervention and is a negative prognostic factor [10].

Currently, there are four views on the problem of surgical treatment of NRTs with invasion into the main blood vessels: refusal to attempt to remove the tumor, partial removal of NRTs with the tumor site remaining on the vascular wall, resection of a large vessel without restoring the main blood flow and removal of NRT with resection and plasty of large blood vessels and restoration of proper blood flow [1]. We believe that the option with resection and plastic surgery of large vessels should be a priority. The first 2 options can be used only in extremely rare cases due to defects in preoperative diagnosis, the possibility of choosing the third option is also rare.

It should be noted however, that the possible tumor germination of large veins in all cases was determined before surgery, which required the use of a wide range of instrumental diagnostic methods, including X-ray endovascular method. It is necessary to note the effectiveness of endovascular techniques, in particular embolization of tumor blood vessels to reduce intraoperative blood loss.

CONCLUSIONS

Performing surgical operations with locally spread malignant non-organ retroperitoneal tumors requires a proper preoperative examination, determining the possible invasion of large blood vessels, technical readiness to perform angiosurgical interventions, as well as appropriate material support. Tumor invasion of large main blood vessels is not a contraindication for surgical treatment, however, performing an intervention on large vessels of the retroperitoneal space should be recommended only if the operation is an oncological radical one.

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Snezhko A. V. – wrote the paper, performed processing of the material, operations, assisting in operations;

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ABOUT THE BLOOD CHARACTERISTICS AND ADAPTATION STATUS VARIABILITY IN INTACT BALB/C MICE OF DIFFERENT SEX

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ABSTRACT

Purpose of the study. Was to study the values and variability of traditionally determined blood parameters and index of the adaption status in intact mature Balb/c mice of different sex.

Materials and methods. Studies were carried out on 20 animals of mature age with spf-status, males (10) and females (10). Cytological and biochemical parameters of blood and weight characteristics of the organs of the immune system and adrenal glands were studied (considering the constitutional features of mice of this line). Statistica 10.0 software package was used for statistical analysis. The coefficient of variation (CV) was determined. The significance of statistical differences was assessed using the Mann-Whitney test.

Results. Sex differences were found in the ratio of albumins and globulins, the content of urea and alkaline phosphatase, the relative proportion of lymphocytes and monocytes in the blood count, the erythrocytes' count and the level of hemoglobin in erythrocytes. There were also indicators which had values of severe variability (CV up to 51 %), the number of those in males was higher than in females. The information content of the weight characteristics of the studied organs is reduced due to the constitutional features of Balb/c females. The identified sex differences indicated a higher adaptive status of female rodents compared to males and let to propose the prevalence of different activity of T-cell, B-cell and myeloid line in animals of different sexes.

Conclusion. The study of weak and moderate shifts in traditional laboratory parameters reflecting the adaptive status and characteristics of the blood of sexually mature males and females of the Balb/c line under physiological conditions made it possible to identify the sex characteristics of systemic regulatory processes that may be important for the body's resistance to malignant growth and the effectiveness of antitumor treatment. The current areas of further research are also indicated.

Keywords: individual and sex differences, adaptation status, general nonspecific adaptational reactions of the body, hematological parameters, immune processes

For citation: Zhukova G. V., Frantsiyants E. M., Shikhlyarova A. I., Kaplieva I. V., Trepitaki L. K., Galina A. V. About the blood characteristics and adaptation status variability in intact Balb/c mice of different sex. South Russian Journal of Cancer. 2023; 4(4): 13-22.
<https://doi.org/10.37748/2686-9039-2023-4-4-2>, <https://elibrary.ru/dqdkii>

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Compliance with ethical standards: work with animals was carried out in accordance with the rules of the «European Convention for the Protection of Animals Used for Experimental and other Scientific Purposes» (Directive 2010/63/EU), as well as in compliance with the «International Recommendations for Biomedical Research Using Animals» and Order of the Ministry of Health of Russia No. 267 of June 19, 2003 «On Approval of the Rules of Laboratory Practice». The study was approved by the Ethics Committee of the National Medical Research Centre for Oncology (Protocol No. 11/115 of 03/01/2021).

Funding: this work was not funded.

Conflict of interest: the authors declare that there are no obvious and potential conflicts of interest associated with the publication of this article.

The article was submitted 14.02.2023; approved after reviewing 11.10.2023; accepted for publication 09.12.2023.

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О ВАРИАБЕЛЬНОСТИ ПОКАЗАТЕЛЕЙ КРОВИ И АДАПТАЦИОННОГО СТАТУСА ИНТАКТНЫХ МЫШЕЙ ЛИНИИ BALB/C РАЗНОГО ПОЛА

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РЕЗЮМЕ

Цель исследования. Изучение величины и вариабельности традиционно определяемых показателей крови и некоторых показателей адаптационного статуса у интактных половозрелых мышей линии Balb/c разного пола.

Материалы и методы. Исследования проведены на 20 половозрелых животных SPF-статуса, самцах (10) и самках (10). Были изучены цитологические и биохимические показатели крови и весовые характеристики органов иммунной системы и надпочечников (с учетом конституциональных особенностей мышей данной линии). При статистическом анализе использовали пакет программ Statistica 10.0. Определяли коэффициент вариации (CV). Значимость статистических различий оценивали с помощью критерия Манна-Уитни.

Результаты. Были выявлены половые различия по соотношению уровней альбумина и глобулина, содержанию мочевины и щелочной фосфатазы, относительному числу лимфоцитов и моноцитов в крови, количеству эритроцитов и содержанию гемоглобина в эритроцитах. Также были отмечены показатели, отличавшиеся сильной вариабельностью (CV до 51 %), число которых у самцов было большим, чем у самок. Информативность весовых характеристик исследованных органов была снижена вследствие конституциональных особенностей самок линии Balb/c. Результаты анализа показателей указывали на более высокий адаптационный статус самок по сравнению с самцами и позволяли предположить различную активность Т-клеточных, В-клеточных и миелоидных звеньев иммунитета у животных разного пола.

Заключение. Изучение слабых и умеренных сдвигов традиционных лабораторных показателей, отражающих адаптационный статус и характеристики крови половозрелых самцов и самок линии Balb/c в условиях физиологической нормы, позволило выявить половые особенности системных регуляторных процессов, которые могут иметь значение для устойчивости организма к злокачественному росту и эффективности противоопухолевых воздействий. Указаны актуальные направления дальнейших исследований.

Ключевые слова: индивидуальные и половые отличия, адаптационный статус, общие неспецифические адаптационные реакции организма, гематологические показатели, иммунные процессы

Для цитирования: Жукова Г. В., Франциянц Е. М., Шихлярова А. И., Каплиева И. В., Трепитаки Л. К., Галина А. В. О вариабельности показателей крови и адаптационного статуса интактных мышей линии Balb/c разного пола. Южно-Российский онкологический журнал. 2023; 4(4): 13-22. <https://doi.org/10.37748/2686-9039-2023-4-4-2>, <https://elibrary.ru/dqdkii>

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Соблюдение этических стандартов: работу с животными проводили в соответствии с правилами «Европейской конвенции о защите животных, используемых в экспериментах» (Директива 2010/63/EU), а также в соответствии с «Международными рекомендациями по проведению медико-биологических исследований с использованием животных» и Приказом Минздрава России от 19 июня 2003 г. № 267 «Об утверждении правил лабораторной практики». Исследование одобрено этическим комитетом ФГБУ «НМИЦ онкологии» Минздрава России (протокол № 11/115 от 01.03.2021 г.).

Финансирование: финансирование данной работы не проводилось.

Конфликт интересов: все авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Статья поступила в редакцию 14.02.2023; одобрена после рецензирования 11.10.2023; принята к публикации 09.12.2023.

INTRODUCTION

Currently, in experimental oncology, as in other medical sciences, linear animals are widely used to standardize the results of research and preclinical trials of new therapeutic agents and technologies. At the same time, the genetic proximity of the objects of study helps to reduce the variability of changes caused by the development of tumors and the action of antitumor factors, but still cannot completely neutralize it. This is indicated by information about the characteristics of certain lines of experimental animals, which may manifest themselves to different degrees in different individuals [1; 2]. In addition, in mongrel animals and laboratory rodents of some lines, the dependence of several indicators, both under normal conditions and in the malignant process, on age [3; 4] and gender [5; 6] was noted.

At the present stage, when conducting experiments *in vivo*, in most cases it is customary to pay attention, first, to sharp changes in laboratory parameters [7]. In this regard, the question of the severity of individual and sexual differences and the factors that cause the heterogeneity of the condition in physiological conditions and pathology for animals of many widely used lines remains insufficiently studied [2; 4]. Meanwhile, individual and gender differences in the state of regulatory systems and the sensitivity of the body to the action of various antitumor agents and accompanying therapy factors, determining the adaptive status of humans and animals, can cause noticeable variations in the dynamics of the malignant process and the effectiveness of complex antitumor treatment [2; 8; 9]. At the same time, it should be borne in mind that modern means of traditional antitumor treatment, in addition to the direct damaging effect on the tumor, have a systemic effect on the body, thereby modulating their own direct effects on malignized cells. Thus, clarification of the issue of individual and sexual differences in adaptive status is necessary for the correct interpretation of research results in conditions of malignant growth, especially when the malignant process and common comorbid pathologies are combined. In addition, the assessment of the heterogeneity of the condition of animals will allow us to determine approaches to optimizing antitumor treatment and accelerate the search for effective, pathogenetically justified antitumor agents.

As an object of research of this kind, mice of the Balb/c line are of interest, belonging to the "old" at the time of creation and the most widely used lines of laboratory animals [1], which also gave rise to the first line of immunodeficient mice (Balb/c nude) capable of supporting the growth of human malignant tumors [10; 11]. As is known, Balb/c mice are distinguished by a good ability to learn, reproduce and feed offspring, as well as low aggressiveness and the highest brain weight ratio compared to this indicator in mice of other lines. At the same time, animals of this line are characterized by high emotionality and low stress resistance, high sensitivity to the action of ionizing radiation, the carcinogenic effect of certain substances, gastrointestinal infections, susceptibility to diseases of the cardiovascular system, the development of amyloidosis of the spleen, etc. [1]. Thus, the characteristic features of Balb/c mice, manifested in different individuals to varying degrees, create the basis for the heterogeneity of the functional state of these laboratory animals and, therefore, for variations during the pathological process and the body's responses to therapeutic effects. Meanwhile, there is no information in the literature on a comprehensive and consistent study of individual differences in the functional state of Balb/c mice under physiological norm conditions.

The heterogeneity of the state of laboratory animals, as a rule, is reflected, somewhat more or less, in the level of blood characteristics and some other simple system parameters that can be used to objectify research results. This determines the expediency of evaluating variations of such indicators in linear animals in physiological and pathological conditions. Although this problem is broader than the question of the relationship of functional characteristics with the gender of laboratory rodents, nevertheless, when studying the heterogeneity of the initial state of Balb/c mice, the analysis of changes in blood parameters and other parameters should be carried out considering the sex of these animals as the most obvious constitutional difference between individuals of the same line.

The purpose of the study was to study the magnitude and variability of traditionally determined blood parameters and some indicators of adaptive status in intact sexually mature Balb/c mice of different sexes.

MATERIALS AND METHODS

The studies were carried out on 20 intact sexually mature mice of the Balb/c line of the vivarium of the National Medical Research Centre for Oncology. The initial batch of animals was obtained in the nursery "Rappolovo". Prior to inclusion in the study, the animals were kept in an SPF-status vivarium. The weight of males ($n = 10$) ranged from 22.5 – 30 g, the weight of females ($n = 10$) ranged from 27.5 – 34 g. This corresponded to the age of 9–11 weeks in males and the age of 14–16 weeks in females [12]. The specified age difference between animals of different sexes was random and was due to the restriction of the possibility of choosing animals of different sexes that exactly match in age. Thus, in general, taking into account sexual dimorphism, the age difference between males and females for individual individuals could be from 3 to 7 weeks. According to the established standards, the life span and habitat conditions of the studied Balb/c mice should be considered as SPF-status animals at the age corresponding to the first half of the reproductive period [12–14]. During the experiments, the international rules of bioethics were observed.

As the studied indicators, the indicators of general and biochemical blood analysis determined by instrumental methods, as well as the weight and weight coefficients of the immune system organs (thymus, spleen) and adrenal glands characterizing the adaptive status of animals were taken [15; 16]. The animals were euthanized by decapitation. Necropsy, isolation and weighing of organs were performed. To determine the characteristics of the blood obtained after euthanasia, the Exigo EOS vet hemoanalyzer (Boule Medical A. B., Sweden) and the VetScanVS2 biochemical analyzer (ABAXIS Inc., Germany) were used.

Statistical processing of the results of the study was carried out using the software package "Statistica 10". The range of variations of the studied indicators, their medians, averages and errors of averages, as well as the coefficient of variation (CV) were determined. The nonparametric Mann-Whitney criterion was used to evaluate the differences in the variation series.

STUDY RESULTS

Analysis of the studied parameters in intact Balb/c SPF-status mice at the age corresponding to the first

half of the reproductive period revealed a number of differences related to the sex of the animals. At the same time, peculiarities in the variability of the values of the studied blood parameters and characteristics of the adaptive status in males and females were also noted (Tables 1–3).

Differences in the characteristics of the red blood germ in mice of different sexes were associated with the peculiarities of the distribution of hemoglobin (Table 1). The level of red blood cells in the blood of females statistically significantly exceeded this indicator in males by 23 %. At the same time, the average hemoglobin content in the red blood cells of males was slightly higher than that of females, so that the levels of hemoglobin in the blood as a whole in animals of different sexes did not statistically differ. At the same time, most of the characteristics of the red blood germ showed an average variability of values in animals of both sexes (from 10 to 20 %, Table 1).

More pronounced sex differences were noted for the white blood parameters of Balb/c mice (Table 1). Thus, females had noticeably higher (1.2 times) values of the relative number of lymphocytes and significantly lower (almost 2 times) values of the relative number of monocytes than males. At the same time, the percentage of lymphocytes in the group of females was surprisingly stable (CM did not exceed 5 %), while the total number of leukocytes in these animals, on the contrary, was characterized by strong variability (CM more than 40 %). In males, in contrast to females, the most variable indicator was the relative number of monocytes (CV more than 37 %).

The level of platelets in the blood of animals of different sexes was similar. At the same time, a strong variability of this indicator (CV more than 36 %) was noted in females (Table 1).

Table 2 provides information on the biochemical blood parameters available for instrumental determination of Balb/c mice of different sexes. As can be seen from the table, there was a tendency for females to slightly increase the total protein content compared to that noted in males. At the same time, the compared groups of animals did not differ statistically significantly in the content of globulins in the blood. The level of albumins in the blood of females was almost 1.5 times higher than this indicator in males, which caused a pronounced difference between individuals of different sexes also in terms of the albumin-globulin index (1.6 times). In turn, male mice had higher levels

of alkaline phosphatase and urea than females (1.5 and 1.4 times, respectively) (Table 2). It should be noted that the values of most biochemical parameters determined in the blood of males were characterized by strong variability (CV from 22.6 to 43.8 %), whereas in females the lower limit of CV values corresponding to a strong variability of indicators (20 %) was overcome only for the content of alanine aminotransferase and albumin-globulin index (Table 2).

Table 3 provides information on the weight and weight coefficients of the immune system and adrenal glands. As already noted, females were characterized by greater weight than males, which indicated a difference in the age of the animals of the compared groups. At the same time, the weight of females in the group was characterized by low variability (CV less than 7 %). Attention was drawn to a more significant mass of all the studied organs of females compared with these indicators in males (differences of 1.5, 2 and 1.8 times, respectively, for the thymus, adrenal glands and spleen). At the level of the weight coefficients of the thymus, this difference was leveled due to the higher weight of the females. The values of the thymus-adrenal mass ratio had no sex differences.

The weight parameters of the thymus (the mass of the organ and its weight coefficient) were characterized by strong variability in animals of both sexes (CV 32.7–41.7 %). In males, the weight indices of the spleen also differed by very strong variability (CV up to 50 %), whereas in the group of females, on the contrary, both the mass and the weight coefficient of this organ varied slightly (Table 3).

Thus, for each of the three groups of studied indicators (Tables 1–3) Sexual differences were noted in Balb/c mice. At the same time, both males and females had a number of characteristics with strong variability, which could indicate the heterogeneity of the state of animals within the compared groups.

DISCUSSION

Earlier, during long-term studies conducted on white laboratory rats, mongrel and linear (Wistar), as well as in sick and healthy people, criteria of adaptive status were identified, reflecting the nature and intensity of general nonspecific adaptational reactions of the body (AR), which had different values in humans and animals [9; 13; 14]. At the same time, several hematological indicators (primarily, the percentage of

Table 1. Indicators of ROC in intact sexually mature mice of the Balb/c line of different fields
 $(X_{av.} \pm m, Me [X_{min} - X_{max}], CV \%)$

Indicator	Male, <i>n</i> = 10	Female, <i>n</i> = 10
Erythrocytes (10 ⁹ /L)	5.57 ± 0.42 5.8 [3.9–6.4] 22.6 %	6.9 ± 0.42 7.3 [3.8–8.2]* 18.4 %
Hemoglobin (g/L)	119.7 ± 6.8 120 [89–138] 13.9 %	132.3 ± 5.1 137 [97–47] 11.5 %
Mean Corpuscular Hemoglobin (pg)	21.8 ± 0.8 21.4 [19.9–25.2] 11.0 %	19.5 ± 0.7 18.8 [17.5–25.5]* 11.2 %
Platelets (10 ⁹ /L)	808.0 ± 79.7 736 [601–1301] 29.6 %	854.3 ± 103.9 832.5 [443–1363] 36.5 %
Leukocytes (10 ⁹ /L)	4.8 ± 0.44 5.3 [3–6] 27.0 %	3.8 ± 0.54 3.35 [1.8–7] 42.6 %
Lymphocytes %	69.6 ± 4.0 71.4 [49.4–84.7] 17.2 %	83.6 ± 1.33 84.2 [77.4–87.7]* 5.0 %
Monocytes %	13.3 ± 1.7 1.9 [6.5–20.5] 38.3 %	7.24 ± 0.50 7.0 [5.4–10.2]* 20.6 %

Note: * – differs from the indicators in male mice, *p* ≤ 0.01.

lymphocytes) were shown to be related to processes in the neuroendocrine and immune systems that determine the development of a particular AR, the body's resistance to the action of various damaging factors and are reflected in such simple characteristics as the weight coefficients of the thymus and adrenal glands and the ratio of the masses of these organs. Differences in the character and tensions of AR in animals close in age and conditions of existence are determined by differences in the individual sensitivity of the organism to the action of exogenous and endogenous factors [9; 14]. In pathological conditions, such differences can cause differences in the level of nonspecific antitumor resistance of the body, and, consequently, in the clinical course of the disease and the effectiveness of treatment, which requires an individual approach to determining the

parameters (regimens, doses, intensity, etc.) of the therapeutic effect.

Unlike laboratory rats, in mice, mongrel and linear, studies have not been conducted to determine specific values of parameters reflecting the nature and intensity of AR. Nevertheless, given the universal nature of systemic adaptation processes, it is advisable to try to use the previously identified informative indicators of adaptation status for a preliminary, indicative assessment of the differences noted in intact Balb/c mice.

When determining the mass and weight coefficients of the studied organs, attention was drawn to the more significant value of most of these indicators in females compared to males. At the same time, the difference in the mass of the thymus was leveled when switching to the weight coefficient of this organ,

Table 2. Some indicators of biochemical blood analysis in intact sexually mature Balb/c mice of different fields
($X_{av} \pm m$, Me [$X_{min} - X_{max}$], CV %)

Indicator	Male, n = 10	Female, n = 10
Complete protein (g/dL)	4.33 ± 0.23 4.2 [3.4 – 5.4] 15.9 %	5.01 ± 0.18 [†] 5 [3.8 – 5.5] 10.2 %
Albumin (g/dL)	2.30 ± 0.22 2.5 [1.1 – 3.2] 28.7 %	3.37 ± 0.18 3.4 [2.4 – 4.0]* 15 %
Globulins (g/dL)	2.00 ± 0.23 1.7 [1.3 – 3] 34.5 %	1.62 ± 0.10 1.5 [1.4 – 2.2] 17.6 %
Albumin/globulin index	1.30 ± 0.19 1.37 [0.37 – 2.00] 43.8 %	2.14 ± 0.18 2.20 [1.36 – 2.86]* 24.1 %
Alkaline Phosphatase (u/L)	79.70 ± 6.00 83 [50 – 98] 22.6 %	53.44 ± 2.63 55 [47 – 61] 13.9 %
Alanine Aminotransferase (u/L)	38.8 ± 2.1 39 [29 – 48] 16.2 %	31.8 ± 2.3 32 [21 – 44] 20.6 %
Amilase (u/L)	929 ± 101 887 [543 – 1457] 32.6 %	1124 ± 72 1098 [875 – 1502] 18 %
Urea (mg/dL)	21.9 ± 1.9 20 [15 – 33] 26 %	1.8 ± 1.1 16 [11 – 21]* 19.2 %

Note: * – differs from the indicators in male mice, $p < 0.05-0.01$; [†] – differs from the values in male mice as a tendency, $p < 0.1$.

in contrast to similar, but more pronounced differences in the weight characteristics of the adrenal glands and spleen. Considering the known information about an active decrease in thymus mass in laboratory rats and mice over a period of 1.5–6 months [17], as well as a slightly more significant age in females compared to males, one would expect a decrease in their thymus weight characteristics compared to those in males, which, however, did not happen. This suggests a high level of lymphoproliferative activity in the thymus of female mice.

When analyzing the weight characteristics of the adrenal glands of the studied animals, it should be noted that female mice of the Balb/c line have the largest adrenal glands among other mice of widely used lines [1]. At the same time, we were unable to find information with a direct indication of the established or possible cause of the increased size of this organ in these animals compared to the size of the adrenal glands in males of the same line. It is possible that such an increase is to some extent due to the increased pro-

duction of aldosterone, characteristic specifically for females of the Balb/c line [18]. Due to this feature, the weight coefficient of the adrenal glands cannot be fully used as an indicator of the adaptive status of female mice of the line under consideration, which also reduces the informativeness of another indicator – the ratio of thymus mass to adrenal mass. An even greater difficulty is caused by the interpretation of the sex difference in the weight characteristics of the spleen in mice of the Balb/c line, which, as is known [1], is characterized by large sizes exceeding this indicator in mice of most known lines. We have not been able to find any information in the literature devoted to this issue and shedding light on the reason for the larger spleen size in Balb/c females, except for some indications of higher reactivity of the spleen in these animals compared to males of the same lineage [19].

Despite the mentioned difficulties associated with the undeveloped criteria of adaptive status and sexual characteristics of the weight characteristics of the immune system and adrenal glands in mice of

Table 3. Weight and weight coefficients of some internal organs in intact sexually mature mice of the Balb/c line of different fields ($X_{av} \pm m$, Me [$X_{min} - X_{max}$], CV %)

Показатель		Male	Female
Animal weight (g)		27.9 ± 1.5 27.7 [22.5–34] 13.2 %	32.2 ± 0.7 33 [27.5–34]* 6.8 %
Thymus	Mass (mg)	28.4 ± 3.6 30 [13–42] 38.0 %	42.1 ± 5.7 43 [24–68]* 35.7 %
	Weight coefficient ×10 ⁻⁴	10.1 ± 1.1 9.1 [5.8–14.2] 32.7 %	13.3 ± 2.1 13.2 [8.5–24.7] 41.7 %
Adrenal glands	Mass (mg)	14.2 ± 1.15 15 [10–19] 24.3 %	28.9 ± 1.87 28 [21–32]* 18.4 %
	Weight coefficient ×10 ⁻⁴	5.1 ± 0.23 5.1 [4.4–5.9] 13.5 %	9.0 ± 0.6 8.3 [7.6–11.8]* 16.8 %
Thymus mass/Adrenal glands mass		2.0 ± 0.19 2.1 [1.3–2.8] 28.5 %	1.6 ± 0.3 1.6 [0.6–3.2] 51.4 %
Spleen	Mass (mg)	84.2 ± 14 94 [39.4–130] 50.0 %	156 ± 5.0 154 [131–179]* 9.5 %
	Weight coefficient ×10 ⁻⁴	29.4 ± 4.0 36.9 [14.3–39.3] 40.8 %	48.8 ± 1.9 49.7 [39.7–54.2]* 10.3 %

Note: * – differs from the indicators in male mice, $p < 0.05$ –0.001.

the Balb/c line, the results obtained, in our opinion, still allow us to make a number of assumptions about the differences in the condition of intact animals of different sexes belonging to the line under consideration. As already noted, the group of females was somewhat more homogeneous in terms of the values of the studied indicators compared to the group of males and differed in relative stability of animal weight and lower variability of a few indicators compared to the characteristics of males (Tables 1–3). In our opinion, on the day of the examination, the adaptive status of females was slightly higher than the adaptive status of males, because despite a slightly more significant age compared to males, as well as such a constitutional feature of Balb/c females as an increased weight of the adrenal glands, the ratio of thymus mass to adrenal mass in these animals did not statistically significantly differ from the ratio of the mass of these organs in males. In addition, females demonstrated a higher and more stable percentage of lymphocytes in the blood than males, corresponding to the range of maximum values of this indicator for Balb/c mice [1; 20]. This allowed us to assume about the development of AR increased activation in the studied group of females [9; 16].

