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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 Oncological Journal": 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.
The journal contains publications of various categories: literature reviews, meta-analyses, clinical studies, observations of clinical cases, discussions, announcements and descriptions of new treatment methods.
The journal accepts for publication: original articles, health organizations, radiation diagnostics, exchange of experience, reviews, clinical case reviews.

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ORIGINAL ARTICLE

FEATURES OF REPLACEMENT OF EXTENSIVE POST-RESECTION BONE DEFECTS IN PELVIC AND SACRAL TUMORS

L. N. Vashchenko, P. V. Chernogorov, R. G. Luganskaya, A. A. Barashev, E. S. Bosenko, T. V. Ausheva, N. S. Saforyan✉

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ABSTRACT

Purpose of the study. To represent the results of a two-stage surgical treatment in patients with extensive pelvic bone defects.
Materials and methods. Data on 7 patients who underwent surgery for pelvic and sacrum tumors at the National Medical Research Centre for Oncology from 2016 up to 2020 are presented. The average age of the patients was 36 years. Patients with massive tumors that required a major resection of the posterior pelvis and the formation of an extensive bone defect requiring reconstruction with massive allografts and implants were selected for the study. 5 patients underwent different variations of sacrectomies with the resection of the iliac bones; 2 patients – interiliac-abdominal resections. For all these patients, surgical treatment was split into 2 stages.
Results. At the first stage we performed: 5 surgical interventions (total or extended sacrectomy at L5–S1 with lumbar-iliac bilateral stabilization with an 8-screw pedicle system) in patients with sacral tumors. In 2 cases, an interilio-abdominal resection with the defect replacement with cement articulating spacer. Intraoperative blood loss on average was 1.8L. We used autohemotransfusion to compensate the intraoperative blood loss.
The 2nd (reconstructive) stage was completed on average after 3 months. The reconstructive stage was not accompanied by major trauma in all patients. The average blood loss was approximately 800 ml. There were no complications after the reconstructive surgical stage.
Conclusion. The described two-stage technique allowed to avoid severe infectious complications requiring removal of implants and grafts in all patients. Adequate spinal pelvic stabilization and/or spacing of the defect contributed to early functional rehabilitation of patients and the continuation of adequate adjuvant therapy in the interstage period. The delaying of the reconstruction allowed to reduce the duration and invasiveness of the main intervention without affecting the final result of treatment.

Keywords:
sacral tumors, sacrectomy, lumbar-pelvic stabilization, bone defects, infectious complications, augment

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ОРИГИНАЛЬНАЯ СТАТЬЯ

ОПЫТ ДВУХЭТАПНОГО ХИРУРГИЧЕСКОГО ЛЕЧЕНИЯ БОЛЬНЫХ С ОБШИРНЫМИ ПОСТРЕЗЕКЦИОННЫМИ КОСТНЫМИ ДЕФЕКТАМИ ПРИ ОПУХОЛЯХ ТАЗА И КРЕСТЦА

Л. Н. Ващенко, П. В. Черногоров, Р. Г. Луганская, А. А. Барашев, Е. С. Босенко, Т. В. Аушева, Н. С. Сафорьян✉

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РЕЗЮМЕ

Цель исследования. Представить результаты двухэтапного хирургического лечения больных с обширными костными дефектами таза.

Материалы и методы. Представлены данные о 7 пациентах, которым выполнены оперативные вмешательства по поводу опухолей таза и крестца в ФГБУ «НМИЦ онкологии» Минздрава России с 2016 по 2020 гг. Для анализа отобраны пациенты с местно-распространенным процессом, потребовавшим значительной по объему резекции задних отделов таза и с формированием обширного костного дефекта, требующим реконструкции массивными ауто- и аллотрансплантатами и имплантами. 5 пациентов – это сакрэктомии в разных вариантах с резекцией подвздошных костей и 2 пациента – межподвздошно-брюшные резекции. Всем пациентам хирургическое лечение разделено на 2 этапа.

Результаты. На 1 этапе было выполнено: 5 оперативных вмешательств у больных с опухолями крестца – тотальная или расширенная сакрэктомия на уровне L5–S1 с пояснично-подвздошной билатеральной стабилизацией 8 винтовой педикулярной системой, и в 2 случаях – межподвздошно-брюшная резекция с замещением дефекта цементным артикулирующим спейсором. Средняя кровопотеря составила 1,8 л. В качестве возмещения интраоперационной кровопотери нами использовалась методика аутогемотрансфузии.

2-й (реконструктивный) этап выполнен в среднем через 3 мес. У всех пациентов реконструктивный этап не сопровождался большой травматичностью. Кровопотеря в среднем 800 мл. Осложнений после реконструктивного этапа операции не было.

Заключение. Использование двухэтапной методики позволило избежать тяжелых инфекционных осложнений, требующих удаления имплантов и трансплантатов у всех пациентов. Адекватная позвоночно-тазовая стабилизация и (или) спейсирование дефекта способствует ранней функциональной реабилитации больных и продолжению адекватной адъювантной терапии в межэтапном периоде. Отсроченное выполнение реконструкции позволяет уменьшить продолжительность и травматичность основного вмешательства, не влияя на окончательный результат лечения.

Ключевые слова:
опухоли крестца, сакрэктомия, пояснично-тазовая стабилизация, дефекты костной ткани, инфекционные осложнения, трансплантат

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RELEVANCE

Pelvic bones are affected by tumors relatively often: these tumors make up 10–15 % in relation to tumors of other bones of the skeleton [1].

According to a number of authors, pelvic bones are most often affected by malignant tumors (about 50 %), the proportion of benign tumors is 15 %, dysplastic processes – 35 %. Among primary malignant tumors, chondrosarcomas, osteogenic sarcoma, malignant giant cell tumors are most common; among benign ones – chondromas, osteoid osteomas, giant cell tumors [1].

One of the main and distinctive features of malignant formations of the pelvic bones is a long asymptomatic course, this is due to anatomical and topographic features of the pelvis, as well as insufficient oncological alertness of doctors of the general network [2]. The main reason for the patient's visit to the doctor is a pronounced pain syndrome, when the tumor already reaches an impressive size, and sometimes internal organs and intra-phase structures germinate.

Surgical interventions for tumors of the pelvic and sacral bones are associated with a high risk of massive blood loss, damage to the main vessels, visceral organs, damage to nerve structures, as well as often secondary healing or deep suppuration of a postoperative wound [1; 2].

When performing radical oncoresections on the anterior semicircle of the pelvis, including branches of the pubic and sciatic bones, restoration of the integrity of the pelvic ring is not required even in advanced cases [1].

In the area of the acetabulum, reconstruction is usually and quite successfully performed with modular or individual prostheses with a small number of complications.

