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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.

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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South Russian Journal of Cancer: professional medical publication. It publishes news from the medical and pharmaceutical communities, scientific and practical articles for the target audience-oncologists. The editorial board of the journal aims to popularize the research works and achievements of oncologists of the Southern Federal District, to analyze the process of deep reorganization of healthcare in Russia. The editorial board invites as authors all those who are looking for and find interesting solutions to the multifaceted problems facing modern medicine and want to share their thoughts and observations with colleagues.

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

СОДЕРЖАНИЕ

South Russian Journal of Cancer 2023, Vol. 4, No. 3. P. 6-11
https://doi.org/10.37748/2686-9039-2023-4-3-1
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ORIGINAL ARTICLE

EXPERIENCE IN SURGICAL TREATMENT OF VERTEBRAL METASTATIC TUMORS OF CRANIOVERTEBRAL LOCALIZATION

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ABSTRACT

Purpose of the study. Elaboration of a surgical technique to manage patients with metastatic lesions of the craniovertebral region.
Patients and methods. The study included 7 patients with metastatic lesions of the craniovertebral region, who've been operated on for severe instability, pain syndrome, neurological deficit in the period from 01/01/2014 to 09/30/2022. To assess the neurological status and patients' condition the Frankel and Karnofsky scales were used on the day of admission and discharge of the patients from the hospital. Pain intensity was assessed using a visual analog pain scale (VAS). To assess instability in the affected spinal motion segment the SINS scale was used. All patients underwent palliative surgical treatment in the amount of occipitospondylodesis with a biopsy of the neoplasm from the posterior approach.
Results. The average age of patients was 60 [44; 66] years. All patients had a marked pain syndrome prior to the surgery. The average pain intensity according to the visual analog pain scale was 8 points. In the preoperative period, 6 (85 %) patients on the Frankel scale were assigned to group E, 1 (14 %) – to group C. In 6 (85 %) patients there was no dynamics in the neurological status following the surgery, however according to the Karnofsky scale there was an improvement up to 10 points due to the regression of the pain syndrome down to 1 point on the visual analog scale. Hemiparesis developed in 1 (14 %) patient due to malposition of the laminar hook in the postoperative period. The average duration of surgical interventions made up 337.5 [315; 345] min, the average intraoperative blood loss made up 300 [300; 800] ml. In 6 out of 7 patients (85 %) there was no neurological status dynamics after the surgery, and according to the Karnofsky scale an improvement up to 10 points was noted due to regression of the pain syndrome to an average value of 1 [1; 2] VAS score.
Conclusion. The obtained results indicate the clinical application possibilities of minimally traumatic surgical technologies for the treatment of craniovertebral zone metastatic tumors.

Keywords: metastatic tumors, craniovertebral area, surgical treatment

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ОРИГИНАЛЬНАЯ СТАТЬЯ

ОПЫТ ХИРУРГИЧЕСКОГО ЛЕЧЕНИЯ МЕТАСТАТИЧЕСКИХ ОПУХОЛЕЙ ПОЗВОНКОВ КРАНИОВЕРТЕБРАЛЬНОЙ ЛОКАЛИЗАЦИИ

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РЕЗЮМЕ

Цель исследования. Разработка методики хирургического лечения пациентов с метастатическим поражением краниовертебральной области.

Пациенты и методы. В исследование включено 7 больных с метастатическим поражением краниовертебральной области, оперированных по поводу выраженной нестабильности, болевого синдрома, неврологического дефицита в период с 01.01.2014 по 30.09.2022 гг. Для оценки неврологического статуса и состояния пациентов использовали шкалы Frankel и Karnofsky в день поступления и выписки пациентов из стационара. Интенсивность болевого синдрома оценивали по визуально аналоговой шкале боли (ВАШ). Для оценки нестабильности в пораженном позвоночно-двигательном сегменте пользовались шкалой SINS. Всем пациентам выполнено паллиативное хирургическое лечение в объеме окципитоспондилодеза с биопсией новообразования из заднего доступа.

Результаты. Средний возраст больных составил 60 [44; 66] лет. У всех пациентов до операции отмечался выраженный болевой синдром, средняя интенсивность боли по визуально аналоговой шкале боли составиляла 8 баллов. В предоперационном периоде 6 (85 %) больных по шкале Frankel отнесены к группе Е, 1 (14 %) – к группе С. После операции у 6 (85 %) больных динамика неврологического статуса отсутствовала, однако по шкале Karnofsky отмечалось улучшение до 10 баллов вследствие регресса болевого синдрома до 1 балла по визуально аналоговой шкале боли. У 1 (14 %) больного вследствие мальпозиции ламинарного крючка в послеоперационном периоде развился гемипарез. Средняя продолжительность выполненных оперативных вмешательств составила 337,5 [315; 345] мин, средняя интраоперационная кровопотеря – 300 [300; 800] мл. У 6 из 7 больных (85 %) динамика неврологического статуса после операции отсутствовала, а по шкале Karnofsky отмечалось улучшение до 10 баллов вследствие регресса болевого синдрома до среднего значения 1 [1; 2] балл по ВАШ.

Заключение. Полученные результаты свидетельствуют о возможностях клинического применения минимальных по травматичности технологий в хирургическом лечении метастатических опухолей краниовертебральной зоны.

Ключевые слова: метастатические опухоли, краниовертебральная область, хирургическое лечение

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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 № 118 от 02.06.2022 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.

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INTRODUCTION

Metastatic lesion of the upper cervical segments of the vertebral column occurs in no more than 0.5–1 % of cases. Due to the low frequency of lesions of the upper cervical vertebrae, most studies in the available literature are represented by small series of patients. The body of the second cervical vertebra is most often affected due to its size and abundant blood supply. The main problem of this category of patients is spinal instability, which, in addition to severe pain syndrome (in 90 % of patients), can manifest symptoms of compression of the upper spinal cord such as tetraparesis (in 0–20 % of patients), and with the development of ascending edema, even respiratory arrest and death [1; 2]. Thus, the fixation of the spine comes to the fore in the surgery of metastatic lesions of the craniovertebral region. External fixation methods (collar, Halo device) are inconvenient and can cause various complications with prolonged use in an average of 40 % of patients. And no convincing data on their effectiveness has been obtained [3]. Internal fixation methods are preferred.

According to the literature, there are several types of surgical strategies. The first and least radical option involves the use of occipitospondylodesis from the posterior access without resection of the tumor [3; 4]. The advantage of posterior access is explained by the peculiarities of the axial load distribution in the craniovertebral region, where 64 % of it falls on the posterior structures, and not on the anterior ones as in the lumbar spine. The use of occipital plates with bicortical screws and screws for lateral masses are the methods of choice due to the high fatigue strength and torsion resistance of the structure.

The second variant of surgical tactics implies the addition of posterior fixation by vertebroplasty with transpedicular access [5], less often with transoral access [6].

The third and most radical variant of the tactic, in addition to fixation from the posterior access, implies intra-tumor resection of the neoplasm through a retropharyngeal [7; 8] and even a transmandibular [9] approach. Thus, the problem of choosing the optimal tactics in the surgical treatment of metastatic spinal tumors, including craniovertebral localization, still remains insufficiently developed.

The purpose of the study: to develop a technique for surgical treatment of patients with metastatic lesions of the craniovertebral region.

PATIENTS AND METHODS

The study included 7 patients with craniovertebral lesion out of 145 patients operated on for metastatic tumors of the vertebrae in the period from 01/01/2014 to 06/30/2022 in the Department of Neuro-Oncology of the National Medical Research Center for Oncology and the Department of Neurosurgery of the Moscow State University of Medicine and Dentistry named after A. I. Evdokimov.

To assess the neurological status and condition of patients, the Frankel and Karnofsky scales were used on the day of admission and discharge of the patient from the hospital. The intensity of the pain syndrome was assessed by a visually analog pain

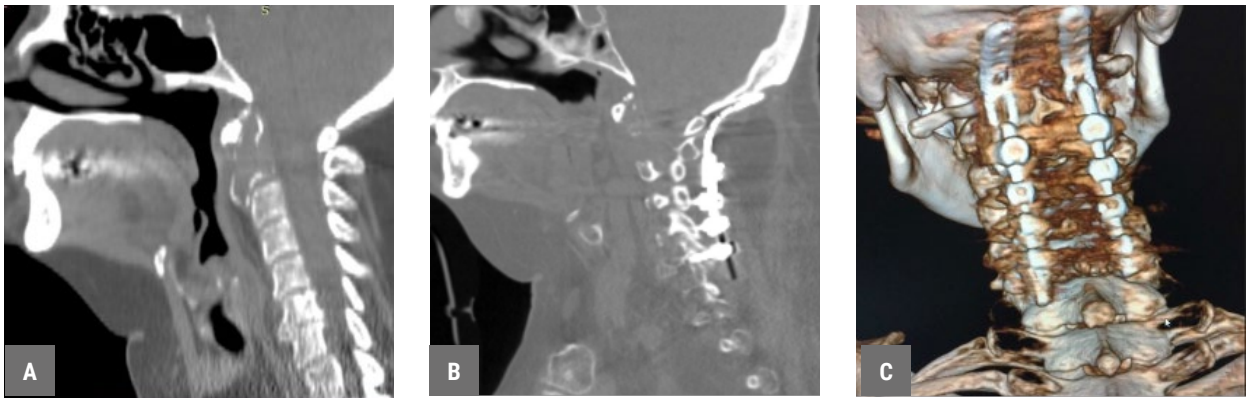

Fig. 1. Occipitospondylodesis in patient No. 3. A – computed tomography data before surgery. B, C – computed tomography data after the surgery.

scale (VAS). The SINS scale was used to assess instability in the affected vertebral-motor segment.

All patients underwent palliative surgical treatment in the volume of occipitospondylodesis with a biopsy of the neoplasm from the posterior access. Occipitospondylodesis was performed using a design consisting of occipital plates and neck screws inserted into the lateral masses, or laminar hooks (depending on surgeon's choice) (Fig. 1).

All the necessary patient data were recorded in the Microsoft Excel electronic database, after which the data was analyzed using the Statistica 7.0 program. For each group of indicators, the type of data distribution was determined (histograms were constructed according to the Kolmogorov-Smirnov agreement criterion). When the distribution differs from the normal one, median values, 1st and 3rd quartiles (Me [Q1; Q3]) were used for the description.

RESEARCH RESULTS

The study group of patients was analyzed according to such signs as: gender, age, histological type of primary tumor, localization of metastatic lesion, indicators on the Frankel, Karnofsky, VAS, SINS scales (Table 1).

The average age of the patients was 60 [44; 66] years. All patients had anterior and anterolateral localization of the neoplasm with a predominant lesion of the vertebral bodies, 2 (28 %) patients had unilateral sprouting of the vertebral artery tumor. In 6 (85 %) patients, the degree of epidural compression corresponded to grade 1, in 1 (14 %) patient – grade 3 (due to pathological dislocation of the vertebra). The average score on the SINS scale among all patients on this scale was 8.5 [7; 9]. All patients had significant pain syndrome before surgery with the average pain intensity scores according to VAS of 8 [7; 8].

Concomitant visceral metastases were detected in 2 (28 %) patients, metastases to other bones – in 2 (28 %) patients, metastatic lesions of the liver, lungs, pelvic bones and spinal column were simultaneously diagnosed in 1 patient.

The average duration of surgical interventions performed was 337.5 [315; 345] minutes, the average intraoperative blood loss was 300 [300; 800] ml. Intraoperative complication in the form of a laminar hook malposition with spinal cord compression and the development of hemiparesis in the early postoperative period was noted in 1 patient.

The dynamics of neurological disorders on the Frankel scale and the functional status of the patient on the Karnofsky scale after surgical stabilization were analyzed in a group of patients. In 6 out of 7 patients (85 %), there was no dynamics of neurological status after surgery, and according to the Karnofsky scale, an improvement of up to 10 points was noted due to the regression of pain syndrome to an average value of 1 [1; 2] point according to VAS.

Table 1. Operated patients' characteristics

Patient	Age, years	Sex	Histology	Tumor localization	By Frankel	Scores by Karnofsky	VAS scores	SINS
Scores	66	F	Carcinoma WAD	C1-C2	E	70	7	11
2	67	M	Carcinoma WAD	C1-C2	C	50	8	9
3	44	F	Renal clear cell carcinoma	C2	E	70	8	7
4	37	M	Renal clear cell carcinoma	C1	E	70	7	7
5	59	F	Renal clear cell carcinoma	C2	E	50	10	8
6	61	M	Lung adenocarcinoma	C2-C3	E	60	8	9
7	60	M	Carcinoma WAD	C2	E	70	7	7

Note: WAD stands for: without additional details.

DISCUSSION

The purpose of surgical intervention in metastatic lesions of craniovertebral localization cannot be radical removal of the tumor. Even block resection is effective only with respect to local control of metastasis, provided it is performed within healthy tissues, but it is unable to prevent the progression of metastatic lesion in general [12]. In our opinion, intra-tumor cytoreduction of secondary neoplasm at the C1-C2 levels can be surgically extremely challenging, according to the data of literature sources (Table 2).

The analysis of publications shows that the addition of posterior fixation in case of C1-C2 segment damage by intra-tumor cytoreduction significantly lengthens the duration of surgery, significantly increases intraoperative blood loss and surgical risks, with similar results of the degree of regression of pain syndrome in the immediate and long-term postoperative period.

The benefits of surgical decompression in the developed phenomena of pronounced epidural compression of the spinal cord by a tumor at first glance seems obvious. However, Uei H. et al. [13] when choosing the volume of decompression, consider the leading factor not to be the degree of epidural compression of the spinal cord, but the severity of limb paresis and recommend the use of decompression with a degree of paresis D2 or more instead. Uei H. et al. [13] believe that there is no direct correlation between the severity of paresis and the degree of epidural compression. Uei H. et al. and other authors report the advantages of stabilization without decompression over decompression-stabilizing interventions in patients with metastatic vertebral lesion, even with 2–3 degrees of epidural compression without neurological deficiency [13; 14].

In this study, it was noted that in patients, the manifestation and clinical picture of the disease caused by motor deficiency (Frankel group C) was only in 1 (17 %) of the patient, in the remaining patients there was no violation of the transverse conduction function of the spinal cord at the levels of C1-C2 segments. An MRI picture of marked epidural compression was also presented only in one case, however in another patient. Thus, there were no indications for decompression of the spinal canal in the group of patients presented by us.

Taking into account the topographic and anatomical features of the craniovertebral region, the high risks of developing instability of the craniospinal zone, accompanied by a intense pain with its metastatic lesion, the use of isolated spine stabilization technology seems to be the most favourable in the treatment of this category of patients. This is indirectly confirmed by the fact that most of the available publications on the topic of surgical treatment of metastatic lesions of the craniovertebral region describe this technology [3-6].

CONCLUSION

The data obtained by us indicate clinically satisfactory results on minimal traumatic technologies utilized in the scenario of craniovertebral zone metastatic leasions surgical treatment.

Table 2. Literature data on various types of surgical interventions performed in patients with metastatic lesions of the craniovertebral region

Intervention type	Credits	Number of patients	Procedure duration (min)	Blood loss (ml)	Complication frequency	Pain regression according to VAS
OSD*	Rustagi T. et al., 2019 [3]	39	235.0 ± 51.9	364.8 ± 252.1	8 %	From 8.3 ± 1.5 to 1.0 ± 1.1 (p < 0.001)
OSD+VP*	Wu X. et al., 2018 [5]	10	182 (120–255)	450 (250–850)	10 %	From 8.2 ± 0.4 to 2.3 ± 0.2 (p <0.001)
OSD+TR*	Wu X. et al., 2016 [7]	15	252 (150–300)	1240 (760–2200)	27 %	From 7.86 ± 1.72 to 2.13 ± 1.40 (p < 0.01)

Note: OSD stands for occipitospondylodesis, OSD+VP is for combination of occipitospondylodesis and vertebroplasty, OSD+TR is for combination of occipitospondylodesis and tumor resection from retropharyngeal/posterior access.

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ORIGINAL ARTICLE

CLINICAL AND MORPHOLOGICAL FEATURES OF BLADDER CANCER COURSE IN HPV-INFECTED PATIENTS

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ABSTRACT

Purpose of the study. To study the histological type, grade of tumor differentiation in patients with primary and recurrent clinically non-muscle-invasive bladder cancer (NMIBC) with highly carcinogenic human papillomavirus (HPV) infection.
Patients and methods. Formalin-fixed and paraffin-embedded bladder tumor tissue samples have been studied in 159 patients who underwent transurethral resection (TUR) of the bladder, for the presence of HPV DNA. To detect, quantify and differentiate DNA of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 genotypes in the samples, the AmpliSense® HPV HRC genotype-titer-FL was used. The result of the study was taken into account when the amount of DNA of the β-globin gene was at least 1000 copies per reaction. In order to statistically analyze our data we used the Fisher exact test and also calculated the odds ratio (OR) and 95 % CI.
Results. According to the results of the study, out of 159 patients, high-risk HPV DNA was detected in the tumor tissue in 59 (37.1 %), of which HPV type 16 was found in 52 patients (89.4 %), HPV 18 was detected in 4 patients type (6.7 %) and type 35 in 3 (5.08 %). In a morphological study of the tissues of HPV-positive patients, the grade of tumor differentiation was G2 in 18 cases (30.5 %), G3 in 37 blocks, and G1 was detected only in 4 cases (6.7 %). In the presence of HPV, the chance of detecting a stage G3 tumor increases by 4.3 times. According to the received data, we can assume that there is a close relationship between detection in HPV patients of high-risk genotypes with moderately differentiated and low-differentiated forms of bladder cancer.
Conclusion. this study may indicate that HPV infection affects the grade of tumor differentiation, and this, in turn, may allow the use of the HPV test to assess the nature of the development of relapse and/or progression of the disease.

Keywords: bladder cancer, human papillomavirus, urothelial carcinoma, transurethral resection of the bladder

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Compliance with ethical standards: the ethical principles presented by the World Medical Association Declaration of Helsinki (1964, ed. 2013) were observed in the work. Informed consent was obtained from every participant of the study.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

КЛИНИКО-МОРФОЛОГИЧЕСКИЕ ОСОБЕННОСТИ ТЕЧЕНИЯ РАКА МОЧЕВОГО ПУЗЫРЯ У ВПЧ-ИНФИЦИРОВАННЫХ БОЛЬНЫХ

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РЕЗЮМЕ

Цель исследования. Изучение гистологического типа, степени дифференцировки опухоли, у больных первичным и рецидивирующим клинически немышечно-инвазивным раком мочевого пузыря (НМИРМП) при инфекции вирусом папилломы человека (ВПЧ) высокого канцерогенного риска.

Пациенты и методы. Изучены образцы фиксированных в формалине и залитых в парафин тканей опухолей мочевого пузыря у 159 пациентов, перенесших трансуретральную резекцию (ТУР) мочевого пузыря на наличие ДНК ВПЧ. Для выявления, количественного определения и дифференциации ДНК ВПЧ 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 генотипов в образцах использовали набор реагентов «АмплиСенс® ВПЧ ВКР генотип-титр-FL». Результаты учитывались при количестве ДНК β-глобинового гена не менее 1000 копий на реакцию. Для проведения статистического анализа полученных нами данных, мы использовали точный критерий Фишера, а также рассчитывали отношение шансов (OR) с 95 % ДИ (CI).

Результаты. По результатам исследования из 159 пациентов ДНК ВПЧ высокого риска была обнаружена в ткани опухоли у 59 (37,1 %), из них ВПЧ 16 типа у 52-ти больных (89,4 %), у 4-х выявлен ВПЧ 18 типа (6,7 %) и 35 типа у 3-х (5,08 %). При морфологическом исследовании тканей ВПЧ-позитивных пациентов степень дифференцировки опухоли в 18 случаях (30,5 %) являлась G2, в 37 блоках- G3 и лишь в 4 случаях (6,7 %) выявлен G1. При наличии ВПЧ повышается в 4,3 раза шанс обнаружения опухоли стадии G3. По полученным данным мы можем предположить, что имеется тесная связь между выявлением у пациентов ВПЧ генотипов высокого риска с наличием умеренно дифференцированных и низкодифференцированных форм рака мочевого пузыря (РМП).

Заключение. Данное исследование может свидетельствовать о том, что ВПЧ инфекция влияет на степень дифференцировки опухоли, а это в свою очередь, может позволить использовать ВПЧ-тест для оценки характера развития рецидива и/или прогрессирования заболевания.

Ключевые слова: рак мочевого пузыря, вирус папилломы чело века, уротелиальная карцинома, трансуретральная резекция мочевого пузыря

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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). 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.

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INTODUCTION

Bladder cancer (BC) is one of the most common pathological conditions, which ranks 7th among men and 17th among women in the world. In Russia, this disease ranks 9th among the male population (4.6 %), and 16th among women population. In recent years, the incidence of bladder cancer has increased, however, the widespread use of visualization diagnostic methods allows diagnosing bladder cancer in 75 % of cases at stage I-II of the disease. Nevertheless, this disease is a growing public health problem due to the frequent recurrence and progression of the tumor process [1].