Signs such as a statistically significant decrease in the percentage of lymphocytes in the blood, monocytosis, an increase in the content of urea with a tendency to decrease the level of total protein compared to the indicators in females indicated a slightly lower adaptive status of males compared to that noted in females. At the same time, the shift of the albumin-globulin index towards globulins, an increase in the level of urea and alkaline phosphatase, as well as signs of moderate monocytosis could be associated with some tension of antistress AR in males [2; 9; 16] and reflect the activation of metabolic and immune processes, which has a compensatory value. The reasons for such a decrease in the adaptive status of males compared to females could be related to their lower stress resistance and greater dependence on social status in the group than in females [1; 2; 21].

A comprehensive examination of the results gives the impression of the existence of sexual differences in the regulatory mechanisms that dominate in females and males in physiological conditions. It can be assumed that in males there is a certain shift in immunoreactivity towards the B-cell and myeloid links

of immunity in comparison with the regulatory circuit in females, who probably have more active T-cell immune processes. This may be evidenced by an increase in the content of immunoglobulins relative to albumins and an increase in the level of alkaline phosphatase, which affects the activity of B-lymphocytes and neutrophils [22], in the blood of males compared with females, while the indicators of the condition of females indicated high lymphoproliferative activity in the thymus, accompanied by a maximum percentage of lymphocytes in the blood. This assumption is consistent with the literature data on the gender-related features of immunity in humans and animals and the greater severity of T-cell processes in female individuals, and B-cell immune processes in male individuals [6; 21; 22]. The high variability of a number of the studied indicators (observed in males of the Balb/c line more often than in females) may indicate the existence of different variants of integration of reactivity mechanisms in animals of the same sex, which may affect the effectiveness of systemic regulation under the action of damaging factors. To clarify this issue, it is necessary to develop criteria for the nature and intensity of AR, similarly used in humans and white laboratory rats [9; 14].

CONCLUSIONS

The study of weak and moderate shifts in traditional laboratory indicators reflecting the adaptive status and characteristics of the blood of mature males and females of the Balb/c line under physiological norm conditions allowed us to identify individual and sexual characteristics of systemic regulatory processes that may be important for the body's resistance to damaging factors, including the malignant process. To objectify the connection of sexual and individual differences of the studied indicators with the systemic mechanisms of nonspecific, including antitumor, resistance, it is advisable to develop criteria for the character and tensions of AR, similar to those used in humans and white laboratory rats. The results obtained indicate the relevance of the issue of sex differences in the reactivity of T-cell, B-cell and myeloid links of immunity and the influence of the severity of immune processes dominating in physiological conditions on carcinogenesis, which may contribute to the development of new effective methods of antitumor treatment.

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MICROBIOTICENOSIS OF ADENOCARCINOMA TISSUE IN COLON CANCER PATIENTS WITH DIFFERENT PREOPERATIVE PREPARATION

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ABSTRACT

Purpose of the study. To assess the effect of inclusion of lactoglobulin in complex preoperative preparation of colon cancer patients on their tumor and resection line tissue microbiota.

Materials and methods. 40 patients with colon cancer stages II–III, in whom the operation was the first stage of treatment, during standard preoperative preparation, were injected with a preparation of antibodies against opportunistic intestinal microorganisms obtained from colostrum of immunized cows, 2 g twice a day orally before surgery for 3 days (total dose of 12 g) (main group); 40 patients received standard antibiotic prophylaxis (control group). The quantitative composition of the microbiota was determined in the samples of the removed tumor and tissue of the resection line.

Results. The total microbial contamination of the tumor was 9.2 times lower in the main group relative to the control group; the frequency of *E. coli* and *Clostridia* excretion was also statistically significantly lower ($p = 0.004$ and 0.03 , respectively). In the tumors of patients of the main group out of twelve studied representatives of microorganisms, the number of six was statistically significantly lower than in control group, and three of those found in the control group were not detected. Since they were potentially pathogenic (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, fungi of the *Candida* spp.), the microbial composition of the tumor of patients in the main group can be considered more favorable than the control group. Similar differences were noted in non-tumor intestinal tissue, in which the content of *Enterobacter* spp, *Streptococcus*, *Clostridia*, *Peptostreptococci* was statistically significantly lower than in the control group.

Conclusion. Thus oral administration of colostrum antibodies caused positive changes in tumor and colon tissue microbiota. We suggest the application of lactoglobulin to be useful for surgical treatment of such patients taking into account the possible impact of microbiota in patients' response to chemo- and immunotherapy.

Keywords: colon cancer, tissue microbiota, tumor and intact colon tissue, lactoglobulin

For citation: Simonenko N. I., Zlatnik E. Yu., Panova N. I., Shulgina O. G., Maksimov A. Yu. Microbiocenosis of adenocarcinoma tissue in colon cancer patients with different preoperative preparation. South Russian Journal of Cancer. 2023; 4(4): 23-31. <https://doi.org/10.37748/2686-9039-2023-4-4-3>, <https://elibrary.ru/etosyk>

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Compliance with ethical standards: the research was carried out according to the ethical principles, set forth by the World Medical Association Declaration of Helsinki, 1964, ed. 2013. The study was approved by the Biomedical Ethics Committee at the National Medical Research Center for Oncology (extract from the protocol of the meeting No. 2 dated 01/22/2021. Informed consent was obtained from all participants of the study.

Funding: this work was not funded.

Conflict of interest: the authors declare that there are no obvious and potential conflicts of interest associated with the publication of this article.

Acknowledgments: the authors express their gratitude to the T. I. Tverdokhlebova, Dr. Sci. (Med.), CEO of the Rostov Scientific Research Institute of Microbiology and Parasitology, Rospotrebnadzor, for providing lactoglobulin.

The article was submitted 15.03.2023; approved after reviewing 08.10.2023; accepted for publication 09.12.2023.

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МИКРОБИОЦЕНОЗ ТКАНИ АДЕНОКАРЦИНОМЫ ОБОДОЧНОЙ КИШКИ В ЗАВИСИМОСТИ ОТ ВАРИАНТА ПРЕДОПЕРАЦИОННОЙ ПОДГОТОВКИ БОЛЬНЫХ

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РЕЗЮМЕ

Цель исследования. Оценка влияния включения препарата лактоглобулина в комплекс предоперационной подготовки больных раком ободочной кишки на состав микробиоты опухоли и ткани, взятой по линии резекции.

Материалы и методы. 40 больным раком ободочной кишки II–III стадий, у которых операция была первым этапом лечения, в курсе стандартной предоперационной подготовки вводили препарат антител против условно-патогенных микроорганизмов кишечника, полученный из молозива иммунизированных коров, по 2 г 2 раза в день перорально перед операцией в течение 3-х дней (суммарная доза 12 г) (основная группа); 40 больных получали стандартную антибиотикопрофилактику (контрольная группа). В образцах удаленной опухоли и ткани линии резекции определяли количественный состав микробиоты.

Результаты. У больных основной группы общая микробная обсемененность опухоли была в 9,2 раза ниже контрольной; частота выделения *E.coli* и *Clostridiaceae* была также статистически значимо ниже ($p = 0,004$ и $0,03$ соответственно). В опухолях больных основной группы из двенадцати исследованных представителей микроорганизмов количество шести было статистически значимо ниже контроля, а три из обнаруженных в контрольной группе не выявлялись. Поскольку они относились к потенциально патогенным (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, грибы рода *Candida*), микробный состав опухоли больных основной группы можно считать более благоприятным, чем контрольной. Подобные различия отмечены и в неопуховатой ткани кишки, в которой содержание *Enterobacter* spp, *Streptococci*, *Clostridiaceae*, *Peptostreptococci* было статистически значимо ниже, чем в контроле.

Заключение. Итак, пероральное применение антительного препарата лактоглобулина вызывает положительные изменения микробиоты опухоли и неопуховатой ткани кишки. Учитывая возможное влияние состава микробиоты на ответ больного на дальнейшую химио- и иммунотерапию, считаем целесообразным использование препарата для подготовки к адъювантному лечению.

Ключевые слова: рак ободочной кишки, микробиота опухоли и интактной ткани кишечника, лактоглобулин

Для цитирования: Симоненко Н. И., Златник Е. Ю., Панова Н. И., Шульгина О. Г., Максимов А. Ю. Микробиоценоз ткани аденокарциномы ободочной кишки в зависимости от варианта предоперационной подготовки больных. Южно-Российский онкологический журнал. 2023; 4(4): 23-31. <https://doi.org/10.37748/2686-9039-2023-4-4-3>, <https://elibrary.ru/etosyk>

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Соблюдение этических стандартов: в работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). Исследование одобрено Комитетом по биомедицинской этике при ФГБУ «НМИЦ онкологии» (выписка из протокола заседания № 2 от 22.01.2021 г.). Информированное согласие получено от всех участников исследования.

Финансирование: финансирование данной работы не проводилось.

Конфликт интересов: все авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Благодарности: авторы выражают благодарность директору ФБУН «Ростовский НИИ микробиологии и паразитологии» Роспотребнадзора д.м.н. Т.И. Твердохлебовой за предоставление лактоглобулина

Статья поступила в редакцию 15.03.2023; одобрена после рецензирования 08.10.2023; принята к публикации 09.12.2023.

INTRODUCTION

In recent years, the attention of researchers around the world has been attracted to the definition of the role of microbiota involved in oncological processes. More and more scientific arguments are accumulating that the imbalance of the intestinal microbiota contributes to carcinogenesis and tumor growth; primarily this applies to colorectal cancer [1–3]. The effect of the microbiota composition on the sensitivity of colorectal cancer to the action of a new generation of antitumor immunopreparations – checkpoint inhibitors [4], as well as cytostatics [5; 6].

In this regard, microbiota correction in cancer patients is the current agenda [7]. With the development of a tumor in the colon, excessive growth of opportunistic bacteria is detected, the accumulating metabolites of which can cause the suppression of normal microflora, which is accompanied by a change in the trophic, protective, metabolic and immunological functions of autochthonous microorganisms of the large intestine [8]. As a result, there is an increase in the biochemical activity of microflora, a change in pH, which creates a favorable environment for the reproduction of opportunistic bacterial species, increased putrefactive processes and inflammation in the colon, i.e., a vicious circle arises. The formation of dysbiosis leads to a decrease in the immune reactivity of the body and contributes to the progression of the tumor process [9; 10]. Even though many papers have been published describing the composition of the microbiota of the colon in various pathologies, including oncological, there are significantly fewer publications on the study of the microbiota of the tumor itself [11; 12].

Modern standard therapeutic technologies for the preparation of patients with colon cancer do not aim to eliminate microecological disorders. On the contrary, due to the appointment of a preventive course of antibiotic therapy, they can contribute to the aggravation of dysbiosis. Meanwhile, the presence of an increased number of opportunistic microorganisms in the colon of patients is an unfavorable background for the postoperative course of the disease. This disadvantage can be leveled by prescribing probiotics in the preoperative period [13].

Lactoglobulin, not being a probiotic in the strict sense, is intended for the correction of the microbiota. It has such an effect due to the presence in its

composition of colostrum antibodies to conditionally pathogenic microorganisms (*Proteus vulgaris*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella thyphimurium*, *Salmonella enteritidis*, *Salmonella dublin*), as well as lactoferrin, bifidogenic factors, as a result of which it contributes to the suppression of conditionally pathogenic and stimulation symbiont microorganisms [14].

Purpose of the study was to evaluate the effect of the inclusion of lactoglobulin in the complex of preoperative preparation of patients with colon cancer on the composition of the tumor microbiota and mucosal tissue taken along the resection line.

MATERIALS AND METHODS

The subject for the study were samples of a tumor and tissue taken by resection during surgery for colon cancer in 80 patients who were treated at the National Medical Research Centre for Oncology. All patients signed an informed consent to participate in the study. The age of the patients ranged from 40 to 80 years, the average age corresponded to 65.6 ± 4.5 years, among them there were 32 men and 48 women (40 and 60 %, respectively). All were diagnosed with stage II–III colon cancer, the operation was the first stage of treatment. In all cases, the histological structure of the tumor was adenocarcinoma. 40 patients were included in the main and 40 in the control group; the groups were comparable in gender, age, and stage of the disease. The control group of patients underwent standard preoperative preparation, including antibiotic prophylaxis of postoperative complications (ceftriaxone 1 g 2 times a day, metrogil 500 mg 2 times a day); the main group of patients, in addition, received 12 g of lactoglobulin before surgery for 3 days (2 g 2 times a day orally). All 80 patients underwent standard antibiotic therapy in the postoperative period. In the future, all patients received adjuvant chemotherapy according to the FOLFOX scheme. During the operation, a fragment from the tumor tissue and mucosa from the intact intestine taken along the resection line were excised in patients for microbiological examination, microbiocenosis in the tissues was studied and its comparative analysis was carried out between two groups of patients.

Microbiological methods of quantitative analysis for dysbiosis were used to assess the composition

of the microbiota (OST 91500.11.0004-2003). The tissue suspension was washed from the lumen microflora, dissected and its internal parts were used for further research. Sample preparation was carried out on a Medimachine device. Serial dilutions were prepared from the obtained suspension of biological material, which were seeded with 0.1 ml per petri dish with differential diagnostic media. Chromogenic Uriselect agar (Bio-Rad, France), blood agar (Columbia blood agar base with the addition of 5 % defibrinated horse blood, yolk-salt agar (salt agar with mannitol/Mannitol Salt Agar, with the addition of egg yolk emulsion/Egg Yolk Emulsion), medium were used for sowing Saburo (Saburo Agar with glucose and chloramphenicol/ Sabouraud Chloramphenicol), as well as a medium for the isolation of lactobacilli lactobacilli, Wilson-Blair agar for clostridium; all media, except chromogenic Uriselectagar, were manufactured by HiMedia Laboratories Pvt. Limited (India). Incubation in a thermostat at 37 °C was carried out for aerobic microflora for 24–48 hours, for anaerobic under anaerostat conditions for 6–7 days, except for clostridium, which were grown for 24 hours. Next, the grown colonies were counted, if necessary, screening was carried out to isolate a pure culture and subsequent identification of microorganisms, which was carried out on an automatic bacteriological analyzer Vitek 2 (BioMerieux, France).

Microorganisms related to opportunistic pathogens were identified to the following strains: *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *Enterobacter*,

Proteus. The results were expressed in lg/g of tissue, and the total microbial contamination was expressed in colony-forming units (CFU/g of tissue).

Statistica 12 (Stat Soft, USA) and MedCalc 19.3.0 (MedCalc Software bv, USA) programs were used for statistical processing of the results. The estimation of the distribution of values and the normality of the distribution were analyzed according to the Shapiro-Wilk criterion (Statistica 12 frequency analysis module). When calculating variational statistics, the Statistica 12 descriptive statistics module was used with the calculation of the average value (*M*), its error (*m*), median (*Me*) and interquartile range [*Q25*; *Q75*]. In the presence of a normal distribution of indicators, the Student-Fisher criterion was used to assess the statistical significance of differences, in the absence of a normal distribution, the Mann-Whitney criterion was used. When comparing the average values of independent samples, the criterion for the significance of differences was the value $p \leq 0.05$.

STUDY RESULTS AND DISCUSSION

Several quantitative and qualitative differences were observed after preoperative preparation with and without the inclusion of lactoglobulin in the studied tissues of patients of the main and control groups. Representatives of the microflora of the gastrointestinal tract were found in the tumor tissue of patients of both groups, however, the degree of microbial contamination differed. Thus, in the tumor

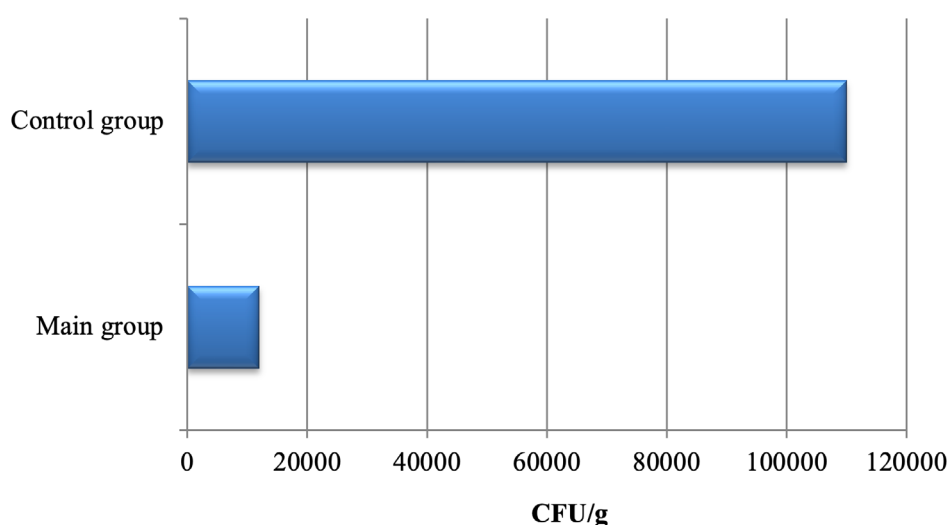


Fig. 1. Microbial contamination of colonic tumor tissue (CFU/g) in the main and control groups.

tissue in patients of the main group, it varied from 10^3 to 10^5 ($M \pm m 1.2 \pm 0.3 \times 10^4/g$), and in the control group – in the range of 10^4 to 10^7 ($M \pm m 1.1 \pm 0.2 \times 10^5/g$), that is, it was 9.2 times higher (Fig. 1).

The contamination of the mucosa of the intact intestine did not exceed 10^3 and mainly ranged from 10^1 – 10^2 .

The frequency of isolation of individual microorganisms in the samples of the studied tissues is reflected in Table 1, which presents a comparative characteristic of tumors of patients of both groups, as well as tumors and intact intestine in each of them.

In the tumor tissue of patients of both groups, *E.coli* aerobes were most often found, and *Bacteroides* spp and *Clostridia* anaerobes. Statistically significant differences between the groups were noted only in the content of *E.coli* and *Clostridia* in the tumor, which were less frequently represented in the tumor tissue of patients of the main group.

The frequency of detection of the remaining studied representatives of the microbiota was also lower in the main group, although these differences were not statistically significant. Of the 12 identified microorganisms, 9 were found in the tumors of the patients of the main group, 6 in the tissue of the intact intestine.

In the colon mucosa taken by resection in patients of the main group, bacteroids were detected in almost half of the observations (47.5 %), in 10 % – *E.coli*, in a small percentage of observations – *Clostridia*, *Peptostreptococci*, *Klebsiella pneumoniae*, *Proteus* spp. In the main group, *Bacteroides* spp. were statistically significantly more often detected in the tumor tissue compared to the intact intestine, whereas *E.coli* and *Clostridia* were statistically significantly more often detected in the tumors of patients in the control group, in addition to bacteroids.

In the control group, the spectrum of microorganisms in the tumor tissue was wider than in the intact

Table 1. Frequency of detection of microorganisms obtained from tumor tissues and mucosa of the intact intestine in patients of the main and control groups

Indicator	Tissue samples									
	Main group					Control group				
	Tumor tissue		Intact intestines		<i>p</i>	Tumor sample		Intact intestines		<i>p</i>
	abs.	%	abs	%		abs	%	abs	%	
<i>E.coli</i>	6	15	4	10	> 0.05	19	47.5	7	17.5	0.04*
<i>Klebsiella pneumoniae</i>	4	10	2	5	> 0.05	8	20	3	7.5	> 0.05
<i>Proteus</i> spp.	3	7.5	2	5	> 0.05	6	15	3	7.5	> 0.05
<i>Enterobacter</i> spp.	2	5	0	0	-	5	12.5	2	5.0	> 0.05
<i>Streptococci</i>	2	5	0	0	-	5	12.5	2	5.0	> 0.05
<i>Bacteroides</i> spp	33	82.5	19	47.5	0.002*	36	90	15	37.5	0.03*
<i>Clostridia</i>	8	20	3	7.5	> 0.05	18	45	8	20.0	0.04*
<i>Peptostreptococci</i>	3	7.5	2	5	> 0.05	7	17.5	2	5.0	> 0.05
<i>Peptococci</i>	1	2.5	0	0	-	2	5	1	2.5	> 0.05
<i>Pseudomonas aeruginosa</i>	0	0	0	0	-	3	7.5	0	0	-
<i>Staphylococcus aureus</i>	0	0	0	0	-	3	7.5	0	0	-
<i>Candida</i> spp. fungi	0	0	0	0	-	1	2.5	0	0	-

Note: * – statistically significant differences between the parameters of the tumor tissue and the resection line in each of the compared groups ($p < 0.05$); "–" – *p* cannot be determined due to the actual absence of representatives of the microbiota in the tissue samples

intestine: 12 pathogens were identified in the tumor tissue, and 9 in the intact intestine (Table 1). The frequency of detection of microorganisms in the tumor tissue was statistically significantly higher compared with the intact intestine for *E.coli*, *Bacteroides* spp.,

Clostridia. Noteworthy is the presence of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* fungi in the tumor tissue and the absence of their intact intestinal mucosa, which did not allow us to assess the reliability of the differences. In the

Table 2. The content of microorganisms in the tumor tissue and mucosa of the intact intestine in patients of the main and control groups (lgCFU/g, M ± m)

Indicator	Tissue samples					
	Main group		Control group		p_{1-3}	p_{2-4}
	Tumor tissue	Intact intestine	Tumor tissue	Intact intestine		
	1	2	3	4		
<i>E.coli</i>	3.5 ± 0.3*	2.3 ± 0.2	6.3 ± 0.7	2.4 ± 0.02	0.015	> 0.05
	$p = 0.027$		$p < 0.001$			
<i>Klebsiella pneumoniae</i>	1.7 ± 0.2*	0.7 ± 0.08	4.6 ± 0.5	0.5 ± 0.02	0.002	> 0.05
	$p = 0.023$		$p < 0.001$		>0.05	> 0.05
<i>Proteus</i> spp.	3.1 ± 0.4	2.2 ± 0.3	4.5 ± 0.8	2.0 ± 0.07	>0.05	> 0.05
	$p = 0.046$		$p = 0.004$			
<i>Enterobacter</i> spp.	1.3 ± 0.1*	0.9 ± 0.05**	2.7 ± 0.4	1.3 ± 0.02	0.03	0.04
	$p = 0.048$		$p = 0.005$			
<i>Streptococci</i>	0.6 ± 0.1*	0.3 ± 0.09**	2.6 ± 0.3	0.7 ± 0.03	0.008	0.035
	$p = 0.067$		$p < 0.001$			
<i>Bacteroides</i> spp.	4.2 ± 0.7	2.5 ± 0.1	5.2 ± 0.4	2.4 ± 0.2	> 0.05	> 0.05
	$p = 0.02$		$p < 0.001$			
<i>Clostridia</i>	2.9 ± 0.3*	1.5 ± 0.06**	5.5 ± 0.5	2.0 ± 0.1	0.04	0.045
	$p = 0.01$		$p < 0.001$			
<i>Peptostreptococci</i>	0.3 ± 0.07*	0.2 ± 0.03**	1.7 ± 0.3	0.5 ± 0.08	0.009	0.04
	$p = 0.26$		$p < 0.01$			
<i>Peptococci</i>	1.0	0.1 ± 0.02	1.5 ± 0.2	1.0	-	-
	$p = 0.57$					
<i>Pseudomonas aeruginosa</i>	0	0	0.4 ± 0.03	0.1 ± 0.03	-	-
			$p < 0.01$			
<i>Staphylococcus aureus</i>	0	0	1.1 ± 0.02	0.2 ± 0.001	-	-
			$p < 0.001$			
<i>Candida</i> spp. fungi	0	0	2.5 ± 0.2	0.5 ± 0.01	-	-
			$p < 0.001$			

Note: * – statistically significant differences between the parameters of the tumor tissue; ** – statistically significant differences between the parameters of the intact intestine tissue ($p < 0.05$); "-" – p cannot be determined due to the actual absence of representatives of the microbiota in the samples of the main group of patients

intact intestine, *Bacteroides* spp. (37.5 %), *Clostridia* (20 %), *E.coli* (17.5 %) were more common than others, and in isolated cases, coccoid microflora (*Peptococci* – 2.5 %, *Peptostreptococci* – 5 %, *Streptococci* – 5 %) (Table 1).

Quantitative indicators of the content of various microorganisms in the studied tissue samples of patients of the main and control groups are shown in Table 2.

As seen from Table 2, in the tumor tissue, the quantitative content of almost all the studied microorganisms in patients of the main group was statistically significantly lower ($p < 0.05$) than in the control group (Fig.3). Similar contamination of tumor tissue in the main and control groups was noted only for bacteroids.

Worth noting, that after preoperative use of lactoglobulin from the tumor tissue of patients, it was not possible to isolate such representatives of opportunistic microflora as *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida* fungi, and the number of other species, in particular, coccoid microflora, was significantly lower compared to the control group (*Peptococci* 1.5 times, *Peptostreptococci* by 82 %, *Streptococci* by 77 %) (Tables 1, 2, Fig. 2).