One of the main and most dangerous complications of extensive oncoresections is infection. In orthopedic operations, the frequency of infectious complications after endoprosthetics is 1.5–2.5 %, but in oncological patients this indicator is significantly higher, according to literature data from 10.0 to 66.0 % [3–5]. And if the issue of two-stage tactics of prosthetics of bones and joints in peri-implant infection is beyond doubt, then the defects of bone tissue obtained during the removal of infected implants, after resection of bone tumors, represent a significant problem of modern orthopedics. With diaphyseal and articular traumatic and oncological defects, we are completely satisfied with the available modular and individual megaprostheses. The replacement of extensive defects of the iliac bones with the hip complex and sacrum does not always lead to the desired satisfactory results. A large percentage of complications (up to 40 %) [4; 6–8]. it

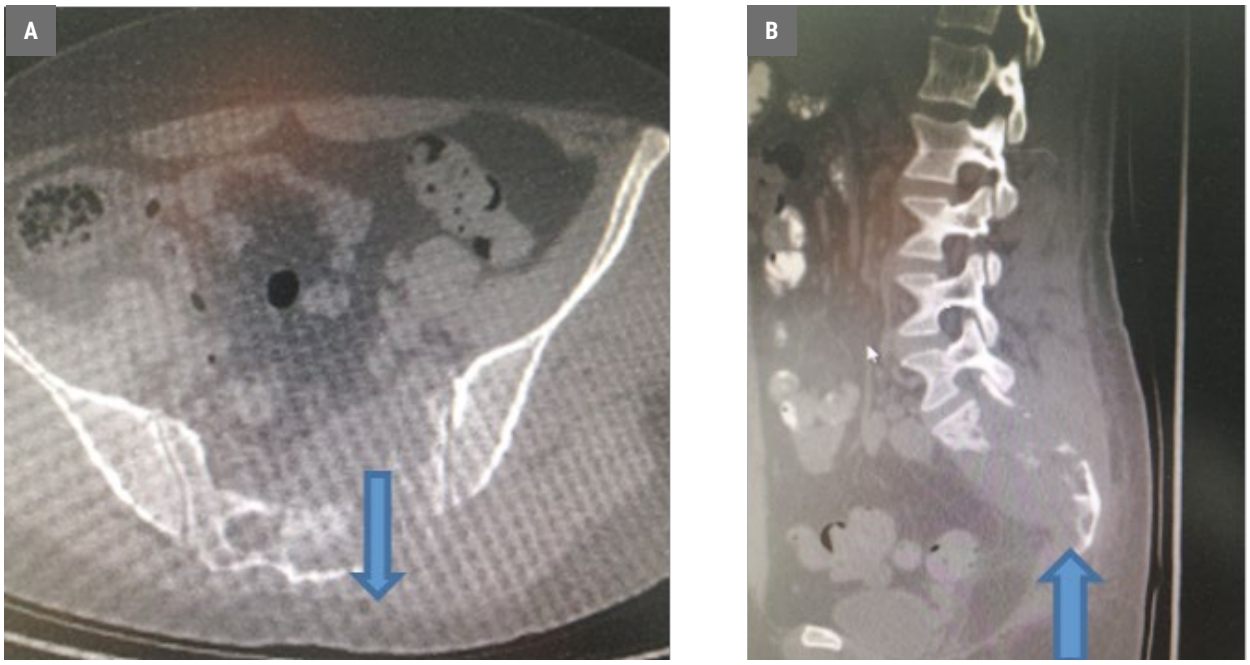

Fig. 1. Pelvic SCT, sacral liposarcoma (arrows showing the tumor).

requires analysis, individual correction of tactics and search for new solutions. The main problem is infectious complications due to the duration of the operation, the volume of surgical intervention, intraoperative blood loss, traumatization of the altered surrounding soft tissues, the presence of extensive metal structures and often allografts [4; 6, 7, 9–12]. Also, in the postoperative period, lymphorrhea is possible, which can last from several days to several weeks, which is supported by the presence of allografts and massive implants installed in the defect. The risk of deep suppuration increases significantly. After a number of similar complications, we began to separate (if possible) the stage of extensive pelvic resection and stabilization (if necessary, with the spacing of the defect with cement with antibiotics) and the reconstructive stage using auto- and allografts, the final vertebral-pelvic stabilization. The time between the stages depends on the specific clinical situation, ranging from one to several months. During this period, it is possible to carefully prepare for reconstruction, prepare implants, if necessary, perform modeling and an individual prosthesis exactly according to the shape of the resulting defect.

The purpose of the study: to analyze the results of two-stage surgical treatment of patients with extensive bone defects of the pelvis.

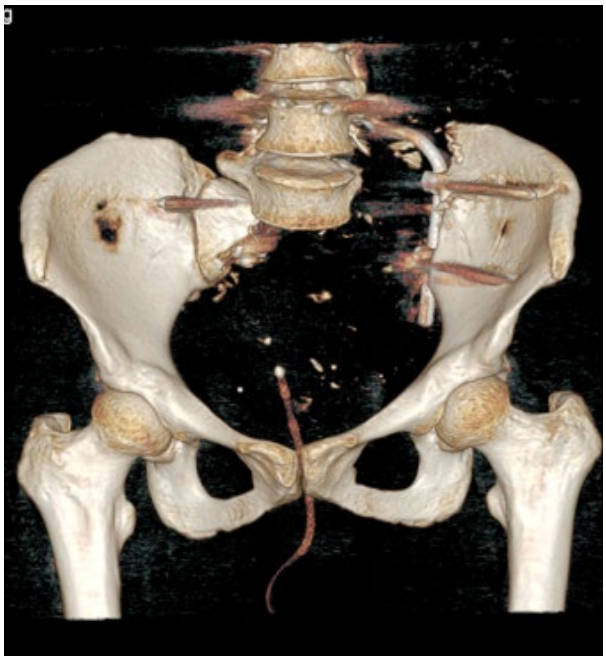

Fig. 2. 3D SCT pelvic reconstruction after the 1st stage.

MATERIALS AND METHODS

Data on 7 patients who underwent surgical interventions for pelvic and sacral tumors at the National Medical Research Centre for Oncology of the Ministry of Health of Russia from 2016 to 2020 are presented. For this analysis, patients were selected with a locally widespread process that required a significant volume of resection of the posterior parts of the pelvis and with the formation of an extensive bone defect requiring reconstruction with massive auto- and allografts and implants. The average age of the patients was 36 years, all women.

All patients at the prehospital stage underwent spiral computed tomography (CT), magnetic resonance imaging (MRI), trepanobiopsy. All patients have given informed consent for this type of surgical intervention, for the study.

Five patients underwent sacrectomy in different variants with resection of the iliac bones (1 patient had sacral liposarcoma, G3T3N0M0, 1 patient had malignant chordoma, G2T3N0M0, 2 patients had sacral chondrosarcoma, T3N0M0.

Two patients underwent ilio-abdominal resections, one patient had iliac fibrosarcoma, G3T2N0M0, and one patient had Ewing's sarcoma, T3N0M0.