Urothelial carcinoma, which accounts for about 90 % of all cases of bladder cancer, is so far the most common histological type worldwide. The stratification of bladder cancer can be binary, based on the depth of penetration, i. e. muscle-invasive (MIBC) and non-muscle-invasive (NMIBC) bladder cancer. NMIBC accounts for about 75 % of newly diagnosed urothelial cell carcinoma of the bladder [2]. Due to the high mitotic activity of urothelial bladder carcinoma (UBC), despite the radical transurethral resection of the bladder tumor and adjuvant intravesical therapy, after performing transurethral resection of the primary tumor in NMIBC, relapse occurs in 30–60 % of cases [3].

Well-known risk factors for bladder cancer include cigarette smoking, several occupations associated with exposure to aromatic amines (for example, industrial production of dyes), cyclophosphamide and frequent use of the analgesic phenacetin. Possible carcinogens for bladder cancer are parasitic (schistosomiasis) and bacterial agents (nonspecific urinary tract infections, gonorrhea), as well as viral infections such as human papillomavirus (HPV) [4; 5].

One of the most common sexually transmitted infections is HPV. During the examination of patients in medical centers of the Russian Federation (RF) in 2019 the HPV deoxyribonucleic acid (DNA) was discovered in 5015 (39 %) of 12946 examinees. It was also noted that 3509 patients had one type of HPV, and 1957 patients had several types of HPV, and among 5015 people 8584 had HPV of different types [6]. There are more than 200 different types of HPV affecting human mucous membranes and skin, of which 14 types belong to the high-risk group (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 73). Those are detected in 98 % of cases of cervical, vaginal and vulvar cancers. HPV 6 and 11 are the most common cause of genital warts, whereas HPV types 16 and 18 lead to the development of intraepithelial neoplasia and cervical cancer [7]. HPV is also a well-known mucosotropic carcinogen and a common cause of cancer in the anogenital region.

HPV DNA replication occurs only in the cells of the basal layer, and in the cells of other layers of the epidermis, viral particles only persist, including the transition zones of the multilayer epithelium into the cylindrical one. This process is controlled by virus proteins, which disrupt the normal process of cell differentiation, leading the death of the cellular nucleus and, as a result, the alteration of the epidermis.

A feature of HPV is its ability to persist in the human body for a long time, affecting only the basal layer of the epithelium, while it does not penetrate into the blood. The virus resides in the form of an episome in the cell, and due to this infection course is often benign [8].

When comparing groups of HPV-positive and HPV-negative patients, higher cellular anaplasia was found in people with BC infected with HPV, due to which primary cancer is more often HPV-positive in contrast to recurrent [9].

According to a study by the Department of Human Pathology of Wakayama Medical University (Japan), using the in situ miRNA hybridization (RISH) RNAscope method, high-risk HPV E6/E7 mRNA was analyzed in shear of BC tissues filled with paraffin. Low-grade and high-grade urothelial cancer (UC) were detected in 61 (26.8 %) and 167 (73.2 %) cases, respectively. Noninvasive UC was the most common tumor (39.5 %, including 37.3 % pTa and 2.2 % PTIs), followed by invasive pT1 (21.9 %), pT2 (18.0 %), pT3 (11.4 %), pT4 (3.1 %) and metastatic tumor (6.1 %) [3].

Despite the experimental and theoretical data accumulated up to date, many oncogenic properties of HPV, their involvement in the pathological process and influence on the processes of relapse, progression of bladder cancer remain poorly understood.

Purpose the study: to study the histological type and the grade of tumor differentiation in patients with primary and recurrent clinically muscle- noninvasive BC with HPV infection of high carcinogenic risk.

PATIENTS AND METHODS

Our study involved patients with confirmed BC (*n* = 159) who underwent transurethral resection (TUR) of the bladder. The average age of the patients was 63.7 years ± 11.6 years. Among them 136 men and 23 women were. All patients (*n* = 159) included in the study had a preoperative clinical stage cT1N0M0. The criteria for inclusion in the research work were the following: morphologically confirmed non-muscle-invasive urothelial bladder cancer in the clinical stage cT1N0M0, where can be performed transurethral resection. The patients voluntary participation in all stages of the study was confirmed by informed consents they have signed. We also put forward criteria according to which we excluded patients from the study, and those are: the presence of non-urothelial BC; the presence of therapeutic or psychiatric reasons that could potentially challenge the participation in the study; pregnancy or lactation; inability to perform transurethral resection of BC. Transurethral resection of the bladder for primary BC was performed in 97 patients, and 62 patients underwent surgery for recurrent BC.

HPV status of patients was determined by PCR test. The presence of HPV DNA was determined in tumor tissue, which was fixed in formalin and filled with paraffin (FFPE tissue). Before the analysis, the paraffin was removed with xylene and 96 % ethanol. HPV DNA of high cancerogenic risk was determined (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 types) by PCR. The cross-binding of formalin to DNA was eliminated by incubation at a temperature of 90 °C after cleavage with proteinase. The result of the study was taken into account when the amount of DNA of the β-globin gene was at least 1000 copies per reaction. To carry out statistical analysis of the data obtained by us, we used the Chi-square Criterion with the Yates correction, the exact Fisher criterion, and also calculated the odds ratio (OR) with 95 % CI. To assess the strength of the relationship between the risk factor and the histotype, stage or relapse, a normalized value of the Pearson coefficient or Kramer's criterion V was used.

STUDY RESULTS

According to our study, in samples from 159 FFPE DNA blocks, HPV of high oncogenic risk was detected in tumor tissue in 59 patients (37.1 %). HPV type 16 of those was present in 52 patients (89.4 %), HPV type 18 (6.7 %) in four patients, and HPV type 35 was detected in three patient (5.08 %). During morphological examination of the tumor tissue of HPV-positive patients, only 43 (72.6 %) patients had a typical transitional-cell BC, and 15 (24.4 %) patients had squamous cell differentiation and 1 (1.7 %) patient had micropapillary BC (Table 1). The last two histological variants refer to tumors of high malignant potential (high-grade), which is a poor prognostic criterion. While in HPV-negative patients, 96 (96 %) patients had a typical transitional cell BC and 4 (4 %) patients had squamous cell differentiation

Evaluation of the significance of histotype differences depending on the exposure to risk factor (HPV) showed that statistically significantly the absence of HPV infection leads to the development of transitional cell BC (Chi-squared criterion with Yates correction = 15.994, $p < 0.001$; Fisher's exact

Table 1. Urothelial cancer morphological type depending on HPV presence

	HPV-positive BC patients (*n* = 59)	HPV-negative BC patients (*n* = 100)
Transitional cell BC (*n* = 139)	43 (72.9 %)	96 \ 100 (96 %)
Squamous cell BC (*n* = 19)	15 (25.4 %)	4 \ 100 (4 %)
Miscropapillary BC (*n* = 1)	1 (1.7 %)	Not detected

criterion (bilateral) = 0.00004, $p < 0.05$). At the same time, the strength of the relationship between the risk factor and the histotype was relatively strong (the normalized value of the Pearson coefficient = 0.451).

Histological examination of the tumor tissue of HPV-positive patients according to the degree of tumor differentiation obtained the following results: G2 was detected in 18 cases (30.5 %), G3 – in 37 blocks (62.7 %) and only in 4 cases (6.7 %) G1 was detected (Table 2)

The statistical analysis showed that the presence of HPV reduces the chance of detecting a tumor of stage G1 (Lower limit, 95 % CI 0.140 Upper limit 95 % CI (CI) 1.427) and G2 (Lower limit 95 % CI 0.161. Upper limit 95 % CI 0.629). In the presence of HPV the chance of detecting a G3 stage tumor increases by 4.3 times (The lower limit is 95 % CI 2,180, the upper limit is 95 % CI 8,578), according to the obtained data, we can assume that there is a close relationship between the detection of high-risk HPV genotypes in patients with the presence of moderately differentiated and low-differentiated forms BC.

We also found that HPV does not significantly affect the development of grade G1, but affects the development of grade G2, as well as G3 (Fisher's exact criterion (bilateral) = 0.00003, $p < 0.05$).

In the structure of patients, recurrent HPV infection was detected more often (in 46.8 % of cases) than in patients with primary BC (in 30.9 % of cases) (Table 3).

According to the criteria for assessing the significance of differences in outcomes, our value (4.07) exceeds the critical one, which means that based on the application of Pearson's criterion $\chi 2$, the null hypothesis about the absence of a statistical relationship between the studied risk factor and the outcome can be rejected at a critical significance level of 5 % ($p = 0.044$). At the same time, the value of the Yates-adjusted $\chi 2$ criterion is 3.419, which is less than the critical value (3.841), which means that we cannot reject the null hypothesis about the absence of a statistical relationship between the risk factor and the outcome ($p = 0.065$). It is also shown that there is a weak link between the development of relapse of BC and HPV infection (Kramer's criterion V = 0.160).

However, an analysis of the effect of HPV infection on disease recurrence when calculating the odds

Table 2. The quantitative relations among HPV-positive and HPV-negative morphologically confirmed BC cases of different tumor grades of differentiation

	HPV-positive BC patients (*n* = 59)	HPV-negative BC patients (*n* = 100)
G1 (person)	4 (6.8 %)	14 (14 %)
G2	18 (30.5 %)	58 (58 %)
G3	37 (62.7 %)	28 (28 %)

Table 3. HPV status of the patients with primary and recurrent BC

	HPV-positive BC patients (*n* = 59)	HPV-negative BC patients (*n* = 100)
Primary BC (*n* = 97)	30 (30.9 %*)	67 (69.1 %*)
Recurrent BC (*n* = 62)	29 (46.8 %**)	33 (53.2 %**)
G3	37 (62.7 %)	28 (28 %)

Note: * – out of all patients with primary BC, ** – out of all patients with recurrent BC.

ratio with a 95 % confidence interval showed that HPV infection increases the chances of relapse by 2 times (Odds Ratio (OR) = 1.963, the standard error of the odds ratio (S) = 0.336, the lower limit 95 % CI = 1.015, the upper limit 95 % CI = 3.793).

After pathomorphological examination of the surgically removed material migration of the stage from clinical preoperative T1 to stage T2 in the postoperative material in HPV-positive patients with BC was observed 4.5 times more often (13.5 % of cases) than in HPV-negative patients (3 % of cases of BC), (Odds ratio (OR) = 5.072, S = 0.699, lower limit 95 % CI = 1.289, upper limit 95 % CI = 19.951) (Table 4).

Statistical analysis also showed the presence of an average strength of the relationship between the development of stage T2 and the presence of HPV infection (Kramer's Criterion V = 0.201). At the same time, the stage of the disease statistically significantly depended on the presence of HPV (Chi-squared with Yates correction = 4,890, p = 0.028).

DISCUSSION

Many scientists believe that HPV type 16 is often actually involved in the process of BC formation. HPV-affected tumor cells are able to influence the microenvironment, causing tumor recurrence from normal urothelial cells that were in close proximity to the area of the removed tumor. The direct effect on the HPV microenvironment of an infected tumor during its removal may further contribute to a prognostic factor [9].

HPV infection in most cases is cured spontaneously. In other cases, with the persistence of a high-risk virus, the risk of malignant neoplasm increases.

The confirmation of a specific type of HPV is necessary because different types of this virus have different potential to participate in carcinogenesis: oncogenic types of HPV are 16 and 18 for instance. The detection of several types of virus is a negative outcome and a more severe course of the disease with a high risk of persistence.

Given the contradictory nature of the literature data on the role of human papillomavirus in the pathogenesis of bladder cancer, we sought to investigate the frequency of their involvement in a cohort of patients with BC of varying degrees of invasion and differentiation. DNA was determined in 159 paraffin blocks for the presence of human papillomavirus. (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 types) of high oncogenic risk were determined by real-time PCR. Due to the limits of analytical sensitivity, our data cannot exclude the presence of extremely low levels of HPV16 or HPV18 in bladder tumors. Another factor proposed to explain the differences in the prevalence of bladder tumors is that the virus may not infect all parts of the tumor tissue in the same way. Thus, if the samples are not taken from an infected area, the test may give a false negative result. Similarly, contamination during sampling can lead to false positive results [5].

Out of 59 HPV positive results, histological examination revealed squamous cell differentiation of the tumor in 15 (25.4 %). This type of differentiation often indicates poor sensitivity to radiation and systemic chemotherapy. HPV infection was also detected in a single patient with micropapillary BC. One of the aggressive variants of the morphological component is micropapillary, and due to the low degree of cell differentiation and invasion into the muscle layer, the five-year survival rate in this variant of BC is 51 %. Of 59 HPV-positive patients, 37 (62.7 %) had a low degree of differentiation, 18 (30.5 %) had a moderate degree, and only 4 (6.8 %) had a high degree of differentiation.

It should be noted that in all patients with a positive HPV test, a low viral load was detected on average 2.1 ± 0.8 lg HPV\$10^5$ × β-globin, which we explain by the fact that all HPV tests used to date have been

Table 4. Postoperative stages of BC patients following the TUR of the bladder

	HPV-positive BC patients (n = 59)		HPV-negative BC patients (n = 100)	
Stage before the surgery/ number of patients	T1 (n = 59)	T2 (n = 0)	T1 (n = 100)	T2 (n = 0)
Stage after the surgery/ number of patients	T1 (n = 51, 86.4 %)	T2 (n = 8, 13.5 %)	T1 (n = 97, 97 %)	T2 (n = 3, 3 %)

validated for the cervical epithelium and the viral load is estimated due to the severity of cervical intraepithelial neoplasia. Clinically significant for cervical cancer and cervical intraepithelial neoplasia is the load from 3 to 5 lg 10^5 × β-globin, a high probability of developing cervical cancer is observed with an amount of more than 5 lg 10^5 × β-globin. At the same time, the assessment of viral load in bladder tumors has not been determined.

Thus, we found HPV DNA in tumor tissue in patients with a low or moderate degree of tumor differentiation, which is consistent with the literature data and may indicate an unfavorable course of the disease, as well as the possibility of using this relapse prediction test after complex treatment.

Previously published studies have shown the important role of HPV infection and the development of various types of malignant neoplasms, including cancer of the cervix, vagina, vulva, oropharyngeal zone, anogenital cancer. HPV type 16 is a papilloma virus with the highest oncogenic risk and is found in about 55–60 % of cases of cervical cancer. HPV type 18 is the second most oncogenic papilloma virus, which is found in about 10–15 % of cases of breast cancer [10].

CONCLUSION

According to the given study, in HPV-positive patients with a clinically diagnosed non-muscle-invasive BC, migration of the stage up to T2 was observed 4 times more often than in HPV-negative patients. At the same time, the prevalence of HPV in tumors of low and moderate malignancy was 93.2 %. Our results indicate a link between HPV infection and a lower degree of differentiation and a higher stage of the tumor process, as well as aggressive forms of urothelial cancer (squamous cell, micropapillary variants). Thus, this study may indicate that HPV infection affects the degree of tumor differentiation, and this, in turn, may allow the use of an HPV test to assess the nature of relapse and/or further progression of the disease.

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ORIGINAL ARTICLE

PROSPECTS OF DIFFERENTIAL DIAGNOSIS OF FOCAL LESION OF PANCREAS BY THE MICRORNA ASSESSMENT

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ABSTRACT

Purpose of the study. Identification of potential miRNA markers in material of focal pancreatic lesions.

Materials and methods. Samples of focal pancreatic lesions after histological evaluation were enrolled in the study including chronic pancreatitis (ChP) (*n* = 23), low-grade pancreatic intraepithelial neoplasia /PanIN-1/2 (*n* = 19), high-grade pancreatic intraepithelial neoplasia /PanIN-3 (*n* = 8), and invasive pancreatic ductal adenocarcinoma PDAC (*n* = 26). Workflow of research included the profiling of cancer-associated miRNA in pooled samples, the selection of potential marker miRNAs, the assessment of selected miRNAs expression in total collection of specimens, the identification of differentially expressed miRNAs, and the approbation of new algorithm of data interpretation via ratio of "reciprocal miRNA pair". Consequent reactions of revers transcription and quantitative teal-time PCR were used.

Results. The expression levels of miR-216a and miR-217 were decreased in the following order: PanIN-1/2 > PanIN-3 > PDAC. Moreover, miR-375 was up-regulated while miR-143 was down-regulated in the PDAC. Differential diagnostics of PDAC versus focal chronic pancreatitis might be performed with high accuracy (AUC > 0.95) by assessment panel of four molecules: miR-216a, miR-217, miR-1246 and Let-7a.

Conclusion. The assessment of microRNAs in pancreatic lesions is a promising approach for the differential diagnosis of PDAC, but this technology requires further validation with an increase in the number of samples.

Keywords: microRNA, pancreatic cancer, ductal neoplasia, chronic pancreatitis, RT-PCR, diagnostics

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Compliance with ethical standards: the ethical principles presented by the World Medical Association Declaration of Helsinki (1964, ed. 2013) were observed in the work. The study was approved by the local Ethics committee of the N. N. Petrov National Medical Research Center of Oncology (extract 27/27 from the protocol of meeting No. 1 dated 01/28/2021). Informed consent was received from all participants of the study.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

ВОЗМОЖНОСТЬ ДИФФЕРЕНЦИАЛЬНОЙ ДИАГНОСТИКИ ОЧАГОВЫХ ОБРАЗОВАНИЙ ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ ПУТЕМ АНАЛИЗА МИКРОРНК

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РЕЗЮМЕ

Цель исследования. Поиск потенциально маркерных молекул микроРНК в материале узловых образований поджелудочной железы.

Материалы и методы. В исследование были включены образцы ткани очаговых образований поджелудочной железы с гистологическим заключением: хронический панкреатит (*n* = 23), интраэпителиальная неоплазия low grade / PanIN-1/2 (*n* = 19) и high grade степени /PanIN-3 (*n* = 8), инвазивная протоковая карцинома (*n* = 26). В рамках работы был проведен широкий профайлинг пулов образцов разных гистологических типов, выбор потенциально маркерных микроРНК, анализ экспрессии выбранных молекул микроРНК во всех образцах, включенных в исследование, поиск статистически значимых различий между группами образцов, апробация нового алгоритма интерпретации полученных результатов путем вычисления соотношений концентраций «реципрокных пар» микроРНК. Метод анализа: обратная транскрипция с последующей количественной ПЦР в режиме реального времени.

Результаты. Уровень экспрессии молекул miR-216a и miR-217 снижается в ряду: PanIN-1/2 > PanIN-3 > протоковая карцинома ПЖ. Также в клетках инвазивной протоковой карциномы поджелудочной железы повышена экспрессия miR-375 и снижена экспрессия miR-143. Высокая точность дифференциальной диагностики (AUC > 0,95) очагов хронического панкреатита и инвазивной протоковой карциномы ПЖ может быть обеспечена с помощью панели из четырех молекул miR-216a, miR-217, miR-1246 и let-7a.

Заключение. Оценка экспрессии молекул микроРНК в материале очаговых образований ПЖ с целью дифференциальной диагностики протоковой карциномы имеет диагностический потенциал, но метод требует валидации с использованием большей коллекции биологических образцов.

Ключевые слова: микроРНК, рак поджелудочной железы, протоковая неоплазия, хронический панкреатит, ОТ-ПЦР, диагностика

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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 локальным этическим комитетом ФГБУ «НМИЦ онкологии им. Н.Н. Петрова» Минздрава России (выписка 27/27 из протокола заседания № 1 от 28.01.2021 г.). Информированное согласие получено от всех участников исследования.

Финансирование: исследование выполнено в рамках государственного задания Министерства Здравоохранения Российской Федерации «Разработка и клиническая апробация методов диагностики и прогнозирования эффекта терапии онкологических заболеваний на основе анализа микроРНК в биопсийном материале» (№ 121032300206-4) 2021–2023 гг. и в рамках проекта по соглашению № 075-15-2021-709, уникальный (идентификатор проекта RF-2296.61321X0037), финансируемого Министерством науки и высшего образования Российской федерации.

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

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INTRODUCTION

In the structure of oncological morbidity in Russia, pancreatic ductal adenocarcinoma (PDAC) accounts for 3.4 % of cases, this disease ranks 11th in incidence. During the period from 2010 to 2020, there was an increase in the absolute number of incidences of PDAC from 7522 to 9275 cases. In the structure of oncological mortality of the Russian population, the proportion of diagnoses of PDAC was 6.8 %, which corresponded to the 5th position in the list of the most "lethal" diagnoses. The absolute number of deaths from PDAC in the period 2010-2020 increased from 7783 to 9625 [1]. The cumulative five-year survival rate of patients with PDAC (M/W) is 22.2 / 33.3 % – at stage I; 14.9 / 13.7 % – at stage II; 8.4 / 5.9 % – at III; 3.4 / 3.9 % – at stage IV of the first diagnosis [2]. The presented statistical data confirm the well-known fact of low "curability" of PDAC and indicate a deterioration in the epidemiological situation observed despite the development of diagnostic and therapeutic technologies.