In patients of the control group, the microflora contamination of tumor tissue was significantly higher compared to the mucosa of the intact intestine for all pathogens. The main differences were formed for *Klebsiella rheimopiae* (9.2 times higher compared to the intact intestine), *Pseudomonas aeruginosa* (4 times), *Candida* spp. fungi (5 times) (Table 2).

Figure 2 shows the excess of the titer of microorganisms (in %) in the tumor tissue compared with the intact intestine along the resection line in patients of the main and control groups. In patients of the control group, these differences were significantly higher compared to the main group; they were mainly characteristic of *Klebsiella rheimopiae*, *Streptococci*, *Peptococci* (Fig. 2).

CONCLUSIONS

Preoperative administration of lactoglobulin preparation against conditionally pathogenic microorganisms contributes to the formation of a more favorable microbial landscape of the tumor and tissue taken along the resection line in patients with colon cancer, which manifests itself in a decrease in the total microbial contamination of the studied tissue samples, as well as in a decrease in the frequency

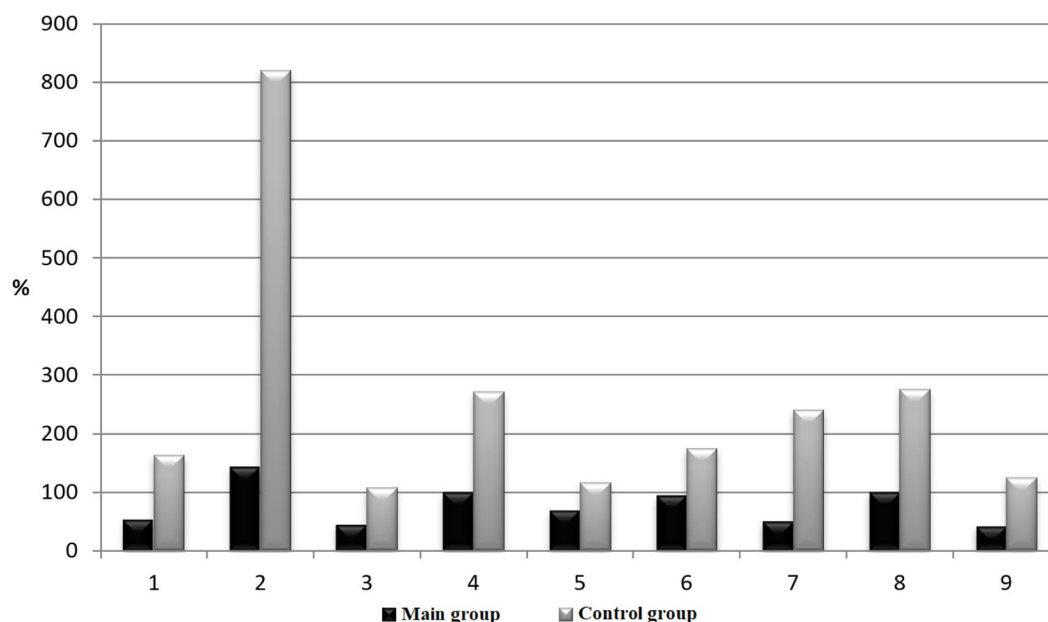


Fig. 2. Comparative characteristics of the content of microorganisms in the tumor tissue in patients of the main and control groups (% in the tumor tissue compared with the tissue of the intact intestine).
 Note: 1 – *E.coli*, 2 – *Klebsiella pneumoniae*, 3 – *Enterobacter* spp., 4 – *Streptococci*, 5 – *Bacteroides* spp., 6 – *Clostridia*, 7 – *Peptostreptococci*, 8 – *Peptococci*, 9 – *Proteus* spp.

of detection and the number of several conditionally pathogenic microorganisms. We found a high number of studied microbiota representatives in the tumor tissue of patients, which corresponds to the literature data on their potentially pro-oncogenic effect, which is described in *Klebsiella pneumonia* [15], streptococci [16], peptostreptococci [17], clostridium [18; 19]. Since among the mechanisms of the growth-stimulating effect of these microorganisms, attention

is paid to the production of toxins, the maintenance of local inflammation, and the imbalance of the immune microenvironment, it seems important to correct the microbiota with the help of an immune drug, which especially applies to the tissue of the resection line, since it remains in the patient's body after surgery, and the composition of its microbiota can contribute not only to postoperative tissue regeneration, but also affect the further course of the disease.

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Panova N. I. – conducting of microbiologic studies;
Shulgina O. G. – statistical analysis, text formatting;
Maksimov A. Yu. – development of the concept.

MAGNETIC RESONANCE IMAGING RELEVANCE IN DIAGNOSIS AND PROGNOSIS OF EARLY POSTOPERATIVE PERIOD FOLLOWING PANCREATIC CANCER SURGICAL TREATMENT

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ABSTRACT

Purpose of the study. Improvement of the prognostic outcomes for the pancreatic fistula development in surgical treatment for pancreatic cancer by implementation of new diagnostic algorithms for magnetic resonance imaging (MRI) assessment of supposed pancreatic stump.

Materials and methods. We performed a retrospective analysis of MRI results of 1136 patients from the medical data base of National Medical Research Centre for Oncology for 2009–2020. An original scanning technique, trans- and cross-pancreatic imaging and MR spectroscopy of the pancreas in patients with pancreatic cancer, was developed and applied. Preoperative examinations were compared retrospectively: a standard MRI protocol without anatomical orientation of the series; MRI protocol using the developed technology.

Results. 717 patients were diagnosed with advanced pancreatic cancer. Lymph nodes were affected in 302 patients among 419 patients with radical surgeries. All cases were confirmed morphologically. In the group of 419 patients with pancreatic resections, based on the analysis of morphological and clinical data and preoperative MRI data, we developed an original preoperative scale for assessing the risk of pancreatic fistula development and compared its accuracy with other intraoperative scales, FRS and modified FRS. Lactate and lipid complex were selected from a wide range of metabolites. The surgical protocols and results of histological examination of the surgical material were used to prove the accuracy of the study. The overall accuracy of the technique in predicting fistula development was 97.5 %. New visual predictors ("domino" and "white on white" symptoms) based on MRI data were used to improve the scale accuracy.

Conclusion. The developed method of using a modified scale for risk assessment of pancreatic fistula development allows predicting the onset of early postoperative complications already at the preoperative stage. When comparing the calculated risks of pancreatic fistula according to the developed scale with the results by the known scales (FRS and modified FRS), statistical analysis showed a significant difference for the better when compared with FRS ($p = 0.0477$), and a tendency when compared with modified FRS ($p = 0.0544$).

Keywords: pancreatic cancer, MRI, MRI assessment, tumor tissue, supposed pancreatic stump

For citation: Kolesnikov E. N., Iozefi D. Ya., Kit O. I., Maksimov A. Yu. Magnetic resonance imaging relevance in diagnosis and prognosis of early postoperative period following pancreatic cancer surgical treatment. South Russian Journal of Cancer. 2023; 4(4): 32-43. <https://doi.org/10.37748/2686-9039-2023-4-4-4>, <https://elibrary.ru/hetxqy>

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Compliance with ethical standards: The research was carried out according to the ethical principles, set forth by World Medical Association Declaration of Helsinki, 1964, ed. 2013. The study was approved by the Biomedical Ethics Committee at the National Medical Research Center for Oncology (extract from the protocol of the meeting No. 118 dated 03/11/2019). Informed consent was obtained from all participants of the study.

Funding: this study was funded by a state assignment, registry number 122032300207-0.

Conflict of interest: the authors declare that there are no obvious and potential conflicts of interest associated with the publication of this article.

The article was submitted 07.08.2023; approved after reviewing 06.10.2023; accepted for publication 09.12.2023.

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ЗНАЧЕНИЕ МАГНИТНО-РЕЗОНАНСНОЙ ТОМОГРАФИИ В ДИАГНОСТИКЕ И ПРОГНОЗЕ ТЕЧЕНИЯ РАННЕГО ПОСЛЕОПЕРАЦИОННОГО ПЕРИОДА ПРИ ХИРУРГИЧЕСКОМ ЛЕЧЕНИИ БОЛЬНЫХ РАКОМ ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ

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РЕЗЮМЕ

Цель исследования. Совершенствование результатов прогнозирования развития панкреатической фистулы при оперативном лечении рака поджелудочной железы путем внедрения новых диагностических алгоритмов магнитно-резонансной томографии (МРТ)-оценки предполагаемой ее культи.

Материал и методы. Проведен ретроспективный анализ результатов МРТ-исследований 1136 пациентов из базы данных медицинской информации ФГБУ «НМИЦ онкологии» Минздрава России за 2009–2020 гг. Разработана и применена оригинальная методика сканирования – транс- и кросспанкреатическая визуализация и методика МР-спектроскопии у пациентов раком поджелудочной железы. Выполнено ретроспективное сравнение предоперационных исследований: стандартный протокол МРТ без анатомического ориентирования серий; протокол МРТ с применением разработанной технологии.

Результаты. Генерализованный рак поджелудочной железы был у 717 пациентов. Поражение лимфатических узлов – у 302 больных среди 419 радикально оперированных. Все случаи были подтверждены морфологически. В группе 419 пациентов, перенесших резекцию ПЖ, на основании анализа морфологических и клинических данных, а также данных предоперационного МРТ мы разработали оригинальную предоперационную шкалу оценки риска развития панкреатического свища, которую сравнили по точности с интраоперационными шкалами: шкалой риска развития панкреатической фистулы (FRS) и модифицированной FRS. Из широкого спектра метаболитов выбраны лактат и липидный комплекс. Для доказательства точности исследования мы использовали протоколы операций и результаты гистологических исследований операционного материала. Общая точность методики в возможности предсказать развитие свища – 97,5 %. Для повышения точности разработанной шкалы использовались новые визуальные предикторы (симптом «домино» и «белое на белом»), основанные на данных МРТ.

Заключение. Разработанный метод использования модифицированной шкалы риска развития панкреатической фистулы позволяет уже на дооперационном этапе предсказать развитие ранних послеоперационных осложнений. При сравнении рассчитанных рисков развития панкреатической фистулы по разработанной шкале с результатами их оценки по известным шкалам (FRS и модифицированной FRS) статистический анализ показал достоверное отличие в лучшую сторону при сравнении с FRS ($p = 0,0477$), и тенденцию – при сравнении с модифицированной FRS ($p = 0,0544$).

Ключевые слова: рак поджелудочной железы, МРТ-исследование, МРТ-оценка, опухолевая ткань, предполагаемая культи поджелудочной железы

Для цитирования: Колесников Е. Н., Иозефи Д. Я., Кит О. И., Максимов А. Ю. Значение магнитно-резонансной томографии в диагностике и прогнозе течения раннего послеоперационного периода при хирургическом лечении рака поджелудочной железы. Южно-Российский онкологический журнал. 2023; 4(4): 32-43. <https://doi.org/10.37748/2686-9039-2023-4-4-4>, <https://elibrary.ru/hetxqy>

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Соблюдение этических стандартов: в работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). Исследование одобрено Комитетом по биомедицинской этике при ФГБУ «НМИЦ онкологии» Минздрава России (выписка из протокола заседания № 10 от 11.03.2019 г.). Информированное согласие получено от всех участников исследования.

Финансирование: финансирование за счет госзадания: регистрационный № 122032300207-0.

Конфликт интересов: все авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Статья поступила в редакцию 07.08.2023; одобрена после рецензирования 06.10.2023; принята к публикации 09.12.2023.

INTRODUCTION

The incidence of malignant neoplasms in Russia has been growing for decades, in 2021, oncological diseases were diagnosed in more than 580,415 thousand residents. The "rough" indicator of pancreatic cancer incidence in Russia in 2021 was at the level of 13.1 per 100,000 population, the increase in this indicator since 2011 was 31.08 %, while mortality per 100,000 population was 13.7, with an increase of this indicator by 26.01 % over 10 years [1].

Diagnosis of pancreatic cancer is playing an increasingly important role, allowing you to quickly respond to the extent of the spread of the tumor and choose a reasonable therapeutic strategy for patients. Various imaging methods, such as computed tomography – CT [2; 3], magnetic resonance imaging – MRI [4], endoscopic ultrasound [5] and positron emission computed tomography – PET [6] are becoming increasingly important and are widely used for the diagnosis of pancreatic cancer.

MRI can potentially be a sufficient method for assessing pancreatic fibrosis, which is confirmed by histological studies [4; 7] and is extremely important when planning surgical treatment. Thus, the inclusion of MRI results in a set of techniques that determine the risks of the course of the postoperative period in patients after radical resections of the pancreas is an urgent task.

The surgical method remains the leading one in the treatment of resectable pancreatic tumors [8]. We analyzed the literature sources concerning the calculation of the risk of postoperative complications, especially pancreatic fistulas (PF) after pancreatic resections [9–14]. For example, D. S. Gorin (2022) [15] focused on the following main risk factors: the nature of the tumor lesion, CT signs of "soft" pancreas (accumulation coefficient greater than 1); intraoperative visual and palpatory assessment; the proportion of functioning acinar structures during urgent histological examination of the pancreatic section and the features of its angioarchitectonics.

The scales known to date allow predicting the risk of pancreatic fistula only in the early postoperative period. At the same time, if the risk of developing a fistula could be judged at the preoperative stage, the patient in this case would receive sufficient individualized preoperative preparation.

Purpose of the study was to improve the results of predicting the development of pancreatic fistula in the surgical treatment of pancreatic cancer by introducing new diagnostic algorithms for MRI evaluation of its presumed stump.

MATERIALS AND METHODS

A retrospective analysis of the results of MRI studies of 1136 patients from the database of medical information of the National Medical Research Centre for Oncology, was carried out in the period from 01/01/2009 to 12/31/2020. MRI was performed both at the National Medical Research Centre for Oncology (78.6 %, 893 cases), and on the basis of other medical institutions. It was counted as one case when performing both a biopsy and an operation on a patient.

We considered pancreatic fistula to be the drainage of the discharge from 3 days after surgery, in which amylase was increased by 3 times relative to blood amylase. In the study group of 419 patients who underwent radical surgery for pancreatic cancer, pancreatic fistulas developed in 102 patients (24.3 % of cases).

Taking into account the complex anatomical structure of the pancreas and the spatial location of the organ, we have developed an original scanning technique called trans- and cross-pancreatic imaging.

We performed magnetic resonance examination of abdominal organs in T2 sequences to position the pancreas and determine the direction of the pancreatic and common bile ducts. The peculiarity of the protocol was that we used a technique involving a trans and cross-pancreatic T2-weighted sequence, sections of which were laid through the pancreas parallel and perpendicular to the location of the Virsung duct (Fig. 1).

To obtain an optimal image of the pancreas and the Virsung duct, an oblique T2-weighted image with TE in the range of 80–90, TR 9400–9500 was used, the resolution of the matrix was not lower than 320 × 192, while the thickness of the slice should be 3–4 mm, and the distance between the slices was set to 0 mm. The function of starting data collection at coinciding phases of respiration or holding the breath on exhalation was used, the number of slices was sufficient to visualize the main array of pancreatic parenchyma. A T2-weighted image of the pancreas with the main pancreatic (Virsung) duct was obtained.

Then an isovoxel diffusion weight imaging (DWI) with $b = 0 \text{ s/mm}^2$ and $b = 1000 \text{ s/mm}^2$ was diagonally laid in the same way parallel to the Virsung duct. Then, by mathematical summation of polychrome diffusion-weighted images with monochrome T2 images, a FUSION image of the pancreas was obtained at the tomograph workstation using DWI sequence images and a transpancreatic T2 sequence.

The localization of the pancreatic tumor, which often violates the patency of the Virsung duct with its supragenotic expansion caudal to the tumor, the expansion of small ducts, the condition of the alleged pancreatic stump, was determined.

In the concept of "the alleged stump of the pancreas" we put the following. After determining the position of the tumor in the pancreas, we focused on the mesenteric portal trunk. When the tumor was located proximally to the right of it, we analyzed the distal parts of the gland to the left of it (tail and body) as a supposed stump. When the tumor was located distally to the left of the mesenteric-portal trunk as a supposed stump, we analyzed the distal parts of the gland to the right of it (the head of the pancreas).

The domino criterion was used as an additional criterion for the diagnosis of pancreatic cancer and evaluation of its alleged stump. We first developed this criterion for the differential diagnosis of pancreatic tumors, and then, with a retrospective analysis of images, we began to observe it in the perifocal zone. The visualization pattern represents small rounded

hyperintensive in T2 multiple areas of dilated collateral pancreatic ducts against the background of an isointensive tumor in conditions of obstruction of the Virsung duct. The ductal system of the pancreas includes small lobular ducts flowing into the main and additional ducts, hyperintensive in T2, hypointensive in T1 due to the liquid component in their lumen. The domino pattern introduced by us is found, according to our data, in 92 % of cases of adenocarcinomas in the head of the pancreas.

Pancreatic MR spectroscopy technique

Lactate and lipid complex were selected from a wide range of metabolites available for determination under conditions of a magnetic field strength of 1.5 T during preliminary research work. We evaluated both the tumor tissue and the presumed pancreatic stump.

The procedure was carried out as follows: after laying the patient in the supine position on the abdominal coil of the diagnostic table of the tomograph, marking (localizer) and calibration series were carried out. T2-weighted series in three planes crossing the pancreas were performed, and axial T1FS LAVA and DWI ($b = 0$, $b = 1000$) and T1 double gradient echo in phase and antiphase (dual gradient echo in-phase/out-of-phase) allowing to evaluate its anatomy. In the RadiAnt software package, the area of the most pronounced changes was identified (in normal glands, indicators for the head or body of

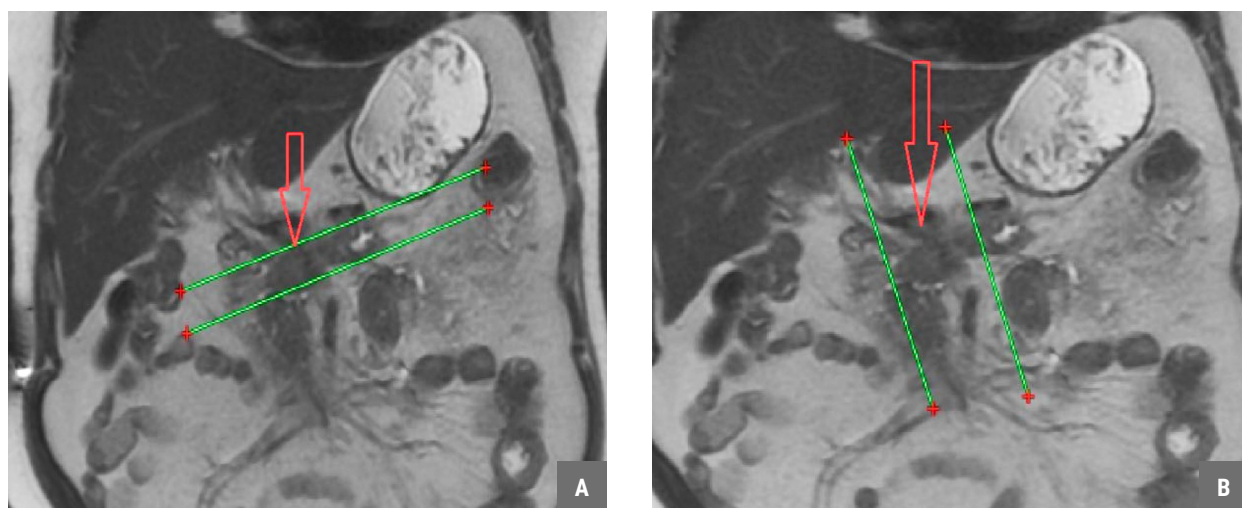


Fig. 1. A – the direction of the slices when planning axial transpancreatic series parallel to the axis of the Virsung duct. The red arrow points to the tumor, the green lines are the boundaries of the most important visualization area. B – the direction of the slices when planning cross-pancreatic series is perpendicular to the course of the Virsung duct. The red arrow points to the tumor, the green lines are the boundaries of the most important visualization area.

the organ were studied). This area was outlined with an oval or round border, the structural heterogeneity coefficient (HC) was calculated as the ratio of the standard deviation value (stdO or SD) to the average signal intensity (SI), according to the formula: $HC = stdO/SI$. The amount of lipids in the dual echo is measured. The lipid content was quantified using the Dixon method. The sequence of double gradient echo in phase and antiphase (dual gradient echo in-phase/out-of-phase) was used for evaluation. The indicators of the signal intensity from the pancreatic parenchyma and spleen in the corresponding zones in the images in phase (SIP) and antiphase (SOP) were used and subsequent calculations using the Dixon formula: $Fat\ fraction = (SIP - SOP) / 2 * (SIP)$, where SIP is the ratio of the pancreatic signal to the spleen signal in the images in phase; SOP-the ratio of the pancreatic signal to the spleen signal in the images in the opposite phase.

Also, for a better diagnosis of the prevalence of the tumor process, proton single-pixel spectroscopy of the same area of changes suspected of cancer was performed. The data was transmitted to a computer with an installed program for processing spectra. The values of the spectrum peaks were determined in the Tarquin program and the value of the lactate signal was measured in atomic units a. u. The value of the accountable diffusion coefficient (ADC) was measured by DWI.

To assess the statistical reliability of the results, criteria were used to assess the significance of differences in outcomes depending on the impact of the inclusion factor of the studied developed techniques: Chi-square criterion, Fisher's exact criterion (two-sided).

STUDY RESULTS AND DISCUSSION

Generalized PC was found in 717 patients, among whom 3 had bone metastases. Lymph node lesion was diagnosed in 302 patients among 419 radically operated. All cases were confirmed morphologically.

According to our study, operations for tumors of the pancreatic head prevailed over corporocaudal resections 254 vs. 165, while biopsies were performed more often for pancreatic body cancer than for the head (386 vs. 287). Moderate differentiation of tumors prevailed in most histological findings.

In our sample, the most patients were from 50 to 79 years old, and among them from 60 to 69 years old – 471 patients (41.46 %).

In the analyzed group, men 295 (25.97 %) were more likely to suffer from pancreatic head cancer than women 246 (21.65 %). When the tumor is localized in the body of the pancreas, women are more often 265 (23.32 %) than men 232 (20.43 %). When the tumor was localized in the tail of the gland, women (71; 6.25 %) significantly prevailed over men (27; 2.37 %) ($\chi^2=18.94$; $p < 0.00001$).

The patients were distributed according to the TNM classification (8th edition), the IV stage of the process prevailed in our study – 585 (51.5 %), the results are shown in Table 1.

A retrospective comparison of preoperative studies was performed to study the diagnostic value of the anatomically oriented series technique: a standard MRI protocol without anatomical orientation of the series (205 cases from 2009 to 2014 inclusive); an MRI protocol using the above technology (from 2014 to 2020 inclusive 214 cases). To prove the accuracy of the study, we used the protocols of

Table1. Distribution of the PC patients according to the TNM classification ($n = 1136$)

TNM	Stage	Patients' number	Patients' share
T2N0M0	IB	25	2.20
T3N0M0	IIA	92	8.1
T1-3N1M0	IIB	128	11.26
T1-3N2M0	III	174	15.32
T4NanyM0	III	132	11.62
TanyNanyM1	IV	585	51.50

operations and the results of histological studies of the surgical material.

The overall accuracy of the technique for diagnosing the prevalence of pancreatic cancer using anatomically oriented series, which was compared with standard methods, intraoperative picture and the results of postoperative morphological examination, reaches 97.5 %, $p < 0.005$.

Subsequently, we applied the developed technique on 205 operated patients, whom we had previously examined using the standard method. Thanks to the proposed method, 20 additional episodes of perineural extrapancreatic invasion were evaluated and described in detail, the nature and degree of contact of the tumor with the main vessels were clarified. Statistical analysis confirms the advantages of the method. The reliability of the advantages of the high accuracy of the method is confirmed by the low sum of false-positive and false-negative results when using anatomically oriented series compared with standard methods in relation to the local prevalence of the tumor. The exact Fisher criterion (two-sided) is 0.03169.

The study of the diagnostic value of determining the degree of local tumor spread by MR spectroscopy, performed by retrospectively comparing preoperative studies using the standard MRI protocol (205 cases from 2009 to 2014 inclusive) and the MRI protocol using the MRI technology developed by us (from 2014 to 2020 inclusive 214 cases) showed an overall accuracy of 98.75 %.

The method of adding MR spectroscopy demonstrates high accuracy in assessing the tumor, surpassing the standard scanning protocol in terms of the main indicators of diagnostic significance (tumor size, vascular and perineural invasion, retropancreatic spread) – overall accuracy, sensitivity and specificity, which is confirmed statistically (Fisher criterion; $p = 0.00917$).

The method of metabolically oriented visualization, determination of the proportion of lipids by chemical shift and lactate peak during spectroscopy requires the presence of a solid bulk formation with a homogeneous structure without cavities of decay, necrosis, sinters with a total size of at least $20 \times 20 \times 20$ mm due to the peculiarities of MR spectroscopy.