The group did not include patients with sacral resection below the S2 level and paraarticular resection of the hip joint, when the reconstructive-stabilizing stage was performed immediately. Surgical treatment is divided into 2 stages for all patients.

At the 1st stage, we performed: 5 surgical interventions in patients with sacral tumors – total or extended sacrectomy at the level of L5–S1 vertebrae with lumbo-iliac bilateral stabilization by an eight-screw pedicular system. In 2 cases, ileo-abdominal resection was performed with replacement of the defect with a cement articulating spacer (2–3 doses of polymethylmethacrylate) with vancomycin. The spacer was manufactured in the operating room and installed on spongy 6.5–7.0 screws inserted into the remains of the pubic, sciatic bones and sacrum from the sawdust side.

In order to reduce the volume of blood loss, an intraoperative system was used to return autologous blood with Cell-Saver Sorin, thanks to which it was possible to significantly improve the quality of infusion-transfusion therapy and obtain an acceptable level of hematocrit. As compensation for

intraoperative blood loss, we used the autohemotransfusion technique. In the preoperative period, autologous blood components were harvested in one or two stages, which had a pronounced positive effect on the course of the postoperative period, and also allowed to reduce the number of allogeneic (donor) blood transfusions and related complications. In order to reduce intraoperative blood loss, three patients underwent angiography with superselective embolization of tumor vessels before surgery.

The reconstructive stage was completed in an average of 3 months. In 1 case, a massive individual prosthesis of half of the pelvis with a hip joint. In other cases, combined auto- and alloplasty. Additional stabilization (installation, rewiring) of the vertebral-pelvic pedicular system was required in 4 patients.

Patients (according to indications) received adjuvant chemo-radiation therapy according to clinical recommendations [13].

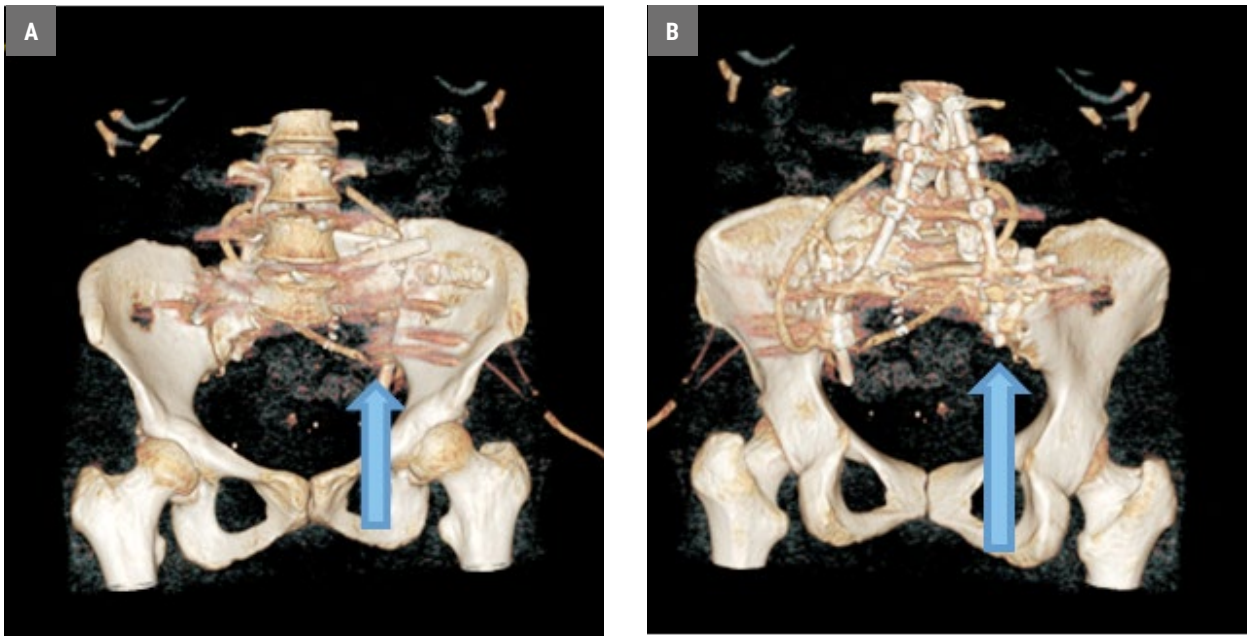

Fig. 3. 3D SCT reconstruction of the pelvis after stage 2 (arrows indicate the zone of additional stabilization of the pelvis).

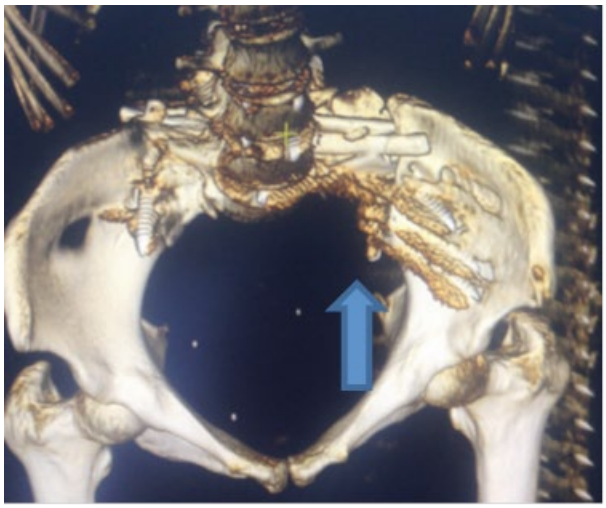

Fig. 4. 3D SCT pelvic reconstruction six months after stage 2 (arrows indicate the emerging spondyloidesis).

Fig. 5. SCT of the right hip joint (indicated tumor).

RESEARCH RESULTS AND DISCUSSION

The presented cases of two-stage surgical treatment with extensive postresection bone defects in pelvic and sacral tumors were evaluated according to the following criteria: surgical, orthopedic, oncological.

Surgical result. At stage 1, the average duration of the operation was 3.5 hours, intraoperative blood loss was 1–4.5 l. The average length of hospital stay was 18 days. All patients were prescribed adequate antibiotic therapy, anticoagulant therapy, and accompanying therapy.

Complications after stage 1: 1 patient had necrosis of the wound edges, infection of seroma, which required necrectomy and wound rehabilitation with preservation of implants.

In all patients, stage 2 (reconstructive stage) was not accompanied by great traumatism. The average blood loss is 800 ml. The average duration of the operation was 2 hours. There were no complications after the reconstructive stage of the operation.

Orthopedic result. Patients were activated with lifting on crutches for 2–4 days (at each of the 2 stages). Rehabilitation doctors and psychologists worked with patients every day.

The functional result was evaluated according to the Enneking scale [13]. In 1 patient after reconstruction of the acetabulum and hip joint with an endoprosthesis, the functional result was assessed as good. In patients after sarcectomy, the functional result was assessed as good and satisfactory.