Modern diagnostic standards are based on methods of physical examination, laboratory and instrumental research methods, such as endoscopic ultrasound (EUS), dynamic multispiral computed tomography (MSCT) and magnetic resonance imaging (MRI) of the abdominal cavity [3]. Biopsy with subsequent morphological examination of the biopsy material is recommended for all patients with suspected PDAC, while surgical intervention in some cases can be performed without morphological confirmation; the use of conservative treatment requires mandatory morphological verification. Modern diagnostic algorithms do not fully solve the problem of timely diagnosis of PDAC. The task of introducing screening for certain risk groups and improving the effectiveness of the diagnosis of PDAC seems important and involves various approaches [4], including predictive analytics methods [5], innovative liquid biopsy technologies [6; 7], high-tech methods of pathology visualization. Modern instruments allow you to combine endo-ultrasound with contrast technologies [8], elastography, fine needle aspiration biopsy [9]. Endoscopic ultrasound-guided fine needle aspiration biopsy (EUS-FNA) is gradually entering into wide clinical practice, opening up prospects, firstly, for expanding the scope of morphological analysis methods and, secondly, for developing new technologies for molecular diagnostics of biopsy material.

The main advantage of the EUS-FNA method with subsequent production of cytological smears and cell block is relatively low invasiveness. The Russian authors showed high sensitivity (93 %) and specificity (100 %) of a complex morphological study of the EUS-FNA material using the technology of manufacturing cell blocks calculated taking into account the results of subsequent histological diagnostics of the surgical material [10]. Despite the obvious advantages, the technology and effectiveness of EUS-FNA is accompanied by known objective and subjective difficulties of subsequent cytological diagnosis. A fine needle puncture allows you to obtain microfragments of the tissue of the gland formations and a cellular suspension, the effectiveness of the analysis of which is determined by the volume and structure of the aspirate. Difficulties also arise in assessing the proliferative processes of the ductal epithelium, which are observed both in areas of intraepithelial neoplasia (primarily PanIN3) and in areas of reactive hyperplasia associated with the pancreatitis; cellular signs are similar and differential diagnosis is often difficult due to the small amount of material. In contrast to the histological examination of the surgical material, it is more difficult to assess the condition of the basement membrane, the structure and non-glandular patterns of the organ in the FNA material. In this regard, additional technologies of molecular diagnostics of epithelial changes can increase the informative value of a complex morphological diagnosis.

MicroRNAs are short regulatory molecules that control the stability of protein-coding RNAs and the efficiency of protein synthesis. The transformation of the ductal epithelium of the pancreas reflects a multi-stage process of development of ductal adenocarcinoma (the dominant morphological form of PDAC); this process is accompanied by a change in the expression profile (or composition) of microRNA molecules. Therefore, the analysis of the microRNA composition in the biopsy material seems to be a promising method for diagnosing focal lesions of the pancreas, which is confirmed by the activity of research and the number of relevant publications. A search in the PubMed scientific literature database for the keywords "pancreatic cancer AND miRNA" yields more than 2.8 thousand publications. The analysis of the available scientific information

is presented in a number of review articles [11-15], but in clinical practice, there are no examples of diagnostic test systems based on microRNA analysis of the material of pancreatic lesion biopsy.

Purpose of the study: To select potential microRNA markers of PDAC, to evaluate the diagnostic significance of the method and to develop an algorithm for PCR data interpretation, based on the analysis of the expression of tumor-associated microRNAs. To evaluate the possibility and expediency of developing and introducing into clinical practice the method of differential diagnosis of PDAC pathological patterns based on the analysis of microRNA in the material of EUS-FNA.

MATERIALS AND METHODS

Patients

The study plan was approved by the local Ethics Committee of the N. N. Petrov National Medical Research Center of Oncology, extract 27/27 No. 1 dated 01/28/2021. All patients signed an informed consent to participate in the study. Before being included in the study, biological samples and clinical data were depersonalized.

The study included patients who were treated at the L. G. Sokolov Federal State Medical Center and the N. N. Petrov National Medical Research Center of Oncology. In accordance with the standards of medical care and clinical recommendations (ID:355), all patients underwent pancreato-duodenal resection or pancreatectomy. The material of the pancreatic formations was immediately placed in a buffered solution of 10 % formalin in a ratio of 1:10 and after 24 hours of fixation was cut into cassettes for further histological wiring and subsequent light microscopy. Based on histological examination of hematoxylin-stained eosin preparations, the study included material from 47 patients. In most cases, the analysis of the preparations allowed us to identify areas of tissue with different morphological structures, so 76 representative samples were prepared for further study.

Isolation of RNA

Sections with a thickness of 3–4 microns were prepared from the tissue samples, the sections were dewaxed by incubation in 1 ml of mineral oil (MP Biomedicals, USA) at 65 °C – 15 min, then the oil and paraffin were removed using two washings with 96 % ethanol. Proteolysis was carried out in 100 µl of proteinase K solution, 2 mg/ml (activity: 30 units/mg, Algimed-Techno, Belarus) at 60 °C – 1 hour. The remaining tissue after proteolysis was precipitated by centrifugation (10,000 G, 4 °C – 10 min), the supernatant (~100 µl) was transferred to a clean test tube, 200 µl of buffer was added (0.8 M sodium acetate; pH 4.0; 0.5 % octanoic acid) and 100 µl of guanidine isothiocyanate (3M), mixed, and incubated for 5 minutes at room temperature. The sample was transferred to a spin column filled with sorbent (BioSilica, Russia), washed twice with buffer for washing No. 1 (500 µl; 0.5M guanidine isothiocyanate; 10mM tris-acetate; pH 6.5; 50 % ethanol; 1 % 2-mercaptoethanol) and twice with buffer for washing No. 2 (500 µl; 75 % ethanol; 0.1M sodium chloride, 10mM tris hydrochloride; pH 7.5. RNA from the surface of the sorbent was eluted using 50 µl of an elution buffer (10mM NaHCO3, 10 mM EDTA). The concentration and quality of the isolated RNA were evaluated using a NanoDrop 2000C spectrophotometer (Thermo Scientific, USA).

"Profiling" of tumor-associated microRNAs

In order to select potentially marker molecules, the expression analysis (profiling) of cancer-associated 85 microRNAs was carried out. For this purpose, "pools" were formed, which were mixtures of RNA samples of similar morphology: chronic pancreatitis (ChP), intraepithelial neoplasia of the pancreas (PanIN), invasive ductal adenocarcinoma of the pancreas (PDAC). The analysis was carried out using a set of reagents manufactured by Exiqon (miScript II RT Kit, miRCURY LNA miRNA Cancer-Focus PCR Panel) the analytic procedure involved poly-adenylation reaction, reverse transcription (RT) of all RNA molecules using a poly-T primer followed by real-time quantitative PCR reaction for 85 microRNA molecules. The intensity of amplification was evaluated using the DNA intercolating reagent SYBR-green. All reactions were carried out in accordance with the manufacturer's protocol on the CFX96 Touch™ amplifier (Bio-Rad, USA).

Analysis of individual microRNA molecules expression

The expression of individual microRNA molecules in the material of each sample (n = 76) was analyzed by RT-PCR using kits manufactured by

Algimed-Techno (Belarus). The set for the analysis of each microRNA involved conducting a "two-tailed" microRNA-specific reverse transcription reaction using an RT-primer, the "flanks" of which complementarily annealed on the 3' and 5' ends of the microRNA molecule, orienting "towards" each other. This technology of priming the reverse transcription reaction ensured its high specificity [16]. Then, a real-time quantitative PCR reaction was performed using two microRNA-specific PCR primers. The amplification intensity was evaluated using a fluorescently labeled PCR probe. All RT-PCR reactions were carried out in accordance with the manufacturer's protocols, each analysis was performed in three repeats on the CFX96 Touch™ amplifier (Bio-Rad, USA).

Statistics

Normalization of the "profiling" data was carried out according to the manufacturer's recommendations: the results of the analysis of each pooled sample were normalized versus averaged Ct values of the three reference molecules. Before comparing the results of the analysis of individual samples (pools), normalization was carried out versus "inter-plate" calibrators. The molecules for subsequent analysis were selected by simply comparing the normalized Ct values.

Normalization of the results of the individual molecules (n = 24) analysis in individual samples (n = 76) was carried out in two alternative ways: relative to the averaged Ct values of all analyzed samples (24 × 76 = 1842), so called total Ct, and relative to one of the 24 studied molecules, which was characterized by the most stable level of expression in the studied samples. To select the optimal normalizer, the stability (or variability) of the expression of the analyzed molecules was evaluated using the NormFinder algorithm [17]. Normalization was carried out according to the standard method of counting (formula 1):

$$dCt=2^{Ct(miRNA)-Ct(normalizer)}$$

The statistical significance of the difference in the expression levels of individual microRNAs in the compared groups was assessed using the nonparametric Mann-Whitney U-test. The prognostic significance of each molecule was evaluated using ROC analysis (constructing a ROC curve and calculating the AUC value).

We also searched for optimal ratios of the relative concentrations of the so-called "reciprocal pairs" of microRNA molecules (molecules with multidirectional and associated with the process of neoplastic transformation changes in expression levels). A software algorithm has been developed for the automated solution of this problem. Reciprocal pairs were formed as all possible combinations of 24 tested microRNAs. The total number of analyzed pairs, determined by the formula 2:

$$P_n^r = \frac{n!}{(n-r)! \times r!}$$

(where P – quantity of unique miRNAs combinations, n – quantity of analyzed miRNAs (24), r – quantity of miRNA in any particular combination (2)).

Then, for each mRNAs pair, the amplification efficiency ratio parameter (Ratio miR-1/miR-2) was determined by the formula 3:

$$dCt=2^{Ct(miR-1)-Ct(miR-2)}$$

The evaluation of the diagnostic significance of Ratio miR-1/miR-2 parameters was evaluated similarly to the significance of individual molecules: using ROC analysis and calculating the AUC value.

RESEARCH RESULTS

Research design, sample preparation

In the practice of analyzing the material of EUS-FNA focal formations of the pancreas, differential diagnosis between conditions having similar clinical and sonographic patterns is of clinical importance: chronic pancreatitis (ChP), intraepithelial lesions of varying degrees of malignancy (pancreatic intraepithelial neoplasia, PanIN), and invasive ductal adenocarcinoma (pancreatic ductal adenocarcinoma, PDAC). Taking into account the task of searching for markers of differential diagnosis of the listed conditions, the research plan assumed the sequential implementation of the following stages: 1) histological analysis of pancreatic pathology (surgical material), 2) selection and microdissection of sites with the appropriate morphological pattern and isolation of RNA, 3) formation of "pools" of RNA from samples of typical morphology and analysis of 85 tumor-associated microRNAs ("profiling") in these pools, 4) selection and analysis of potentially marker

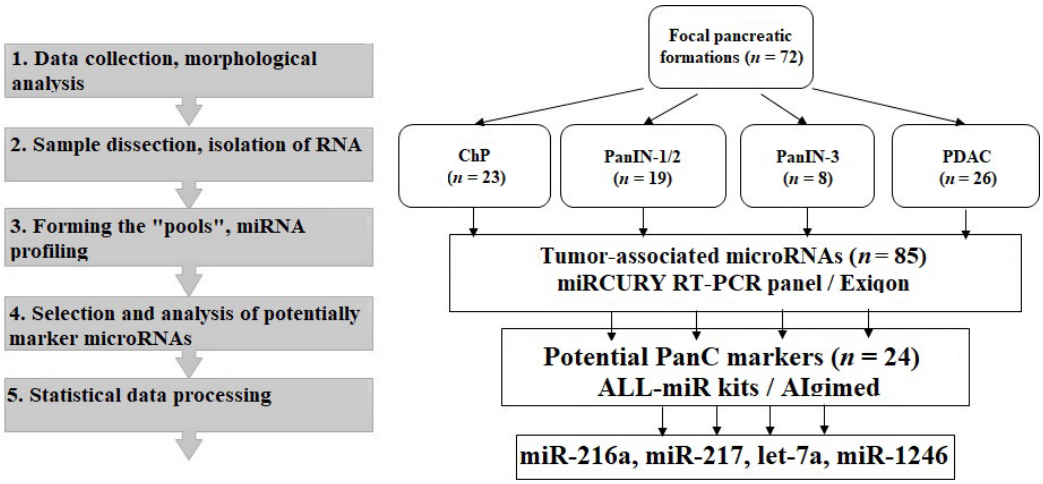

Fig. 1. Study design.

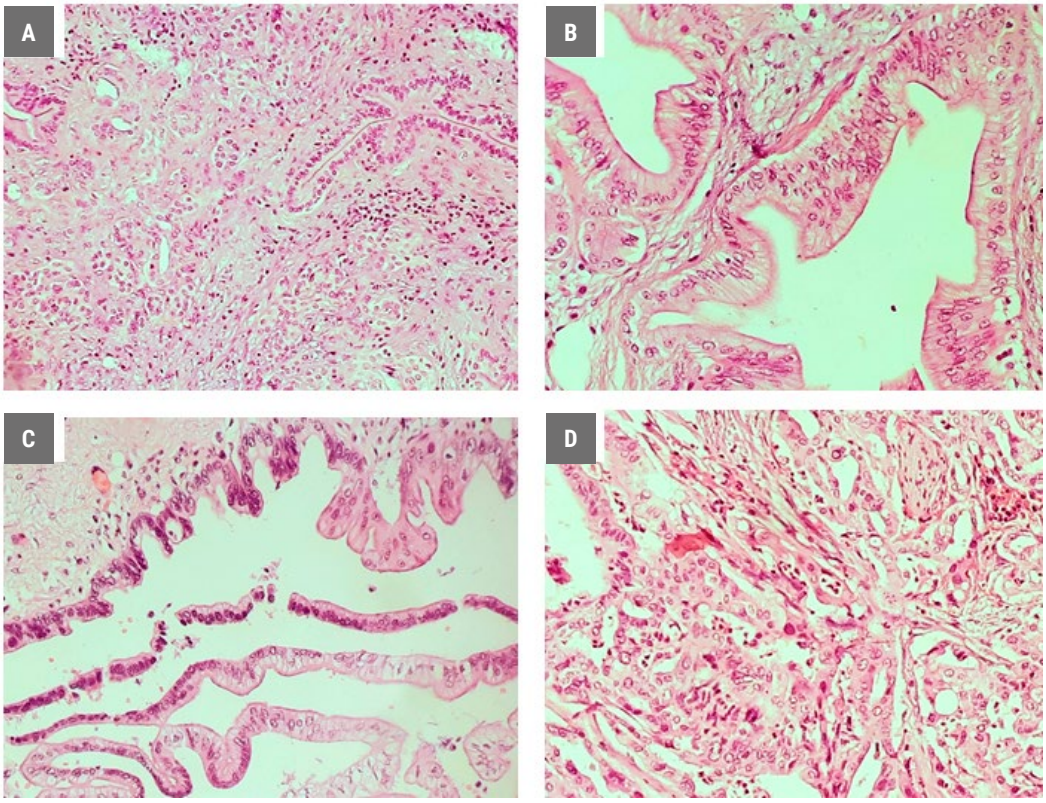

Fig. 2. Representative examples of sites of pathological formations of the pancreas.
Note: A – fibrosing chronic pancreatitis (ChP): the structure of the gland with pronounced structural changes, loss of acinar cells, pronounced fibrosis with inflammatory infiltration, compressed deformed ducts and areas of proliferation of small ducts. B – intraepithelial lesion (PanIN1/2): small pancreatic ducts lined with a single–layer cylindrical ductal epithelium with predominantly basally located nuclei (PanIN1), as well as areas with signs of pseudostratification – nuclei located at different levels (PanIN2). B – intraepithelial lesion (PanIN3): a large pancreatic duct lined mainly with cells with cellular and nuclear atypia, high nuclear-cytoplasmic ratio, hyperchromia of the nuclei, uneven nuclear contours, loss of polarity of the nuclei, mitosis figures, partly foamy-gland pattern (foamy cells), partly with mucus formation and oncocytic differentiation. Atypical cells are located within the basement membrane. G – invasive ductal adenocarcinoma (PDAC) with widespread perineural invasion. Staining in all cases with hematoxylin-eosin.

Table 1. The "profile" of 18 potentially marker microRNA molecules expression in normal pancreatic tissue and cells of focal formations of various morphologies

No.	microRNA	Relative expression level in pathological sample (miRNA Cancer-Focus PCR Panel)			Relative expression level in intact pancreatic cells (miRNATissueAtlas2)
		ChP	PanIN1/2/3	PDAC	
1	hsa-let-7a-5p	9.13	14.41	7.80	512.4
2	hsa-miR-10b-5p	0.50	0.40	0.60	111.5
3	hsa-miR-15a-5p	0.80	1.29	0.82	143.6
4	hsa-miR-23a-3p	6.96	4.43	5.21	462.7
5	hsa-miR-24-3p	10.03	7.67	8.31	177.2
6	hsa-miR-26b-5p	2.22	2.73	1.70	415.5
7	hsa-miR-27a-3p	10.25	9.31	7.08	229.1
8	hsa-miR-29c-3p	2.21	5.91	2.86	1298.3
9	hsa-miR-125b-5p	32.56	17.24	17.46	426.3
10	hsa-miR-126-3p	13.02	7.50	5.39	532.6
11	hsa-miR-141-3p	2.99	10.13	3.02	82
12	hsa-miR-143-3p	3.20	3.92	6.22	94.6
13	hsa-miR-145-5p	23.79	12.19	23.60	509.2
14	hsa-miR-146b-5p	0.51	0.80	0.61	82.1
15	hsa-miR-155-5p	0.32	0.39	0.28	74.7
16	hsa-miR-192-5p	1.38	4.25	4.08	24.6
17	hsa-miR-200a-3p	0.38	1.01	0.90	22.8
18	hsa-miR-200c-3p	7.56	10.96	5.16	17.4

Table 2. The list of molecules additionally included in the study

No.	microRNA	Relative expression level in intact pancreatic cells (miRNATissueAtlas2)	Literature sources
1	hsa-miR-375-3p	20.4	[19]
2	hsa-miR-451a-5p	4650.4	[13]
3	hsa-miR-1246	3416.7	[20]
4	hsa-miR-1290	886.3	[21]
5	hsa-miR-216a-5p	42.3	[22]
6	hsa-miR-217-5p	20.1	[23]

mircoRNA molecules, 5) statistical processing of the results. The design of the study is schematically presented in Figure 1.

The samples of ChP, when selected on the area of the entire slides, did not contain tumor tissue, the morphological picture varied from mild inflammatory infiltration with moderate fibrosis (both intra-lobular and periductal) to a pronounced inflammatory reaction with areas of fibrosing Ch P. The PanIN1/2 group included samples of intraductal papillary mucinous neoplasia (intraductal papillary mucinous neoplasm, IPMN) and mucinous cystic tumors (mucinous cystic neoplasm, MCN) with a low degree of dysplasia. Specimens of this group could include small fragments of normal pancreatic tissue. The PanIN3 group included images of severe dysplasia, including cases of IPMN and MCN, the presence of PDAC sites was excluded. The PDAC group was represented by typical histotypes and two cases of mucinous cystadenocarcinoma with moderate or low degree of differentiation. This category can contain small fragments of the PanIN3 and PDAC combination.

During light microscopy, zones with the corresponding morphological pattern were identified, microdissection of selected tissue fragments was performed for subsequent RNA isolation. In some cases, material of different morphology was obtained from the same sample and categorized in different groups. As a result, the following samples were included in the study: ChP (*n* = 23), PanIN1/2 (*n* = 19), PanIN3 (*n* = 8), PDAC (*n* = 26). Representative examples of sites with a characteristic morphological pattern selected for the study are shown in Figure 2.

Selection of potentially "marker" microRNAs

Total RNA was isolated from all samples. There was a large variation in RNA concentrations (0.5-350 ng/μl), which could be a result of tissue heterogeneity and/or different degrees of cellular RNA integrity. RNAs isolated from histologically similar samples were combined in equal mass ratio into so-called "pools" for preliminary analysis of the expression profile of potentially marker molecules. This approach makes it possible to conduct an inexpensive comparative analysis of groups of samples, but without the possibility of assessing the statistical significance of the results obtained. The analysis made it possible to assess the difference between three conditions: chronic pancreatitis (ChP), intraepithelial neoplasia of any degree (PanIN1/2/3) and invasive ductal adenocarcinoma of the pancreas (PDAC). MicroRNA molecules with relatively high and significantly different levels of expression in three groups were selected for subsequent analysis. An additional selection criterion was information on the previously estimated expression of these molecules in the pancreatic cell, presented

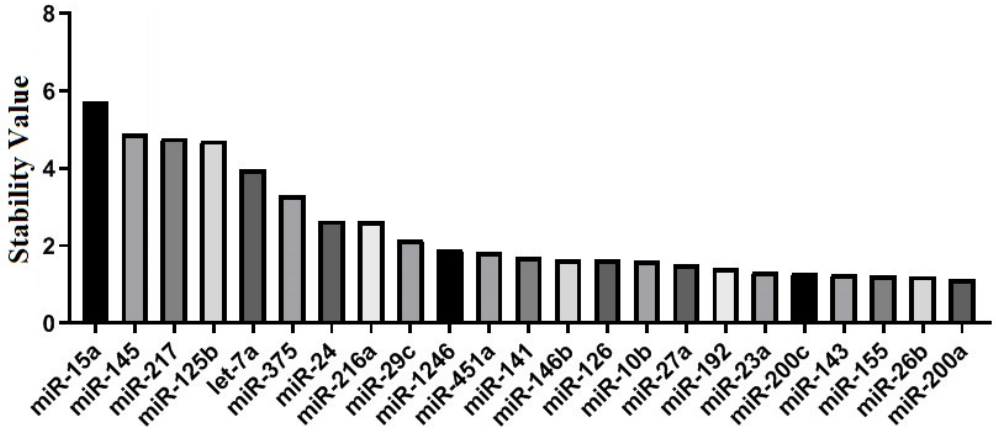

Fig. 3. Results of evaluation of expression variability of microRNA molecules (n.23) in the analyzed samples (n.76) using the NormFinder algorithm.
Note: the NormFinder algorithm [17] allows ranking the analyzed molecules according to the level of stability/variability of their expression in the group of samples under study. The stability coefficient = 1 corresponds to a molecule whose expression level is identical in all samples, such molecules can be used as normalizers. As part of the experiment, the most stable expression level was shown for miR-200a.

in the miRNATissueAtlas2 database [18]. A total of 18 molecules were selected. Table 1 presents normalized expression levels of selected molecules in samples of three variants of pathological focal formations: ChP, panIN1/2/3, PDAC and data on the expression of these molecules in cells of normal pancreatic tissue (according to miRNATissueAtlas2). It should be noted that the comparison of the presented results of the analysis of groups of pathological and normal samples is not adequate, because these are the results of different studies performed by different methods. But a comparative assessment of different molecules within a certain study can be informative. In general, the presented results suggest that the selected 18 molecules are indeed actively expressed in pancreatic cells, and the formation of focal formations by chronic inflammatory reaction or malignancy is probably associated with a change in their expression and functional activity.