In a standard MRI examination of the pancreas, isointensive tissue with a smoothed lobular structure was visualized in 28 patients, which did not allow

unambiguously indicating the presence of a tumor in the pancreas. The calculated diffusion coefficient ADC was in the range of $0.0013\text{--}0.0021\text{ mm}^2/\text{s}$. With MR spectroscopy, Lac values in the range of 850 a.u. indicate active processes of anaerobic glycolysis. According to the measured coefficients of signal intensity and heterogeneity in T2 SI 750; SD 141.4; HC 0.185, according to T1FS SI 611; SD 61.8 HC 0.101. The proportion of lipids according to the Dixon method was in the range of 1.61–1.72 %, which is significantly less than in normal parenchyma and inflammatory changes. Thus, the patients were diagnosed with pancreatic cancer, which was subsequently confirmed by morphological examination of preoperative punctures and the removed tumor.

The technique also made it possible to differentiate formations located outside the tumor in the area of the alleged stump of the gland. Below is an example when a small tumor of the head of the pancreas (14 mm in diameter) and an indistinct formation in its body was diagnosed, which was initially perceived as a metastasis or a second tumor. However, according to the measured signal intensity and heterogeneity coefficients SI T2 445; SD 81; HC 0.182; SI T1FS 674 SD 57 HC 0.084, the proportion of lipids according to the Dixon method is 22.2 %, increased, which is not typical for tumor pathology. With a spectroscopy of Lac 0.0000 a.u. (Fig. 2).

The mentioned above changes were regarded by us as pancreatic steatosis without significant expansion of the Virsung duct, which was confirmed during subsequent surgical treatment during intraoperative revision and ultrasound.

While working on the spectra of pancreatic tissue metabolites in various conditions, we made an interesting observation. The "lipid complex" of Lip 13a peaks at 1.25–1.28 ppm is due to the presence of glycerol and triacyl glyceride analogues. With adenocarcinoma, the average value of this peak reaches 6.9 a.u., with pancreatitis 4.4 a.u. in a normal gland 1.4 a.u.

Literature sources devoted to their significance for the diagnosis of pancreatic adenocarcinoma have not been found at present, however, experience with neuro-oncological spectra demonstrates the presence of this peak in the spectra of cerebral metastases similar to those obtained in the studies of V. Sawlani, M. D. Patel, N. Davies (2020) [16], performed on a magnetic resonance tomograph with

magnetic field intensity fields of 3T. These changes were used by us to study the condition of the perifocal zone of the tumor and the planned stump of the pancreas. To choose surgical tactics already at the preoperative stage, it is necessary to have a judgment about the "tissue density" of the pancreas. Life-threatening complications with pancreatoduodenal resections (pancreatitis of the stump, bleeding, failure of anastomosis sutures) develop just with an "unchanged" gland. Their prevention should be carried out both preoperatively and intraoperatively [11; 17].

Based on the diagnostic techniques we have developed, we have identified a number of new signs,

the determination of which during the assessment of the alleged pancreatic stump in both pancreatoduodenal and distal resections allows us to assume a possible intraoperative situation at the preoperative stage that will affect surgical tactics: the location of the pancreatic duct depending on the resection zone, the nature of drainage of the duct (external or internal), the probability of eruption of the stitches being applied. In order to determine their actual meaning, we compared the data obtained with morphological micro-preparations of the perifocal zone of the tumor and intraoperative macroscopic description of the stump, which we performed together with the surgical team (Table 2).

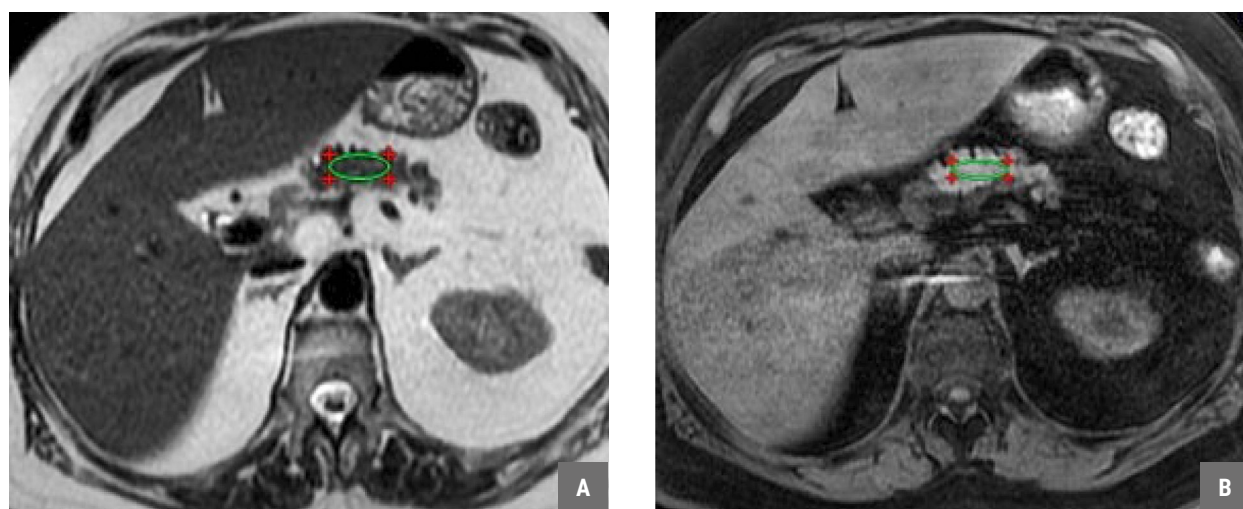


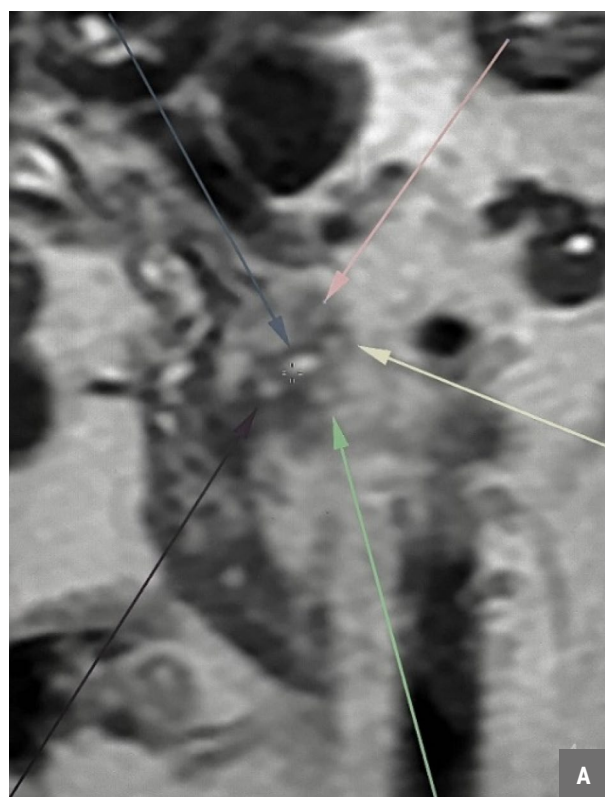
Fig. 2. A – the marker in T2 marks the area of heterogeneity measurement and the voxel placement area for MR spectroscopy; B – the marker in T2 fatsat marks the area of heterogeneity measurement and the voxel placement area for MR spectroscopy.

Table 2. Average values of imaging parameters for various conditions of the pancreatic stump in the studied patients (n = 419)

Indicator	Pancreatic stump		
	Intact tissue (n = 114)	Inflammatory and dystrophic alterations (n = 127)	Fibrous and indurative alterations (n = 178)
Signal intensity in T2	654.71 ± 6.7	690.29 ± 9.8	640.52 ± 8.6
HC value T2	0.0744 ± 0.005	0.1117 ± 0.003	0.0653 ± 0.004
Signal intensity in T1FS	1282.27 ± 10.3	461.40 ± 12.8	1476.11 ± 7.4
The value of HC in T1FS	0.0792 ± 0.003	0.2234 ± 0.04	0.0543 ± 0.005
Diffusion (SD/ADC mm ² /s)	0.00035/0.0015	0.0004/0.0018	0.00027/0.00011
The proportion of lipids, %	11.22	16.47	8.12
Lactate, a.u.	0	0.1	0

Signs of fibrous indurative changes in the putative stump of the pancreas.

Most often, we considered the signs of fibrous indurative changes in the supposed pancreatic stump at the preoperative stage to be positive, since the pancreatic anastomosis formed during the operation was reliable (due to the absence of thread eruption). One of the most characteristic manifestations of fibrosis and induration was the presence of a domino symptom. In cases where the MRI picture fit into the symptom complex of induration, surgeons applied pancreatic anastomoses on the "lost" drainage. Most often, the operator confidently detected an enlarged pancreatic duct, information about the location of which was also obtained during an MRI examination. The duct was sewn into the lumen of the small intestine. The regions of heterogeneous signal were determined in the supposed stump of the pancreas (arrows are marked in Figure 3A). Microscopic examination determines the enlarged pancreatic duct, cystically altered small ducts against the background of fibrosis in the pancreatic tissue (Fig. 3B).



MRI-picture of inflammatory and dystrophic changes in the supposed stump of the pancreas

This symptom complex was characterized by the presence of blurred contours of the alleged pancreatic stump, swelling, inflammatory changes, or the inability to determine its boundaries from the underlying adipose tissue, when visually, when describing an MRI study, the area of interest was perceived by us as "white on white". The "white on white" symptom was more characteristic of dystrophic changes, when the pancreatic tissue itself was replaced by fat cells (Fig. 4).

Later, during the joint intraoperative evaluation with surgeons, we encountered a loose, inflamed gland. The possibility of determining the pancreatic duct in the stump was difficult, which led to the need to form an anastomosis either with the entire section of the gland (23 cases), or when installing drainage into the duct by performing external drainage through the enterostome. These cases were accompanied by the greatest number of complications (38 %). Extremely unfavorable were cases when, during the MRI examination in the preoperative period, the contours

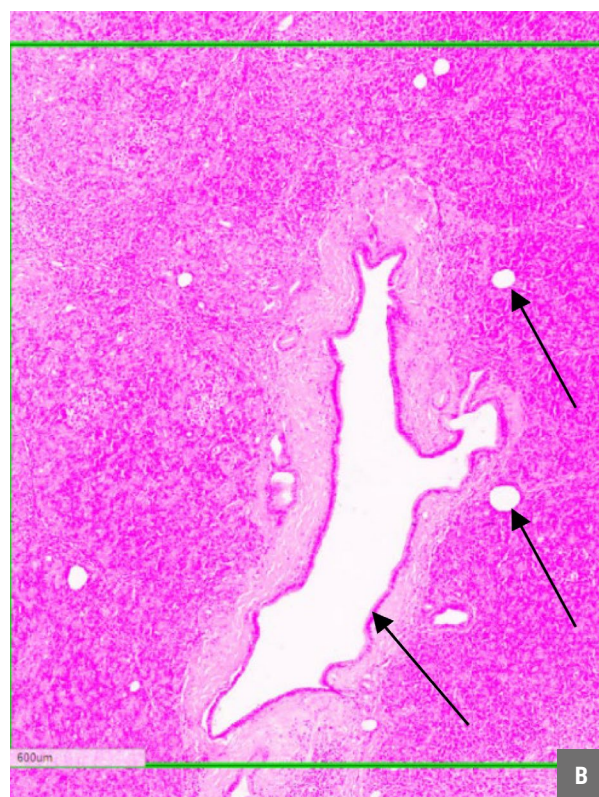


Fig. 3. The "domino" symptom in the perifocal zone of the tumor. In the pancreatic tissue outside the tumor, areas of fibrous tissue with cystic duct expansion are determined. H&E Staining, x 200.

of the gland were not determined, and intraoperative surgeons characterized its density as "soft" during the revision of the pancreas.

Development of a scale for calculating the risk of pancreatic fistula following a radical resections of the pancreas based on MRI data.

In 2013, based on a multicenter study led by M. Callery and including the results of treatment of 445 patients who underwent pancreateo-deodunal resections (PDR), a 10-point prognostic scale for the occurrence of postoperative pancreatic fistula (Fistula Risk Score) was developed and adopted [11]. Risk factors on this scale were the soft texture of the gland, pathomorphological data – ampullary or duodenal cancer, neuroendocrine neoplasia, cystic tumors; pancreatic duct diameter less than 5 mm, intraoperative blood loss more than 400 ml. In 2017, the risk scale was modified by American pancreatologists: the texture of the gland and the diameter of the pancreatic duct remained, and gender, body mass index and bilirubin level were added [17]. All the scales described above can only be used intra-

operatively. However, prevention of postoperative pancreatitis of the stump of the gland and pancreatic fistula should begin in the preoperative period.

When using known scales, risk stratification is possible only intraoperatively, which does not allow the use of preoperative methods for the prevention of fistula formation. This also applies to the appointment of synthetic analogues of somatostatin in the preoperative period, the duration of preoperative preparation. The scale developed by us, including MRI criteria, allows us to predict the course of the postoperative period at the preoperative stage. The MRI-symptom complexes proposed by us (fibrous indurative changes in the pancreatic stump, unchanged pancreatic tissue, inflammatory and dystrophic changes in the pancreatic stump) have a clear morphological confirmation. During the preoperative period, repeated MRI examination is possible to objectify the effectiveness of therapeutic preparation (reduction of the picture of inflammation) and its effect on the structure of the alleged pancreatic stump (Table 3). The scale does not require statistical calculations, which makes

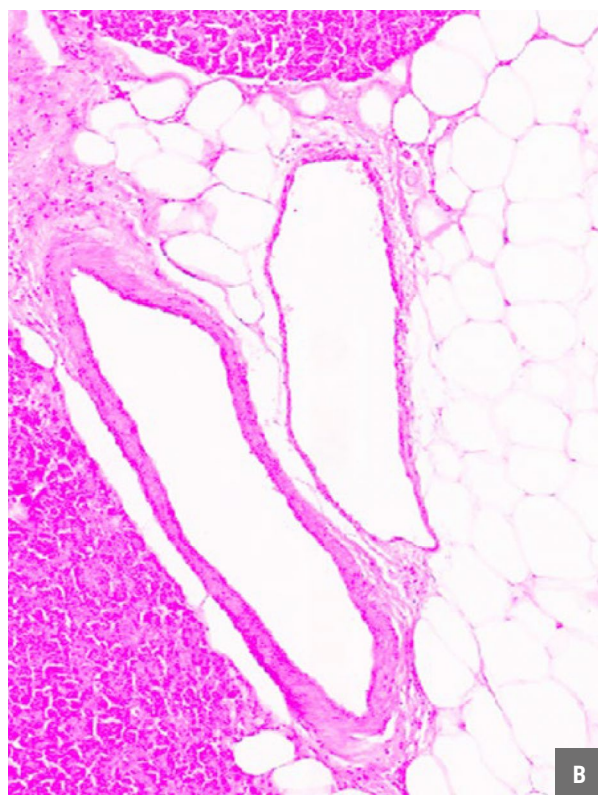
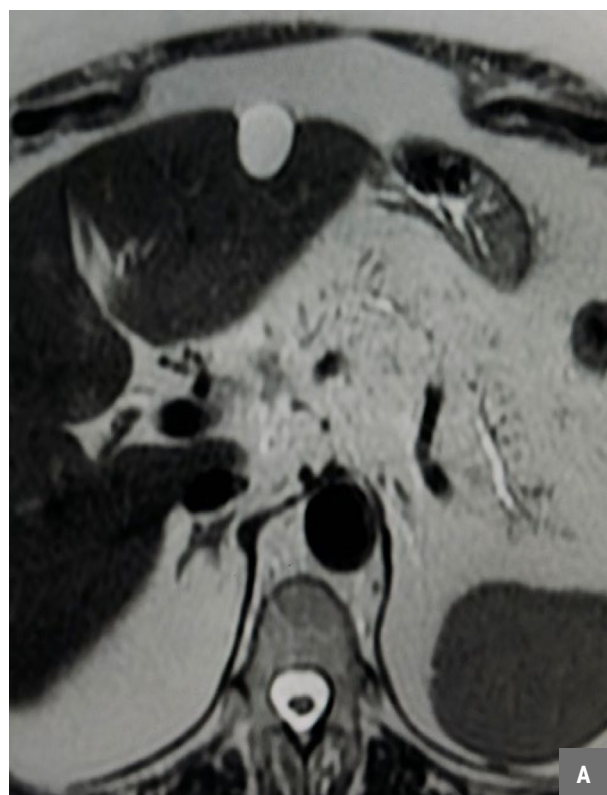


Fig. 4. The "white on white" symptom. There is an almost complete absence of the contours of the pancreas, the tissue of which seems to merge with the surrounding adipose tissue. H&E Staining, × 200.

it available for use. The experience of using such scales shows that their popularity among practitioners is due to the lack of complex mathematical calculations, and the results obtained are understandable.

To predict the possible development of pancreatic fistula retrospectively, we applied 3 risk scales for each patient. At the same time, in the case of calculation on the FRS scale, prognosis mismatch occurred in 52 patients (12.41 %), on the modified FRS scale – in 45 (10.74 %), and on the MRI risk assessment scale – in 48 (11.45 %) patients. In the future, we analyzed each specific case of forecast mismatch. When using the FRS scale, fistulas did not develop in 15 (3.58 %) patients, although they were predicted, in 37 (8.83 %) they developed, although

they were not predicted; with modified FRS – 13 (3.1 %) and 32 (7.66 %), respectively. In the case of using MRI for prediction, 31 (7.4 %) patients did not develop fistulas, although they were predicted, 17 (4.06 %) developed fistulas, but were not predicted. Since we were more interested in the sensitivity of the calculation in favor of predicting a severe complication, analyzing the above figures, the risk calculation turned out to be more accurate when using MRI readings: with the first two scales, the discrepancy was 37 (8.83 %) and 32 (7.64 %), with the third – 17 (4.06 %). Further statistical analysis showed a significant difference (criterion χ^2) when comparing the first (FRS) and third (MRI) scales ($p = 0.0477$), and a trend when comparing the second (modified FRS) and third (MRI) scales ($p = 0.0544$).

Table 3. Modified risk scale for the development of pancreatic fistula according to the results of preoperative studies

Risk factor		Points
Sex	male	2
	female	0
Body Mass Index, kg/m ²	< 25	0
	≥ 25	1
Total bilirubin, mcMol/L	< 20	0
	≥ 20	1
Pancreatic duct diameter identified with the use of MRI, mm	< 3	4
	3–6	3
	≥ 6	0
The texture of the parenchyma of the gland, determined by MRI	Pancreatic stump fibrous and indurative alterations	0
	Intact pancreatic tissue	1
	Inflammatory and dystrophic alterations in the pancreatic stump	2
Risk, summary of points	minimal	0–2
	low	3–6
	intermediate	7–8
	high	9–10

CONCLUSION

The scale developed by us with the help of additional MRI criteria based on a clearer description of the perifocal zone of the tumor and the entire

pancreatic tissue can be used for the subsequent selection of surgical treatment methods, which generally reduces the number of surgical complications by reducing the number of pancreatic fistulas.

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Kit O. I. – study concept, conclusions;

Maksimov A. Yu. – material processing, text revision and scientific editing.

FEATURES OF BLOOD PARAMETERS AND ADAPTATIONAL STATUS OF BALB/C AND C57BL/6 MICE LINES IN THE ABSENCE OF SPECIAL INFLUENCES

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ABSTRACT

Purpose of the study. A comparative analysis of blood parameters and some characteristics of the adaptation status of intact Balb/c and C57Bl/6 mice of both sexes.

Materials and methods. We investigated intact mice of both sexes belonging to the C57Bl/6 ($n = 18$) and Balb/c ($n = 20$) lines. The age characteristics of these animals corresponded to the first half of the reproductive period. We studied the parameters of the complete and biochemical blood tests, the weight characteristics of the thymus, spleen and adrenal glands. The character and tension of general nonspecific adaptational reactions of the body (AR) were assessed as well. In statistical analysis we used the coefficient of variation (CV), Student's t-test, Mann-Whitney test.

Results. In mice of both studied lines, the dominance of females over males was noted in terms of the weight characteristics of the thymus and spleen, the development of the most favorable antistress AR, and the number of indicators with low variability. At the same time, in C57Bl/6 mice, animals of different sexes had a similar nature of AR (AR of elevated activation), but differed in signs of tension, this might indicate the difference in the range of levels of reactivity, appropriate to AR in males and females C57Bl/6. Unlike C57Bl/6, mice Balb/c mice of different sexes were distinguished with the predominant antistress AR. Differences between C57Bl/6 mice and Balb/c mice in terms of amylase and ALT activity indicated a shift towards carbohydrate metabolism in Balb/c mice and a shift towards protein metabolism in C57Bl/6 mice. Animals of the C57Bl/6 line had an advantage over Balb/c mice (especially pronounced in females) in some indicators of the adaptation status.

Conclusion. The results of the study indicated possible difference in the ratio of carbohydrate and protein metabolism in the animals of the studied lines and testified a more favorable state of the regulatory systems in C57Bl/6 mice compared to animals of the Balb/c line. The revealed regulatory and metabolic interlinear differences can determine the features in the reaction of the body of animals belonging to different lines to the malignant process and efficiency of antitumor therapy.

Keywords: hematological parameters, adaptation status, antistress adaptational reactions, metabolism

For citation: Zhukova G. V., Frantsiyants E. M., Shikhlyarova A. I., Kaplieva I. V., Trepitaki L. K., Kachesova P. S., Galina A. V., Ushakova N. D., Shalashnaya E. V., Ishonina O. G. Features of blood parameters and adaptational status of Balb/c and C57Bl/6 mice lines in the absence of special influences. South Russian Journal of Cancer. 2023; 4(4): 44-56. <https://doi.org/10.37748/2686-9039-2023-4-4-5>, <https://elibrary.ru/hogdam>

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Compliance with ethical standards: work with animals was carried out in accordance with the rules of the «European Convention for the Protection of Animals Used for Experimental and other Scientific Purposes» (Directive 2010/63/EU), as well as in compliance with the «International Recommendations for Biomedical Research Using Animals» and Order of the Ministry of Health of Russia No. 267 of June 19, 2003 «On Approval of the Rules of Laboratory Practice». The study was approved by the Ethics Committee of the National Medical Research Centre for Oncology (Protocol No. 11/115 of 03/01/2021).

Funding: this work was not funded.

Conflict of interest: the authors declare that there are no obvious and potential conflicts of interest associated with the publication of this article.

The article was submitted 25.05.2023; approved after reviewing 25.10.2023; accepted for publication 09.12.2023.

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ОСОБЕННОСТИ ПОКАЗАТЕЛЕЙ КРОВИ И АДАПТАЦИОННОГО СТАТУСА МЫШЕЙ ЛИНИЙ BALB/C И C57BL/6 ПРИ ОТСУТСТВИИ СПЕЦИАЛЬНЫХ ВОЗДЕЙСТВИЙ

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РЕЗЮМЕ

Цель исследования. Сравнительный анализ показателей крови и некоторых характеристик адаптационного статуса интактных мышей линий Balb/c и C57Bl/6 обоего пола.

Материалы и методы. Исследования проводили у интактных мышей обоего пола линий C57Bl/6 ($n = 18$) и Balb/c ($n = 20$). Возрастные характеристики животных соответствовали первой половине репродуктивного периода. Изучали показатели общего и биохимического анализа крови, весовые характеристики тимуса, селезенки и надпочечников. Оценивали характер и напряженность общих неспецифических адаптационных реакций организма (АР). При статистическом анализе использовали коэффициент вариации (CV), t-критерий Стьюдента, критерий Манна-Уитни.

Результаты. У мышей обеих изученных линий было отмечено доминирование самок над самцами по весовым характеристикам тимуса и селезенки и частоте развития наиболее благоприятной антистрессорной АР повышенной активации. Вариабельность изученных показателей в целом у самок была ниже, чем у самцов. При этом у мышей линии C57Bl/6 животные разного пола имели сходный характер АР (АР повышенной активации), но отличались признаками напряженности этой реакции, указывающими на разный диапазон уровней реактивности, на которых развивалась эта АР у самцов и самок. У мышей линии Balb/c животные разного пола различались характером преобладавших антистрессорных АР. Сравнение биохимических показателей крови самок разных линий указывало на более активный углеводный обмен у мышей линии Balb/c и более активный белковый обмена – у мышей линии C57Bl/6. Животные линии C57Bl/6 имели преимущество над мышами Balb/c (особенно выраженное у самок) по некоторым показателям адаптационного статуса.

Заключение. Результаты исследования указывали на возможное различие в соотношении углеводного и белкового обмена у самок Balb/c и C57Bl/6 и свидетельствовали о более благоприятном состоянии регуляторных систем у мышей линии C57Bl/6 по сравнению с животными линии Balb/c. Выявленные регуляторные и метаболические межлинейные различия могут обусловить особенности в реакции организма животных, принадлежащих к разным линиям, на злокачественный процесс и эффективность противоопухолевой терапии.