Oncological result. All patients underwent radical surgical intervention – according to the morphological conclusion, the resection edges are negative (R0) in all patients. Patients (according to indications) received adjuvant chemo-radiation therapy according to clinical recommendations [14].

The follow–up period for patients is 6–36 months. Relapse in 1 patient (after 1 year), 1 patient died – generalization of the process (metastases to the lungs after 12 months), in 5 patients there are no signs of relapse and metastases.

Clinical case No. 1. Patient R., 39 L., in IX-2017, complained of pain in the sacrum, weakness in the n/ extremities, pelvic disorders and impaired walking function (previously, she had been treated for polyosteochondrosis for more than six months). She moved in a wheelchair and for short distances with a walker. During the examination, a tumor lesion of the sacrum was diagnosed with a soft-tissue intraphase component and compression of the roots of the sacral canal and extra-channel on the left (Fig. 1). According to the conclusion of trepan biopsy and IHC – a malignant tumor of sarcomatous nature, the morphological picture and immunophenotype most correspond to pleomorphic liposarcoma. She refused the proposed surgical treatment. The patient underwent 8 courses of polychemotherapy radiation therapy in II-2018 (50Gr) – there is a slight positive effect with partial regression of pain and radicular syndrome.

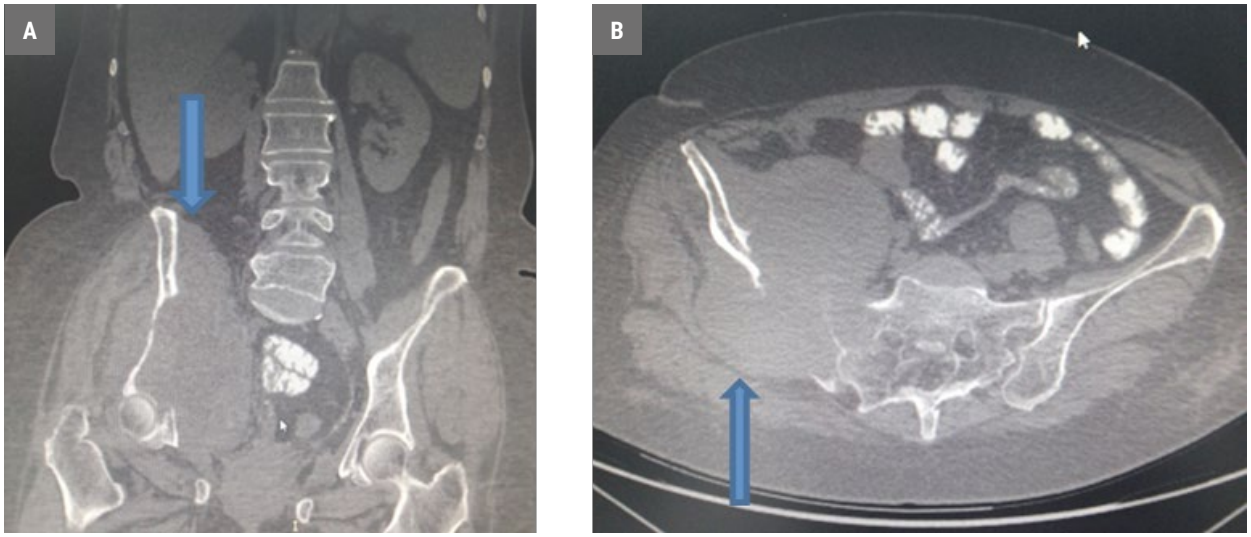

Fig. 6. Pelvic SCT, fibrosarcoma of the iliac bone after 4 weeks (the tumor is indicated).

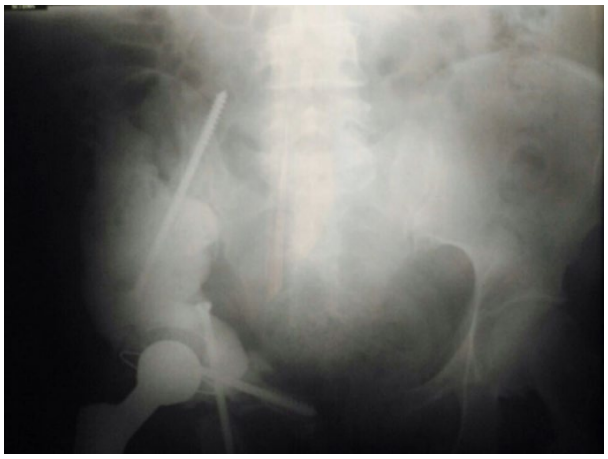

Fig. 7. Pelvic X-ray in direct projection after stage 1.

Taking into account the volume and duration of the intervention, radiation changes in the access area, and the high risk of infectious complications, it was decided to divide surgical treatment into two stages. In IX-2018, the first stage was sacrectomy with resection of the left sacroiliac joint, lumbo-pelvic stabilization (Fig. 2). The duration of the operation was 4 hours 50 minutes, blood loss was 3.0 liters; the Cell-Saver Sorin system was used.

In III–IV-2018, the 2nd reconstructive stage of intervention was planned, but the patient applied only in V-2019. She complained of pain in the area of surgery. There is no data for a relapse on SCT. Fatigue fracture of the locking rod, instability of the structure on the side of the greatest defect was revealed. 05/20/2019 – stage 2 reconstructive surgery: fusion with auto-allografts, supplemented with lumbar-pelvic stabilization (Fig. 3).

XI-2019, control examination of the patient. The data for tumor recurrence is not determined, it is already possible to see the reconstruction of transplants and the emerging spondyloidesis (Fig. 4).

Clinical case No. 2. Patient K., 49 years old. Diagnosis: (C41.4) Fibrosarcoma of the right iliac bone, G3T-2N0M0, Stil, cl. gr. 2. Applied in March 2017 (Fig. 5).

After trepanobiopsy (HA – fibrosarcoma), chirurgical treatment was offered, which the patient refused. She reapplied after 4 weeks due to increased pelvic pain. According to the CT data, rapid progression of the disease was revealed (Fig. 6). Surgical treatment (two-stage) was re-proposed.

05/17/2017 resection of the right half-ring of the pelvis with the right hip joint was performed, the defect was replaced with a cement articulating spacer and a femoral component of the endoprosthesis (Fig. 7).

The tumor was removed in one block. The edges of the resection R0. Then 3 courses of adjuvant polychemotherapy were conducted.