An analysis of previous studies has shown that a number of mircoRNA molecules not included in the miRCURY LNA miRNA Cancer-Focus PCR Panel play an important role in the development of PDAC and may have diagnostic potential; they (n = 6) were also included in the study. The level of expression of these molecules in normal pancreatic tissue according to "miRNATissueAtlas2" and links to relevant studies are presented in Table 2. Thus, a "panel" of 24 potentially marker microRNA molecules was formed.

Table 3. The results of a comparative analysis of the expression of potentially marker microRNAs in samples of focal formations of pancreas of different morphology

MicroRNA	ChP (n = 23)	PanIN1/2 (n = 19)	PanIN3 (n = 8)	PDAC (n = 26)	Kruskal-Wallis test
hsa-miR-143-3p	0.1	0.16	0.09	0.04	0.0007 ***
hsa-miR-217-5p	0.87	0.42	0.28	0.07	0.001 ***
hsa-miR-216a-5p	1.48	0.84	0.83	0.38	0.0032 **
hsa-miR-375-3p	0.2	0.32	0.13	0.48	0.0137 *
hsa-miR-200c-3p	0.18	0.23	0.24	0.10	0.0259 *
hsa-miR-1246	0.07	0.09	0.09	0.03	0.0336 *
hsa-miR-155-5p	0.4	0.38	0.31	0.22	0.1098
hsa-miR-146b-5p	1.4	2.56	2.5	0.84	0.1131
hsa-miR-26b-5p	0.42	0.23	0.58	0.22	0.1485
hsa-miR-192-5p	0.5	0.38	0.19	0.29	0.1538
hsa-miR-125b-5p	0.09	0.17	0.34	0.20	0.2127
hsa-miR-451a-5p	0.11	0.12	0.04	0.52	0.2323
hsa-miR-29c-3p	0.84	5.97	17.65	0.62	0.2599
hsa-miR-24-3p	0.09	0.1	0.12	0.09	0.3167
hsa-let-7a-5p	0.34	0.31	0.27	0.24	0.3674
hsa-miR-126-3p	0.96	1.05	0.66	1.00	0.4304
hsa-miR-10b-5p	51.04	70.6	50.47	74.01	0.4911

Analysis of potentially marker molecules

The expression level of each of the selected 24 molecules was assessed using a microRNA-specific two-flank reaction and subsequent PCR in each sample included in the study. The specificity of RT was provided by an RT primer having two flanks for binding to a microRNA molecule. As a result of the RT reaction, a complementary DNA molecule was synthesized, both flanks of which had microRNA-specific sites for binding to PCR primers. The possibility of assessing microRNA expression levels and the high specificity of this approach were shown earlier [24]. On the base of obtained result, miR-1290 was excluded in further analysis since expression level of this molecules appeared under the limit of detection of used method in majority of samples.

In order to determine the optimal normalizer of RT-PCR data, the variability of expression of each of the 23 molecules was evaluated using the NormFinder algorithm, the results are shown in Figure 3.

The most stable expression level (stability value → 1) was shown for microRNA-200a, which was later used as a normalizer. An alternative normalization method involves calculating the averaged (total) Ct value of all molecules tested in all samples (76 samples × 24 molecules = 1824 values in total), and normalizing each particular results relative to this value [25]. The results of RT-PCR were normalized by two methods: relative to the value of total Ct and relative to Ct (miR-200a) for each individual sample. The normalized values were combined into groups (ChP, PanIN 1/2, PanIN 3, PDAC), which made it pos-

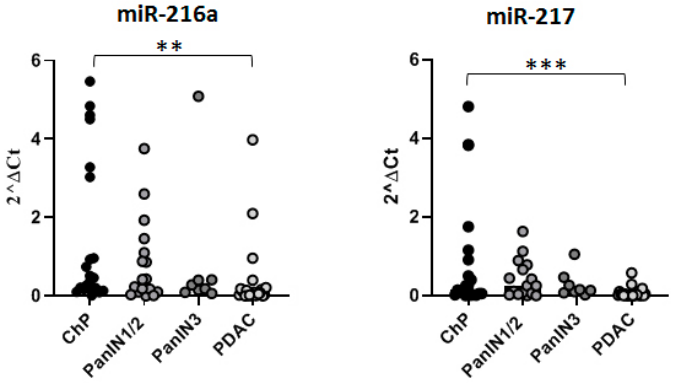

Fig. 4. Comparative analysis of miR-216a and miR-217 expression levels in tissue samples of pancreatic focal formations of various histological structures.
Note: groups of samples of chronic pancreatitis / ChP (n = 23), mild intra-epithelial neoplasia / PanIN-1/2 (n = 19), and severe / PanIN-3 (n = 8), ductal carcinoma / PDAC (n = 26). The statistical significance of the observed difference was estimated by calculating the Kruskal-Wallis criterion (** p < 0.005; *** p < 0.0005).

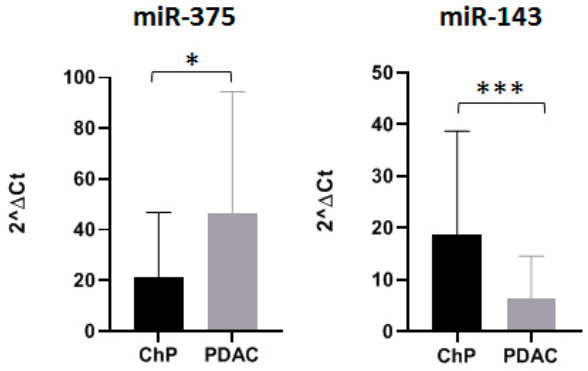

Fig. 5. Comparative analysis of miR-375 and miR-143 expression levels in the tissue of foci of chronic pancreatitis and PDAC.
Note: The graph represents arithmetic averages for a group of samples of chronic pancreatitis /ChP (n = 23) and ductal carcinoma/PDAC (n = 26). The statistical significance of the observed difference was estimated by calculating the Mann-Whitney criterion (* p < 0.05; *** p < 0.0005).

sible to evaluate the difference in the expression of individual molecules in the studied groups of samples. For a number of molecules, contradictions were observed between the results obtained using different normalization methods. Such molecules were excluded from the further analysis. Only those results that were similar with different normalization methods, i. e. minimally dependent on them, were taken into account; there were 17 of 24 such molecules.

The next step was to evaluate the statistical significance of the difference in the expression levels of individual microRNAs between clinical groups. The results are presented in Table 3.

For a number of molecules, a statistically significant change in expression activity was observed in the PanIN-1/2 – PanIN-3 – PDAC series. For example, an increase in the severity of ductal epithelial dysplasia was accompanied by a decrease in the expression level of miR-216a and miR-217 (Fig. 4).

The obtained results suggest that the low expression of these molecules in the material of the EUS-FNA of the focal lesion of the pancreas may be a marker of its malignant nature. Statistically significant difference between the studied groups was obtained for a number of molecules: miR-143, miR-375, miR-200c and miR-1246.

Interestingly, when comparing groups of ChP and PDAC samples, without taking into account the data obtained for samples of intraepithelial lesions of varying degrees of malignancy (PanIN1/2/3), an increase

Table 4. Indicators of efficacy of differential diagnosis of chronic pancreatitis (ChP) versus PDAC by means of individual microRNAs assessment

MicroRNA	AUC	Sens.	Spec.	PPV, %	NPV, %	Accuracy, %
hsa-miR-143-3p	0.75	70.59	82.61	79.17	75.00	77.50
hsa-miR-216a-5p	0.81	76.19	78.26	78.26	76.19	77.27
hsa-miR-217-5p	0.81	76.19	78.26	78.26	76.19	77.27
hsa-miR-375-3p	0.78	71.43	71.43	71.43	71.43	71.43

Table 5. Indicators of effectiveness of chronic pancreatitis (ChP) and PDAC differential diagnosis based on the assessment of the concentration ratios of microRNA "reciprocal pairs"

MicroRNA pair	AUC	Sens.	Spec.	PPV, %	NPV, %	Accuracy, %
miR-1246/miR-217	0.95	95.24	82.61	95.00	83.33	88.64
miR-1246/miR-216a	0.95	90.00	91.30	91.30	90.00	90.70
miR-1246/miR-375	0.81	85.00	73.91	85.00	73.91	79.07
miR-143/miR-216a	0.83	81.25	82.61	86.36	76.47	82.05
let-7a/miR-216a	0.97	94.44	94.74	94.74	94.44	94.59
miR-155/miR-217	0.93	88.89	90.48	90.48	88.89	89.74
let-7a/miR-217	0.96	88.24	100.00	90.48	100.00	94.44
miR-155/miR-216a	0.91	83.33	90.48	86.36	88.24	87.18
miR-192/miR-216a	0.93	83.33	100.00	87.50	100.00	92.31
miR-192/miR-217	0.91	80.95	95.24	83.33	94.44	88.10
miR-143/miR-217	0.85	68.75	95.65	81.48	91.67	84.62
miR-200c/miR-216a	0.88	80.95	78.26	81.82	77.27	79.55
miR-200c/miR-217	0.88	75.00	78.26	78.26	75.00	76.74
miR-451a/miR-216a	0.81	80.00	78.26	81.82	76.19	79.07
miR-451a/miR-217	0.82	85.71	73.91	85.00	75.00	79.55

in the expression level of miR-375 and a decrease in the expression level of miR-143 associated with the process of chronic inflammation was observed (Fig. 5).

In order to assess the diagnostic value of the individual potentially marker microRNAs (miR-143, miR-217, miR-216a, miR-375) for differential diagnostic of ChP and PDAC, a ROC analysis and calculation of standard indicators were carried out. The results presented in Table 4 and Figure 6 indicate a high diagnostic potential of these markers.

Differential diagnosis algorithm

Normalization of the results of RT-PCR analysis of microRNA and clinically applicable interpretation of the results of such analysis is a non-trivial task. One of the possible approaches is the search for molecules with a reciprocal (opposite) trend of tumor-associated expression changes and the evaluation of the ratio of concentrations of such molecules in tested samples. The effectiveness of this approach has been proven earlier: the values of such expression ratios may have a higher diagnostic potential than the diagnostic values of individual molecules [26].

As part of this work, a previously developed computational algorithm was used to search for promising "reciprocal pairs" of microRNAs. So, this algorithm assumed the selection of all possible pairs of microRNA molecules, calculation of their concentration ratios (miR-1/miR-2 Ratio) and ROC analysis of these parameters as markers of differential diagnosis of ChP vs. PDAC. Thus, diagnostic values were estimated for 276 pairs of molecules, including AUC, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy. The results presented in Table 5 demonstrated the high diagnostic potential of many "reciprocal pairs" of microRNAs (AUC > 0.8).

From the data in the table, it can be seen that the composition of "reciprocal pairs" includes molecules

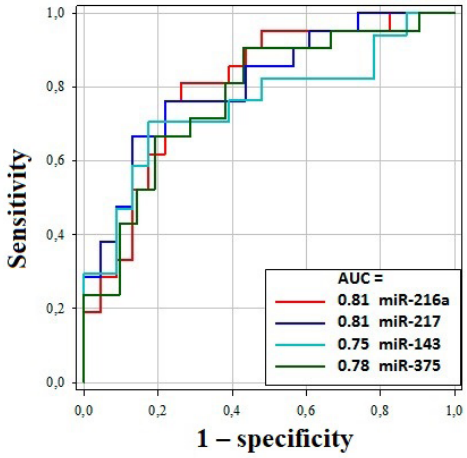

Fig. 6. Evaluation of individual microRNA diagnostic value in the framework of differential diagnosis of chronic pancreatitis (ChP) and PDAC.
Note: The results of the ROC (Receiver operating curve) analysis and the values of the area under the AUC (area under the curve) curves for four molecules are presented.

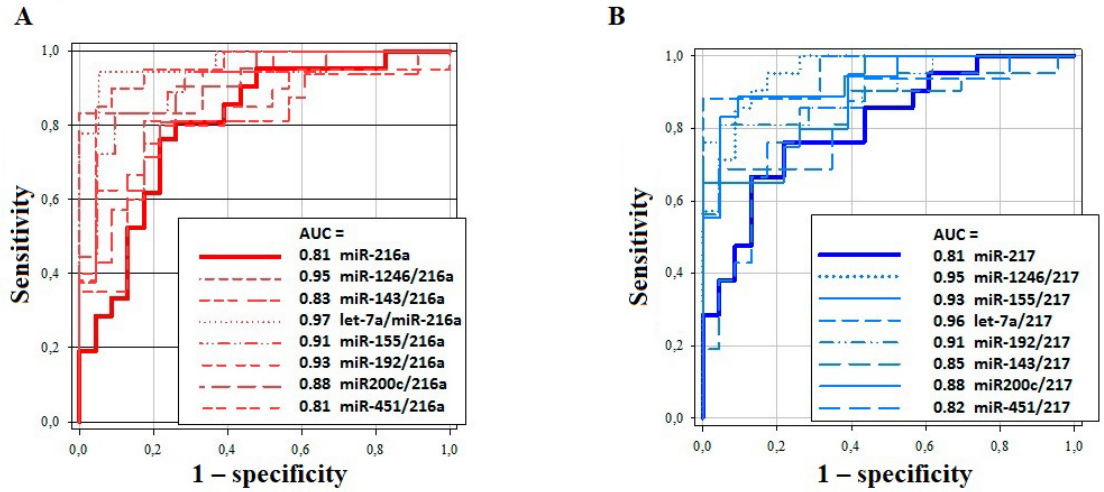

Fig. 7. Evaluation of the diagnostic value of the analysis of "reciprocal pairs" of microRNA molecules in the framework of differential diagnosis of chronic pancreatitis (ChP) and PDAC.
Note: A – the results of the evaluation of the diagnostic potential of miR-216a and several "reciprocal pairs" formed by this molecule. B – the results of the evaluation of the diagnostic potential of miR-217 and several "reciprocal pairs" formed by this molecule. The results of the ROC (Receiver operation curve) analysis and the values of the area under the AUC (area under curve) curves are presented.

with a relatively high diagnostic potential as individual markers. However, the ratio of the concentration of these molecules and molecules with a "reciprocal" expression behavior have significantly higher diagnostic value. Figure 7 shows the ROC curves confirming this conclusion for the "reciprocal pairs" that form the miR-216a and miR-217 molecules.

Thus, the AUC value for miR-216a is 0.81, and for the concentration ratios of this molecule and miR-192, miR-1246 or let-7a, the AUC values increase to 0.93, 0.95 and 0.97, respectively. Similarly, the AUC value for miR-217 is 0.81, and for the concentration ratios of this molecule and miR-155, miR-1246 or let-7a, the AUC values increase to 0.93, 0.95 and 0.96, respectively. In general, the analysis of a "panel" of four molecules, miR-216a, miR-217, miR-1246 or let-7a, provides the ability to calculate four parameters for the differential diagnosis of chronic pancreatitis and PDAC, and the AUC value of these parameters is in the range of 0.95–0.97, sensitivity: 0.88–0.95, specificity: 0.82–1.

DISCUSSION

The article presents the results of a pilot study conducted to assess the prospects for the development of a method for differential diagnosis of focal pancreatic pathology based on microRNA analysis in biopsy material. The materials of postoperative histological examination were used in the work. The authors suggest that the obtained results will serve as the basis for the development of an innovative method of molecular analysis of the EUS-FNA material, which will be able to complement and increase the diagnostic potential of a standard morphological study.

The study analyzed the expression of 85 cancer-associated microRNA molecules in the material of 76 samples obtained from 47 operated patients. Histological examination made it possible to form four groups of samples corresponding to the diagnoses: chronic pancreatitis (ChP), mild pancreatic intraepithelial neoplasia (PanIN-1/2), severe intra-epithelial neoplasia (PanIN-3) and the most common form of pancreatic cancer (invasive ductal adenocarcinoma, PDAC). MicroRNA expression analysis revealed statistically significant differences in expression levels of miR-143, -217, -216a, -375, -200c, -1246 between assayed groups of samples. The results obtained have both fundamental and applied significance.

Involvement of miR-217 [23], -216a [22], -375 [19], -1246 [20] in the process of malignant transformation of the ductal epithelium of the pancreas was shown earlier; these data were confirmed by the our results. Suppression of miR-143 in PDAC cells is an interesting finding, which is also in a line with the scientific literature. Thus, a comparative analysis of normal pancreatic tissue and PDAC, conducted by Chinese researchers using the material of 37 patients, showed similar results to ours [27]. In addition, ectopic expression of this molecule in various pancreatic cancercell lines (MIA PaCa-2 and PANS-1) had a therapeutic effect, inhibiting cell proliferation and metastatic potential [27; 28]. *In situ* experiments have shown that a decrease in the concentration/functional activity of miR-143 in PDAC cells is associated with activation of the expression of a number of oncogenes (ARHGEF1 (GEF1), ARHGEF2 (GEF2), K-RAS), which confirms the therapeutic potential of synthetic analogues of this molecule.

No statistically significant changes in the expression of miR-1246 were detected in our study, but the ratio of the concentration of this molecule and the concentration of miR-216a or miR-217 showed a high diagnostic potential. The results obtained indicate the possibility of over-expression of this molecule in PDAC cells, and this process is associated with inhibition of miR-216a or miR-217 expression. Whether this association is random or biologically justified event is still unknown. The analysis of scientific literature does not form an unambiguous idea of the participation of miR-1246 in the development of PDAC. A number of publications show the diagnostic potential circulating in the plasma miR-1246 [6; 29], but what is the relationship between the concentration of this molecule in plasma and its role in PDAC cells is not clear. The analysis of the biogenesis of this molecule in tumor cells was recently carried out by a group of American researchers [30]. It has been shown that miR-1246 is a product of degradation of small nuclear RNA (RNU2-1), which functions as part of the nuclear complex of the spliceosome. In this case, miR-1246 is a product of a non-canonical microRNA synthesis pathway, and an increase in intracellular concentration and/or secretion of this molecule by PDAC cells may not be related to its role in the process of post-transcriptional regulation of target genes expression. Our results shown that the expression of the let-7a molecule has a "reciprocal"

character relative to the expression of miR-216a and miR-217. The biological meaning of this phenomenon remains still unknown, but it can be used for clinically acceptable interpretation of RT-PCR results. In general, the results obtained complement the existing ideas about the role of individual microRNA molecules in the development of PDAC.

The practical value of the results obtained is important as well. We achieved to demonstrate, firstly, the technical possibility of isolating and analyzing microRNAs in biopsies of pancreatic formations using domestic reagents, and, secondly, the diagnostic potential of such an analysis. In terms of developing a technology suitable for clinical use, additional efforts should be made (1) to develop a method for preserving the EUS-FNA material and subsequent isolation of small RNAs and (2) to determine the optimal (minimum) set of marker microRNAs for inclusion in the diagnostic "panel" for differential diagnosis of focal pancreatic lesion. In order to ensure high and, most importantly, reliable indicators of the diagnostic significance of the test system, an important aspect is the development of an algorithm for interpreting RT-PCR data. This issue was successfully solved previously in the frame of test-system for the assessment the severity of cervical epithelial dysplasia [31]. Thus, the following analytical algorithm seems optimal: semi-quantitative analysis of a set of microRNA molecules with reciprocal (opposite) PDAC-associated expression changes, calculation of concentration ratios of "reciprocal pairs" of microRNAs, determination of the significance of each such parameter using a machine learning algorithm and calculation of the final diagnostic criterion reflecting the risk of pancreatic malignancy. After evaluating diagnostic parameters on a large collection of biopsy material, the developed test system can be registered as a medical device and offered for practical use.

CONCLUSION

In presented study we identified several potentially marker microRNA molecules and shown that the assessment of their expression in the sample of focal pancreatic formations is a promising diagnostic approach.

The development of a reliable and clinically-applicable diagnostic technology will require continued research in two main areas: (1) expanding the panel of marker molecules and developing an algorithm for their complex analysis, and (2) validating the diagnostic potential of the new technology using a larger collection of biological samples and developing approaches to clinically convenient and effective interpretation of the RT-PCR results.