Ключевые слова: гематологические показатели, адаптационный статус, антистрессорные адаптационные реакции, метаболизм

Для цитирования: Жукова Г. В., Франциянц Е. М., Шихлярова А. И., Каплиева И. В., Трепитаки Л. К., Качесова П. С., Галина А. В., Ушакова Н. Д., Шалашная Е. В., Ишонина О. Г. Особенности показателей крови и адаптационного статуса мышей линий Balb/c и C57Bl/6 при отсутствии специальных воздействий. Южно-Российский онкологический журнал. 2023; 4(4): 44-56. <https://doi.org/10.37748/2686-9039-2023-4-4-5>, <https://elibrary.ru/hogdam>

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Соблюдение этических стандартов: работу с животными проводили в соответствии с правилами «Европейской конвенции о защите животных, используемых в экспериментах» (Директива 2010/63/EU), а также в соответствии с «Международными рекомендациями по проведению медико-биологических исследований с использованием животных» и Приказом Минздрава России от 19 июня 2003 г. № 267 «Об утверждении правил лабораторной практики». Исследование одобрено этическим комитетом ФГБУ «НМИЦ онкологии» Минздрава России (протокол № 11/115 от 01.03.2021 г.).

Финансирование: финансирование данной работы не проводилось.

Конфликт интересов: все авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Статья поступила в редакцию 25.05.2023; одобрена после рецензирования 25.10.2023; принята к публикации 09.12.2023.

INTRODUCTION

Research on animal models is aimed at identifying the fundamental mechanisms of carcinogenesis and experimental substantiation of new drugs and methods of treatment. At the same time, there is a need to identify and separate universal patterns and features of tumor and organism reactions due to molecular genetic characteristics, the state of regulatory systems of specific individuals [1; 2], sex [3–5] and age of animals [6; 7], their linear affiliation [5; 8]. Mice of the Balb/c and C57Bl/6 lines are among the laboratory rodents most widely used in biomedical research, including preclinical trials of new drugs and technologies for antitumor treatment [1; 9]. There is information about several anatomical, reproductive and behavioral features of these animals that need to be taken into account when conducting experimental studies [10–12]. At the same time, the question of interlinear differences in the functioning of their regulatory systems and adaptive status remains little studied, which, considering the different stress resistance of these animals [1; 13; 14] and their highly probable origin from different subspecies of mice *Mus musculus* [15; 16], seems very important in the development of effective methods of antitumor effects and the identification of universal pathogenetically significant processes. The relevance of this issue is enhanced due to the rather long existence of the experimental animal lines under consideration, since an increase in the number of closely related crosses could lead to an increase or, conversely, to a weakening of the previously known immunological and physiological characteristics of mice of these lines [15].

The informative value of biochemical and cytological indicators of blood for the general assessment of the state of the body, as well as the correlation of the adaptive status with the processes in the organs of the immune and neuroendocrine systems [17; 18] allows the use of hematological indicators and shifts in the weight characteristics of internal organs to objectify the results of studies and identify universal systemic rearrangements under the action of damaging factors. One of the stages of solving these problems is a comparative analysis of indicators reflecting the state of regulatory systems, the adaptive status and features of the metabolism of animals of these lines in the conditions of the physiological norm.

Purpose of the study was the comparative analysis of blood parameters and some characteristics of the adaptive status of intact Balb/c and C57Bl/6 mice of both sexes.

MATERIALS AND METHODS

The parameters characterizing the condition of intact mice of both sexes belonging to the C57 Black/6 ($n = 18$) and Balb/c ($n = 20$) lines were studied. We used animals of our own breeding vivarium of NMRC for Oncology, Russian Ministry of Health, originally purchased from the Rappolovo nursery. Each of the four groups (control and main groups of animals of the two studied lines) included 9–10 animals. Their age characteristics corresponded to the first half of the reproductive period. According to the known dependencies between weight and life expectancy of laboratory rodents of various lines [9; 19] the age of mice of the C57Bl/6 line was 9–12 weeks, and in mice of the Balb/c line this indicator corresponded to 9–16 weeks. The differences in life expectancy were random in nature, were due to the limited choice of mice with matching weight characteristics, the possibilities of their own breeding, and were not critical of their belonging to the category of young animals capable of intensive reproduction [9].

To assess the features of the general condition and adaptive status of intact animals of different lines, a study of the complete blood count test (CBC) and biochemical blood analysis, as well as the weight characteristics of the thymus, spleen and adrenal glands (weight and weight coefficients of these organs) was carried out. At the same time, special attention was paid to the percentage of lymphocytes in the blood, the mass and weight coefficients of the thymus and adrenal glands, as well as to the ratio of the masses of these organs as indicators of the nature and intensity of the general nonspecific adaptive reactions of the AP organism [17; 18]. Necropsy, isolation and weighing of organs of animals subjected to euthanasia by decapitation were performed. The weight coefficient of the organ was the ratio of the mass of the organ to the mass of the animal in grams multiplied by 10^4 . To determine the blood parameters obtained after euthanasia, the Exigo EOS vet hemoanalyzer (Boule Medical A. B., Sweden) and the VetScanVS2 biochemical analyzer (ABAXIS Inc., Germany) were

used. At the same time, in the case of C57Bl/6 mice, biochemical blood parameters were determined only in females (due to technical difficulties that prevented the determination of these indicators in males). Statistical processing of the results of the study was carried out using the software package Statistica 10.0. The range of variations of the studied indicators, their medians, averages and errors of averages, as well as the coefficient of variation (CV) were determined. The Student's t-test (with a normal distribution of indicator values) and the nonparametric Mann-Whitney test were used to evaluate statistical differences in the variation series.

STUDY RESULTS

As a result of a comparative analysis of the studied hematological parameters, weight characteristics of the immune system and adrenal glands, as well as the ratio of thymus and adrenal mass in animals of both sexes, the peculiarities of the nature and severity of sexual differences, as well as several interlinear differences in the value of individual indicators in mice of the same sex were revealed. Table 1 shows the indicators that had sex differences in the animals of the studied lines.

Earlier we've discussed sex differences in cytological and biochemical blood parameters and weight characteristics of the internal organs of the immune system and adrenal glands of intact Balb/c mice [20] (Table 1). In these animals, with close values of hemoglobin levels in the blood, there were sex differences in the number of red blood cells and their saturation with hemoglobin. At the level of the weight characteristics of the studied organs, the differences between female mice and male mice of this line consisted in a slightly more active proliferation in the thymus (revealed by the weight of the organ, taking into account the slightly larger age of female mice than males), noticeably larger adrenal glands (which was a well-known constitutional feature of female mice of the Balb/c line) [1] and the spleen (Table 1). These differences, along with the distinctive features of blood biochemical parameters and a noticeably higher percentage of lymphocytes, allowed us to make an assumption about a more pronounced activation of the T-cell link of immunity and a higher adaptive status of females, the development of AR increased activation in them. As is known, it is this

AR that is distinguished by the most noticeable increase in the activity of regulatory systems and the level of nonspecific resistance of the body [18]. At the same time, the combination of signs observed in male mice could indicate the development of AR training and quiet activation [2; 18] and a relatively higher activity of the B-cell link and neutrophils compared to that observed in females (a shift of the albumin-globulin index towards globulins, higher than in females, the activity of alkaline phosphatase, capable of influencing the state of B-lymphocytes and neutrophils) [21; 22].

As can be seen from Table 1, in mice of the C57Bl/6 line, similar to that noted for animals of the Balb/c line, there were sexual differences in the weight coefficient of the spleen and adrenal glands, as well as in the saturation of erythrocytes with hemoglobin. The relative weight of the spleen of female mice of both lines was at least 1.5 times higher than this indicator in males, while the differences in the other two indicators had the opposite direction. Thus, females of the Balb/c line differed from males by larger adrenal glands (a well-known constitutional feature of these animals) [1] and a lower average hemoglobin level in the erythrocyte. In contrast, in female mice of the C57Bl/6 line, the relative weight of the adrenal glands was lower, and the saturation of erythrocytes with hemoglobin was slightly higher than in males (Table 1).

In addition, male mice of the C57Bl/6 line differed from females with a higher platelet count and larger adrenal glands. At the same time, the weight coefficients of the thymus and the ratio of the mass of the thymus and adrenal glands of females significantly exceeded these indicators in males (1.9 and 2.8 times, respectively), while the percentage of lymphocytes in both cases corresponded to the maximum values of the reference interval for this line and had no sex differences (Table 1). Thus, in animals of the C57Bl/6 line, the dominance of females over males in terms of thymus weight characteristics was expressed more clearly than in mice of the Balb/c line, which could reflect a higher level of lymphoproliferative activity in the thymus of females.

Table 2 shows the indicators that differ in male mice of the studied lines. Males of C57Bl/6 were superior to same-sex mice of the Balb/c lineage in terms of adrenal gland weight, leukocyte level and percentage of lymphocytes in the blood. The

Table 1. Blood counts and weight characteristics of internal organs that differ in mice of the C57Bl/6 and Balb/c line of different sexes. ($X_{av.} \pm m$, $Me [X_{min} - X_{max}]$, CV %)

Parameter	Male	Female
C57Bl/6	Thymus weight 35.2 ± 5.7 32 [23–59] 32.5 % n = 9	56.2 ± 7.7 [†] p = 0.064 58 [41–79] 27.3 % n = 9
	Thymus weight coefficient 14.4 ± 2.4 13.1 [9.6–19.6] 33 %	27.6 ± 3.4* p = 0.016 27.3 [20–38.5]* p < 0.01 24.9 %
	Adrenal glands weight 18.1 ± 1.1 17 [16–21] 12.4 %	10.5 ± 0.7* p = 0.00006 11 [8.5–12]* p < 0.01 12.6 %
	Adrenal glands weight coefficient 7.5 ± 0.8 6.9 [5.9–9.5] 21.0 %	5.2 ± 0.2* p < 0.001 5 [4.6–5.8]* p < 0.01 8.8 %
	Spleen weight 81.6 ± 4.0 80 [71–91] 9.8 %	102.4 ± 7.1* p = 0.038 99 [88–126]* p < 0.05 14.3 %
	Spleen weight coefficient 33.4 ± 2.2 32.3 [27.8–38.3] 12.9 %	51.3 ± 6.4* p = 0.03 46.8 [42.9–74.1]* p < 0.01 25.1 %
	Thymus mass/adrenals mass 1.9 ± 0.4 1.6 [1–3.1] 42.8 %	5.4 ± 0.55* p < 0.001 5.1 [4.8–7.2]* p < 0.01 20.6 %
	Mean Corpuscular Hemoglobin (pg) 17 ± 0.3 16.8 [16.6–17.9] 3.2 %	18.6 ± 0.4* p = 0.015 18.5 [17.3–19.7]* p = 0.01 4.7 %
	Platelets' count. (10 ⁹ /n) 994 ± 18.5 1006 [943–1030] 3.7 %	735 ± 77.5* p = 0.014 755 [515–905]* p < 0.01 21.1 %
	Thymus weight 28.4 ± 5.3 30 [13–42] 37.3 % n = 10	4.1 ± 5.7 [†] p = 0.098 43 [24–68] 35.7 % n = 10
	Thymus weight coefficient 10.1 ± 1.6 9.1 [5.8–14.2] 32.8 %	13.3 ± 2.1 13.2 [8.5–24.7] 41.7 %
	Adrenal glands weight 14.2 ± 1.7 15 [10–19] 24.1 %	28.9 ± 1.9* p = 0.00003 28 [21–32]* p < 0.01 18.4 %
	Adrenal glands weight coefficient 5.1 ± 0.23 5.1 [4.4–5.9] 13.5 %	9.0 ± 0.6* p = 0.00003 8.3 [7.6–11.8]* p < 0.01 16.8 %
	Spleen weight 84.2 ± 14 94 [39.4 – 130] 50.0 %	156 ± 5.0* p = 0.0002 154 [131–179]* p < 0.01 9.5 %
Balb/c	Spleen weight coefficient 29.4 ± 4.0 36.9 [14.3–39.3] 40.8 %	48.8 ± 1.9* p = 0.00003 49.7 [39.7–54.2]* p < 0.01 10.3 %
	Erythrocyte count (10 ⁹ /n) 5.57 ± 0.42 5.8 [3.9–6.4] 22.6 %	6.9 ± 0.42* p = 0.042 7.3 [3.8–8.2]* p < 0.01 18.4 %
	Mean Corpuscular Hemoglobin (pg) 21.8 ± 0.8 21.4 [19.9–25.2] 11.0 %	19.5 ± 0.7* p = 0.048 18.8 [17.5–25.5]* p < 0.01 11.2 %
	Lymphocytes, % 69.6 ± 4.0 71.4 [49.4–84.7] 17.2 %	83.6 ± 1.33* p = 0.0050 84.2 [77.4–87.7]* p < 0.01 5.0 %
	Monocytes, % 13.3 ± 1.7 12.9 [6.5–20.5] 38.3 %	7.24 ± 0.50* p = 0.0041 7.0 [5.4–10.2]* p < 0.01 20.6 %

Note: * – it is statistically significantly different from the indicators in male mice; [†] – is the difference from the indicator in male mice at the tendency-like levels (p < 0.1).

hemoglobin content in the red blood cells of males C57Bl/6 was less significant compared to Balb/c males and was compensated by a higher number of red blood cells in the blood of these animals, which caused a tendency to a higher hemoglobin level in these animals compared to Balb/c males (Table 2).

Attention was drawn to the lower variability of a number of indicators in males of the C57Bl/6 line compared with Balb/c mice of the same sex. Thus, all three characteristics of the erythrocyte blood germ and the relative content of lymphocytes in males C57Bl/6 had CV values below 10 %. At the same time, the percentage of lymphocytes in these animals, similar to that noted earlier for Balb/c females [20], was practically constant (CV % = 2.2 %, Table. 2), was in the range of the maximum values of this indicator in animals of the considered line [10] (Table. 2) and could indicate the development of AR increased activation in these males [18].

Data on the interline differences in the indicators of female mice of the studied lines are presented in Table 3. It should be noted that in females these differences were expressed to a greater extent than in males. Thus, female C57Bl/6 mice were distinguished by a significantly larger thymus and significantly smaller adrenal glands than Balb/c females. As a result, the ratio of thymus and adrenal gland mass in C57Bl/6 females was 3.4 times higher than this indicator in Balb/c female mice (Table 3).

In addition, in C57Bl/6 females, significantly higher values were also noted for such indicators as the level of leukocytes in the blood, as well as the activity of the enzymes alanine aminotransferase (ALT) and alkaline phosphatase (1.4 and 2.7 times, respectively). At the same time, the indicator of amylase activity in the blood of these animals was almost 2 times lower than in female mice of the Balb/c line (Table 3). The interlinear differences in the activity of amylase and ALT could indicate the distinctive

Table 2. The studied indicators differ in male mice of the lines C57Black/6 and Balb/c. ($\bar{X}_{av} \pm m$, Me [$X_{min} - X_{max}$], CV %)

Parameters	Balb/c, n = 10	C57Bl/6, n = 9
Adrenal glands weight coefficient	5.1 ± 0.34 5.1 [4.4–5.9] 13.4 %	7.5 ± 0.8* $p = 0.0280$ 6.9 [5.9–9.5]* $p < 0.01$ 21.0 %
Blood hemoglobin levels (g/L)	119.7 ± 6.8 120 [89–138] 13.9 %	135.2 ± 3.3 [†] $p < 0.07$ 136 [128–143] 4.9 %
Erythrocyte count (10 ⁹ /L)	5.57 ± 0.42 5.8 [3.9–6.4] 18.4 %	7.9 ± 0.3* $p = 0.0014$ 8.2 [7.2–8.5]* $p < 0.01$ 6.9 %
Mean corpuscular hemoglobin (pg)	21.8 ± 0.8 21.4 [19.9–25.2] 8.6 %	17 ± 0.3* $p = 0.00033$ 16.8 [16.6–17.9]* $p < 0.01$ 3.2 %
Leukocyte count (10 ⁹ /L)	4.8 ± 0.5 5.3 [3–6] 27.6 %	10.7 ± 1.0* $p = 0.0005$ 10.8 [8.2–12.2]* $p < 0.01$ 18.5 %
Relative lymphocyte count, %	69.6 ± 4.8 71.4 [49.4–84.7] 17 %	85.8 ± 1.0* $p = 0.0092$ 86.3 [83–87.8]* $p < 0.01$ 2.2 %
Relative monocyte count, %	13.3 ± 2.02 12.9 [6.5–20.5] 37.3 %	6.5 ± 0.4* $p = 0.0092$ 6.2 [5.8–7.6]* $p < 0.01$ 12.5 %

Note: * – statistically significantly differs from the indicators in male mice of the Balb/c line; [†] – differs from the indicators in male mice of the Balb/c line at the trend level, $p < 0.1$.

features of metabolism in Balb/c and C57Bl/6 mice – a higher level of carbohydrate metabolism in female Balb/c mice and a shift towards protein metabolism in C57Bl/6 animals.

In female mice of the studied lines, stable (CV 3.1 and 5.0 %, respectively, in animals of the C57Bl/6 and Balb/c lines) values close to the maximum and almost identical values of the percentage of lymphocytes in the blood (84.0 and 83.6 %, respectively) were observed, which could indicate the development of AR in these animals increased activation [2; 18]. At the same time, as in the case of comparing males of different lines, attention was drawn, in general, to the lower variability of indicators in animals of the C57Bl/6 line (Table 3). Thus, in the females of this line, the CV values of adaptive status indicators did not exceed 25 %, whereas in the same-sex mice of the Balb/c line with them, 3 of the 7 indicators presented in Table 3 differed in high variability (CV 41.7–56 %).

DISCUSSION

As it is known, the adaptive status of humans and animals can be assessed by determining the nature and intensity of AR, which have an integral character and reflect the state of the regulatory systems of the body [2; 17; 18]. The percentage of lymphocytes in the blood, the weight coefficients of the organs of the immune and endocrine systems, the ratio of the masses of the thymus and adrenal glands allowed us to give an approximate assessment of the adaptive status of the animals of the studied lines. The maximum for this line and stable values of the percentage of lymphocytes in C57Bl/6 mice (CV 2.2 and 3.1 %, respectively, in males and females) indicated the development of AR increased activation in animals of both sexes. At the same time, a significantly larger thymus in females and at the same time noticeably smaller adrenal glands compared to these indicators in males could indicate a higher level of reactivity

Table 3. The studied indicators, differing in female mice C57Bl/6 and Balb/c. ($X_{av.} \pm m$, $Me [X_{min} - X_{max}]$, CV %)

Parameters	Balb/c, $n = 10$	C57Bl/6, $n = 9$
Thymus Weight Coefficient	13.3 $\pm$ 2.1 13.2 [8.5–24.7] 41.7 %	27.6 $\pm$ 3.4* $p = 0.016$ 27.3 [20–38.5]* $p < 0.01$ 24.9 %
Adrenals Weight Coefficient	9.0 $\pm$ 0.6 8.3 [7.6–11.8] 18 %	5.2 $\pm$ 0.2* $p = 0.001$ 5.0 [4.6–5.8]* $p < 0.01$ 8.8 %
Thymus Weight / Adrenals Weight	1.6 $\pm$ 0.3 1.6 [0.6–3.2] 56 %	5.4 $\pm$ 0.55* $p = 0.000016$ 5.1 [4.8–7.2]* $p < 0.01$ 20.6 %
Leukocyte count ($10^9/L$)	3.8 $\pm$ 0.54 3.35 [1.8–7] 42.6 %	10.3 $\pm$ 1.1* $p = 0.000071$ 10.5 [7.6–13]* $p < 0.01$ 22.4 %
Alkaline phosphatase	53.44 $\pm$ 2.63 55 [47 – 61] 13.9 %	104 $\pm$ 11* $p = 0.00095$ 100 [83–123]* $p < 0.01$ 21.2 %
ALT	31.8 $\pm$ 2.3 32 [21 – 44] 20.6 %	46.4 $\pm$ 3.8* $p = 0.0072$ 49 [38–56]* $p < 0.01$ 16.6 %
Amilase	1124 $\pm$ 72 1098 [875–1502] 19 %	586 $\pm$ 33* $p = 0.00003$ 606 [495–662]* $p < 0.01$ 11 %

Note: * – statistically and significantly differs from the indicators in male mice.

compared to males, at which AR increased activation developed in females of the C57Bl/6 line [2; 18]. As is known, the concept of the level of AR reactivity is associated with the previously identified periodic pattern of the development of these reactions, which consists in the dependence of the nature and intensity of AR on the absolute and relative magnitude of the effect causing them [18]. It has been shown that as the intensity of the active factor increases or decreases, the AR tetrads (AR of training – AR of calm activation – AR of increased activation – AR stress) naturally repeat. At the same time, the same-named ARS of different levels of reactivity caused by effects of different intensity, along with similar features (percentage of lymphocytes, ratio of effects of gluco- and mineralocorticoid hormones, etc.), have differences in the severity of signs of tension in the functioning of the regulatory systems of the body. At the same time, the most physiological antistress AR, which most contribute to the activation of systemic mechanisms of nonspecific resistance, develop in response to low-intensity effects of various nature, that is, at high levels of reactivity of the body.

The lower levels of reactivity at which AR increased activation developed in males of the C57Bl/6 line, compared with the reactivity levels of the same AR in females, could be due to the psychosomatic state formed in males upon reaching puberty and causing their aggressive behavior. As is known, this behavior is accompanied by activation of ergotropic processes, an increase in the level of catecholamines and testosterone in blood and tissues, as well as a more significant level of cellular energy [23; 24].

As previously described, Balb/c mice showed sex differences in the percentage of lymphocytes and some other indicators [20]. Based on the totality of these changes, it was possible to judge the sexual differences in the adaptive status associated with the nature of AR – the development of AR increased activation in females and the development of AR training and quiet activation, including signs of tension of these AR – in males. As already mentioned earlier, such differences in the nature of AR in animals of different sexes could be due to the dependence of the psychosomatic state of males on their social status in the group, and could also reflect the presence of any other constitutional features associated with the reduced stress resistance of males of the Balb/c line compared to females [1; 5; 25].

A characteristic feature of the animals of both studied lines was a very significant excess of the weight coefficient of the spleen of females of a similar indicator in males. Unfortunately, we were unable to find a detailed explanation of this sexual difference in the literature, except for information about the more significant functional activity of the spleen of female Balb/c lines than in males [10; 26]. Another common feature of the animals of the studied lines was the presence of signs indicating more pronounced lymphoproliferative activity in the thymus of females compared to what was observed in males. These results are consistent with the ideas available in the literature about the different ratio of the activity of T- and B-cell processes in animals of different sexes – a shift in the ratio towards T-cell mechanisms in females and a shift in the ratio towards B-cell processes in males [5; 27; 28].

The results of comparing the studied indicators in same-sex animals of the C57Bl/6 and Balb/c lines indicated a greater severity of interline differences in females compared to males. In addition to constitutional differences in the total content of leukocytes, the males of the studied lines had features in the percentage of mononuclears (lymphocytes and monocytes) in the blood, as well as in the characteristics of the red blood germ. In our opinion, these features could reflect a higher adaptive status of animals of the C57Bl/6 line, which are known to be more stress-resistant compared to mice of the Balb/c line [1; 13; 14]. At the same time, the lower variability of indicators in male mice of the C57Bl/6 line could be due to the fact that all these animals developed AR increased activation, whereas in males of the Balb/c line the spectrum of antistress AR was wider.

The interlinear differences revealed in females also indicated a higher adaptive status of animals of the C57Bl/6 line. At the same time, the maximum values of the percentage of lymphocytes in the blood of females of both lines in combination with the dominance of mice of the C57Bl/6 line over animals of the Balb/c line in the weight characteristics of the thymus and the ratio of thymus and adrenal masses indicated that it was found that in females of the C57Bl/6 line, the development of AR increased activation occurred in the range of higher levels of reactivity of the organism than in Balb/c females. Moderate and low variability of indicators in mice of the C57Bl/6 line could reflect a high degree of

coincidence of the reactivity levels of these animals within the range, whereas high variability of indicators in females of the Balb/c line could, on the contrary, indicate noticeable differences in the reactivity levels at which AR increased activation developed in female mice of this line. It is likely that differences in the ranges of AR reactivity levels of females C57Bl/6 and Balb/c could to some extent affect the stress resistance of animals of the studied lines.

The lower variability of adaptive status indicators in mice of both sexes of the C57Bl/6 line compared to the variability of these indicators in animals of the Balb/c line could reflect the presence of more stable regulatory relationships in mice of the C57Bl line/6. According to the nonlinear dynamics of complex open (dissipative) systems, which include biological systems, such stable states are characterized by optimization of regulatory and metabolic processes, which ensures an energy-efficient mode of their functioning [29; 30].

The results of a comparative analysis of the biochemical parameters of the blood of female mice of the C57Bl/6 and Balb/c lines are of interest. They point to the peculiarities of the metabolism of the studied animals, which may be important for the reaction of mice of different lines to antitumor therapy. Thus, the interlinear differences in the activity of ALT and amylase could reflect the peculiarities of the ratio of carbohydrate and protein metabolism in animals belonging to different lines. It is known that carbohydrate metabolism differs the fastest. At the same time, the brain, characterized by a high intensity of energy metabolism and depleted of glucose and glycogen reserves compared to other tissues, consumes at least 50 % of free glucose from arterial blood [31]. In this regard, the shift towards carbohydrate metabolism in Balb/c mice obviously contributes to achieving a high level of brain energy metabolism and the realization of such a well-known feature of Balb/c mice as good learning ability [1].