Histological conclusion: Low–grade fibrosarcoma with extensive foci of necrosis, hemorrhages, with destruction of bone beams (G3), in some areas – undifferentiated fibrosarcoma with the presence of

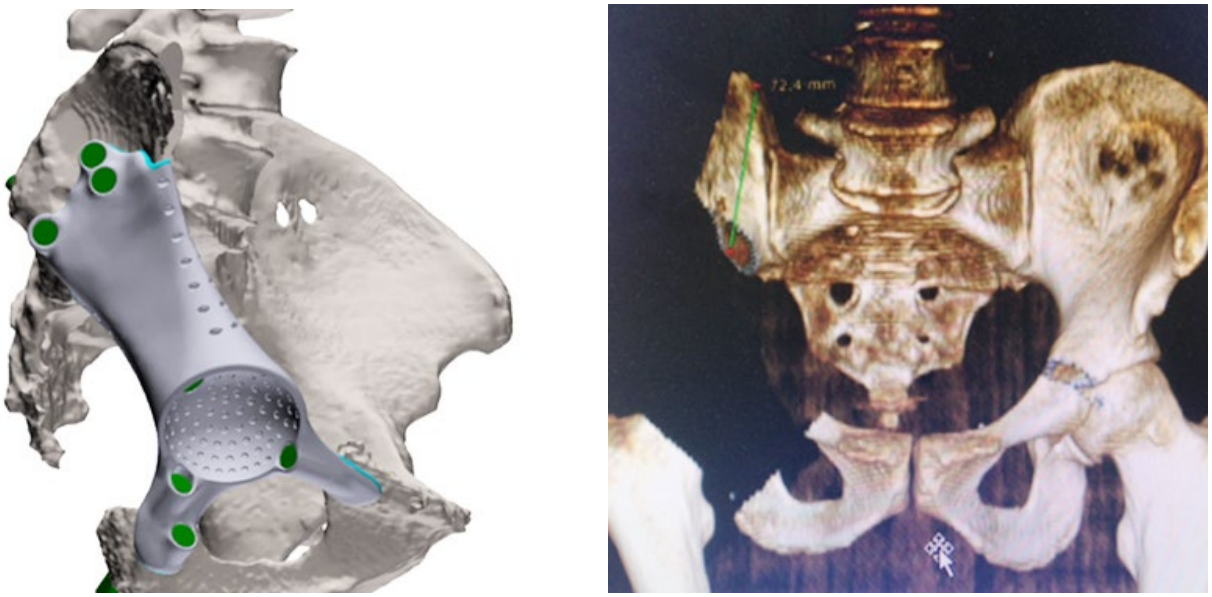

Fig. 8. 3D SCT-pelvic reconstruction, preoperative planning of an individual endoprosthesis.

multinucleated cells; resection lines without signs of tumor growth; in the lymph node – hyperplasia of lymphoid tissue, focal fibrosis.

4 courses of adjuvant polychemotherapy were performed (doxorubicin 75 mg/m^2 in the form of a 72-hour infusion, ifosfomide 2.5 g/m^2 in the form of a 3-hour infusion in 1–4 days).

During this time, an individual pelvic endoprosthesis was made (Fig. 8).

09/05/2017 by stage 2 was performed pelvic reconstruction with an individual titanium implant with hip replacement (Fig. 9).

CONCLUSION

1. The use of the described 2-stage technique allowed avoiding severe infectious complications requiring removal of implants and grafts in all patients.

2. Adequate vertebral-pelvic stabilization and (or) spacing of the defect contributes to the early functional rehabilitation of patients and the continuation of adequate adjuvant therapy in the inter-stage period, and also gives the surgeon time to prepare implants for the reconstructive stage of treatment.

3. The delayed reconstruction makes it possible to reduce the duration and traumatism of the main intervention without affecting the final result of treatment.

Fig. 9. A direct radiograph of the pelvis after stage 2 II-2019. According to the CT data, there is no data on local relapse.

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ORIGINAL ARTICLE

APPLICATION OF SILICONE COATING TO OPTIMIZE THE PROCESS OF OBTAINING CELLULAR SPHEROIDS BY THE HANGING DROP METHOD

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ABSTRACT

Purpose of the study. To study the effect of SIEL 159–330 coating on the cell clusters formation rate in a hanging drop method in combination with the use of methylcellulose (MC) and collagen as cell aggregation improving agents.
Materials and methods. BT20 breast cancer cells were cultured in drops of 20 µL (10^4 cells per drop) on the lid of a polystyrene Petri dish coated with SIEL 159–330 silicone elastomer (GNIIKHTEOS, Moscow, Russia) or without coating. The study tested three concentrations of MC (0.1 %, 0.25 % and 0.4 %) and collagen (150 µg/ml, 300 µg/ml and 600 µg/ml). The rate of formation of cell conglomerates was assessed by evaluating their area after 4, 24, 48, and 72 hours of cultivation.
Results. The use of SIEL 159–330 coating made it possible to obtain spheroids of the same size as the addition of 0.4 % MC over a time interval of 72 hours. The silicone coating additionally reduced the size of cell spheroids in the medium with 0.1 % MC at all time points; however, this effect disappeared with increasing concentration of MC. In addition, the use of SIEL 159–330 reduced the relationship between the size of cellular spheroids and the concentration of MC, which allows us to consider the use of this coating as an alternative to MC or a way to reduce its concentration. In the experiment with the addition of collagen to the culture medium, the sizes of cell conglomerates formed on the silicone coating were significantly smaller than on uncoated plastic in all variants of the experiment and time points. The effect was more pronounced for a collagen concentration of 600 µg/ml. The use of SIEL 159–330 coating, in addition, reduced the variability in the size and shape of the resulting cell conglomerates.
Conclusion. Accelerated aggregation of cells and fibers of the extracellular matrix in hanging drops, as well as a reduction in the variability in the size and shape of the resulting cell clusters on SIEL 159–330, allows us to reduce the time of experiments and material costs, as in experiments with the addition of substances that accelerate the formation of spheroids (MC and collagen), as well as in their absence.

Keywords:
3D cell culture, cell spheroid, hanging drop method, methylcellulose, collagen, silicone elastomer

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ОРИГИНАЛЬНАЯ СТАТЬЯ

ПРИМЕНЕНИЕ СИЛИКОНОВОГО ПОКРЫТИЯ ДЛЯ ОПТИМИЗАЦИИ ПРОЦЕССА ПОЛУЧЕНИЯ КЛЕТОЧНЫХ СФЕРОИДОВ МЕТОДОМ ВИСЯЧЕЙ КАПЛИ

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РЕЗЮМЕ

Цель исследования. Исследовать влияние покрытия из СИЭЛ 159–330 на скорость и характер образования клеточных скоплений в висячей капле в сочетании с применением метилцеллюлозы (МЦ) и коллагена в качестве агентов, улучшающих агрегацию клеток.

Материалы и методы. Клетки культуры рака молочной железы ВТ20 в количестве 10^4 помещали в каплях объёмом 20 мкл на крышку полистироловой чашки Петри с покрытием из силиконового эластомера СИЭЛ 159–330 (АО «ГНИИХТЭОС», г. Москва, Россия) или без покрытия. В исследовании тестировали по три концентрации МЦ (0,1 %, 0,25 % и 0,4 %) и коллагена (150 мкг/мл, 300 мкг/мл и 600 мкг/мл). Скорость формирования клеточных конгломератов оценивали через изменение их площади спустя 4, 24, 48 и 72 часа культивирования.