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Shestopalova T. M. – histological diagnostics, selection and preparation of material, microdissection, preparation of microphotographs;

Zabegina L. M. – development and implementation of an algorithm for calculating the concentration ratio of "reciprocal pairs" of miRNAs, participation in laboratory stages of work, analysis of the results;

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ORIGINAL ARTICLE

GASTRIC CANCER MODELING IN IMMUNODEFICIENT MICE WITH ORTHOTOPIC XENOTRANSPLANTATION

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ABSTRACT

Purpose of the study. Creation of a transplantable orthotopic PDX model of gastric cancer in Balb/c Nude immunodeficient mice using implantation and injection.
Materials and methods. Two methods, that are injection and implantation, were used to create an orthotopic PDX model of human gastric cancer. The first method involved injections of a suspension of a mechanically disaggregated patient's tumor after filtration into the gastric wall of Balb/c Nude mice. For the second method, small fragments (3 × 3 × 3 mm) of patients' tumors were implanted in the gastric wall of mice along the greater curvature with a dissection of the serous muscular layer.
Results. Control laparotomy in Balb/c Nude immunodeficient mice showed a successful engraftment of the tumor material at the 1st and 3rd procedures when using the implantation method for the creation of a PDX model of gastric cancer. The injection method was ineffective, and no models were created. The histological type of the obtained PDX models was compared to the type of the donor tumor by histological examination (hematoxylin and eosin staining). The tumor grade remained stable and did not change during xenograft passage, which showed that the obtained model was identical to the histotype of the donor tumor.
Conclusion. The presented implantation method for the model creation results in effective tumor engraftment. The developed model can be used to test the effectiveness of anticancer or antimetastatic drugs, for studying the functions of biomarkers, or in assessing the microenvironment of a gastric cancer.

Keywords: orthotopic xenotransplantation, gastric cancer, immunodeficient mice, PDX models, transplantation

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Compliance with ethical standards: all manipulations with laboratory animals were carried out in accordance with the "Rules for carrying out work using experimental animals" when performing the study. The study was approved by the Ethics Committee of the National Medical Research Centre for Oncology (Protocol No. 22/126 of 08/10/2021).

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ОРИГИНАЛЬНАЯ СТАТЬЯ

МОДЕЛИРОВАНИЕ РАКА ЖЕЛУДКА НА ИММУНОДЕФИЦИТНЫХ МЫШАХ ПУТЕМ ОРТОТОПИЧЕСКОЙ КСЕНОТРАНСПЛАНТАЦИИ

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РЕЗЮМЕ

Цель исследования. Создание перевиваемой ортотопической PDX-модели рака желудка на иммунодефицитных мышах линии Balb/c Nude при помощи имплантационного и инъекционного способов.
Материалы и методы. С целью создания ортотопической PDX-модели рака желудка человека были применены 2 способа – инъекционный и имплантационный. Первый способ заключался в инъекции суспензии механически дезагрегированной опухоли пациента после фильтрации в стенку желудка мышей линии Balb/c Nude. Для второго способа мелкие фрагменты (3 × 3 × 3 мм) опухоли пациентов были имплантированы мышам в стенку желудка по большой кривизне с рассечением серозно-мышечного слоя.
Результаты. При применении имплантационного способа получения PDX-модели рака желудка на иммунодефицитных мышах линии Balb/c Nude в ходе процедур контрольной лапаротомии, был обнаружен положительный результат приживления опухолевого материала при 1-ой и 3-ей процедурах. Инъекционный способ не дал эффективного результата – не была получена ни одна модель. Гистотип полученных PDX-моделей сравнивали с донорской опухолью и подтверждали при помощи гистологического исследования (окрашивание гематоксилином и эозином). Степень дифференцировки оставалась стабильной и не менялась в результате пассирования ксенографта, что показало идентичность полученной модели гистотипу донорской опухоли.
Заключение. Представленный имплантационный метод создания модели дает эффективный результат приживления опухоли. Полученная модель позволяет использовать её для проверки эффективности противоопухолевых или антиметастатических препаратов, возможных исследований функций биомаркеров, а также для оценки микроокружения опухоли желудка.

Ключевые слова: ортотопическая ксенотрансплантация, рак желудка, иммунодефицитные мыши, PDX-модели, трансплантация

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Соблюдение этических стандартов: при выполнении данного исследования все манипуляции с лабораторными животными проводились в соответствии с «Правилами проведения работ с использованием экспериментальных животных». Исследование одобрено этическим комитетом ФГБУ «НМИЦ онкологии» Минздрава России (протокол № 22/126 от 08.10.2021 г.).

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

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INTRODUCTION

Every year, gastric cancer is diagnosed for the first time in more than 1 million people. At the moment, gastric cancer remains the fourth cause of cancer mortality [1; 2]. Treatment of patients diagnosed with gastric cancer consists in the use of surgical methods, chemotherapy, immunotherapy. However, all these methods have limited effectiveness. Over a 20-year period, the proportion of patients with metastases increased from 24 to 44 %, which indicates an urgent need for an optimized approach to both treatment and diagnosis [3]. More and more efforts are being made to find effective ways to research and understand the biology and therapeutic features of gastric cancer.

One of such approaches is the use of orthotopic cancer models obtained from patients (PDX stands for: patient derived xenograft) [4; 5]. The main advantage of PDX models is that tumor cells tend to consistently repeat the features of the original tumor, and the orthotopic implantation method can better simulate the natural environment of the tumor. It has been shown that for some types of tumors, the subcutaneous model has a lower rate of engraftment than the orthotopic one [6]. Orthotopic xenografts of tumors are one of the best experimental models for representing the mechanisms of spontaneous metastasis [7; 8].

This model is an important tool for providing scientific substantiation of the relevance of new therapeutic combinations in gastric cancer. To study the growth and metastasis of gastric cancer, as well as to test the effectiveness of treatment and therapy, as well as testing new pharmacological substances, various methods of orthotopic transplantation to bestimus mice (Balb/c Nude) have been developed [9; 10]. Although several methods are used to develop an orthotopic model, the optimal way to create it has not yet been determined.

The purpose of the study was to create an orthotopic PDX model of gastric cancer in immunodeficient Balb/c Nude mice using implantation and injection methods.

MATERIALS AND METHODS

Tumor sample

The tumor samples required for orthotopic transplantation to laboratory animals were obtained from patients diagnosed with gastric cancer who were treated in 2022, from whom written permits were obtained for the use of samples for research purposes.

Laboratory animals

All procedures related to *in vivo* studies on mice were carried out in accordance with the "Guidelines for the maintenance and use of laboratory Animals" [11]. 39 female immunodeficient Balb/c

Table 1. Characteristics of tumor material donor patient, and evaluation of xenotransplantation results to immunodeficient Balb/c Nude mice

Method of xenotransplant isolation	Characteristics of tumor material donor patient						Assessment of implantation outcomes (1st generation)
	Procedure number	Donor patients sex	Method of sample isolation	TNM stage	Micromorphology	Prior therapy	Total number of transplantations / number of successful implantations
Tumor fragment implantation	1	F	Distal subtotal resection	$T_3N_2M_0$	Low-differentiated adenocarcinoma	-	7/6
Injection of tumor suspension	2						7/0
Tumor fragment implantation	3	F	Distal subtotal resection	$T_3N_1M_0$	Low-differentiated adenocarcinoma	-	7/5
Injection of tumor suspension	4						7/0

Nude mice were used to create tumor models. The animals were kept in the SPF-zone of the vivarium, in individually ventilated cages at a temperature of 21–23 °C. The mice were provided with free access to food and water.

Creating an orthotopic model

The orthotopic PDX model of gastric cancer was obtained in two ways. The first method consisted in implanting a fragment of the patient's tumor into the gastric wall of Balb/c Nude mice along the large curvature. The second method was the injection of a homogenized crushed tumor of the patient into the gastric wall of Balb/c Nude mice along the large curvature. The tumor material was obtained from two patients. General characteristics of patients and evaluation of the results of tumor engraftment to animals are presented in the table 1.

Xenograft engraftment and growth were evaluated by performing control laparotomies 20 and 40 days after implantation and injection of tumor material. Surgical manipulations were performed using injectable anesthesia for laboratory animals using veterinary drugs "Xylazine" and "Zoletil-100".

Assessment of the growth of tumor xenografts

Measurements of tumor nodes were performed during laparotomy using a caliper. The volume of tumor nodes was calculated by the formula: $V = L \times W^2 / 2$, where V is the volume of the tumor (mm^3); L, W are the linear dimensions of the tumor (mm).

Histological examination

Fragments of tumor tissue were fixed in 10 % formalin for 24 hours, then subjected to dehydration, after which they were enclosed in paraffin. After that, microsections were prepared, which were stained with hematoxylin and eosin according to the standard procedure. A donor and xenogenic tumor were subjected to histological examination.

Statistical analysis

Statistical data processing was performed using the STATISTICA 8.0 software package. The results are presented as median values [25th and 75th percentiles].

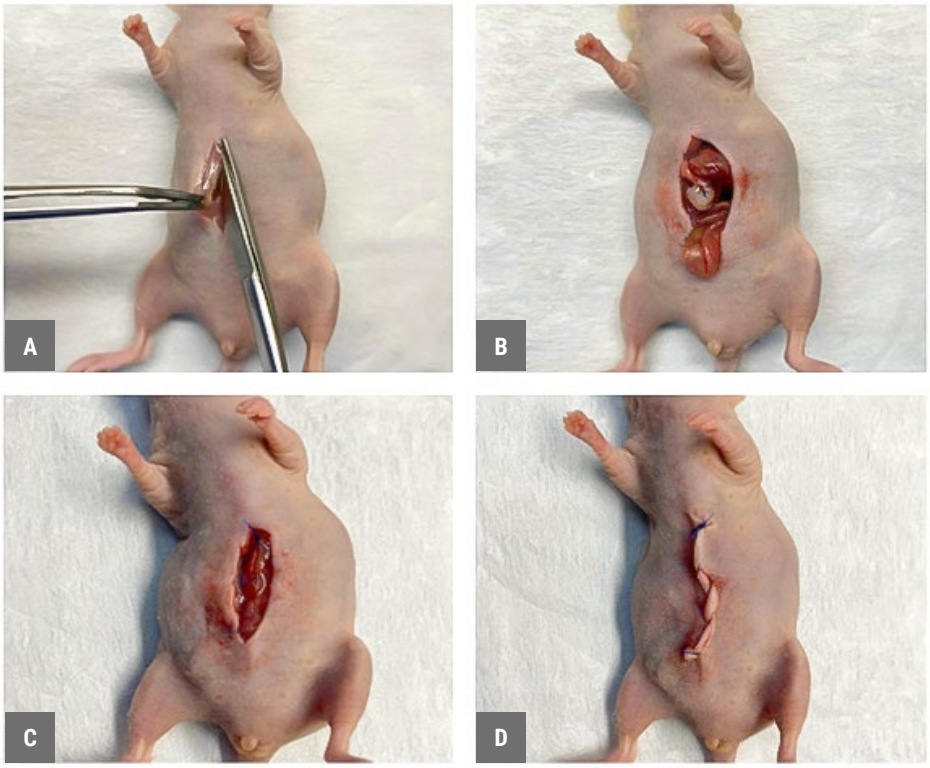

Fig. 1. Generating an orthotopic PDX model by implanting a fragment of a human gastric cancer into the body wall of the immunodeficient mice stomach. A – dissection of the skin, muscles and tissue of the abdominal wall of an immunodeficient mouse; B – implantation of a human tumor fragment into the body of the mouse stomach; C – suturing of the mouse abdominal wall tissue; D – final appearance after transplantation.

RESEARCH RESULTS

Modeling of gastric cancer on immunodeficient Balb/c Nude mice was carried out in two ways – the method of injection of a suspension of the patient's tumor cells; the method of implantation of a fragment of the patient's tumor. To create xenografts, 4 procedures were performed (2 procedures for each method). As part of one procedure, the model was created on a group of seven animals.

After surgery, the patient's tumor fragment was washed with a nutrient medium (DMEM and 1 % penicillin/streptomycin) and areas with signs of necrosis were removed.

The first method of creating a PDX model was carried out as follows. The tumor was cut into small fragments (3 × 3 × 3 mm), then the resulting fragment was implanted into the gastric wall of an immunodeficient Balb/c Nude mouse. After anesthesia of the animal with the sedative "Xylazine" at a concentration of 20 mg/kg and the general anesthesia drug "Zoletil 100" at a concentration of 50 mg/kg, layered dissection of the skin and tissue of the abdominal wall of the mouse was performed. After the expansion of the surgical wound with the help of anatomical tweezers, the stomach was isolated and the serous-muscular layer of the stomach was dissected along a large curvature. Then the resulting fragment of the donor's tumor was sewn with a ligature to the gastric wall at the site of the incision and the abdominal cavity and skin were sewn in layers (Fig. 1).

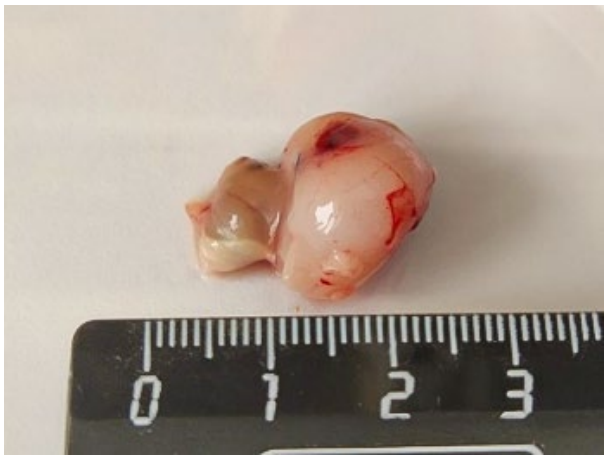

Fig. 2. Orthotopic xenograph of human gastric cancer on the gastric body of an immunodeficient mouse of the Balb/c Nude line.

When using the implantation method for obtaining a PDX model during the control laparotomy procedures, a positive result of the engraftment of tumor material was found (Fig. 2).

The tumors of the obtained PDX models retained identical histological features of the original tumors of the donor patients (Fig. 3).

The second method was as follows: the fragments of the tumor were subjected to mechanical disaggregation, then the resulting suspension was transferred to a vial, passed through a filter and typed into a syringe. After that, an injection was carried out into the corresponding part of the gastric wall of an immunodeficient mouse.

DISCUSSION

When using the injection method to obtain a PDX model, no tumor growth was detected during the control laparotomy procedures. It can be assumed

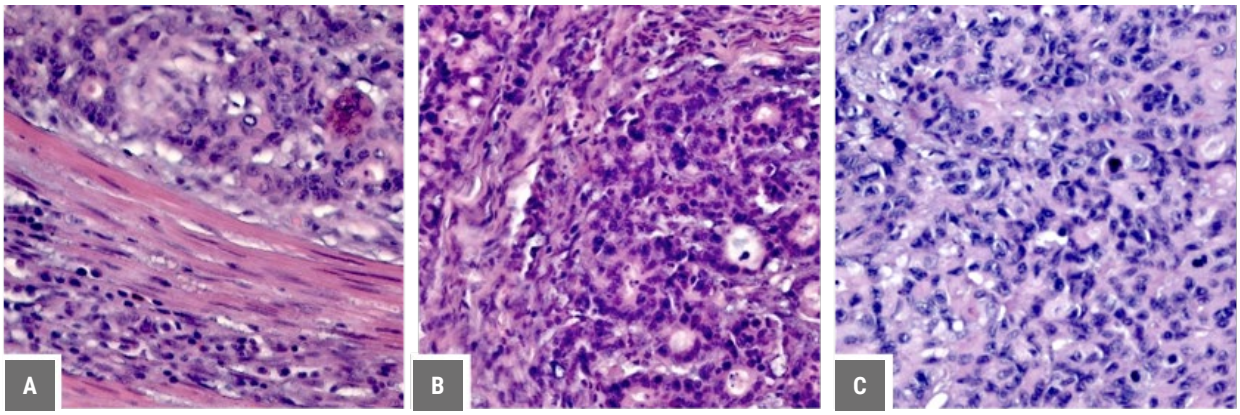

Fig. 3. Histological preparations of gastric cancer. A is the patient's tumor (donor tumor). H&E staining. × 200; B – xenograft, 1st generation; C – xenograft, 2nd generation.

that the absence of a positive result in the form of engraftment of tumor material and the formation of a tumor node was probably due to the phenomenon of anoikis – a form of apoptosis that occurs in response to loss of connection with the matrix or caused by the separation of cells from neighboring cells caused by mechanical disaggregation. Thus, the method of injecting a suspension of tumor cells is insufficiently effective due to the complexity of manipulations and lack of effectiveness, as was shown in our study and in the researches of other authors [12; 13].

Implantation procedure No. 1 showed a result with 86 % engraftment and tumor growth as a result of xenotransplantation during the first generation. The first laparotomy was performed 20 days after the operation. According to the results, the median volume of xenografts was 99.61 [70.44; 138.29]. With laparotomy performed after 40 days, the median was 221.21 [184.27; 202.17]. The second generation showed 100 % engraftment and faster tumor growth. The median volumes of xenografts were 125.56 [106.21; 168.51] and 288.61 [223.48; 344.1] 20 and 40 days after implantation, respectively (Table 2).

As a result of procedure No. 3, the growth of xenografts was observed in five of the seven animals in the group at the 1st generation. At laparotomy on day 20, according to the results of measurements, the median volume of xenografts was 67.37 [55.35; 118.59]. As a result of laparotomy on day 40, the median tumor size was 126.77 [104.76; 169.99].

The second generation was also characterized by higher growth rates. The median volumes of xenografts of the second generation were 157.71 [102.16; 172.96] and 291.5 [251.42; 346.32] 20 and 40 days after implantation, respectively (Table 2).

The tumor pathology in both PDX and patients was a low-grade adenocarcinoma of a solid type. There were no changes in the degree of differentiation as a result of xenograft passage (within the two generations obtained). The observations obtained indicate the ability of xenotransplanted tumors of early generations (1st and 2nd generations) to accurately display the morphological features of donor tumors.

CONCLUSION

Up to the date, a number of methods of orthotopic transplantation have been developed, but each of them has disadvantages that limit its widespread use. As part of our study, 2 methods of creating an orthotopic model of gastric cancer were analyzed: injection and implantation. The injection method proved to be ineffective. The implantation method yielded a result with a high level of tumor engraftment, i. e. 86 % and 100 % of the two patients' tumor materials. The presented method of creating a model allows us to transplant tumor tissue into the stomach orthotopically without additional labor, and also gives an effective result of tumor engraftment. The obtained models can potentially be used for screening and evaluation of known and new drugs.

Table 2. Volumes of orthotopic xenografts of human gastric cancer of two consecutive generations 20 and 40 days after implantation of the tumor fragment to immunodeficient Balb/c Nude mice, presented as median (M) and interquartile span (implantation procedure No. 1, 3)

Procedure No.	Generation	Laparotomy	M	25 percentile	75 percentile
Procedure 1	1	20 days	99.61	70.44	138.29
		40 days	221.21	184.27	202.17
	2	20 days	125.56	106.21	168.51
		40 days	288.61	223.48	344.1
Procedure 3	1	20 days	67.37	55.35	118.59
		40 days	126.77	104.76	169.99
	2	20 days	157.71	102.16	172.96
			291.5	251.42	346.32

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Karasev T. S. – performed review of publications, writing the manuscript;

Goncharova A. S. – developed concept and design of the study;

Kolesnikov E. N. – performed surgical manipulations;

Maksimov A. Yu. – performed surgical manipulations, took the lead in data interpretation;

Averkin M. A. – analyzed the received data, worked out technical details of the paper;

Galina A.V. – conducted the experimental part of the study, performed technical design;

Romanova M. V. – analyzed the received data, interpreted the results;

Gusareva M. A. – performed scientific editing of the paper;

Zinkovich M. S. – arranged bibliography, edited the text of the article.

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CLINICAL CASE REPORT

A RARE CLINICAL CASE OF SYRINGOMYELIA PROGRESSION IN THE PRESENCE OF CHIARI I MALFORMATION FOLLOWING THE SURGERY

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ABSTRACT

Today, an Arnold-Chiari malformation is defined as a developmental pathology of the craniovertebral junction manifested by a discrepancy between the volume and contents of the posterior cranial fossa, which in turn leads to compression of neurological structures and changes in the cerebrospinal fluid circulation. There are several theories of the correlation between Chiari malformation and syringomyelia, but the exact mechanism of syringomyelia development remains unclear.

This article describes a clinical case of treatment of a child with Chiari I malformation and syringomyelia within the cervical and thoracic segments of the spinal cord; after complete posterior fossa decompression, syringomyelia progressed in the early postoperative period with the development of a severe neurological deficiency. Since there is no standard treatment of such postoperative complications, a decision was made on the expectant management of the patient. From the twentieth day of the postoperative period, the patient showed complete regression of the neurological deficiency and positive MRI dynamics of syringomyelia.

The presented clinical case raises such issues as not only the pathophysiology of syringomyelia progression after complete posterior fossa decompression, but also the determination of patient management tactics in case of a complicated postoperative course of the disease.