Higher ALT activity than in animals of the Balb/c line in female mice of the C57Bl/6 line with a markedly reduced level of amylase activity indicated a shift in the metabolism of these animals towards protein metabolism. Thus, in mice of the C57Bl/6 line, a decrease in the provision of the brain with energy substrates was observed with an increase in the plastic resources of the body compared to what was observed in female mice of the Balb/c line. At

the same time, a decrease in the overall body's need for "fast" energy obtained as a result of carbohydrate metabolism could be associated with the establishment of stable energy-efficient regulation regimes, which were mentioned above. This assumption is consistent with the known information about the relatively low energy metabolism and poor learning ability of mice of the C57Bl/6 line [32], characterized by more intense motor activity and more active behavior than mice of the Balb/c line [11; 33], as well as the relatively low variability of adaptation status indicators noted above. At the same time, the dominance of C57Bl/6 females over Balb/c mice in the activity of alkaline phosphatase and ALT, along with a higher level of lymphoproliferative processes in the thymus in these animals, could indicate an increase in the activity of all parts of the immune system (both T cells and B cells and neutrophils) [21; 22], and be important for the higher stress resistance of these animals compared to the stress resistance of Balb/c mice [1; 13; 14].

The revealed features of systemic regulation can have a noticeable effect on the mechanisms of nonspecific antitumor resistance and the effectiveness of antitumor treatment. Thus, the nature and intensity of the most stable ARs for a particular organism, the level of reactivity at which they develop, can significantly determine the functional state of the centers of autonomic regulation and peripheral links of the neuroendocrine and immune systems, modulating the state of tissues. In turn, the ratio of sympathetic and parasympathetic influences in tissue loci, the combination and activity of biochemical factors of the neuroendocrine and immune systems, metabolic products can contribute or, conversely, hinder the development of a malignant process [34; 35].

The peculiarities of metabolism may be important in connection with the important role of energy homeostasis of malignant cells and surrounding tissues in oncogenesis and the realization of the effects of therapy. In this regard, in recent years, attention has been increasing to the issue of metabolic concomitant therapy of oncological diseases [36; 37]. It has been shown that the metabolic background, the presence and type of energy substrates can be essential for the effectiveness of antitumor chemotherapy [38; 39]. At the same time, the means of metabolic therapy used in a number of severe chronic diseases can have an inhibitory effect on the

malignant process. In particular, a number of studies indicate the oncoprotective effect of metformin, which is a first-line drug for the treatment of type 2 diabetes [39; 40].

CONCLUSION

As a result of the study of intact mice of the C57Bl/6 and Balb/c lines of both sexes, along with sexual differences of a similar nature (the dominance of female mice over males in terms of weight characteristics of the thymus and spleen, the development of the most favorable antistress AR and the number of indicators with low variability), a number of interlinear features of the adaptive status and biochemical blood indicators. The interlinear features of the adaptive status indicated that in mice of the C57Bl/6 line, animals of different sexes had a similar character of AR (AR increased activation), which, however, developed at different levels of reactivity, whereas in mice of the Balb/c line, animals of different sexes differed in the nature of the prevailing antistress AR (in females – AR increased activation, in

males, obviously – AR quiet activation and training). At the same time, in general, the revealed interlinear differences indicated a more favorable state of regulatory systems in mice of the C57Bl/6 line, which complements the known information about the higher stress resistance of these animals compared to mice of the Balb/c line. The interlinear features of the studied biochemical parameters of blood testified to the difference between female mice of the C57Bl/6 line and female mice of the Balb/c line in the ratio of carbohydrate and protein metabolism. The data obtained indicate a more active carbohydrate metabolism in Balb/c mice and a more intensive protein metabolism in C57Bl/6 mice.

The results of the analysis allow us to assume about some mechanisms that contribute to or hinder the stress resistance and learning ability of the studied animals. The revealed interlinear differences allow us to determine the directions for further study of the regulatory and metabolic characteristics of linear mice, which must be considered when choosing adequate experimental models for the development of effective methods of complex antitumor treatment.

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LOW ANTERIOR RESECTION SYNDROME AND METHODS OF ITS ASSESSMENT (LITERATURE REVIEW)

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ABSTRACT

Low anterior resection syndrome is a common problem due to the increased incidence of rectal cancer and the high incidence of anorectal dysfunction during sphincter-preserving surgical interventions. The influence of functional disorders on the quality of life of patients and changes in social adaptation makes it possible to attribute the syndrome of low anterior resection to topical and discussed issues.

Purpose of the study: to consider the features of the development of anorectal dysfunction in patients with rectal cancer by studying risk factors and pathogenetic aspects of the development of low anterior resection syndrome, as well as to evaluate the role of objective studies in assessing the syndrome according to published literature.

The etiology of low anterior resection syndrome is multifactorial. Unmodified and modified predictors are distinguished among the risk factors. Unmodified factors include female gender and age over 65 years. Among the modifiable predictors, radiation therapy, surgical intervention, the type of formed colorectal anastomosis and the development of its insolvency, as well as the elimination of intestinal stoma have a negative effect on anorectal function. Nevertheless, the greatest role in its development is played by organ-preserving surgery with total mesorectectomy, radiation therapy and the formation of a preventive ileostomy. The complexity of the pathophysiological mechanism of the syndrome necessitates a detailed study of anorectal function and changes in its parameters in patients during treatment for rectal cancer. Functional disorders in low anterior resection syndrome vary in severity. In the study of the severity of clinical manifestations of the syndrome, the use of the LARS scale is important, and in the development of anal incontinence, the use of the Wexner scale is important. However, the most accurate assessment can be carried out by objective research methods, such as high-resolution anorectal manometry. This research method allows to control the function of the rectal obturator apparatus at different stages of combined treatment and unravels the possibility of searching for new predictors of low anterior resection syndrome.

Keywords: colorectal cancer, low anterior rectal resection syndrome, high-resolution anorectal manometry

For citation: Bondarenko O. K., Gevorkyan Yu. A., Soldatkina N. V., Gusareva M. A., Kosheleva N. G., Solntseva A. A., Duritsky M. N., Savchenko D. A. Low anterior resection syndrome and methods of its assessment (literature review). South Russian Journal of Cancer. 2023; 4(4): 57-71. <https://doi.org/10.37748/2686-9039-2023-4-4-6>, <https://elibrary.ru/lhdwfc>

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Funding: this work was not funded.

Conflict of interest: the authors declare that there are no obvious and potential conflicts of interest associated with the publication of this article.

The article was submitted 10.02.2023; approved after reviewing 11.10.2023; accepted for publication 09.12.2023.

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СИНДРОМ НИЗКОЙ ПЕРЕДНЕЙ РЕЗЕКЦИИ И МЕТОДЫ ЕГО ОЦЕНКИ (ОБЗОР ЛИТЕРАТУРЫ)

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РЕЗЮМЕ

Синдром низкой передней резекции является распространенной проблемой в связи с повышенной заболеваемостью раком прямой кишки и высокой частотой развития аноректальной дисфункции при сфинктеросохраняющих оперативных вмешательствах. Влияние функциональных расстройств на качество жизни пациентов и изменение социальной адаптации позволяет отнести синдром низкой передней резекции к актуальным и обсуждаемым вопросам. Цель исследования: рассмотреть особенности развития аноректальной дисфункции у больных раком прямой кишки путем изучения факторов риска и патогенетических аспектов развития синдрома низкой передней резекции, а также оценить роль объективных исследований в оценке синдрома по данным опубликованной литературы.

Этиология синдрома низкой передней резекции многофакторна. Среди факторов риска выделяют немодифицируемые и модифицируемые предикторы. К немодифицируемым факторам относят женский пол и возраст старше 65 лет. Среди модифицируемых предикторов отрицательное влияние на аноректальную функцию оказывает проведение лучевой терапии, оперативного вмешательства, тип сформированного колоректального анастомоза и развитие его несостоятельности, а также выведение кишечной стомы. Тем не менее наибольшую роль в его развитии играют органосохраняющее оперативное вмешательство с тотальной мезоректэктомией, проведение лучевой терапии и формирование превентивной илеостомы. Сложность патофизиологического механизма синдрома обуславливает необходимость детального изучения аноректальной функции и изменения ее параметров у пациентов в процессе лечения по поводу рака прямой кишки. Функциональные расстройства при синдроме низкой передней резекции варьируются по степени тяжести. В изучении выраженности клинических проявлений синдрома имеет значение использование шкалы LARS, а при развитии анальной инконтиненции – применение шкалы Wexner. Однако наиболее точную оценку позволяют осуществить объективные методы исследования, такие как аноректальная манометрия высокого разрешения. Данный метод исследования позволяет контролировать функцию запирающего аппарата прямой кишки на разных этапах комбинированного лечения и открывает возможности поиска новых предикторов синдрома низкой передней резекции.

Ключевые слова: колоректальный рак, синдром низкой передней резекции прямой кишки, аноректальная манометрия высокого разрешения

Для цитирования: Бондаренко О. К., Геворкян Ю. А., Солдаткина Н. В., Гусарева М. А., Кошелева Н. Г., Солнцева А. А., Дурицкий М. Н., Савченко Д. А. Синдром низкой передней резекции и методы его оценки (обзор литературы). Южно-Российский онкологический журнал. 2023; 4(4): 57-71. <https://doi.org/10.37748/2686-9039-2023-4-4-6>, <https://elibrary.ru/lhdwfc>

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Финансирование: финансирование данной работы не проводилось.

Конфликт интересов: все авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Статья поступила в редакцию 10.02.2023; одобрена после рецензирования 11.10.2023; принята к публикации 09.12.2023.

INTRODUCTION

The functional consequences associated with the formation of a low colorectal anal anastomosis have an adverse effect on the quality of life of patients who underwent this intervention [1; 2]. The change in anorectal function can manifest itself in the form of an increase in imperative urges to defecate and stool frequency (up to 6 times a day or more), sensitivity disorders and associated evacuation disorders, feelings of incomplete emptying, the development of anal incontinence. The described symptom complex is called low anterior resection syndrome (LARS) [3; 4]. Manifestations of this syndrome are observed in 41–90 % of patients after low anterior resection [5–8]. The wide variability in the prevalence of LARS in different studies reflects inconsistencies in its assessment and diagnosis [5].

The problem of anorectal dysfunction remains relevant at the present time, since LARS affects the quality of life of patients and changes social adaptation. The study of risk factors for possible functional disorders can help in predicting LARS, improving methods of its prevention and correction.

The purpose of the study: to consider the features of the development of anorectal dysfunction in patients with rectal cancer by studying risk factors and pathogenetic aspects of low anterior resection syndrome, as well as to evaluate the role of objective studies in assessing this syndrome according to published literature.

MATERIALS AND METHODS

A systematic search of literary sources in databases was carried out on elibrary.ru, PubMed using keywords: "low anterior resection syndrome", "anorectal dysfunction", "rectal cancer", "total mesorectumectomy", "LARS score". The papers devoted to the study of risk factors for the development of LARS and research methods that allow assessing the severity of anorectal dysfunction in patients undergoing combined treatment for rectal cancer were considered. Publications devoted to the occurrence of LARS in other nosologies were excluded. The selected articles have been published over the past 5 years in Russian and English.

An initial search revealed 105 articles. At the next stage of the search, literature reviews and non-original articles were excluded, as a result of which 38 original studies were analyzed.

RESULTS

The incidence of LARS in patients treated for rectal cancer varies widely, and the manifestations of anorectal dysfunction are diverse and variable [1; 5–8]. In most modern studies, LARS is observed on average in 64.8 % of cases, while more than half of patients develop a pronounced clinical picture [8]. In a study by Bao-Jia Luo et al. (2021), 27 % of patients had a stool frequency of more than 5 times a day, and 30 % of patients had imperative urges associated with a change in the evacuation function of the sphincter apparatus [9]. At the same time, the duration of functional disorders can be short-term and last for 6–12 months or long-term with the preservation of symptoms for more than 1 year [10; 11]. In Bryant C. L. et al. up to 71 % of the observed patients noted incontinence of intestinal contents, and 12–74 % of patients reported violations of evacuation [12]. There is also evidence that a violation of rectal sensitivity, manifested in a deterioration in the recognition of gases and intestinal contents, is more common than incontinence, and affects the quality of life of patients to a greater extent [13].

From the point of view of normal physiology, the functioning of the anorectal region is a delicate balance between the level of pressure in the rectum and the tone of the anal sphincters. Defecation is carried out with coordinated contraction of the pelvic floor and anterior abdominal wall muscles and relaxation of the external anal sphincter and puborectal muscle in response to increased rectal pressure [3; 8; 14]. Somatic innervation of the external sphincter and puborectal muscle is carried out by sexual nerves, and pelvic floor muscles – by motor and sensory nerves of segments S3–5. Vegetative innervation of the rectum and the internal anal sphincter is provided by the lower submandibular and pelvic plexuses. The nerves run anterolateral to the rectum between the posterolateral border of the prostate gland or vagina and the lower medial part of the muscle that raises the anus, reaching the anorectal junction [15].

One of the main components of the evacuation function is the rectoanal inhibitory reflex (RAIR), coordinated by neuroregulation of intramural plexuses [16]. The reflex participates in the recognition and retention of intestinal contents due to changes in the rectoanal pressure gradient [17]. Physiologically, the mechanism is a reflex relaxation of the internal anal

sphincter after stretching the walls of the rectum and contraction of the external sphincter, which makes it possible to contact the sensitive transition zone with intestinal contents and differentiate its quality: decorated or liquid stools, gases [16].

Pathophysiological mechanisms of LARS

Various physiological consequences of both surgical intervention and radiation therapy suggest that the pathophysiology of LARS is multifactorial and includes anatomical, sensory and functional changes. The pathophysiological mechanisms of LARS include dysfunction of the internal anal sphincter, decreased sensitivity of the anal canal, violation of RAR, decreased capacity and elasticity of the rectal stump [3; 14].

According to published studies, low anterior resection leads to a change in the functioning of the internal anal sphincter, responsible for maintaining resting pressure and participating in the implementation of RAR. An increase in the relaxation coefficient of the internal anal sphincter is recorded in 40 % of cases of incontinence in the form of a decrease in pressure in the anal canal [16]. Changes in this structure were studied by endosonographic examination, while all 39 patients showed signs of damage after surgical treatment, 7 patients persisted after 2 years of follow-up [17].

Postoperative changes may affect not only the function of the internal anal sphincter, but also the sensory ability of the anal canal. The functioning of the anorectal region is provided by information from the mucous membrane distal to the anastomosis and the side walls of the pelvis. Damage to afferent innervation under the influence of radiation therapy leads to an altered perception of the urge to defecate [18]. Research by Tomita R. and co-authors (2008) demonstrated a statistically significant decrease in the sensitivity of anal canal prescriptions in patients with clinical manifestations of LARS. At the same time, sensitivity correlates with the distance between the level of colorectal anastomosis and the edge of the anus with a tendency to greater disruption of gas and stool recognition at lower levels of anastomosis [19]. In addition, in the work of Haas S. and co-authors (2020), abnormal integration of anorectal stimuli on encephalograms was recorded in patients after combined treatment of rectal cancer, which may also play a role in the pathogenesis of LARS [20].

A decrease in RAIR is an independent predictor of deterioration of the functional state of the anorectal region after surgery for rectal cancer. Observation of patients at different stages of treatment made it possible to note a decrease in RAIR in a quarter of patients a year after low anterior resection. Nevertheless, 85 % of the subjects had the reflex present after 2 years, which is probably due to the restoration of the innervation of the colorectal anastomosis. Studying the RAIR and the maximum threshold volume, Kupsch J. and co-authors (2018), using anorectal manometry and balloon proctometry, noted the most satisfactory functional results in patients with a length of the remaining rectum > 4 cm [21].

The capacity of the neorectum also contributes to the development of the LARS. According to some studies, the large capacity of the neorectum was associated with a significant decrease in the number of bowel movements per day. However, chemoradiotherapy and anterior resection reduce the capacity and elasticity of the rectum, which in turn leads to an increase in false urges [19].

Risk factors

According to the data of the reviewed publications, a significant number of predictors that increase the risk of developing LARS are currently being identified [8; 11; 14; 18; 22; 23]. After the literature review, a number of modifiable and unmodifiable risk factors were identified. Unmodified risk factors include female gender and age over 65. Among the modifiable factors, first of all, radiation therapy and surgical intervention have a negative effect on anorectal function. The type of formed colorectal anastomosis, the development of its insolubility, the formation of a preventive ileostomy, the patient's body mass index of more than 30 kg/m² are also considered by some authors as possible predictors [18; 24–29].

Low anterior rectal resection with total mesorectumectomy leads to a change in reservoir function and impaired evacuation of intestinal contents [3; 18]. In 2019, Keiji Koda et al. It has been shown that damage to the internal anal sphincter as a result of removal of most of the rectum can play a role in the development of LARS [23]. Features of surgical intervention in the form of dilation of the anal pulp can affect both the external and internal sphincter with a transient zone and a "hemorrhoidal pillow" [30].

The location of the tumor and, accordingly, the level of colorectal anastomosis after sphincter-preserving surgical interventions was one of the most frequent factors analyzed. The proportion of patients suffering from this LARS increases as the level of anastomosis approaches the anal sphincter. Analysis of the height of the colorectal anastomosis revealed that an anastomosis at a level up to 5–6.5 cm from the anal margin can be considered a risk factor for the development of LARS [8; 13; 22].

The potential pathophysiological mechanisms of LARS also include a violation of autonomic innervation due to the rupture of intrahepatic neuronal connections between the rectal ampoule and the internal sphincter, causing loss of RAR and impaired stool differentiation. The removal of the rectal ampoule, the loss of the reservoir and evacuation function of the rectum, which in turn leads to accelerated transit of unformed intestinal contents, are considered among the reasons for LARS [3; 31].

Combined treatment according to the results of randomized trials is associated with a higher frequency of defecation compared to performing only total mesorectumectomy [10; 15; 32]. Despite the current use of conformal radiation therapy aimed at reducing the area of irradiation, its effect causes ischemic and fibrous changes in blood vessels, pelvic and musculoskeletal nerve plexuses, muscle fibers of the sphincter apparatus [18; 32–34].

The formation of a preventive ileostomy and the occurrence of diversionary colitis due to bacterial recolonization of the colon are also considered by some authors as possible factors for the development of LARS [35]. Enteral deprivation of nutrients associated with the formation of a preventive ileostomy leads to a deficiency of nutrients. Among the structural and functional changes, there may be atrophy of the muscles of the anal sphincter, atrophy of the villi and inflammation of the mucous membrane, leading to dysfunctional colitis. This factor has an adverse effect on the metabolism and microflora of the colon due to changes in the oxidation of butyrate and increased formation of free radicals [36]. Post-operative dysbiosis with a tendency to a significant decrease in the diversity of the intestinal microbiota correlates with increased stool frequency regardless of the endoscopic activity of the inflammatory process. According to some sources, increased defecation occurs when the composition of the microbiota

is unbalanced with a decrease in the relative number of Bacteroidetes and an increase in Enterobacteriaceae [37].

Methods of assessment of LARS

The complexity of the pathophysiological mechanism of LARS necessitates a detailed study of anorectal function and changes in its parameters in patients during treatment for rectal cancer. To study the severity of symptoms of anorectal dysfunction, the most used and proven method is the LARS scale [38]. This questionnaire separately examines the frequency of defecation, imperative urges, cases of incontinence of gases and liquid stools. According to the scale, the severity of functional disorders is classified as the absence of LARS (0–20 points), small LARS (21–29 points) and large LARS (30–42 points) [26].

Among the analyzed sources, some authors associate the female sex with higher rates of LARS [18; 25]. Juul T. et al. (2019) demonstrated that in the general population of Denmark, significant symptoms (≥ 30 points on the LARS scale) were observed in women in the age group from 50 to 79 years compared with men ($p = 0.001$). In this article, the age of patients was not associated with impaired anorectal function, however, the work of Sandberg S. et al. (2020) showed that among the 334 studied within 2 years after the completion of treatment, elderly patients had a more pronounced clinic of LARS [18]. In the study by Benli S et al. (2021), age (more or less than 65 years) and gender did not affect the incidence of LARS ($p = 0.14$, $p = 0.69$, respectively) [39].

In a study by Bohlok et al. (2020) conducted among 43 patients with rectal cancer after combined treatment, a body mass index of more than 30 kg/m² was a prognostic predictor of pronounced LARS ($p = 0.047$) [26]. However, no statistical significance of the negative influence of this factor was found in other analyzed sources [8; 39]. According to the results of the paperwork of Nafedzova I. O. (2021), there was also no effect on the occurrence of pronounced LARS of such parameters as elderly age (OR = 0.99 (95 % CI: 0.95–1.02), $p = 0.44$), gender (OR = 1.04 (95 % CI: 0.74–1.47), $p = 0.82$) and elevated index body weight (OR = 0.99 (95 % CI: 0.9–1.08), $p = 0.8$) [8].

The LARS scale was also used in the Trenti L. et al. (2018) study conducted in Spain among 152 pa-

tients to assess anorectal function within a year after sphincter-preserving intervention for rectal cancer. According to the data obtained by the authors, the risk of developing pronounced clinical symptoms in 80 % of the subjects increased with a smaller anastomosis distance from the anal margin ($p < 0.05$). Multivariate analysis revealed the statistical significance of a low level of colorectal anastomosis (OR = 3.82 (95 % CI: 1.46–12.01; $p = 0.005$) and neoadjuvant radiation therapy (OR = 2.38 (95 % CI: 0.98–5.96; $p = 0.048$) as independent risk factors for LARS [13].

According to the analysis of the ROC curve of a retrospective research study by Miaci FLC et al. (2020), the threshold distance between the colorectal anastomosis and the anal margin, which increases the risk of anorectal dysfunction, is 6.5 cm ($p < 0.001$). The study of anorectal function using the LARS scale made it possible to distinguish among statistically significant predictors in addition to the level of colorectal anastomosis ($p < 0.001$). The same applies to radiation therapy ($p = 0.0014$) and ileostomy formation ($p = 0.0023$) [22]. At the same time, such studied parameters as gender, age, the presence of bad habits, the type of formed anastomosis did not have a significant impact on the development of LARS.

In the work of Nafedzova I. O. (2021), the relationship between the level of colorectal anastomosis below 5 cm from the outer edge of the anal canal was also demonstrated by an increase in the frequency of development of LARS by 2.6 times (OR = 2.61 (95 % CI: 1.47–4.62), $p = 0.001$ [8].

In the work of Rasulov and co-authors (2021), the degree of anorectal dysfunction after total mesorectumectomy with various methods of reconstruction of the rectum was assessed. According to the LARS scale, the least pronounced manifestations of LARS were noted in the group of patients with the formation of a J-shaped reservoir in comparison with end-to-end and side-to-side anastomoses. The preservation of the functional advantage of the J-reservoir design was also observed after 12 months in the postoperative period [27]. However, in other studies comparing anorectal function in patients with side-to-end and end-to-end anastomosis, no statistical significance was revealed ($p > 0.05$) [26; 28; 33].

There are also data on the effect of colorectal anastomosis failure on postoperative functional results [3; 15; 35]. The work of Hain E et al. (2017)

showed that the occurrence of anastomosis failure is associated with pronounced clinical symptoms of anorectal dysfunction on the LARS scale in 46 studied patients after laparoscopic low anterior resection ($p = 0.02$) [28]. The results of other studies also demonstrate the statistical significance of anastomosis failure in increasing the risk of developing LARS ($p < 0.001$) [8; 29; 33].

The effect of radiation therapy on the functioning of the anorectal zone has reached statistical significance not only in the works of Trenti L. et al. (2018) and Miaci FLC et al. (2020), but also in most of the analyzed publications [11; 15; 22]. A study by Danish scientists Hughes D. L. et al. (2017) showed that the use of non-adjuvant radiation therapy in 85 patients was associated with an almost 20-fold increase in the risk of LARS ($p < 0.01$) [11]. In 18 % of cases, the symptoms were insignificant, and in 56 % of cases, signs of a large LARS were detected. The average score on the LARS scale a year after the treatment was 35.5, after 4 years – 27.9. However, the difference in these indicators did not reach significance ($p = 0.19$), which demonstrates the continued negative effect of radiation therapy on anorectal function in the long term.

According to the results of a study conducted by van der Sande M. E. et al. (2019), the relationship between the dose of radiation therapy and the severity of anorectal dysfunction in patients with rectal cancer was tracked ($p < 0.01$). 2 years after radiation therapy, 11 patients had pronounced clinical symptoms of anorectal dysfunction (30–39 points on the LARS scale), 10 patients had minor manifestations of LARS (21–29 points) [40]. Based on the above, surgical trauma is significant in the early postoperative period, but the negative impact of surgery on anorectal function is leveled in the long term, which cannot be said about radiation therapy.

The use of combined treatment of rectal cancer improves oncological results, however, patients in the long term may be burdened with persistent symptoms of anorectal dysfunction. The manifestations of LARS may decrease during the first 2 years, however, in almost 60 % of patients, symptoms persist for more than 24 months [41]. It is reliably known that the quality of life correlates with the severity of this syndrome [15].