Результаты. Применение покрытия из СИЭЛ 159–330 позволило получить сфероиды таких же размеров, что и добавление 0,4 % МЦ на временном промежутке 72 часа. Силиконовое покрытие дополнительно уменьшило размеры клеточных сфероидов в среде с 0,1 % МЦ во всех временных точках, однако с ростом концентрации МЦ данный эффект исчезал. Кроме того, использование СИЭЛ 159–330 уменьшило связь размеров клеточных сфероидов с концентрацией МЦ, что позволяет рассматривать применение данного покрытия, как альтернативу МЦ или способ сократить её концентрацию. В опыте с добавлением в среду культивирования коллагена размеры клеточных конгломератов, образующихся на силиконовом покрытии, были достоверно меньше, чем на пластике без покрытия во всех вариантах опыта и временных точках. При этом эффект был более выраженным для концентрации коллагена 600 мкг/мл. Применение покрытия из СИЭЛ 159–330, кроме того, сократило вариативность размеров и формы образующихся клеточных конгломератов.

Заключение. Ускоренная агрегация клеток и волокон внеклеточного матрикса в висячих каплях, а также сокращение вариативности в размерах и форме образующихся клеточных скоплений на СИЭЛ 159–330 позволяет сократить время проведения экспериментов и материальные затраты, как в опытах с добавлением веществ, ускоряющих формирование сфероидов (МЦ и коллаген), так и в их отсутствие.

Ключевые слова:
трёхмерная клеточная культура, клеточный сфероид, метод висячей капли, метилцеллюлоза, коллаген, силиконовый эластомер

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RELEVANCE

High-performance methods of drug screening traditionally rely on two-dimensional cell cultures grown on plastic in the wells of multipath tablets. Recently, the trend has shifted towards three-dimensional drug screening, especially in cancer therapy, due to the unique characteristics provided by these cultivation platforms. It is known that the cultivation of cancer cells in two-dimensional cultures leads to a change in their phenotype and a loss of the properties that these cells possess in vivo in the body of patients, in particular, to a loss of expression of molecules of key signaling pathways [1]. Nevertheless, the phenotype of tumor cells is able to recover in three-dimensional cultures due to increased adhesion and signaling interactions between cells and the extracellular matrix, a decrease in the rate of proliferation to the corresponding physiological growth rate, the formation of limited nonlinear metabolic gradients corresponding to the natural growth environment of tumor cells and other factors [2].

One of the most common types of three-dimensional cell culture are cellular spheroids. At the moment, there are many methods for obtaining spheroids, of which the "hanging drop" method is considered the most affordable, which does not require special equipment or consumables [3]. The essence of this method is that the cells are placed in a suspended drop of the medium, and as a result of the action of gravity and the meniscus that occurs at the air-liquid interface, the cells are localized at the bottom of the hanging drop [4]. One of the weak points of the culture of spheroids obtained by the "hanging drop" method is the variability of the morphology and structure of the resulting cellular conglomerates, which reduces the accuracy of reproduction of natural profiles of metabolic gradients and makes it difficult to compare the effectiveness of drugs. The introduction of additives that accelerate cell aggregation contributes to improving the reproducibility of compact round spheroids. Currently, both biological and synthetic additives are used, which are based on the ability to cross-link cells with each other, stimulate cell adhesion or modify the rheological properties of the medium to accelerate the sedimentation of cells in the lower part of the drop. Extracellular matrix components such as collagen, fibronectin or a preparation of the basement membrane formed by the culture of mouse sarcoma cells – Matrigel® are usually used as crosslinking agents [5]. Various cellulose derivatives, in particular, methylcellulose (MC), are most often used as additives that change the rheological properties of the media [6].

The disadvantages of MC and collagen include the possible effect on the interaction of cells with each other and other components of the medium. Thus, collagen is an active participant in cell signaling [7], so its presence can change the behavior of cells that interact with the extracellular matrix of a different chemical composition under natural conditions, such as brain tumor cells [8]. Methylcellulose, according to the general idea, is an inert agent that does not interact with cells, however, due to the large number of coordination bonds with other molecules of the medium, MC can lead to an unpredictable change in their properties [9].

Due to the existing disadvantages of additives accelerating cell aggregation, researchers pay attention to the modification of the surface and giving it hydrophobic properties, which contributes to the creation of a higher curvature of the drop surface, leading to accelerated aggregation of cells in its lower part. For these purposes, Parafilm® laboratory film is most often used [10; 11] and a coating of polydimethylsiloxane (PDMS) [12] – one of the varieties of silicone. Earlier we showed that the coating made of biologically inert silicone elastomer SIEL 159–330, produced for medical purposes (GNIIKHTEOS, Moscow, Russia) [13] after modification of the curing mode for working with culture plastic does not have cytotoxic properties and is not inferior to Parafilm® film in its ability to accelerate the formation of cellular spheroids [14]. In this paper, we continued to study the possibilities of this silicone coating in the context of improving the protocol for obtaining spheroids by the hanging drop method.

The purpose of the study was to investigate the effect of SIEL 159–330 coating on the rate and nature of the formation of cell clusters in a hanging drop in combination with the use of methylcellulose and collagen as agents that improve cellular aggregation.

MATERIALS AND METHODS

BT20 breast cancer culture cells were grown in DMEM (Gibco, USA) with the addition of 10 % fetal cow serum (HyClone, USA) without the addition of antibiotics. Spheroids from BT20 culture cells were obtained by the hanging drop method, namely by

applying droplets of culture medium with cells to the inner surface of the Petri dish lid, followed by turning over and covering the bottom of the cup, into which a phosphate buffer was introduced to create a wet chamber and thereby prevent the droplets from drying out too quickly. The volume of applied drops was 20 µl, each drop contained 10^4 VT20 culture cells. The study tested the coating of culture plastic (polystyrene) with silicone elastomer SIEL 159–330, cured at a temperature of 60 °C for 18 hours, in combination with the addition of methylcellulose in 3 concentrations (0.1 %, 0.25 % and 0.4 %), as well as collagen in 3 concentrations (150 µg/ml, 300 µg/ml and 600 µg/ml). In total, 35 repetitions were laid for each version of the experience and controls. Petri dishes with applied droplets were kept in a CO_2 incubator at a temperature of 37 °C and a CO_2 content of 5.0 % without changing the medium for 72 hours and photofixation of the resulting cell conglomerates was performed after 4, 24, 48 and 72 hours using an inverted Axio Vert microscope. A1 (Carl Zeiss Microscopy, Germany). The resulting images were used to measure the area of the resulting conglomerates. The data is given as an average value of ± 95 % confidence interval for the average value.