The presented clinical case is of interest due to the rarely described aggravation of syringomyelia with enhancing neurological symptoms in the early postoperative period after complete posterior fossa decompression.

Our observation suggests that the expectant management of the patient, despite syringomyelia progression with neurological deficiency aggravation after posterior fossa decompression, allowed a favorable long-term outcome of Chiari I malformation.

Keywords: syringomyelia progression, Chiari I malformation, craniovertebral junction anomaly, posterior fossa decompression

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КЛИНИЧЕСКОЕ НАБЛЮДЕНИЕ

РЕДКИЙ КЛИНИЧЕСКИЙ СЛУЧАЙ ПРОГРЕССИРОВАНИЯ СИРИНГОМИЕЛИИ НА ФОНЕ АНОМАЛИИ КИАРИ I ТИПА ПОСЛЕ ОПЕРАТИВНОГО ВМЕШАТЕЛЬСТВА

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РЕЗЮМЕ

На сегодняшний день мальформация Арнольда-Киари определяется как патология развития краниовертебрального перехода, проявляющаяся несоответствием объема и содержимого задней черепной ямки, что в свою очередь приводит к компрессии неврологических структур и изменению характера ликворотока. Существует несколько теорий корреляции между мальформацией Киари и сирингомиелией, однако точный механизм развития сирингомиелии остается предметом для дискуссии.

В статье описан клинический случай лечения ребенка с аномалией Киари I типа и сирингомиелией шейных и грудных сегментов спинного мозга, у которого после проведенной декомпрессии задней черепной ямки «полного объема» в раннем послеоперационном периоде возникло прогрессирование сирингомиелии с развитием грубого неврологического дефицита. Учитывая отсутствие стандартных подходов в лечении таких послеоперационных осложнений, принято решение о выжидательной тактике ведения пациента. С двадцатых суток послеоперационного периода у пациента отмечен полный регресс неврологического дефицита и положительная магнитно-резонансная томография (МРТ) динамика сирингомиелии.

Представленный клинический случай поднимает не только вопросы патофизиологии прогрессирования сирингомиелии после выполнения декомпрессии задней черепной ямки «полного объема», но и определение тактики ведения пациента в случае осложнённого послеоперационного течения заболевания.

Интерес представленного клинического случая заключается в том, что описано редко встречающееся усугубление сирингомиелии с нарастанием неврологической симптоматики в раннем послеоперационном периоде после выполнения декомпрессии задней черепной ямки «полного объема».

Основываясь на данных нашего наблюдения, выжидательная тактика ведения пациента, несмотря на прогрессирование сирингомиелии с усугублением неврологического дефицита, после декомпрессии задней черепной ямки позволила добиться благополучного отдаленного результата течения заболевания, связанного с аномалией Киари I типа.

Ключевые слова: прогрессирование сирингомиелии, аномалия Киари I, аномалия краниовертебрального перехода, декомпрессия задней черепной ямки

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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). 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.

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INTRODUCTION

Currently, Arnold-Chiari malformation is defined as a pathology of the development of the craniovertebral junction, manifested by a mismatch in the volume of the posterior cranial fossa and brain structures, which in turn leads to prolapse of the cerebellar tonsils below the Chamberlain line, compression of neurological structures within the cranio-vertebral junction and impaired cerebrospinal fluid dynamics of varying severity [1].

In clinical practice, the most common is type I Chiari anomaly. According to the literature, it occurs with a frequency of 1 per 1000 newborns, with a female prevalence of 1.3 to 1 [2]. In 20–85 % of cases, Chiari I is potentially associated with syringomyelia, more often affecting the cervical region, followed by a combined lesion of the cervical-thoracic region (Fig. 1.). However, there is currently no consensus explaining the etiology and progression of syringomyelia.

In the neurological aspect, Chiari type I anomaly remains asymptomatic in most cases, which creates certain difficulties in diagnosing the disease and is detected when performing magnetic resonance imaging (MRI) for other reasons. When the disease manifests, the most common symptom in both adults and children is headache and segmental violations of temperature sensitivity are much less common [3].

According to modern concepts, MRI of the central nervous system is the main and widely used diagnostic method that allows to obtain an image of the anatomy of the craniovertebral junction with the detection of hydrocephalus and/or syringomyelia, as well as fixed spinal cord syndrome [4].

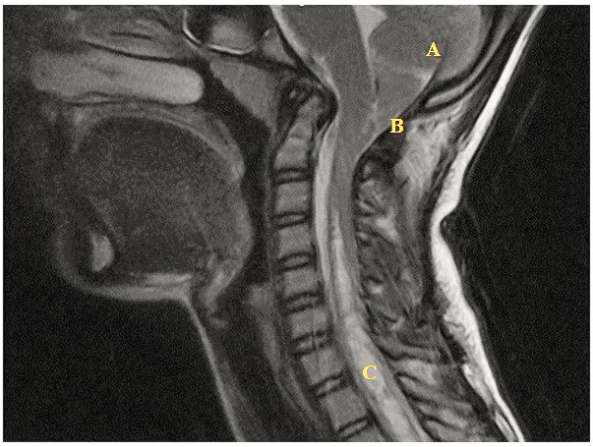

Fig. 1. Arnold-Chiari anomaly type I: A – cerebellum; B – prolapse of the cerebellar tonsils below the foramen magnum; C – syringomyelia at the cervical level.

The main method of treatment of patients with craniovertebral junction anomaly is surgical intervention, which is indicated by the presence of neurological symptoms associated with both syringomyelia and the insertion of the tonsils of the cerebellum [5]. Surgical intervention is aimed at restoring cerebrospinal fluid dynamics at the level of the craniovertebral junction. Optimal access consists in performing a suboccipital craniectomy with decompressive expansion of the foramen magnum, often with laminectomy of the posterior arch C1, and if necessary, the arch C2 of the vertebra, plastic surgery of the dura mater is performed.

Thus, the intervention, designated as decompression of the posterior cranial fossa of the "full volume", is the most common neurosurgical approach aimed at restoring the flow of cerebrospinal fluid at the level of the foramen magnum.

However, there are many questions here: what amount of decompression is required to successfully change the pathology of the cerebrospinal fluid flow, what treatment results are considered satisfactory, what tactics to choose with the further progression of syringomyelia in the postoperative period, what mechanism is responsible for the ineffectiveness of treatment, what is the need and priority of additional surgical interventions [6]?

The clinical example we have given shows the difficulty of determining tactics with an extremely rare aggravation of syringomyelia with the development of a gross neurological deficit after performing a "full-volume" surgical intervention.

Description of the clinical case

Patient T., 17 years old, was admitted to the clinical diagnostic department of the National Medical Research Centre for Oncology with complaints of severe headache in the occipital region. Pain syndrome according to the visual-analog pain scale is 7 points. In the neurological status: cerebral syndrome; tendon and periosteal reflexes in the extremities symmetrical; muscle strength 5 points; segmental sensory disorders were not detected. According to MRI of the central nervous system (Fig. 2): omission of the tonsils of the cerebellum

by 18 mm below the occipital foramen magnum (Fig. 2A). There are no data for hydrocephalus. Syringomyelia of the cervical and thoracic spinal cord with expansion of the central spinal canal up to 6 mm (Fig. 2 B, C). Lack of fixation of the spinal cord at the lumbar level.

The patient underwent surgical intervention in the volume of resection of the posterior edge of the foramen magnum and the posterior semicircle of the atlas (Fig. 3); Y-shaped opening of a significantly hypertrophied dura mater and its subsequent plasty using an artificial dura mater plate from Medtronic. The arachnoid meninges were not opened in connection with the performed tasks of surgical intervention, in order to avoid further formation of the adhesive process.

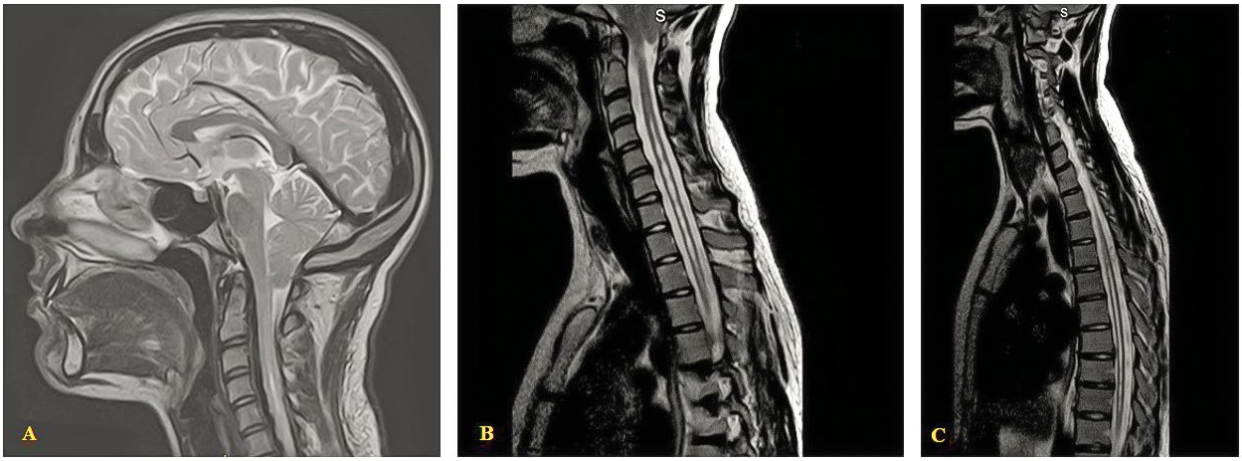

Fig. 2. Data of preoperative MRI of the central nervous system (T2 mode) of the A – brain, B – cervical spinal cord, C – thoracic spinal cord.

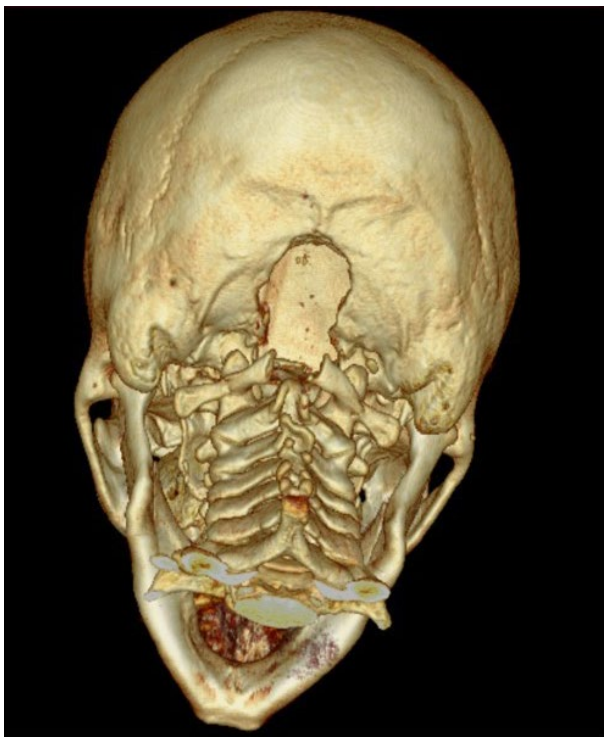

Fig. 3. 3D model of bone decompression reconstruction based on the results of postoperative CT scan of the brain.

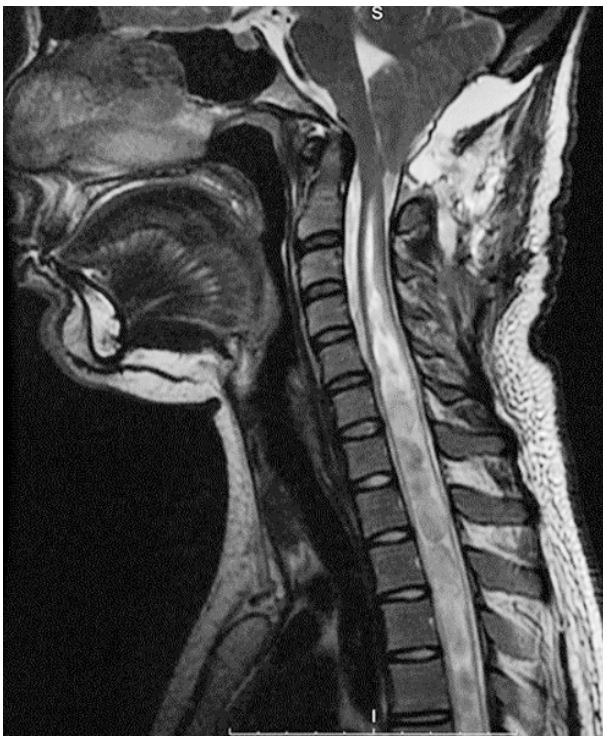

Fig. 4. MRI data (T2 mode) of the brain, cervical spinal cord – 3 days after surgery.

In the early postoperative period, the patient had an increase in cerebral symptoms, the appearance of pronounced muscle weakness in the upper extremities up to 2 points and pronounced hypesthesia in the hands. A control MRI of the central nervous system was performed on the 3rd day after the operation. To exclude the potentially reversible state of "presyrinx", MRI was performed using T1 and T2-weighted sequences [7]. MRI data of the brain and cervical spinal cord: no hydrocephalus; prolapse of the cerebellar tonsils into the surgically expanded occipital foramen magnum remains 18 mm below the large occipital foramen; negative dynamics of syringomyelia of the cervical spinal cord with the expansion of the central spinal canal to 14 mm (versus 6 mm before surgery) (Fig. 4).

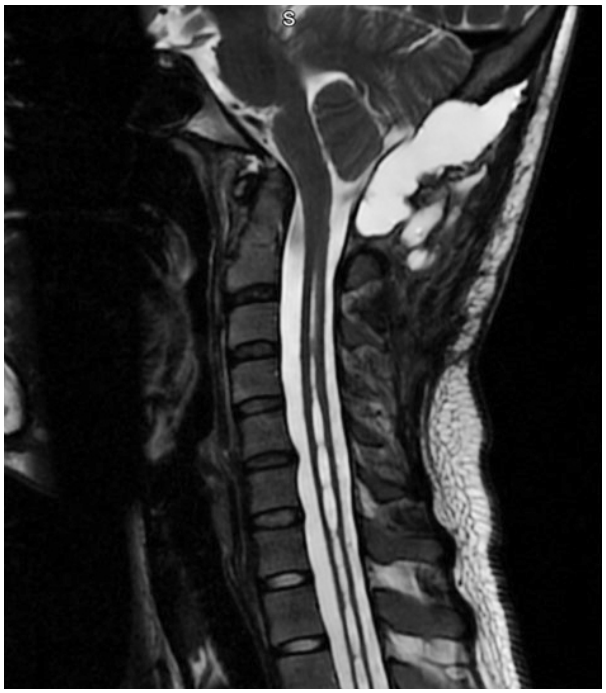

Fig. 5. MRI data (T2 mode) of the brain, cervical spinal cord performed 23 days after surgery.

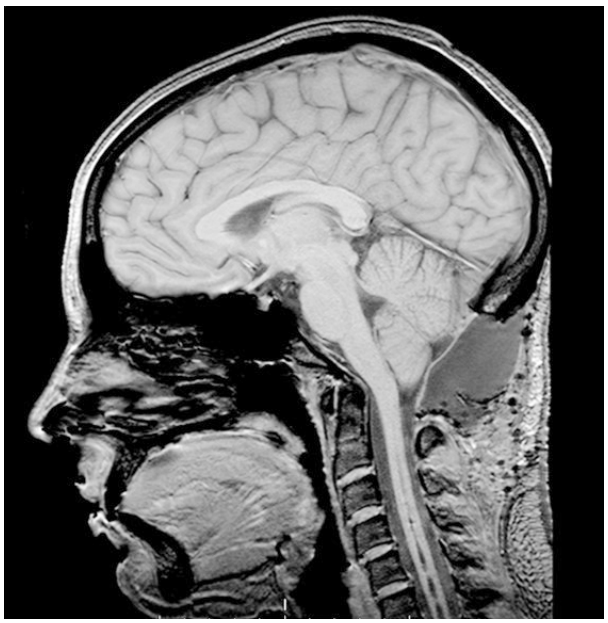

Fig. 6. MRI data (T1 mode) of the brain, cervical spinal cord performed 3 months after surgery.

Given the lack of standard treatment tactics, in this case, a decision was made on conservative management of the patient. Acetazolamide is prescribed in a daily dose of 250 mg. Since the twentieth day after the operation, a positive dynamics of the neurological status was noted in the form of an increase in muscle strength to 5 points, a complete regression of the cerebral syndrome and sensitive disorders.

MRI data of the brain and cervical spinal cord performed on the 23rd day after surgery revealed positive dynamics of the disease: omission of the tonsils of the cerebellum by 11 mm (vs. 18 mm on the 3rd day after surgery); syringomyelia of the cervical spinal cord from the cerebrospinal junction and caudal with the expansion of the central spinal canal to 3 mm (vs. 14 mm on the 3rd day after surgery) (Fig. 5).

Clinically, in the late postoperative period, there is a complete regression of the cerebral syndrome and the absence of focal manifestations.

On the control MRI 3 months after the operation, further positive dynamics was noted: lowering of the tonsils of the cerebellum by 8 mm (versus 11 mm on the 23rd day after surgery); syringomyelia of the cervical spinal cord with expansion of the central spinal canal to 2 mm (versus 3 mm on the 23rd day after surgery) (Fig. 6).

DISCUSSION

Suboccipital craniectomy, resection of the posterior semicircle of the atlas with subsequent plasty of the dura mater without opening the arachnoid medulla is a highly effective method of treating Chiari type I anomaly associated with syringomyelia. However, this method does not exclude a complicated course of the disease with further progression of syringomyelia and aggravation of neurological deficit, which makes it difficult for the neurosurgeon to choose the patient's treatment tactics.

In search of an answer to the question about the possible mechanisms of the progression of syringomyelia after performing a decompressive operation of "full volume", it is worth referring to the work of Aboulker J. (1979), who suggested that the displacement of the cerebellar tonsils downwards causes stenosis of the subarachnoid space at the level of the foramen magnum with the possible formation of syringomyelia. After decompression of the posterior cranial fossa, the tonsils are further displaced downwards due to the weakening of the "support" of the cerebellum [6]. This is also confirmed by the theory of Oldfield E. H. et al. (1994) on the pulsating aggravation of the dislocation of the cerebellar tonsils during each respiratory cycle and cardiac systole, followed by the development of dynamic blockade of the cerebrospinal fluid pathways in the region of the foramen magnum and aggravation of syringomyelia [8-10]. A number of authors, investigating this issue, identified as the basis of the persistent course of the disease: syringomyelia in this group of patients does not always occur due to obstruction of the cerebrospinal tract in the area of the foramen magnum, or the operation did not eliminate obstruction of the cerebrospinal tract in the area of the foramen magnum, which initiates the pathophysiological mechanism of progression of syringomyelia [11].

Based on our observation data, the patient's wait-and-see management tactics, despite the progression of syringomyelia with an aggravation of neurological deficit after decompression of the posterior cranial fossa, made it possible to achieve a safe long-term outcome of the course of the disease associated with type I Chiari anomaly.

CONCLUSION

The given clinical case demonstrates that patients after neurosurgical treatment for type I Chiari anomaly associated with syringomyelia should undergo thorough clinical and instrumental dynamic monitoring for potential progression of syringomyelia. In case of an increase in syringomyelia and aggravation of neurological symptoms in the postoperative period, conservative management of the patient is permissible.

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Kuznetsova N. S. – designed research framework, analysed the data, drafted the manuscript;

Maslov A. A. – edited the text of the paper, analysed the results;

Hatyushin V. E. – reviewed the publications on the topic of the article, analysed the data;

Matevosyan B. V. – processing and analysis of the results;

Reznik G. A. – reviewed the publications on the topic of the article;

Pandova O. V. – reviewed the publications on the topic of the article;

Shalashnaya E. V. – edited the text of the paper.

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CLINICAL CASE REPORT

CLINICAL OBSERVATION OF PATIENTS WITH PRIMARY MULTIPLE MALIGNANT TUMORS, INCLUDING PRIMARY MULTIPLE MELANOMA

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ABSTRACT

Recently, there has been an increase in the number of patients with primary multiple malignant tumors, which not only affect one or more organs, but also differ in their histological structure. At the same time, melanoma of the skin is a rare localization among primary malignant neoplasms. This nosology accounts for only 3–5 % of all skin tumors. Melanoma is associated with high mortality due to the development of a pronounced metastatic potential, and therefore the study of this malignant formation is of the greatest relevance. Over the past 50 years, the incidence of multiple primary melanoma has increased significantly. At the same time, the number of patients with more than 2 lesions has increased to 18 % of the number of primary multiple melanomas over the past 50 years. This emphasizes the importance of monitoring patients with melanoma and regularly examining patients for new lesions. This article demonstrates a clinical case of a patient with a confirmed diagnosis of a primary multiple disease with melanoma of the skin and rectum. For skin melanoma, the patient underwent a wide excision of the tumor with inguinal-femoral lymph node dissection on the right. Subsequently, radiation therapy and chemotherapy were performed. Further, during a comprehensive examination, the patient was diagnosed with a malignant neoplasm of the lower ampullar rectum with a transition to the anal canal. The patient underwent laparoscopic-assisted abdominoperineal extirpation of the rectum. Histological analysis revealed nodular melanoma. From the anamnesis of the patient, among the comorbidities, breast cancer, uterine myoma, hemangioma of the liver and lung hamartoma were also identified. The clinical course of all malignant tumors was favorable, without the development of relapses and metastases. The greatest interest in this situation is the primary multiple melanoma in connection with successful treatment with the most unfavorable prognosis. The described clinical observation indicates the need for an in-depth study of cases of primary multiple malignant tumors and the search for mechanisms for a favorable course of malignant neoplasms in this case.