Combined treatment according to the results of randomized trials is associated with a higher fre-

quency of defecation compared to performing only total mesorectumectomy [9; 15; 26; 42]. In the work of Sun W et al. (2019), within 40 months after combined treatment, 119 (54.1 %) of 220 observed patients reported the occurrence of a large LARS. At the same time, the group of patients without radiation therapy showed fewer signs of functional disorders compared to the group of patients undergoing combined treatment (38.6 and 64.4 %, respectively, $p < 0.001$). The effect of neoadjuvant irradiation (OR = 2.20 (95 % CI: 1.24–3.91), $p = 0.007$) on anorectal function was a statistically significant predictor of the development of pronounced LARS [34].

A logistic regression analysis conducted in Luo B. et al. (2021) showed that preoperative chemoradiotherapy ($p = 0.003$) and the proximity of the lower edge of the tumor to the dentate line ($p = 0.015$) are independent risk factors for LARS [38].

Benli S. et al. (2021) analyzed the clinical manifestations of anorectal dysfunction on the LARS scale in 276 patients after combined treatment of rectal cancer. The authors present a low level of anastomosis (OR = 42.40 (95 % CI: 11.14–161.36), $p < 0.0001$) and radiation therapy (OR = 2.51 (95 % CI: 1.38–4.57), $p = 0.003$) as important predictors of the development of LARS (OR = 42.40 (95 % CI: 11.14–161.36), $p < 0.0001$). In addition to the above data, the study made it possible to distinguish among statistically significant predictors of anorectal dysfunction the formation of a preventive ileostomy (OR = 12.83 (95 % CI: 6.58–25.0), $p < 0.0001$). At the same time, the term of closure of the ileostomy (early less than 6 months and late more than 6 months) did not affect the incidence of LARS ($p = 0.56$) [39].

The LARS scale helped to demonstrate the negative effect of preventive ileostomy on postoperative functional results of the rectal sphincter apparatus and in other sources. Sun W et al. (2019) describe ileostomy as an independent risk factor for pronounced LARS (OR = 2.59 (95 % CI: 1.27–5.30), $p = 0.009$) [37]. In a study by Hughes D. L. et al. (2017), the presence of ileostomy within 6 months after completion of treatment was associated with a 3.7-fold increase in the risk of LARS ($p = 0.03$) [42]. Formation of a preventive ileostomy in a meta-analysis conducted by I. O. Nafedzov. (2021), was associated with a three-fold increase in the risk of severe functional problems after surgery (OR = 3.32 (95 % CI: 1.99–5.55), $p < 0.00001$) [8].

Nuytens F. et al. (2018) evaluated the effect of the time of performing reconstructive surgery on the small intestine in 100 patients over 50 months. However, the study did not reveal a relationship between the time of closure of the ileostomy and the severity of symptoms of anorectal dysfunction ($p = 0.38$) [33].

The Wexner scale allows to expand the understanding of anorectal dysfunction when signs of incontinence occur. This questionnaire evaluates the severity of anal incontinence by studying the frequency of incontinence of gases, liquid or solid intestinal contents. The score varies from 0 to 20 points.

In the study of A. A. Medvednikov and co-authors (2020), the functional activity of the anorectal zone after low anterior rectal resection was evaluated in 100 patients [41]. During the year after surgical treatment, when analyzing the degree of incontinence, I degree of insufficiency was observed in 15 % of patients (up to 5 points on the Wexner scale), II degree of insufficiency – in 33 % (6–10 points on the Wexner scale), III degree – in 52 % of cases (11–20 points on the Wexner scale). After a year of sphincter-preserving intervention, an improvement in anorectal function was observed, and in 45 % of patients there were no significant violations of the locking function ($p < 0.01$).

With respect to the type of anastomosis, the Wexner scale demonstrates better anal retention 6 months after surgery with the formation of a J-shaped reservoir compared with anastomoses "end-to-end" and "side-to-side" ($p = 0.01$). However, by 12 months, the clinical manifestations of anal incontinence decreased and became the same between the groups under consideration ($p > 0.05$) [40].

Another incontinence scale, the use of which has been found in literary sources, is the Vaizey scale, consisting of 7 points, including incontinence of solid and liquid stools, flatulence, the severity of lifestyle changes, the need to wear pads, the use of antidiarrheal drugs. The score is built by summing up each item (range from 0 to 24), where 0 indicates the absence of incontinence, and 24 indicates complete incontinence of intestinal contents [21].

In the work of Trenti L. et al. (2018), the median incontinence index on the Vaizey scale was higher in patients with colorectal anastomosis by 5 cm or less compared to its higher level (7.5, range 3–12; $p = 0.036$) [13].

In a study by Kupsch J. et al. (2018), the Vaizey scale was used as an additional indicator to the

LARS scale in assessing anorectal function in patients undergoing treatment for rectal cancer [21]. Among 144 (55.2 %) of the examined patients, signs of small (51 patients (19.5 %)) or large (93 patients (35.6 %)) LARS were noted. In the group of patients undergoing radiation therapy, more than 20 points were scored on the LARS scale (64.6 %), which reflected less satisfactory functional indicators of the anorectal zone compared to patients who did not receive radiation therapy (43.1 %) ($p = 0.001$). The Vaizey scale also demonstrated significant differences between groups $10.0 (\pm 6.7)$ and $6.3 (\pm 6.1)$, respectively ($p < 0.001$), which correlated with the LARS scale data ($r = 0.81, p < 0.001$).

However, these questionnaires are based solely on the patient's assessment of the severity of symptoms and do not accept changes in the functional parameters of the anorectal zone. For full-fledged counseling, objective research methods should be included in the examination plan.

The first methods of studying the tonic and neuro-reflex activity of the anorectal region were based on the use of rectal cylinders that register pressure and RAIR created by internal and external sphincters [16].

To date, anorectal manometry is an objective method in assessing postoperative anorectal function and diagnosing functional disorders [43; 44]. For a long time, there were two types of systems in the equipment of anorectal manometry: air (balloon catheters) and water-perfusion (open catheter method) [3]. Improved diagnostic accuracy has improved the appearance of high-resolution anorectal manometry (HRAM).

HRAM is a complex research method that provides an opportunity to create a spatio-temporal model of pressure distribution. The advantage of HRAM is the use of a higher physiological resolution created by the increased density of sensitive sensors and their location around the circumference [45; 46; 47]. The HRAM system can consist of miniature semiconductor pressure indicators on 2D and 3D solid-state catheters with a diameter of 4 mm, capable of providing functional and morphological indicators of the anorectal region [48]. HRAM displays changes in anorectal activity at rest and with various functional tests in the form of a color contour graph.

According to the recommendations of the International Working Group of Experts on Anorectal Phys-

iology, the examination of patients is carried out in a supine position on the left side with the hip and knee joints bent at right angles [49]. There is also an opinion in the literature about the effectiveness of determining the anal resting pressure and compression pressure in the lithotomic position. However, this modification has not been introduced into clinical practice at the moment, since it did not reflect the benefits for monitoring rectal sensitivity [50].

Recommended measurements of anorectal manometry assess the following parameters: anal resting pressure, anal canal pressure and fatigue time during voluntary contraction, rectal pressure during straining and coughing, rectal sensitivity, RAR, rectal capacity and extensibility [49].

According to anorectal manometry, anal resting pressure is maintained by the work of the internal sphincter. While the parameter of maximum compression pressure depends on the functioning of the external sphincter and is responsible for the retention of intestinal contents when intra-abdominal pressure increases under stressful conditions. The literature describes a significant decrease in both indicators in the postoperative period [22; 30].

HRAM can also reflect the myogenic activity of smooth muscle cells of the anal sphincter by evaluating rhythmic pressure fluctuations called slow and super slow waves. Slow waves in the anal sphincter arise from interstitial Cajal cells, and in healthy people are recorded with a frequency of approximately 16–18 cycles per minute [51].

According to the data of the analyzed studies, the indicators of high-resolution anorectal manometry are associated with the degree of severity of LARS [15; 32]. In patients who have undergone sphincter-preserving surgery for rectal cancer, the indicators of anal pressure at rest and maximum rectal volume decrease, while RAIR may be absent in 80 % of cases a month after completion of treatment [52]. It was also noted that defecation dysfunction, manifested by a violation of rectal sensitivity, is more common than incontinence, and affects the quality of life to a greater extent [13].

In a study by Luo B. et al. (2021), a decrease in the physiological parameters of the anorectal zone was observed after low anterior rectal resection in 146 patients [9]. The greatest changes were manifested in a decrease in the indicators of anal resting pressure, maximum compression pressure, maximum

volume transferred ($p < 0.001$). The threshold volume of the first sensation was significantly higher in healthy people than in the surgical group ($p < 0.001$).

Liu L. et al. (2017) to assess the effect of laparoscopic anterior resection on anorectal function, high-resolution anorectal manometry was performed in 51 patients. The patients were divided into two groups depending on the level of anastomosis more or less than 5 cm from the edge of the anus. 3 months after surgery, a decrease in anal resting pressure and maximum compression pressure ($p < 0.05$) was registered in both groups, while after 6 and 9 months there was an improvement in indicators to the preoperative level. The inter-group comparison noted higher values in the group of patients with a high level of anastomosis: the maximum compression pressure was significantly higher 3 months after surgery ($p < 0.05$), and after 6 months – a higher level of average and maximum pressure at rest ($p < 0.05$) was observed. A similar trend was also observed with respect to changes in the volume of the rectum, but all these parameters remained significantly lower compared to preoperative values after 9 months [53].

The work of Fratta C. et al. (2022), conducted among 48 patients who received a course of neo-adjuvant radiation therapy, demonstrates a decrease in manometric indicators of anal pressure at rest and average compression pressure ($p < 0.05$). At the same time, there were no statistically significant changes between the initial values of the maximum compression pressure and their value after radiation therapy ($p = 0.05$). From the side of clinical manifestations, the authors report a higher frequency of loose stools and urge to defecate after radiation therapy, but the assessment of the degree of incontinence on the Wexner scale also revealed no significant differences ($p > 0.05$) [43].

According to the work of Baichorov A. B. (2019), prolonged radiation therapy leads to a significant decrease in manometric indicators: resting pressure and contraction, endurance of the sphincter apparatus of the rectum ($p < 0.05$) [54].

In a study by Ilnát P. et al. among 65 patients, the effect of combined treatment was manifested in the form of a change in the normal ratio of manometric parameters: there was a significant increase in the threshold level of the first sensation and a significant decrease in anal pressure at rest and with maximum

contraction, extensibility and maximum volume of the rectum ($p < 0.001$) [32].

There is also information in the literature about the correlation of manometric indicators with the clinical picture of LARS 3 and 6 months after surgery. A higher LARS index was observed in patients with focal pressure defects in the anal canal and with the appearance of spastic peristaltic waves from the colorectal anastomosis to the anus. Spastic hypermobility of the rectal stump, probably caused by external denervation, is associated with the severity of imperative urges to defecate [15]. Patients who developed spastic peristaltic contractions in the postoperative period had a higher stool frequency both 3 and 6 months after surgery.

Anorectal profilometry, which is a type of anorectal manometry, is based on the operation of a thin water-perfusion catheter with a radial arrangement of channels and registers the pressure along each channel throughout the entire process of moving the sensor. The clinical value of this method lies in a quick and objective assessment of the activity of the structures of the internal anal sphincter and the contractility of the pelvic floor muscles, especially in patients with painful sensations when using a sphincteromanometric sensor [31]. Anorectal sphincterometry, as one of the methods of studying the anorectal zone, allows you to diagnose the work of the pelvic floor muscles, taking into account the study of reservoir and evacuation function indicators.

A segmental sphincterometer is used to measure the absolute value of the pressure created by the rectal locking apparatus [55]. The study is carried out by determining the tone of the anal pulp in a relaxed state and with maximum compression. The sensor of the device increases the accuracy of the pressure measurement results by registering indicators from the segments of the sphincter apparatus.

In the modern literature there are data on the use of anorectal sphincterometry and profilometry to study anorectal function in the formation of different types of anastomoses. Rasulov and co-authors (2021) noted significantly higher rates of neorectal sensitivity, the first and constant urge to defecate in patients with a J-shaped reservoir [27]. The greater ability of this type of anastomosis to accumulate and retain intestinal contents is reflected in the highest indicator of the maximum tolerated volume (by 12 months of the postoperative period – 224, 204 and 190 ml in groups

with a J-shaped reservoir, "side-to-end" and "end-to-end" anastomoses, respectively, $p < 0.0001$). There were no significant differences between the compared groups with respect to the indicators of maximum resting pressure and contraction ($p > 0.05$).

Another way to study the functional state of the anorectal zone is defectofluometry with a weight optical sensor that reflects the readings in the form of a curve on the monitor screen. This method makes it possible to evaluate the reservoir function by registering sensitivity thresholds and urge to defecate with slow introduction of filler, as well as to evaluate the evacuation function by residual volume [56].

In the Ssu-Chi Chen et al. (2021) study, physiological variables were evaluated in patients with LARS using a Fecobionic device. Testing was carried out during the evacuation of a balloon with three sensors located in front, behind and inside, which made it possible to measure not radial pressure in a fixed position, but axial pressure during defecation. Obtaining the defecation index allows the parameters of anorectal manometry to better correlate with the Wexner scale and the manifestations of LARS, which in the future may provide a more detailed analysis of anorectal dysfunction [26].

In the work of Liu L. et al. (2017), endoanal ultrasound examination was used, which allowed us to note a tendency to decrease the thickness of the internal and external sphincters and to decrease the volume of the rectum after surgery [53].

Intraanal electromyography is one of the most common methods and allows to obtain a quantitative and qualitative assessment of the work of the external anal sphincter and pelvic floor muscles [57]. By studying background and arbitrary bioelectric activity, the study reflects the indicators of the total contractility of the rectal locking apparatus. The measurement is carried out at rest and arbitrary contraction, as well as during straining tests and with an increase in intra-abdominal pressure. The tonic activity of the external sphincter at rest is 15.2 ± 2.1 mv, and with a decrease and increase in intra-abdominal pressure increases to 74.3 ± 13.7 mv. A normal physiological reaction during straining is observed with synchronous inhibition of the bioelectric activity of the external sphincter and pelvic floor muscles, while an increase in this indicator is regarded as a paradoxical reaction of the puborectal muscle [58].

A study by Vollebregt P. F. et al. (2021) expanded the understanding of the functional activity of the anorectal region by comparing the frequency of slow waves of the anal canal in 21 patients who underwent anterior rectal resection with 37 healthy subjects. The authors were able to demonstrate a decrease in anal slow-wave activity in the first group of patients. In 52.4 % of patients in the postoperative period, the frequency of slow waves of 6–8 cycles per minute without activity at higher frequencies was noted. Changes in the observed pressure activity may reflect damage to pelvic nerves innervating cells of the smooth muscles of the anus [51].

Battersby N. J. et al. (2018) developed a POLARS preoperative nomogram to predict the severity of anorectal dysfunction after low anterior rectal resection. A nomogram is a graphical representation of the function of the variables under study, based on the analysis of parameters such as the patient's gender and age, the height of the tumor, radiation therapy, partial or total mesorectomy, ileostomy formation. After entering the data, the program calculates the number of predicted points on the LARS scale [59].

In the work of Nafedzova I. O. (2021), based on the analyzed predictors of anorectal function disorders, a nomogram was created that allows predicting the occurrence of pronounced LARS in patients in the postoperative period. The probability of the development of significant functional disorders was calculated with a combination of different risk factors: chemoradiotherapy, the height of the anastomosis level, the failure of the anastomosis, the removal of preventive ileostomy and chemotherapy. The authors draw attention to the need to inform patients with a high probability of pronounced LARS according to nomogram data during the formation of a low colorectal anastomosis in order to carry out conservative rehabilitation in the form of complex BOS therapy and tibial modulation [8].

CONCLUSION

LARS is a socially significant problem due to the increasing morbidity and high frequency of development with low sphincter-preserving surgical interventions. Surgical treatment and radiation therapy can improve the oncological results of patients with rectal cancer. However, radiation therapy and the formation

of a low colorectal anastomosis are independent risk factors for functional disorders that have the greatest impact on the development of LARS. A more thorough

study of LARS at different stages of treatment of patients can help in the search for new approaches to the prevention of anorectal dysfunction.

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Kosheleva N. G., Solntseva A. A. – data collection, analysis and interpretation;

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MODERN APPROACHES TO ESOPHAGEAL SQUAMOUS CELL CARCINOMA THERAPY: PARADIGM SHIFT?

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ABSTRACT

Esophageal cancer (EC) is one of the most aggressive malignant neoplasms, ranking sixth among oncological causes of death. According to GLOBOCAN, more than half a million people die from this disease every year, and by 2040 this indicator is expected to increase almost twice. In most patients, esophageal cancer is diagnosed at stages III–IV of the disease. Currently, the standard of treatment for inoperable patients with EC is simultaneous chemoradiotherapy.

One of the main methods of treatment of patients with non-metastatic esophageal lesion remains surgical intervention in the volume of esophagectomy with radical lymph dissection, accompanied by quite frequent serious postoperative complications. However, the results of surgical treatment of locally advanced esophageal cancer alone remain unsatisfactory, and the five-year survival rate is less than 20 %. In order to improve the oncological results of treatment, various combinations of drug and radiation therapy are used (preoperative chemotherapy or chemoradiotherapy, independent chemoradiotherapy). To date, recommendations for the treatment of locally advanced esophageal cancer vary from country to country. Trimodal therapy (preoperative chemoradiotherapy up to TFD – 46 Gy with 5 cycles of weekly chemotherapy according to the carboplatin + paclitaxel scheme followed by surgical treatment) is the standard in operable patients with non-metastatic squamous cell carcinoma of the esophagus in our and European countries. In Asian countries, preference is given to neoadjuvant chemotherapy, based on the data of the JCOG1109 (NExT) study, in which it was shown that the addition of docetaxel to neoadjuvant therapy with cisplatin and fluorouracil is accompanied by an improvement in overall survival and acceptable toxicity, compared with the CF regimen and chemoradiotherapy.

A separate issue is the place of lifesaving esophagectomy in patients who have received a course of radical chemoradiotherapy. Unfortunately, according to several researchers, recurrent or persistent esophageal cancer remains an urgent problem with a risk of relapse of the disease in up to 60 % of cases.

We have studied the data of the Russian and global literature concerning the treatment of squamous cell carcinoma of the esophagus.

Keywords: squamous cell carcinoma, esophageal cancer, combined treatment, neoadjuvant treatment, chemotherapy, immunotherapy, chemoradiotherapy, esophagectomy

For citation: Smolenov E. I., Mironova D. Yu., Kolobaev I. V., Ryabov A. B., Ivanov S. A., Kaprin A. D. Modern approaches to esophageal squamous cell carcinoma therapy: paradigm shift? South Russian Journal of Cancer. 2023; 4(4): 72-84. <https://doi.org/10.37748/2686-9039-2023-4-4-7>, <https://elibrary.ru/ymqzsm>

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Funding: this work was not funded.

Conflict of interest: the authors declare that there are no obvious and potential conflicts of interest associated with the publication of this article.

The article was submitted 10.05.2023; approved after reviewing 18.10.2023; accepted for publication 09.12.2023.

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СОВРЕМЕННЫЕ ВОЗМОЖНОСТИ ТЕРАПИИ ПЛОСКОКЛЕТОЧНОГО РАКА ПИЩЕВОДА: СМЕНА ПАРАДИГМ?

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РЕЗЮМЕ

Рак пищевода (РП) является одним из самых агрессивных злокачественных новообразований, занимая шестое место среди онкологических причин смертности. По данным GLOBOCAN, более полумиллиона человек ежегодно умирает от данного заболевания, а к 2040 г. ожидается увеличение данного показателя практически в 2 раза. У большинства больных рак пищевода диагностируется на III–IV стадиях заболевания. В настоящее время, стандартом лечения неоперабельных больных РП является одновременная химиолучевая терапия.

Одним из основных методов лечения пациентов с метастатическим поражением пищевода остается оперативное вмешательство в объеме эзофагэктомии с радикальной лимфодиссекцией, сопровождающееся довольно частыми серьезными послеоперационными осложнениями. Однако, результаты только хирургического лечения местно-распространенного рака пищевода остаются неудовлетворительными, и показатель пятилетней выживаемости составляет менее 20 %. В целях улучшения онкологических результатов лечения используются различные комбинации лекарственной и лучевой терапии (предоперационная химиотерапия или химиолучевая терапия, самостоятельная химиолучевая терапия). На сегодняшний день, рекомендации по лечению местно-распространенного рака пищевода различаются в разных странах. Тримодальная терапия (предоперационная химиолучевая до СОД – 46 Гр с 5 циклами еженедельной ПХТ по схеме карбоплатин + паклитаксел с последующим хирургическим лечением) является стандартом у операбельных пациентов с метастатическим плоскоклеточным раком пищевода в нашей и европейских странах. В азиатских странах предпочтение отдается неоадьювантной химиотерапии, базируясь на данных исследования JCOG1109 (NExT), в котором было показано, что добавление доцетаксела к неоадьювантной терапии цисплатином и фторурацилом сопровождается улучшением показателей общей выживаемости и приемлемой токсичностью, по сравнению со схемой CF и химиолучевой терапией.

Отдельным вопросом стоит место спасительной эзофагэктомии у больных, получивших курс радикального химиолучевого лечения. К сожалению, по данным ряда исследователей, рецидивирующий или персистирующий рак пищевода остается актуальной проблемой с риском развития рецидива заболевания до 60 % случаев.

Мы изучили данные российской и общемировой литературы, касающиеся вопроса лечения плоскоклеточного рака пищевода.

Ключевые слова: плоскоклеточный рак, рак пищевода, комбинированное лечение, неоадьювантное лечение, химиотерапия, иммунотерапия, химиолучевая терапия, эзофагэктомия

Для цитирования: Смоленов Е. И., Миронова Д. Ю., Колобаев И. В., Рябов А. Б., Иванов С. А., Каприн А. Д. Современные возможности терапии плоскоклеточного рака пищевода: смена парадигм? Южно-Российский онкологический журнал. 2023; 4(4): 72-84. <https://doi.org/10.37748/2686-9039-2023-4-4-7>, <https://elibrary.ru/ymqzsm>

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Финансирование: финансирование данной работы не проводилось.

Конфликт интересов: все авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Статья поступила в редакцию 10.05.2023; одобрена после рецензирования 18.10.2023; принята к публикации 09.12.2023.

INTRODUCTION

Esophageal cancer ranks 9th in terms of the number of new cases detected and 6th in terms of cancer mortality. Currently, neoadjuvant polychemotherapy and chemoradiotherapy are the standard treatment for locally advanced esophageal cancer in combination with subsequent surgical intervention. However, to date, the optimal regimen and radiation dose have not been developed, as well as the time period between the end of neoadjuvant treatment and surgical intervention, and the frequency of relapses remains high. Currently, immunotherapy is being actively introduced into general clinical practice. Many authors suggest that the inclusion of this component in the neoadjuvant treatment regimen may increase survival rates and increase the frequency of pathomorphological response in patients with locally advanced esophageal cancer.

The most common histological subtypes of esophageal cancer are squamous cell carcinoma and adenocarcinoma. The incidence of esophageal adenocarcinoma has doubled in recent decades and prevails in the structure of the incidence of this localization in North America and European countries. In Asian countries and the Russian Federation, squamous cell carcinoma is currently the leading histological type.

The purpose of the study was to study the modern possibilities of therapy of localized and locally advanced squamous cell carcinoma of the esophagus, based on the analysis of publications in the Russian (e-library) and worldwide (PubMed; Cochrane) databases of literature.

Standards for the treatment of localized and locally advanced squamous cell carcinoma of the esophagus.

Today, according to the international classification of diseases, it is customary to divide esophageal cancer into a disease of the cervical and intra-thoracic. The term "cancer of the cervical esophagus" refers to the location of the tumor within 5 cm from m. cricopharyngeus. However, this definition has been expanded to any tumor of the esophagus located above the upper part of the chest. Cervical cancer accounts for 2 to 10 % of cases of esophageal cancer, with a predominant histological picture of squamous cell type [1].

In the combined treatment of cervical esophageal cancer, historically, remote radiation therapy or surgical intervention have been local methods of exposure. However, a number of studies have demonstrated equivalent results between chemoradiotherapy and surgery, which has changed the treatment paradigm [2]. The three-year survival rate of patients with cervical esophageal cancer ranges from 50 to 65 % [3]. To date, the surgical stage of treatment for cancer of the cervical esophagus is considered as a life-saving operation in the development of relapse after radical chemoradiotherapy. Additional problems with surgical treatment of cervical esophageal cancer are associated with the spread of the tumor to nearby structures, which may require an extension of the operation, for example, to pharyngolaryngectomy [4].

There are still no optimal regimens and regimens of chemotherapy as a component of chemoradiotherapy for tumors of the cervical esophagus. The best results, apparently, are obtained by a doublet based on platinum preparations with simultaneous radiation therapy [5]. Extrapolation of literature data on head and neck tumors demonstrated that radiation doses up to 60–70 Gy can be used, however, when using higher doses of radiation, there was no increase in survival rates [6; 7].