RESEARCH RESULTS AND DISCUSSION

The use of a SIEL 159–330 coating by itself led to a reduction in the size of the formed cell conglomerates, which is clearly seen in the graphs in samples without MC (Fig. 1A, B) The result obtained corresponds to the data we previously published [14] and is confirmed by statistical analysis (Table 1). The addition of methylcellulose further reduced the size spheroids on both tested surfaces, which is also consistent with the literature data [6; 15]. Nevertheless, after 72 hours of cultivation on a silicone coating, the difference between the control and the medium with the addition of 0.4 % MC became statistically insignificant ($t = 0.68$, $t_{crit} = 1.995$, $df = 68$, $\alpha = 0.05$), therefore, at this time interval, SIEL 159–330 can be a replacement for MC.

The minimum size and stabilization of the size of the spheroid were not observed on uncoated plastic and without the addition of MC, up to 72 hours

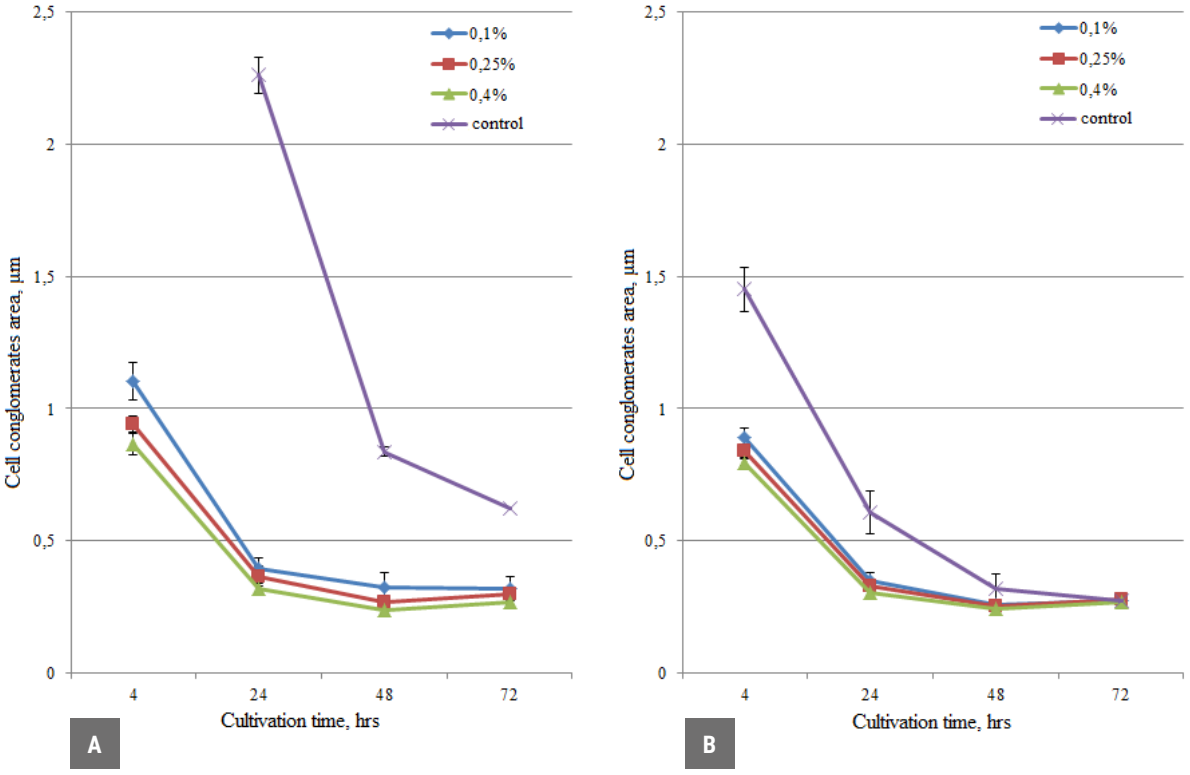

Fig. 1. Size of cell conglomerates with the addition of various concentrations of methylcellulose. A – uncoated polystyrene. B – coated with SIEL 159-330. Mean ± 95 % conf. interval.

of cultivatio (Fig. 1A, control). The use of a coating from SIEL 159–330 reduced the formation time of a compact spheroid to 48 hours (Fig. 1 B, control), and the additional introduction of MC accelerated the stabilization of the sizes of spheroids to 24 hours. At the same time, the addition of a coating from SIEL 159–330 did not affect the final size of the spheroids – after 72 hours of cultivation, there was no statistically significant difference in the values of the area of the spheroids between the two coatings (Table 1).

The data obtained by us also indicate that the silicone coating makes a significant contribution to accelerating the formation of spheroids at low concentrations of MC. This can be judged by the difference between the average values of the area of spheroids formed on uncoated plastic and silicone coating after 4 hours of cultivation (t = 6.53) and after 48 hours of cultivation (t = 2.32) at a concentration of 0.1 % MC. At other time points, the Student's criterion value, although close to the critical value (t_{crit} = 1.995, df = 68), nevertheless did not exceed it (Table 1). For concentrations of 0.25 % and 0.4 % MC, significantly significant differences in the average sizes of spheroids were observed only after 4 hours of cultivation ($t_{0.25\,\%}$ = 2.48, $t_{0.4\,\%}$ = 3.09), with a sharp decrease in the Student's criterion value at subsequent time points. Shortening the time for the formation of spheroids in the hanging drop method would make it possible to move earlier to manipulations with them – transfer, replacement of the medium, introduction of additional components, etc.

The graphs show that on uncoated plastic, the size of the spheroid clearly correlates with the concentration of MC – the higher the concentration, the smaller the size of the spheroids at each time point (Fig. 1A). At the same time, the difference was statistically significant, since the values of the Fisher criterion for all variants exceeded the critical value ($F_{crit.}$ = 3.087, k_1 = 2, k_2 = 102, α = 0.05) with a gradual decrease by 72 hours (Table 2). On the SIEL 159–330 coating, the difference between variants with different concentrations of MC is reduced, but also remains reliably significant.

The observed pattern corresponds to the data known from the literature [15]. These observations

Table 1. The Student's t test value of for pairwise comparison of the average values of the area of spheroids formed on the plastic coated with SIEL 159-330 and uncoated plastic

Cultivation media	Cultivation time, hrs.			
	4	24	48	72
Control	n/a	**71.03**	**11.4**	**31.18**
MC 0.1 %	**6.53**	1.91	**2.32**	1.98
MC 0.25 %	**2.48**	1.87	0.75	1.86
MC 0.4 %	**3.09**	1.56	0.66	0.37
Collagen 150 μg /ml	**6.46**	**10.55**	**10.41**	**9.05**
Collagen 300 μg/ml	**6.0**	**12.57**	**3.15**	**3.02**
Collagen 600 μg/m	**4.17**	**13.58**	**8.31**	**5.1**

Note: values of the t criterion exceeding the critical value for the significance level adopted in the study α = 0.05 (t_{crit} = 1.995, df = 68) are highlighted in bold.