Keywords: primary multiple malignant tumors, primary multiple melanoma, melanoma of the skin

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Compliance with ethical standards: the ethical principles presented by the World Medical Association Declaration of Helsinki (1964, ed. 2013) were observed in the work. Informed consent was obtained from all participants of the study.

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КЛИНИЧЕСКОЕ НАБЛЮДЕНИЕ

КЛИНИЧЕСКОЕ НАБЛЮДЕНИЕ ПАЦИЕНТКИ С ПЕРВИЧНО-МНОЖЕСТВЕННЫМИ ЗЛОКАЧЕСТВЕННЫМИ ОПУХОЛЯМИ, ВКЛЮЧАЮЩИМИ ПЕРВИЧНО-МНОЖЕСТВЕННУЮ МЕЛАНОМУ

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РЕЗЮМЕ

В последнее время отмечается увеличение числа пациентов с первично-множественными злокачественными опухолями, которые не только поражают один или несколько органов, но и отличаются между собой по гистологической структуре. При этом среди первичных злокачественных новообразований редкой локализацией является меланома кожи. Данная нозология составляет всего 3–5 % от всех опухолей кожи. Меланома связана с высокой смертностью из-за развития выраженного метастатического потенциала, в связи с чем изучение данного злокачественного образования представляет наибольшую актуальность. За последние 50 лет встречаемость первично-множественной меланомы значительно возросла. При этом количество пациентов, имеющих более 2 очагов, возросло до 18 % от числа первично-множественных меланом в течение последних 50 лет. Это подчеркивает важность наблюдения пациентов с меланомой и регулярных осмотров больных на предмет возникновения новых очагов. В данной статье продемонстрирован клинический случай пациентки с установленным диагнозом первично-множественного заболевания с поражением меланомой кожи и прямой кишки. По поводу меланомы кожи пациентке было выполнено широкое иссечение опухоли с пахово-бедренной лимфодиссекцией справа. В последующем проводилась лучевая терапия и химиотерапия. В последующем при комплексном обследовании больной был установлен диагноз злокачественного новообразования нижне-ампулярного отдела прямой кишки с переходом на анальный канал. Пациентке была выполнена лапароскопически-ассистированная брюшно-промежностная экстирпация прямой кишки. По данным гистологического анализа выявлена узловая меланома. Из анамнеза больной среди сопутствующих заболеваний также были выявлены рак молочной железы, миома матки, гемангиома печени, гамартома легкого. Клиническое течение всех злокачественных опухолей было благоприятным, без развития рецидивов и метастазов. Наибольший интерес в данной ситуации представляет первично-множественная меланома в связи с успешным лечением при наиболее неблагоприятном прогнозе. Описанное клиническое наблюдение свидетельствует о необходимости углубленного изучения случаев первично-множественных злокачественных опухолей и поиска механизмов благоприятного течения при этом злокачественных новообразований.

Ключевые слова: первично-множественные злокачественные опухоли, первично-множественная меланома, меланома кожи

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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). 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.

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INTRODUCTION

Recently, there has been an increase in the number of patients with primary multiple malignant tumors having different histological structure and affecting one or more organs. The cases of two primary malignant neoplasms are the most common, while the cases of a greater multiplicity of malignant tumors are less than 0.5 % [1-3].

At the same time, among primary malignant tumors, a rare localization is skin melanoma, which accounts for only 3-5 % of all skin tumors, but the relevance of this disease is due to high mortality due to pronounced metastatic potential [4].

Primary multiple melanoma is understood as the occurrence of two or more separate melanomas in one patient. Over the past 50 years, the incidence of primary multiple melanoma has increased significantly (in the 1960s, its rate was less than 1 % among men and women; in the 2000s – 6.4 % among women and 7.9 % among men). The number of patients with more than 2 lesions over the past 50 years has increased to 18 % of the number of primary multiple melanomas [5].

According to a study by Menzies S et al. (2017), 4.8 % of all melanomas are primarily multiple, and the average period between the detection of the first and second foci is 33.7 months. At the same time, in 70 % of patients, the second focus is diagnosed within about 2 years after the first one is detected [6]. This underlines the importance of monitoring patients with melanoma and regular examinations for the appearance of new foci. Among the most frequent localizations, the authors note the lesion of the lower lip. There was no significant difference in the age of patients with solitary and multiple melanomas. According to other authors, 1–8 % of all melanomas are primarily multiple [7]. About 6–12 % of all melanomas are familial and 12 % of familial melanomas are primarily multiple [8]. Risk factors for hereditary and primary multiple forms include mutations of the breast cancer 1 (BRCA1), BRCA1-associated protein 1 (BAP1), CDKN2A and telomerase reverse transcriptase (TERT) genes [9-10]. Despite the available information about melanoma, there is no significant data on the management of patients with primary multiple melanoma to date.

The purpose of the study is to improve the diagnosis and treatment results of primary multiple malignant neoplasms by applying careful dynamic monitoring of oncological patients.

Description of the clinical case

As the matter of information above, the following clinical observation is of interest.

Patient S., female, 64 years old, in September 2021, was admitted to the National Medical Research Centre for Oncology with complaints of an admixture of blood and mucus in the feces, pain during defecation, general weakness for 3 months. Fibrocolonoscopy was performed at the patient's place of residence, during which a tumor of the anal canal was detected, a biopsy was taken. According to the results of histological examination of the biopsy G2 squamous cell carcinoma has been confirmed.

It is known from the anamnesis that in 1990 the patient received treatment for melanoma of the skin of the right thigh (the stage of the disease is not known due to the loss of discharge documents). The patient underwent a wide excision of the tumor with inguinal-femoral lymph dissection on the right, radiation therapy and chemotherapy were performed. In 1991, for the second stage of right breast cancer (pT2N0M0), the patient underwent combined treatment, including remote gamma therapy and radical mastectomy. In 2001, supravaginal amputation of the uterus with appendages was performed due to uterine fibroids of the patient.

The patient's closest relatives have no oncological diseases.

A follow-up examination of the patient was conducted at the National Medical Research Centre for Oncology. A revision of histopreparations and IHC No. 11832-33/21 was performed: in biopsies of rectal tumors, the morphological picture and immunophenotype of tumor cells (S-100+, Vimentin +, panCK-) correspond to pigmented melanoma.

With spiral computed tomography (CT) of the chest and abdominal organs, focal formation of the middle lobe of the right lung of tumor genesis and hemangioma of the right lobe of the liver were revealed.

During magnetic resonance imaging (MRI) of the pelvic organs, a semicircular tumor involving the mucous and submucosal layer without signs of damage to the condyle subserous and serous layers with exophytic growth and narrowing of the rectum to 2/3 of

its lumen is determined by 25 mm from the anodermal junction in the anal canal and by continuation in the lower ampullary section. MR-signs of extramural growth, invasion of mesorectal tissue, mts lesions of lymphatic collectors in the pelvic and retroperitoneal tissues were not detected.

According to the results of a comprehensive examination, the patient was diagnosed with malignant neoplasm (MN) of the lower ampullary rectum with a transition to the anal canal T3N0M0, art. II, clinical group 2.

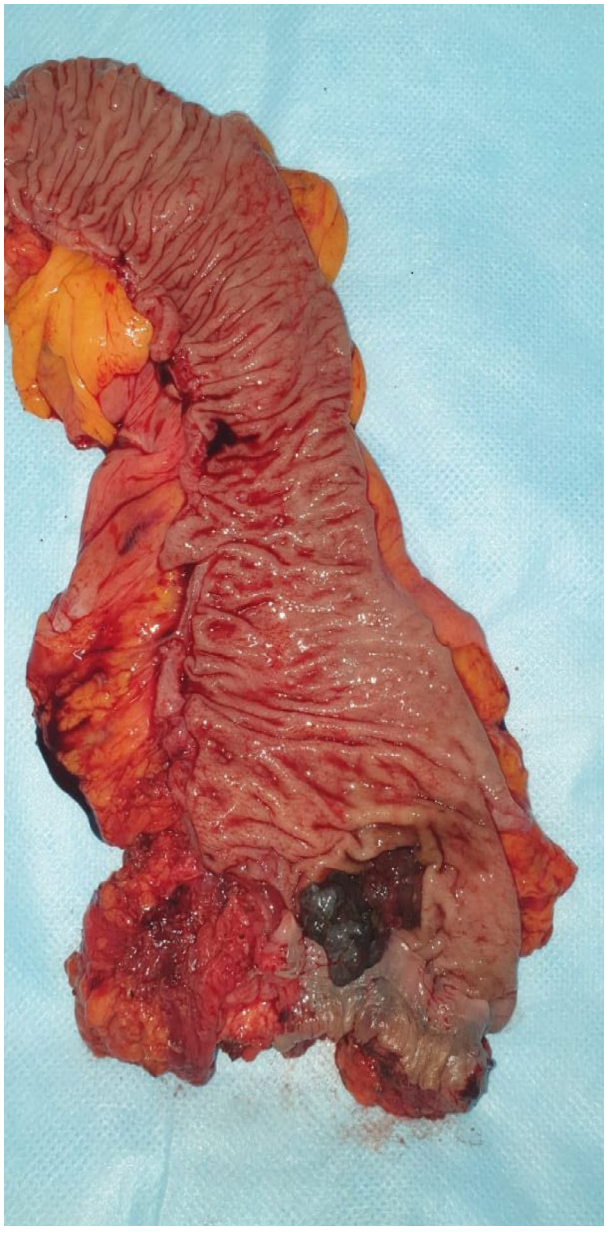

Fig. 1. Postoperative tissue with rectal melanoma.

laparoscopically assisted abdominal-perineal extirpation of the rectum was performed on 09/13/2021. The postoperative preparation is shown in figure 1.

Postoperative pathomorphological examination No. 98743-60/21: nodular melanoma of the lower ampullary rectum with a transition to the anal canal, with ulceration, fusiform and epithelioid cell variants of the structure, with a high content of unevenly distributed brown pigment, with pronounced lymphocytic infiltration along the periphery of the tumor, with invasion to the muscular membrane, with a Breslow thickness of 7 mm. There is sinus histiocytosis in 2 lymph nodes; 10 fragments are represented by fatty tissue with dilated full-blooded blood vessels. There were no signs of tumor growth along the resection lines.

No V600 mutation in exon 15 of the BRAF gene was detected during the DNA study

The postoperative period proceeded without complications.

On 10/27/2021, a videothoracoscopic atypical resection of the middle lobe of the right lung was performed. Postoperative pathomorphological examination No. 118321-23/21: morphological picture of pulmonary (chondoromatous) hamartoma.

With further follow-up and control examination every 3 months, no data for the progression of the disease was revealed.

DISCUSSION

Thus, one patient had six tumor locations: three of them benign (uterine fibroids, liver hemangioma, lung hamartoma) and three malignant (skin melanoma, breast cancer, rectal melanoma). The clinical course of all malignant tumors was favorable, without the development of relapses and metastases. Primary multiple melanoma is of the greatest interest in this observation due to successful treatment with the most unfavorable prognosis.

CONCLUSION

The described clinical observation indicates the need for an profound study of cases of primary multiple malignant tumors and the search for mechanisms of a favorable course of malignant neoplasms in this case.

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Dashkov A. V. – performed material processing.

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REVIEW

IMMUNOTHERAPY FOR EPITHELIAL TUMORS OF THE THYMUS

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ABSTRACT

Thymomas and carcinomas of the thymus gland, also known as epithelial tumors of the thymus (TT) are rare malignant neoplasms, but are the most common solid tumors of the anterior mediastinum. The incidence does not exceed 1.3–1.7 per million inhabitants per year. In Europe, about 1,500 new cases are registered annually, and the average age of patients is around 40–50 years.

Originating from the epithelial component of the thymus, the primary lymphoid organ, they are accompanied by a high risk of developing autoimmune disorders due to their unique biology. Indeed, up to 30 % of TETS patients suffer from autoimmune disorders (AID), the most common of which is myasthenia gravis (MG). AID are detected not only during the diagnosis of a tumor, but also during follow-up. With rare exceptions, there are no specific targets for targeted therapy in TETS. Immune checkpoint inhibitors (ICIs) halt the ability of tumor cells to evade immune surveillance, enhancing their killing. Unprecedented achievements of immunotherapy (IT) in the treatment of metastatic non-small cell lung cancer (NSCLC) and melanoma have made it reasonable to study the effectiveness of prescribing ICI in patients with TETs. The prevalence of AIR in different morphological subtypes of TETs may influence the decision to conduct IT due to the increased risk of toxicity. The review summarizes current data on the effectiveness of IT in thymoma and thymus cancer (TC) and discusses several unresolved problems associated with the use of ICI in TETs.

The purpose of this review is to present up-to-date data on the issue under discussion and possible prognostic biomarkers for IT, and to highlight the problems associated with autoimmune disorders (AID).

In our opinion, a deep understanding of the molecular genetic and immune landscape of thymus epithelial tumors and the interaction of ICI with the immune system is the key to improving the effectiveness and preventing the side effects of autoimmune IT. A comprehensive solution to existing problems will undoubtedly open up new possibilities for the drug treatment of this rare and difficult disease.

Keywords: thymus epithelial tumors (TETs), thymoma, thymic carcinomas (TC), immune checkpoint inhibitors (ICIs), autoimmune disorders (AID), immunotherapy (IT) toxicity

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ОБЗОР

ИММУНОТЕРАПИЯ ЭПИТЕЛИАЛЬНЫХ ОПУХОЛЕЙ ВИЛОЧКОВОЙ ЖЕЛЕЗЫ

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РЕЗЮМЕ

Тимомы и карциномы вилочковой железы, также известные как эпителиальные опухоли тимуса (ОТ), являются редкими злокачественными новообразованиями, но также наиболее частыми солидными опухолями переднего средостения. Заболеваемость не превышает 1,3–1,7 на миллион жителей в год. В Европе ежегодно регистрируется около 1500 новых случаев, а средний возраст заболевших составляет от 40 до 50 лет.

Происходя из эпителиального компонента тимуса – первичного лимфоидного органа, они из-за своей уникальной биологии сопровождаются высоким риском развития аутоиммунных расстройств. Действительно, до 30 % больных ОТ страдают аутоиммунными расстройствами (АИР), наиболее частым из которых является миастения гравис (МГ). АИР выявляются не только при диагностике опухоли, но и во время последующего наблюдения. За редким исключением в ОТ отсутствуют специфические мишени для таргетной терапии. Ингибиторы иммунных контрольных точек (ИИКТ) подавляют способность опухолевых клеток уклоняться от иммунного надзора, усиливая их киллинг. Беспрецедентные достижения иммунотерапии (ИТ) в лечении метастатического немелкоклеточного рака легкого (НМРЛ) и меланомы сделали обоснованным изучение эффективности назначения ИИКТ пациентам с ОТ. Распространенность АИР при разных морфологических подтипах ОТ может повлиять на решение о проведении ИТ из-за повышенного риска токсичности. В обзоре обобщены современные данные об эффективности ИТ при тимоме и раке тимуса (РТ) и обсуждаются несколько нерешенных проблем, связанных с использованием ИИКТ при ОТ.

Цель данного обзора – представить современные данные по обсуждаемому вопросу и возможные прогностические биомаркеры для ИТ и осветить проблемы, связанные с аутоиммунными расстройствами (АИР).

По нашему мнению, глубокое понимание молекулярно-генетического и иммунного ландшафта эпителиальных опухолей вилочковой железы и взаимодействия ИИКТ с иммунной системой является ключом к повышению эффективности и предотвращению побочного аутоиммунного действия ИТ. Всестороннее решение существующих проблем, несомненно, позволит открыть новые возможности лекарственного лечения этого редкого и трудного заболевания.

Ключевые слова: эпителиальные опухоли тимуса (ОТ), тимомы, рак тимуса (РТ), ингибиторы иммунных контрольных точек (ИИКТ), аутоиммунные расстройства (АИР), токсичность иммунотерапии (ИТ)

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INTRODUCTION

Epithelial tumors of the thymus (TETs) are rare and potentially aggressive malignant neoplasms of the anterior mediastinum. The incidence does not exceed 1.3–1.7 per million inhabitants per year; in Europe, about 1,500 new cases are registered annually, and the average age of patients is from 40 to 50 years [1]. Based on the morphological structure, namely the proportion of two components of the thymus gland – immature lymphocytes and epithelial cells – the WHO classification distinguishes two types of epithelial tumors: thymoma and thymus cancer and six main morphological subtypes: thymomas A, AB, B1, B2, B3 and TC [2]. The epithelial component is the only one in which a malignant tumor develops. At the consensus meeting of the ITMIG (International Thymic Malignancy Interest Group) in 2011, the WHO classification was approved as a standard for clinical practice [3]. Less common than thymoma, thymus cancer accounts for about 10–15 % of and is largely associated with the development of distant metastases and poor prognosis. TCis most often represented by squamous cell carcinoma. Tumor cells of thymus carcinomas express cluster determinants CD5+ and CD117/KIT+, determined by immunohistochemical examination (IHCE) and have specific molecular features that distinguish them from thymoma and squamous cell lung cancer [4].

The clinical course of TETs is determined by the nature of the growth of the neoplasm. Encapsulated tumors corresponding to stage I according to the Masaoka-Koga (M-K) surgical classification account for 65 %, while invasive tumors of stage II-IV account for 35 % of cases [5; 6]. Surgical intervention is a key stage of treatment for TETs stage I, II and even III, since radical removal of the tumor, along with stage [5; 6] and morphological structure [3], is the most important independent prognostic factor in terms of survival [6-8]. Tumor recurrence, depending on the stage of the disease, should be expected in 8–33 % of patients with thymoma and in 25–59 % of patients with TC [9]. Regimens of combined chemotherapy (CT) based on platinum preparations remain the standard method of treatment of inoperable, refractory and metastatic forms of the disease. The possibilities of modern CT are known to be limited, while the response rate ranges from 69 % for thymoma to 42 % for TC [9; 10]. In addition, some effectiveness of targeted drugs from the group of tyrosine kinase inhibitors, such as sunitinib [11] and everolimus [12], has been demonstrated. Less often, the c-KIT and PI3KCA genes, as well as epigenetic signaling pathways, are used as targets [13]. The expected 5-year overall survival(s) for thymoma is 80 %, and for TC – 40 % [14].

ICIs have changed the paradigm of cancer treatment, becoming the standard treatment for melanoma, NSCLC and bladder cancer [15]. The role of immunotherapy (IT) in the treatment of TETs has not been definitively studied, primarily due to the high frequency of autoimmune conditions leading to a high risk of toxicity.

The purpose of this review is to present up-to-date data on the issue under discussion and possible prognostic biomarkers for IT, and to highlight the problems associated with autoimmune disorders (AID).

Thymus physiology and oncogenesis

The thymus gland is the central organ of the immune system that ensures the development of immune tolerance. After formation in the bone marrow, immature thymocytes mature in the thymus as a result of interaction between cortical and medullary epithelial cells of the thymus gland. The presentation of tissue-specific autoantigens through the main histocompatibility complex of class II (MHC-II) is regulated by two transcription factors: the AIRE genes (autoimmune regulator) and Fez (Fez family zinc finger 2) [16]. The passage of T-thymocytes through the thymus cortex and corticomedullary junction implies phenotypic modifications affecting the functioning of T-lymphocytes. Immature T-lymphocytes reacting with MHC-II are able to penetrate into the thymus medulla, while non-interacting T-cells are eliminated. Both dendritic cells and medullary epithelial cells are found in the medulla of the thymus gland. Medullary epithelial cells expressing AIRE undergo various changes and undergo apoptosis, releasing tissue-specific autoantigens for thymus dendritic cells. T-lymphocytes reacting against tissue-specific autoantigens undergo apoptosis, providing immune tolerance [16]. This process occurs mainly in childhood, but is sometimes present in adults and probably goes further into the process of carcinogenesis [4].

Autoimmune disorders and TETs

Autoimmune disorders (AID) need to be differentiated from paraneoplastic syndromes. Their patho-

physiology, clinical course and impact on survival are different. Paraneoplastic syndromes, as a rule, arise as a result of the production of hormones, cytokines or peptides by tumor cells, which leads to metabolic disorders and the induction of autoantibodies produced by tumor cells. Thus, the successful treatment of the tumor should eliminate the clinical manifestations of paraneoplastic syndromes, regardless of whether airs can stop due to a violation of the regulation of the function of the thymus gland and imperfect selection of immature T-lymphocytes.