In patients with early forms of squamous cell carcinoma of the esophagus (intra-thoracic), including in-situ carcinoma (Tis) and tumors that grow into their own plate of the mucous and muscular membranes (T1a), without lymph node damage, endoscopic resection of the mucous membrane (EMR) or endoscopic dissection of the submucosal layer (ESD) is recommended [8].

ESD allows resection of the mucous and submucosal layers as a single unit, which allows for a higher resection frequency of R0, which is reflected in satisfactory long-term survival rates [8]. Adjuvant chemoradiotherapy is advisable in patients with poor prognostic factors, such as low-grade tumors, positive resection margins [9]. In patients after endoscopic resection of the mucous and submucosal layers of the esophagus, with morphological verification of invasion to the submucosal layer (T1b), further additional treatment is indicated, such as esophagectomy or chemoradiotherapy [10].

Patients with the absence of lymphatic collector damage, without the tumor spreading to the muscle

membrane proper ($\leq T2N0$) and a low risk of progression may be offered surgical treatment at the first stage in the volume of esophagectomy with lymph dissection [11]. However, it is worth noting that according to the literature, esophageal tumors with invasion into the deep mucous membrane (endosonographically corresponds to the lesion of M3) have an approximately 10 % risk of metastasis to regional lymph nodes. Squamous cell tumors penetrating beyond the upper third of the submucosa have a frequency of metastasis to the lymph nodes from 36 to 55 % [12].

When the tumor spreads to the muscle membrane itself and deeper ($\geq T2$), or when the lymphatic collectors are affected (N+), patients are shown multimodal therapy. In 2012, the results of the CROSS study (chemoradiotherapy of esophageal cancer followed by surgical intervention) were published, which showed an improvement in overall survival and the frequency of complete pathomorphological response in patients with both adenocarcinoma and squamous cell carcinoma of the esophagus, compared only with the surgical treatment option. This was a step towards the introduction of neoadjuvant chemoradiotherapy with the subsequent surgical stage of treatment in the clinical recommendations for the treatment of locally advanced esophageal cancer. In the CROSS cohort of patients, the frequency of complete pathomorphological response after induction 2 cycles of chemotherapy was 23 % for adenocarcinoma and 49 % for squamous cell carcinoma. It should be noted that 75 % of the patients in this study had adenocarcinoma. It is also worth noting that patients with tumor spread to the T4 level were not included in the trial [13]. After completion of neoadjuvant treatment and in the absence of progression, according to the results of a control examination, patients may be offered a surgical stage of treatment, in the volume of esophagectomy (McKe-won operation or IvorLewis operation) with standard two- or three-zone lymph dissection [14].

Several authors conducted a comparative analysis of the results of treatment of patients who received a radical course of chemoradiotherapy with patients who underwent combined treatment together with the surgical stage of treatment. Thus, in the French study FFCD 9102, which included 259 patients with locally advanced cancer of the intra-thoracic esophagus, there was no significant difference in overall

survival between these two groups. It should be noted that in 88.8 % of cases, a squamous histological variant of esophageal cancer was registered. However, the authors noted that esophagectomy after induction chemoradiotherapy reduces the frequency of locoregional relapses when compared with a radical course of chemoradiotherapy [15]. This study was criticized because patients did not undergo endosonography and the total dose of LT was 30 Gy, which is less than the standard induction dose. It should also be noted that patients who did not respond to treatment were excluded from the study [16].

In a study from Memorial Sloan Kettering, which included 232 patients with squamous cell carcinoma of the esophagus, Barbetta et al. They demonstrated an improvement in overall survival in patients who underwent trimodal therapy (neoadjuvant chemoradiotherapy followed by surgical treatment), compared only with radical chemoradiotherapy [17].

When analyzing the clinical recommendations of the USA (NCCN), the European Society of Medical Oncology (ESMO), the Russian Federation and Japan, several differences in the approach to the treatment of locally advanced cancer of the intra-thoracic esophagus are visible. The NCCN recommendations prefer trimodal therapy with preoperative chemoradiotherapy [18], which intersects with the clinical recommendations of the Russian Federation, the ESMO recommendations indicate that neoadjuvant chemoradiotherapy followed by esophagectomy is equivalent to radical chemoradiotherapy [19]. According to clinical guidelines in Japan, induction chemotherapy with subsequent surgical treatment is recommended in the absence of contraindications in patients [20].

Is there a place for a lifesaving esophagectomy after a radical course of chemoradiotherapy?

Analyzing the recommendations of European and Asian countries, it can be concluded that most authors adhere to the following tactics – conducting neoadjuvant therapy followed by surgery or performing radical chemoradiotherapy for patients with locally advanced squamous cell carcinoma of the esophagus. Recurrent or persistent esophageal cancer remains an urgent problem with a risk of relapse of the disease up to 60 % of cases [21].

One of the treatment options for patients with a persistent or recurrent form of the disease after

radical chemoradiotherapy is a lifesaving esophagectomy, provided that the patient's general somatic status is satisfactory within 6–12 weeks after the end of treatment if a relapse occurs.

The authors of a multicenter retrospective study, Markar S. et al., conducted a comparative analysis of the treatment results of patients ($n = 308$) who received lifesaving esophagectomy with a group of patients ($n = 540$) who received the surgical stage after induction chemoradiotherapy. In this work, a similar perioperative mortality was shown, while the incidence of anastomosis failure (17.2 % vs. 10.7 %; $p = 0.007$) and infectious complications was higher in the group where a radical course of CLT was performed. The overall three-year survival rate was identical and was 43.3 % versus 40.1 % ($p = 0.542$), respectively [22].

A meta-analysis of four studies involving 219 patients demonstrated the survival advantage of life-saving esophagectomy compared to repeated chemoradiotherapy (HR: 0.42; 95 % confidence interval: 0.21–0.86, $p = 0.017$). Mortality in the postoperative period was 10.3 % (3 out of 36 operated cases). The authors noted that lifesaving esophagectomy has a significant gain in long-term survival compared to repeated chemoradiotherapy but is potentially associated with high postoperative mortality [21; 22]. The data presented above are based on non-randomized studies, which may indicate a high risk of selection bias, since patients with obviously better initial characteristics received surgical treatment.

The FFCD 9102 study is noteworthy, including 451 patients who received induction therapy with a planned subsequent surgical stage of treatment. It is worth noting that 191 (42.3 %) patients out of 451 did not respond to induction therapy and were not further randomized. In 112 cases of this cohort of patients, life-saving surgery was performed, which in these 112 patients, the median overall survival did not differ from the group of randomized patients – 17.3 months versus 18.9 months ($p = 0.58$).

When analyzing subgroups of non-randomized patients, the median overall survival was higher in the cohort of patients who underwent surgery compared to non-operated patients and was 17 versus 5.5 months (HR = 0.39; 95 % CI: 0.25–0.61; $p < 0.0001$) [23]. Thus, the data presented by Vincent J. et al. they point to the advantages of performing lifesaving esophagectomy in patients with

incomplete response after neoadjuvant chemoradiotherapy.

In a retrospective study of Broderick R. C. (2021), which included 97 patients with locally advanced esophageal cancer, a comparative analysis of the treatment results of patients who received a planned (less than 90 days from the end of neoadjuvant treatment) minimally invasive esophagectomy (MIE) with a group of life-saving MIE (resection for recurrent or persistent disease after a complete response to treatment or an operation performed more than 90 days after the completion of the neoCRT). Broderick et al. there were no significant differences in 30-day postoperative mortality, anastomosis failure and duration of hospitalization. Overall survival ($p = 0.39$) and relapse-free survival ($p = 0.71$) were equivalent between the two groups [24].

According to the above studies, most authors adhere to neoadjuvant therapy with subsequent surgery, or performing radical chemoradiotherapy for patients with locally advanced squamous cell carcinoma of the esophagus. A number of studies have shown that the overall and relapse-free survival in patients after life-saving esophagectomy is higher than in patients after repeated chemoradiotherapy, especially in patients with recurrent squamous cell carcinoma of the esophagus.

Neoadjuvant therapy in the treatment of operable locally advanced esophageal cancer

The absence of modern randomized studies comparing different regimens of drug therapy alone with chemoradiotherapy followed by the surgical stage of treatment creates a dilemma of choosing the optimal treatment tactics in patients with satisfactory general somatic status and operable esophageal tumor [25]. To date, induction chemoradiotherapy remains the standard of treatment for squamous locally advanced intra-thoracic esophageal cancer, according to data obtained from the results of the CROSS study and published in 2012. and having a number of limitations described above. Recently, several clinical trials have been conducted on the neoadjuvant treatment of resectable esophageal cancer.

In the JCOG1109 (NExT) study, launched in 2012, the authors conducted a comparative analysis of the results of treatment of patients with locally advanced squamous cell carcinoma of the esophagus, who

underwent various preoperative therapy. The first group included patients who underwent 2 cycles of neoadjuvant chemotherapy with cisplatin 80 mg/m² on day 1 and 5-fluorouracil 800 mg/m² from 1 to 5 days, a cycle every 21 days (CF), in the second group 3 cycles of PCTs were used according to the DCF scheme (docetaxel 70 mg/m² in 1 day; cisplatin 70 mg/m² in 1 day; 5-fluorouracil 750 mg/m² from 1 to 5 days, cycle every 21 days) and in the third group, chemoradiotherapy with 23 fractions up to 41.4 Gy was performed as a neoadjuvant treatment with 2 cycles of radiomodification according to the scheme: cisplatin 75 mg/m² on 1 day and 5-fluorouracil 1000 mg/m² with 1 for 4 days, a cycle every 21 days [26].

In 2022, at the conference of the American Society of Clinical Oncology (ASCO) on diseases of the gastrointestinal tract, the main results of this study were reported for the first time. The results of treatment of 601 patients were analyzed. The CF group included 199 patients, the DCF group included 202, and 200 patients were registered in the chemoradiotherapy group in the period from December 2012 to July 2018. The median age was 65 years (30–75 years), patients with clinical stage III accounted for 62.6 %.

The average follow-up time was 4.2 years (0–8.5 years). The median overall survival in the CF group was 4.6 years, in the chemoradiotherapy group – 6 years, in the DCF group – was not achieved, three-year overall survival was 62.6 %, 68.3 % and 72.1 %, respectively (log-rank test: $p = 0.006$ for CF compared to DCF and $p = 0.12$ for CF compared to CF-RT). According to the stratified Cox regression analysis for the overall survival rate, the risk ratio is 0.68 [95 % CI: 0.50–0.92] in the comparison groups CF with DCF and 0.84 [0.63–1.12] for CF compared to chemoradiotherapy with the CF radiomodification scheme.

When analyzing adverse events, it is noted that grade 3–4 neutropenia, febrile neutropenia and hyponatremia were more common in the DCF group than in the CF and chemoradiotherapy groups. Grade 3–4 esophagitis was more common in the chemoradiotherapy group than in the neoadjuvant chemotherapy groups (Table 1).

Thus, the researchers note that the addition of docetaxel to neoadjuvant therapy with cisplatin and fluorouracil is accompanied by an improvement in overall survival and acceptable toxicity, compared

with the CF regimen. The authors believe that this scheme may be a new standard of treatment for locally advanced intracoracic squamous cell carcinoma of the esophagus [27].

In 2021, Wang et al. The results of a multicenter randomized trial that examined the comparative analysis of the safety and efficacy of neoadjuvant chemotherapy with chemoradiotherapy followed by minimally invasive esophagectomy were published. The study included 264 patients with esophageal squamous cell carcinoma and cT3-T4aN0/1M0 tumor prevalence who received chemotherapy with paclitaxel and cisplatin. The total dose of radiation therapy was 40 Gy (20 fractions of 2 Gy), starting from the first day of chemotherapy.

The authors note that there was no significant difference in the frequency of postoperative complications between both groups: 47.4 % in the neoCRT group (54 out of 114) and 42.6 % in the neoHT group (46 out of 108; $p = 0.48$); the degree of complications according to the Clavien-Dindo classification was the same. Postoperative mortality was 3.5 % (4 out of 114) in the group of neoadjuvant chemoradiotherapy and 2.8 % (3 out of 108) in the group of chemotherapeutic treatment only ($p = 0.94$). When evaluating the results of a remote surgical preparation in patients in the chemoradiotherapy group, a complete morphological response was more common (35.7 % vs. 3.8 %; $p < 0.001$), as well as a smaller number of affected lymph nodes (ypN0: 66.1 % vs. 46.2 %; $p = 0.03$), which directly affects survival rates.

The authors conclude that the difference in the safety profile between neoadjuvant chemotherapy and chemoradiotherapy is insignificant, however, in the neoCRT group, the indicators of pathomorphological response were recorded more often [28]. Of the various chemotherapy regimens, the DCF scheme is the most preferable as a neoadjuvant component, accompanied by an improvement in overall survival rates, which may enter new standards for the treatment of squamous locally advanced esophageal cancer.

Adjuvant therapy possibilities and the introduction of immunotherapy

Although neoadjuvant therapy is associated with improved survival compared to surgery alone, most patients do not have a complete pathomorphological response, which directly affects the prognosis of relapse.

In a retrospective study involving 118 patients treated from 2000 to 2016 with squamous cell carcinoma who received neoadjuvant ($n = 59$) or perioperative chemotherapy ($n = 59$), Yan et al. there were no differences in relapse-free or overall survival [29]. In another randomized study that examined the results of treatment of 346 patients with squamous cell carcinoma of the esophagus treated in hospitals of Xi'an Jiaotong University since January 2005. By April 2007, the effectiveness of preoperative and perioperative chemotherapy was evaluated using the PCF scheme (paclitaxel 100 mg/m² and cisplatin 60 mg/m² on day 1, followed by infusion of 5-fluorouracil (700 mg/m² mg per day for 5 days). Patients were randomized into 2 groups: group A ($n = 175$) included patients who received perioperative chemotherapy (2 + 2), group B ($n = 171$) – 4 neoadjuvant cycles.

Median follow-up was 60 and 61 months in groups A and B, respectively. The development of locoregional relapse was diagnosed in 25 patients (14.2 %) in group A and in 35 (20.5 %) – in group B, distant metastasis – in 41 (23.4 %) and 62 (36.3 %) cases, respectively. The median relapse-free survival was 23 months in group A compared to 15 in group B. Five-year relapse-free survival was 35.0 % (95 % CI: 26.1–47.2) in the perioperative chemotherapy group compared with 19.1 % (95 % CI: 15.3–28.7) in the neoadjuvant therapy group only ($p < 0.01$). In patients receiving perioperative chemotherapy, the

improvement in five-year survival was 16 % (38 % vs 22 %; $p < 0.01$) [30].

A breakthrough study that opens a new adjuvant therapy option for patients with radically operated locally advanced squamous cell carcinoma of the esophagus was the Checkmate 577 study. This randomized double-blind placebo-controlled study included the results of treatment of 794 patients with stage II or III who underwent radical surgical treatment (R0) with incomplete pathomorphological response (ypT1 or ypN1) after induction chemoradiotherapy. Patients were randomized in a 2:1 ratio into groups receiving PD-1 inhibitor (nivolumab) ($n = 532$) or placebo ($n = 262$). Patients were treated with nivolumab at a dose of 240 mg every 2 weeks / placebo for 16 weeks with a transition to a 4-week administration of 480 mg of nivolumab or placebo [31].

According to the results of the study, it was shown that the addition of nivolumab in adjuvant mode is accompanied by a satisfactory safety profile: adverse events of 3–4 degrees were observed in 71 out of 532 patients (13 %) in the PD-1 checkpoint inhibitor group, and in the placebo group this indicator was 6 % (15 out of 260). The most frequent adverse events of any severity were fatigue, diarrhea, itching and rash in patients in the nivolumab group; diarrhea, fatigue – in patients in the placebo group. When assessing the quality of life, the percentage of patients who answered "I am not at all concerned about the

Table 1. JCOG1109 (NExT) research results

	CF ($n = 199$)	DCF ($n = 202$)	CRT ($n = 200$)
Median relapse-free survival rate 5	2.7 years	Not achieved	5.3 years
Three-year relapse-free survival rate	47.7 %	61.8 %	58.5 %
Undesirable events			
Neutropenia level > 3	23.4 %	85.2 %	44.5 %
Febrile neutropenia	1 %	16.3 %	4.7 %
Hyponatremia	6.2 %	26.0 %	11.0 %
Esophagitis, level > 3	1 %	1 %	89 %
Mortality rate	3 (15 %)	4 (20 %)	2 (1.0 %)

side effects of treatment" in the questionnaire was the same in both groups. The quality-of-life indicator (FACT-E and EQ-5D-3L questionnaires) remained satisfactory throughout the treatment period.

There were 396 cases of relapse or death. The incidence of distant foci was lower in the nivolumab group (in 154 out of 532 patients – 29 %) than in the placebo group (in 103 out of 262 patients – 39 %), as was the development of locoregional relapses (12 % vs. 17 %, respectively). The authors note that the risk of long-term relapse or death was 26 % lower during adjuvant therapy with nivolumab than in the placebo group (HR 0.74; 95 % CI: 0.60–0.92). The median relapse-free survival in the nivolumab group was 22.4 months (95 % CI: 16.6–34.0) compared with 11.0 months (95 % CI: 8.3–14.3) of placebo patients ($p < 0.001$) [31].

The results of this study allow us to recommend adjuvant therapy with nivolumab to all patients with squamous cell carcinoma of the esophagus and incomplete morphological response after induction therapy and esophagectomy [18].

It is also worth noting that the number of studies studying the use of checkpoint inhibitors as one of the components of neoadjuvant treatment of patients with esophageal cancer is growing.

In 2022, Liu J. et al. The results of a multicenter, single-group phase II study of ShiCTR1900026240 were published, which studied the addition of a PD-1 inhibitor (camrelizumab) produced in China to carboplatin + paclitaxel chemotherapy in the neoadjuvant treatment of patients with locally advanced squamous cell carcinoma of the esophagus with affected mediastinal lymphatic collectors. All patients underwent 2 cycles of neoadjuvant therapy, including 200 mg of camrelizumab, nab-paclitaxel 100 mg/m² (day 1, 8, 15) and carboplatin AUC-5 on 1 day, every 3 weeks.

The study included 60 patients, of whom the full course of treatment was completed in 55 (91.7 %) patients. 58 patients (96.7 %) were diagnosed with treatment-related adverse events, the most common of which was hematological toxicity (leukopenia) – 86.7 % of cases. It should be noted that 34 patients (56.7 %) had adverse events of the 3rd degree or higher, in 1 (1.7 %) case the patient died of pneumonia and acute respiratory failure. The surgical stage of treatment was received by 51 patients, resection of R0 was achieved in 50 cases. Postoperative complications were diagnosed in 47.1 % of cases (24/51). Hospital and postoperative mortality of 30 and 90 days was not recorded.

A noteworthy factor is that this study was conducted on patients with clinically detectable lymph node lesion N2–3. According to the results of the removed surgical material, a complete pathomorphological response (ypT0N0) was achieved in 20 (39.2 %) patients, and 5 (9.8 %) patients had a complete response of the primary tumor, but with the presence of tumor cells in the lymph nodes (ypT0N+). The authors also note that there was no significant correlation between the status of PD-L1 and the pathological response in squamous cell carcinoma of the esophagus, regardless of the method of evaluating PD-L1 expression [32].

According to the CROSS study, the addition of radiation therapy to chemotherapy in the neoadjuvant mode can significantly contribute to reducing the size of the tumor and increasing the frequency of complete pathomorphosis [13]. According to a number of researchers, immuno-chemoradiotherapy can enhance the body's response to a tumor and increase the frequency of a complete pathomorphological response compared to standard chemoradiotherapy [33; 34]. To date, a number of studies have been

Table 2. Comparative analysis of studies that studied neoadjuvant chemo-immuno-radiation therapy

Research	N	Treatment algorithm	pCR	% pCR
PALACE-1	20	Pembrolizumab + CROSS	10/18	55.6
NCT02844075	28	Pembrolizumab + CROSS	12/26	46.2
CROSS	41	DCT + PCT according to TC scheme	18/37	48.6

Table 3. Comparative analysis of studies about neoadjuvant chemoimmunotherapy [37]

Research paper	Phase	Start-point and endpoint	Median	N	Neoadjuvant therapy	pCR	R0 resections	AEs Grade 3-4 (%)	CTCAE
ChiCTR1900026240 (Liu, 2022)	2	pCR	-	60	Camrelizumab + TC	20/51	50/51	34/60	5.0
TD-NICE (Yan, 2022)	2	MPR	-	45	Tisrelizumab + TC	18/36	29/36	19/45	-
ESONICT-1 (Zhang, 2021)	2	pCR, AEs	6 m	30	Sintilimab + nab-paclitaxel + cisplatin	4/23	23/23	1/30	5.0
Shen, 2021	-	Safety, feasibility	6 m	28	PD-1 inhibitor+ TC	9/27	26/27	2/28	5.0
Zhang, 2020	2	MPR	7.9 m	24	Toripalimab + nab-paclitaxel + S-1	3/18	-	-	-
ChiCTR2000028900 (Yang, 2022)	1	Safety, feasibility	13.8 m	23	Camrelizumab + TC	5/20	20/20	11/23	5.0
SIN-ICE (Duan, 2021)	NA	pCR	-	23	Sintilimab + platinum-containing chemotherapy	6/17	16/17	7/23	4.03
NCT04177797 (He, 2022)	2	Safety, feasibility and MPR	-	20	Toripalimab + TC	3/16	14/16	4/20	4.03
KEEP-G 03 (Gu, 2020)	1/2	Safety, feasibility	-	17	Sintilimab + lipo-paclitaxel + cisplatin + S-1	4/15	15/15	6/17	5.0
Li, 2020	2	pCR, MPR	4.5 m	17	Toripalimab + TC	2/12	12/12	2/17	-
Yang, 2021	-	pCR	-	16	Camrelizumab + TC	5/16	15/16	-	5.0
NCT03985670 (Xing, 2021)	2	pCR	-	15	Toripalimab (day 3) + TP (day 1)	4/11	11/11	3/15	5.0
				15	Toripalimab (day 1) + TP (day 1)	1/13	13/13	7/15	
FRONTIER (Yamamoto, 2021; Matsuda, 2022)	1	Toxicity	-	6	Nivolumab + CF (Group A)	2/6	6/6	-	4.03
				12	Nivolumab + DCF (Group C and D)	4/12	11/12	-	

Note: pCR – complete pathomorphologic response; AEs Grade 3–4 – unwanted events grade 3–4; MPR – maximal pathomorphologic response; TS – chemotherapy according to the scheme: carboplatin + paclitaxel.

conducted examining the addition of immunochemotherapy to radiation therapy (Table 2).

A study examining the effect of the addition of a checkpoint inhibitor (Pembrolizumab) to chemoradiotherapy according to the CROSS-scheme is a single-center, prospective, single-group study of PALACE-1. Of the 20 patients included in the study, 19 (95 %) received a full course of preoperative treatment, one patient was not given a course of CT due to hematological toxicity. In 18 (90 %) cases, patients underwent the surgical stage of treatment (1 patient had metastatic lesion after the end of neoadjuvant therapy and in 1 case death occurred due to arrosive bleeding).

According to the results of the morphological study, the frequency of complete pathomorphological response was 55.6 % for neoadjuvant therapy with pembrolizumab in combination with chemoradiotherapy [35].

In the NCT02844075 study, out of 28 included patients who received Pembrolizumab with neoadjuvant chemoradiotherapy, esophagectomy was performed in 26 cases. A complete pathomorphological response in the primary tumor was achieved in 46.1 % of patients who underwent resection (95 % CI: 28.8–64.6). Overall survival rates after 6, 12 and 18 months were 89.3 %, 80.8 % and 73.1 %, respectively [36].

Analyzing prospective studies examining the attachment of monoclonal antibodies in neoadjuvant mode to patients with trimodal therapy, Zhu J. et al. (2022) it has been shown that immunotherapy does not significantly improve the frequency of complete pathomorphological responses in squamous cell carcinoma of the esophagus but leads to an increase in the frequency of adverse events of 3–4 degrees [37].

The multicenter randomized phase III trials that have begun should show the effect of neoadjuvant immunotherapy on long-term survival.

CONCLUSIONS

To date, the recommendations for the treatment of locally advanced esophageal cancer vary in different countries. Thus, according to ESMO recommendations, preference is given to a radical course of chemoradiotherapy, in the USA and the Russian Federation – trimodal therapy with preoperative chemoradiotherapy. Clinical guidelines from Asian countries recommend induction chemotherapy followed by surgical treatment in operable patients. In addition to economic factors, the histological type of tumor is of leading importance. The CROSS study shows the effectiveness of chemoradiotherapy followed by surgical treatment in patients with esophageal cancer, but it is worth noting that T4 tumors were not included in the trial. Thus, when the tumor spreads to adjacent structures and with potential operability, neoadjuvant polychemotherapy according to the DCF scheme is indicated.

Over the past decade, immunotherapy with monoclonal antibodies has been active in the treatment of patients with esophageal cancer, blocking the interaction between the programmed death receptor (PD-1) and its ligands (PD-L1 and PD-L2). Thus, the addition of nivolumab in adjuvant mode in patients with incomplete pathomorphological response after trimodal therapy is accompanied by a satisfactory safety profile and improved survival rates, which led to the inclusion of this treatment option in clinical recommendations.

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