AIDs are present in more than 30 % of observations from [16]. The most common syndrome is myasthenia gravis (MG), the frequency of which ranges from 17 % in thymoma A to 71 % in thymoma B2 [7]. In addition, endocrine, rheumatological, gastrointestinal, renal, and skin lesions are recorded [17]. The data from RYTHMIC (Réseau tumeurs THYMiques et Cancer), one of the largest TET registries in Europe, demonstrate a 20.2 % prevalence of AIR with more than one disorder in 3.8 % of patients. The majority of patients had MG – 69.6 %, followed by: Hood syndrome – 5.6 %, systemic lupus erythematosus – 4.4 %, thyroiditis – 3.4 % and pure erythrocyte aplasia – 2.8 %. As for the morphological subtypes, the prevalence of AID more than 40 % was established with thymoma B2-45 % and B3-41 % [18]. Thymomas AB, B1 and B2 are rich in lymphocyte agglomerates, which explains their more frequent relationship with AIR compared to thymoma B3 and RT. Nevertheless, several studies have described a high prevalence of autoimmune conditions in thymoma B3 [7; 18]. It is important to note that TC is rarely associated with AID [19]; the absence of MG in thymus carcinoma has been reported [20], possibly due to the presence of immature T cells. The high prevalence of air in TETs and the appearance of autoimmune symptoms after diagnosis [18] requires extremely careful monitoring of the condition of patients during IT.

Molecular features of TETs and AID

The unique biology of thymus tumors gives special interest to the study of autoimmune processes due to the strong association with AID, especially with MG. In fact, knowledge about the molecular characteristics of MG associated with thymoma is limited. It is well known that antibodies against the acetylcholine receptor (AchR) are mandatory for the development of MG, anti-AchR are able to block the postsynaptic membrane, as well as reduce the amount of AchR in the neuromuscular junction, which leads to a decrease in the reaction to acetycholine, clinically manifested by paroxysmal weakness and fatigue of skeletal muscles [21]. Immunoregulation disorders and tolerance caused by the tumor microenvironment, probable mechanisms of the pathogenesis of tumor-related AID, primarily MG.

Several studies have investigated whether various acetylcholine subunits are expressed in the thymus and whether some of them are associated with the development of MG associated with thymoma. Low levels of expression of AIRE (autoimmune regulator) and AID by tumor cells correlate with a higher risk of MG [22]. Moreover, the relative levels of Foxp3 (forkhead box P3) RNA expression were significantly higher in the tumor tissue samples of patients without AID compared with patients suffering from MG and/or other AID. It should be noted that AIRE and Foxp3 are transcription factors that play an important role in the differentiation of T-reg lymphocytes, which play an important role in suppressing immunity, contributing to tumor growth [23]. Interestingly, AIRE may be associated with specific genomic changes, for example, an imbalance of the NF-kappaB/AIRE signaling pathway observed in MG associated with thymoma [23].

One of the largest molecular studies of TETs was conducted within the framework of TCGA (The Cancer Genome Atlas). The analysis of 117 TETs revealed a higher frequency of aneuploidy in the thymomas of patients with MG [13]. In addition, the expression levels of the α-subunit of AchR (CHRNA1 – neuronal acetylcholine receptor subunit alpha-1) were higher in tim samples of patients suffering from MG. A medium-sized neurofilament (NEFM – neurofilament medium chain) is a protein with similar immunogenic properties to CHRNA1 and titin, mainly overexpressed in the tim A and AB subgroups, accompanied by MG. As for TC, despite their more aggressive biological behavior, several tumor suppressor genes were found in them: CYLD, CBFB, CDH1, CDH11, CTCF and ZFHX3, as well as a higher mutational load of the tumor (TMB) compared to thymomas [13]. The presented results confirm the conclusions that TC and thymoma differ in their genetic and epigenetic profiles. Indeed, the results of a grandiose transcriptomic analysis of 2,560 genes in 194 samples TETs the recent one revealed two different clusters of genes that distinguish TC from thymoma [24].

In a Chinese study of 105 patients suffering from MG, an increase in inflammatory responses was found, in contrast to patients without AIR. It should be noted that in the latter, a mutation of the GTF2I gene was detected significantly more often [25]. In fact, the presence of the GTF2I mutation correlates with better survival rates and, perhaps, its carriers can become candidates for IT. The GTF2I mutation was detected in 82 % of tim A and 74 % of tim AB, but rarely in aggressive subtypes, especially in RT, in which repeated mutations of known malignant tumor genes were detected, including TP53, CYLD, CDKN2A, BAP1 and PBRM1 [13]. At the same time, the expression of the GTF2I gene, which is very important, is associated with severe toxicity in IT, so there is an obvious need for further study of GTF2I as a biomarker [26].

Finally, to differentiate thymoma from thymus cancer and to understand the pathogenesis of MG, several other biomarkers have been studied: IGFBP1, KLF15, PDK4 and HIF3A. Other AIRS, such as encephalitis or polymyositis, correlate with an increase in anti-Hu antibodies, Ma2 antibodies and CRP5 antibodies or a violation of the regulation of the T-cell receptor (TCR) and an increase in the expression of MHC-I in muscle fibers, but the landscape of AID and MG is still unknown [27].

The strategy of TETs immunotherapy

In recent years, ICIs have revolutionized the treatment strategy and prognosis of several solid tumors. In previously treated patients, the appointment of ICIs gave a 5-year overall survival rate of 34 % in advanced melanoma, 28 % in renal cell carcinoma and 16 % in NSCLC [15], which led to the approval of anti-PD1 inhibitors (anti-Programmed cell Death protein 1), anti-PDL1 inhibitors (anti-Programmed Death Ligand 1) and anti-CTLA4 inhibitors (anti-Cytotoxic T Lymphocyte Antigen 4) for the treatment of metastatic forms of the disease. Given the prolonged effect of ICIs in many solid tumors, high hopes are pinned on IT of epithelial tumors of the thymus gland [4].

Immune-related predictive biomarkers

Several biomarkers have been tested as predictors of IT effectiveness, however, only two have been approved as biomarkers of response to the appointment of ICIs: I) PD-L1 expression in tumor cells and II) tumor mutation load (TMB), which is determined by the number of non-synonymous single-nucleotide variants in the coding region of the tumor genome. Tumors with high TMB contain more neoantigens that enhance the immune response, which leads to an increase in the effectiveness of IT, as shown in previous studies. In addition, activation of the immune system requires a high content of tumor-infiltrating lymphocytes (TILs) to achieve a better response to treatment [28].

PD-L1 expression is observed in more than 90 % of epithelial cells of the normal thymus gland and has been extensively studied in TET due to the aggressiveness of their biological behavior. On the material from 100 tim and 69 TC, high expression of PD-L1 and FOXP3+T reg was associated with a higher degree of malignancy of neoplasms. In other studies, PD-L1 expression in thymoma varied from 23 % to 92 % of tumor cells, and in TC – from 36 % to 100 % of tumor cells. Indeed, a number of clinical and pathological features, namely: young age, the common stage of the disease according to the M-K classification, the impossibility of radical removal and neoadjuvant therapy of thymoma, correlated with high expression of PD-L1. On the contrary, the correlation with morphological subtypes remains unclear. Reliable data on survival rates have not yet been presented, given that in some studies high PD-L1 expression correlated with better survival, and in others with poor outcomes. In addition to PD-L1 expression, the severity of TILs tumor tissue infiltration was studied, although on limited material. R. Higuchi and colleagues studied the expression of PD-L1 and the severity of TILs in surgical preparations in 39 patients with thymomas and RT. PD-L1 expression above 1 % was registered in 54 % of samples with different distribution among TETs subtypes: B2> B3> PT> B1> AB> A. A high infiltration (84 %) of CD8+ among CD3+ TILs was determined, which was evenly distributed among all cases. High PD-1 expression in TILs was found in 23–62 % of PTCS, without any predictive or prognostic significance [29-31].

Interestingly, TMB in TETs is one of the lowest among malignant tumors. The question of whether PD-L1 is the best predictive biomarker remains controversial due to the deterioration of the condition of many patients, despite IT. A more favorable therapeutic effect is better with aggressive thymomas of B2 or B3 subtypes, although the high prevalence of AID makes it difficult to use ICIs.

IT effectiveness in clinical trials

ICIs have been studied in several clinical studies on TETs. In one phase II group study, 40 patients with recurrent TC were treated with pembrolizumab, a humanized antibody IgG4 targeted at the PD-1 receptor. Patients with a history of air were not included in the study. The overall response rate (ORR) was observed in 22.5 % of cases. Disease control was achieved in 30 (75 %) patients with a median response time of 3 years. The median progression-free survival (PFS – progression free survival) was 4.2 months, and the median overall survival(s) was 24.9 months. One-year PFS and OS reached 29 % and 71 %, respectively, and 5-year OS was 8 %. High, at least 50 %, PD-L1 expression in tumor cells was observed in 10 (25 %) patients, which was associated with longer survival: median PFS 24 vs. 2.9 months; median S was not reached compared to 15.5 months. When PD-L1 was expressed by tumor cells less than 50 %, only 3 out of 27 patients with a response was achieved. The IFN-γ signature evaluated by Nanostring analysis correlated with the response to pembrolizumab therapy, on the contrary, the TP53 mutation registered in 36 % of tumors was associated with lower PD-L1 expression and shorter s. Interestingly, after a relapse of the disease, 4 patients, one of them 2 years after the completion of therapy with pembrolizumab, pembrolizumab was prescribed repeatedly with 2 responses to treatment [32].

J. Cho and colleagues conducted a second clinical trial with a similar design, examining the efficacy of pembrolizumab in 26 patients with recurrent TC and in 7 patients with recurrent thymoma: subtype B1-4, subtype B2/B3-1 and subtype B3-1). Three patients had a history of MG. ORR was 19.2 % in patients with TC and 28.6 % in patients with thymoma. Similarly, out of 26 patients with RT, 5 (19 %) achieved a partial response, and 14 (54 %) stabilized the disease. Tumors with high PD-L1 expression responded better to treatment. The median duration of response in patients with thymoma was not reached, in patients with TC was 9.7 months. Median PFS was 6.1 months in both groups. The median OS was 14.5 months for TC sufferers and was not achieved in patients with thymoma (Table 1) [33].

Avelumab is a human antibody IgG1 against PD-L1 was studied in 7 patients with thymoma and 1 MRI without a history of autoimmune conditions. The following morphological subtypes were registered among patients with thymoma: B3-2, B2/B3-1, B2-2 and B1-1. An objective response was obtained in four (57 %) of 7 patients with thymoma, including a confirmed partial response in 2 (29 %) patients. It should be noted that a significant decrease in tumor size was observed after one dose of avelumab in three patients [34].

Finally, a Japanese phase II study evaluated the role of nivolumab in the treatment of patients with inoperable or recurrent thymus carcinomas. Of these, 11 patients registered stabilization of the disease, including five patients for 24 or more weeks. Median PFS and median S were 3.8 months and 14.1 months, respectively. Further inclusion of patients in the study was terminated prematurely at the first stage due to the fact that none of them achieved an objective response [35].

Enhanced autoimmune toxicity

Activation of immunity increases the risk of developing undesirable side effects associated with IT

Table 1. Clinical studies on the subject of thymus epithelial tumors immunotherapy

Author/ year	*Phase/N*	Therapeutic agent	RR/DCR (%)	Median PFS (mon.)	Median of OS (mon.)	irAEs ≥ 3 st. (%)
Giaccone et al. (33)	II/ 40 TC	Pembrolizumab	23/76 %	4.2	24.9	15 %
Cho et al. (34)	II/ 40 TC и 7T	Pembrolizumab	19/73 % 29/100 %	6.1	14.5 Not achieved	15.4 % 71.4 %
Katsuya et al. (36)	II/ 13 TC	Nivolumab	0/38 %	3.8	11.3	13 %
Heery et al. (35)	I/7 T 1 PT	Avelumab	5 %	50	-	63 %

Notes: N – number of patients; TC – thymus cancer; T – thymoma; RR/DCR – objective response; PFS – progression-free survival; OS – overall survival; irAEs – adverse events associated with immunotherapy.

(irAE – immune-related adverse events). The frequency of treatment-related adverse events in the studies under consideration is relatively high compared to the results of IT of other malignant neoplasms, such as melanoma, NSCLC, squamous cell carcinoma of the head and neck and urothelial carcinoma, where the frequency of irAE of 3 or more severity ranges from 3 % to 9.7 % [36].

Among 40 patients in the study of G. Giaccone et al., who received pembrolizumab, 6 (15 %) developed serious AIDs, and 4 (10 %) had more than one condition: polymyositis and myocarditis in two cases; pancreatitis, hepatitis and diabetes mellitus in one case; bullous pemphigoid in one case (autoimmune exfoliation of the epidermis); in one case, polymyositis and hepatitis; and, finally, one case of a significant increase in the level of hepatic transaminases. Three patients had to stop treatment due to severe toxicity. Patients suffering from myocarditis and polymyositis, as well as bullous pemphigoid, needed the appointment of corticosteroid hormones for the relief of conditions [33]. It should be noted that one patient with developed myositis, myocarditis and initial MG had a complete response for 40 months [32].

In another study, 5 (71 %) of 7 patients with thymomas and 4 (15 %) of 26 patients with TC had irAE of 3 or more severity, including hepatitis – 12.1 %, myocarditis – 9.1 % and MG – 6.1 %, which 1 patient had initially; in addition, thyroiditis was recorded, antineutrophil cytoplasmic antibodies associated with rapidly progressive glomerulonephritis, colitis and myoclonus. Treatment was discontinued by 8 (24.2 %) patients. Therapy of undesirable toxic reactions, as a rule, was based on the appointment of corticosteroid hormones and immunoglobulins [33].

Among patients treated with nivolumab, serious airs were observed in two cases: an increase in the level of transaminases and adrenal insufficiency [35]. Among patients treated with avelumab, undesirable side effects associated with IT of all degrees were noted in 5 (63 %) patients [34].

Of particular concern is the frequency of myocarditis, since myocarditis accompanies TETs in less than 1 % of cases [32]. Myocarditis is observed in 5 % of TC patients and in 43–57 % of patients with thymoma included in clinical trials of ICI therapy [26; 34; 35]. This fact has been confirmed in several ongoing studies of IT of epithelial tumors of the thymus gland [36].

Myositis was observed in 8 % of patients with TC treated with pembrolizumab, and more than half of patients with thymoma included in the study to increase the dose of avelumab [26]. Muscle toxicity is explained by the existence of TCR clones and increased expression of MHC-I in muscle fibers with inflammatory infiltrates of macrophages and lymphocytes after treatment with ICIs. It should be noted that in patients who developed myositis, no specific antibodies were detected before and after ICI therapy [37].

MG often concomitant TETa as undesirable side effects associated with IT was noted in 3–14 % of TET patients treated with pembrolizumab [26; 33]. As explained above, the development of MG requires antibodies against AhR, as well as antibodies to MuSK and Lrp4 [37]. Interestingly, pure aplasia of erythrocytes, described as the most common AIR after MG in studies on IT of epithelial tumors of the thymus gland, was not recorded.

Ongoing clinical trials

With thymoma of the B1/B2 subtype, IT is not prescribed due to the high prevalence of AIR [4] and should not be carried out without a comprehensive discussion of the risks at a multidisciplinary oncological consultation. Currently, several clinical studies are being conducted on the effectiveness of the use of ICI both in monotherapy and in combination. In Europe, EORTC (European Organization for Research and Treatment of Cancer) and the European Platform for Thoracic Oncology have launched a phase II study of NIVOTHYM to study the effectiveness of nivolumab or its combination with ipilimumab in patients with progressive, refractory thymoma B3 subtype or TC with planned strict registration of AIR (NCT03134118). MD Anderson Cancer Center is conducting a Phase I/II study using pembrolizumab for TC and thymomas (NCT03295227). The National Cancer Institute (NCI) has developed a phase II protocol to evaluate the efficacy and toxicity of IT avelumab in thymoma and TC, (NCT03076554) (Table 2).

The preliminary results of the CAVEATT protocol, a study on the combined administration of avelumab with axitinib, have recently been published, showing a partial response and stabilization of the disease in 40 % and 60 %, respectively, with a median PFS of 7.9 months and an acceptable toxicity profile [38]. In addition, combinations of ICI with tyrosine kinase inhibitors sunitinib or lenvatinib, or with an indoleamine 2,3-dioxygenase-1 inhibitor (IDO – indoleamina 2,3-di-

oxigenasa-1) epacadostatot are being studied due to the importance of these signaling pathways in TET. It should be noted that in patients receiving a combination of pembrolizumab and epacadoscate before discontinuation of the study, no unexpected results obtained in melanoma were recorded [32]. A new studied combination in TET therapy is the bispecific single-domain Fc-fused antibody (PD-L1/CTLA4) KN046 (NCT04469725). Finally, data on the advantages of neoadjuvant and adjuvant approaches to IT of solid tumors formed the basis of studies to assess their effectiveness in the treatment of OT, for example, in relation to pembrolizumab (NCT03858582) (Table 2).

Existing IT pitfalls

Since patients with TET have an increased risk of developing treatment-related adverse events, this is an important aspect that should be taken into account when selecting candidates for IT. Some approaches are needed to reduce the risk of irAEs and increase the IT safety of epithelial tumors of the thymus gland.

Depending on the histological characteristics for each morphological subtype, the probability of developing AIR is different. The degree of infiltration by lymphocytes of the tumor differs from B1 – rich in lymphocytes to B3 – poor in lymphocytes. In addition, different molecular profiles are associated with each morphological subtype [13]. It is known that previously detected autoantibodies against AhR and B-cell lymphopenia studied in thymoma correlate with a higher risk of myositis when avelumab is prescribed [39]. In addition, overexpression of CHRNA1 and RYR3 (Ryanodine receptor type 3) is present in thymomas with a MG clinic [13], which is associated with the ability of tumor cells to secrete functional proteins that mimic non-tumor cells [37]. A distinctive feature of such tumors is their association with autoimmunity, carried out through overexpression of

Table 2. Ongoing clinical studies of immunotherapy of thymus epithelial tumors

NCT/ phase	Title, patients number (*N*)	Therapeutic agent	Tumor type	Final study checkpoints
NCT03076554/II	NCI, *N* = 55	Avelumab	TC, thymoma	Safety, response intensity
NCT03134118/II	NIVOTHYM, *N* = 50\50	Nivolumab/ nivolumab + iplimumab	TC, thymoma B3	PFS
NCT03295227/I–II	MD Anderson Cancer Center, *N* = 30	Pembrolizumab	TC, thymoma	Dose limiting toxicity
NCT04321330/ II	ML41253, *N* = 34	Atezolizumab	TC	Response intensity
NCT04417660/II	Maryland, *N* = 38	Bintrafa Alfa	TC, thymoma	Response intensity
NCT03463460/II	NCI, *N* = 40	Pembrolizumab + Sunitinib	TC	Response intensity
NCT04710628/ II	PECATI, *N* = 43	Pembrolizumab + Lenvatinib	TC, thymoma	PFS
NCT02364076/II	Georgetown University, *N* = 45	Pembrolizumab + Epacadostat	TC	Response intensity
NCT03583086/ I–II	Vanderbilt-Ingram Cancer Center, *N* = 177	Nivolumab + Vorolanib	TC	Safety, response intensity.
NCT04234113/ I- Ib	Sotio, *N* = 96	Pembrolizumab and SO-C101 (IL-15/IL-15R α)	TC	Dose limiting toxicity
NCT04469725/II	Jiangsu, *N* = 66	KN046 (PD-L1/CTLA4 bispecific single domain Fc protein antibody)	TC	Response intensity
NCT03858582/II	Samsung Medical Center, *N* = 40	Pembrolizumab in combination with neoadjuvant and adjuvant chemotherapy	TC, thymoma	Marks pathological response

Notes: TC: thymus cancer; PFS (progression-free survival): progression-free survival.

muscle autoantigens and increased aneuploidy [13].

There are data on the problems of re-prescribing ICI to patients with developed irAE against the background of previous IT. In some retrospective studies, up to 55 % of such cases of irAE were noted, but not as pronounced as in the initial treatment [40]. However, this scenario has not been sufficiently studied in thymoma due to the high probability of developing air. Careful monitoring and molecular profiling of AIR in TETs open up opportunities for the inclusion of patients with this pathology in clinical trials. This tactic has been studied in melanoma patients receiving ipilimumab; of the 30 patients with progressive melanoma suffering from various AID, such as Graves' disease, Crohn's disease and rheumatoid arthritis, 27 % had an exacerbation of AID, 33 % had the development of new irAEs, while half of the patients were treated without exacerbation of old and the emergence of new autoimmune conditions [41]. Finally, as a new approach, a combination of ICI with selective immunosuppressants has been proposed to prevent outbreaks of air [42], which should be studied in depth in TETs.

CONCLUSION

Immunotherapy is a new approach to the treatment of common epithelial tumors of the thymus gland, although its introduction into clinical routine practice seems to be a challenging due to the special biology of these malignant neoplasms. Despite the fact that the frequency of treatment-related adverse events is higher in thymoma compared to thymus carcinoma, patients with thymus cancer are also at risk of developing immune toxicity. Nevertheless, the re-appointment of ICIs is possible, but requires very careful monitoring of autoimmune disorders. New combinations of IT and targeted therapy seem promising. In our opinion, a deep understanding of the molecular genetic and immune landscape of thymus epithelial tumors and the interaction of ICIs with the immune system is the key to improving the effectiveness and preventing the side effects of autoimmune IT. A comprehensive solution to existing problems will undoubtedly open up new possibilities for the drug treatment of this rare and difficult disease.

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