Table 2. The Fisher F criterion values of the analysis of variance of the average values of the area of spheroids formed at three concentrations of methylcellulose for each time point

Coating type	Cultivation time, hrs.			
	4	24	48	72
Polystyrene without coating	112.76	26.09	29.8	14.29
SIEL 159-330	67.86	26.94	3.71	8.12

allow us to conclude that the use of a coating from SIEL 159–330 can contribute to a decrease in the concentration of MC for short exposures and a complete rejection of MC during cultivation for 72 or more hours in cases where MC can interfere with the experiment.

In addition to accelerating the formation of spheroids at the early stages of the experiment, the SIEL 159–330 coating had a noticeable effect on the shape of the formed cellular conglomerates in the experiment with MC. So, after 72 hours of cultivation, the cellular spheroids obtained on SIEL 159–330 had a more even contour, which is especially noticeable in the control samples (Fig. 2). Smooth contours indicate uniform formation of the spheroid and, as a consequence, its homogeneous structure. The homogeneous structure of spheroids, in turn, is the key to a smaller spread of data obtained on such cellular models when testing various physical and chemical influences.

In the experiment with the addition of collagen to the culture medium, the sizes of cellular conglomerates formed on silicone coating were significantly smaller than on uncoated plastic in all variants of the experiment and time points (Table 1). Compared with the control, the addition of collagen led to the formation of conglomerates, the size of which directly depended on the concentration of collagen (Fig. 3A, B). First of all, the observed effect is due to the fact that the resulting fibers of the extracellular matrix are themselves the material of the conglomerate, and, therefore, directly determine its volume. To an even greater extent, the concentration of collagen determines the observed area of conglomerates, since the fibers are collected on the surface of the drop and the cluster formed in this case is a disk, not a ball.

In contrast to the MC experiment, the addition of a silicone coating led to a significant reduction in the size of cell clusters at all time points and in all samples with the addition of collagen. Attention is also drawn to the change in the regularity of the formation of cell clusters when using a coating from SIEL 159–330. Thus, in uncoated plastic samples, the size and concentration are linearly related, as

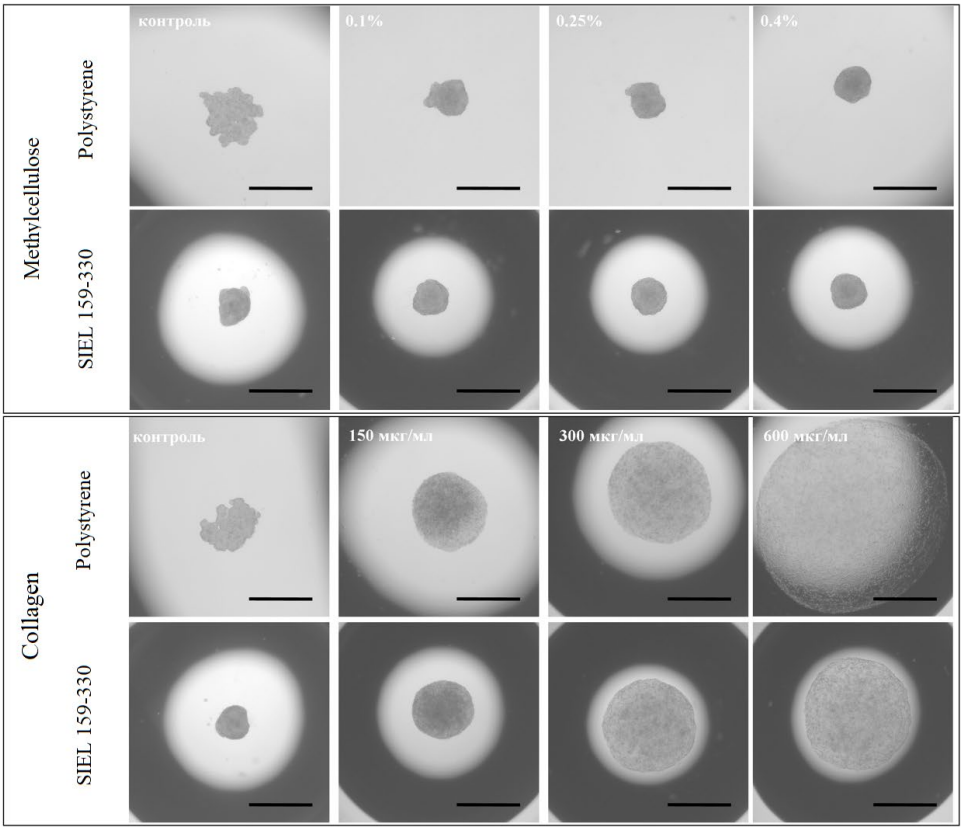

Fig. 2. View of cell spheroids obtained with the addition of methylcellulose or collagen to the medium after 72 hours of cultivation on polystyrene coated with SIEL 159-330 and uncoated polystyrene. Magnification ×20. Scale bar 1000 μm.

indicated by the increase in the size of the disc in proportion to the increase in the amount of collagen, which is most clearly seen after 72 hours of cultivation (Fig. 3A). At the same time, when cultured on a silicone coating, the gap between 300 μg/ml and 600 μg/ml was significantly reduced, with an almost unchanged difference between 150 μg/ml and 300 μg/ml (Fig. 3B). This behavior of cellular conglomerates most likely indicates the accelerated formation of conglomerates in the early hours after the experiment was started, which allowed a more compact cluster to form before the completion of collagen polymerization, all other things being equal. This is also evidenced by the high optical density of the clusters formed on the SIEL 159–330 coating compared to uncoated plastic at the same collagen concentrations (Fig. 2).

One of the disadvantages of collagen observed in our experiment is the long-term stabilization of the size of spheroids. Regardless of the concentration of collagen, the area of aggregates formed on uncoated plastic continues to decrease between 48 and 72 hours of cultivation (Fig. 3A), which increases the time for experiments with such 3-dimensional cell models. However, the use of silicone coating led to a reduction in the stabilization time of the size of aggregates from cells and fibers of the extracellular matrix up to 48 hours, regardless of the concentration of collagen (Fig. 3B).

Attention is also drawn to a sharp decrease in the variation in the size of cellular conglomerates when using a coating from SIEL 159–330 for all time points. Thus, the standard deviation on uncoated plastic averaged 0.9 compared to 0.18 on SIEL 159–330 in all variants of the experiment with the addition of collagen and time points. As in the case of a uniform surface and structure of spheroids, the reduced variability in the size of 3-dimensional cell models makes it possible to reduce the spread of experimental data in subsequent studies.

CONCLUSION

The use of a coating made of silicone elastomer

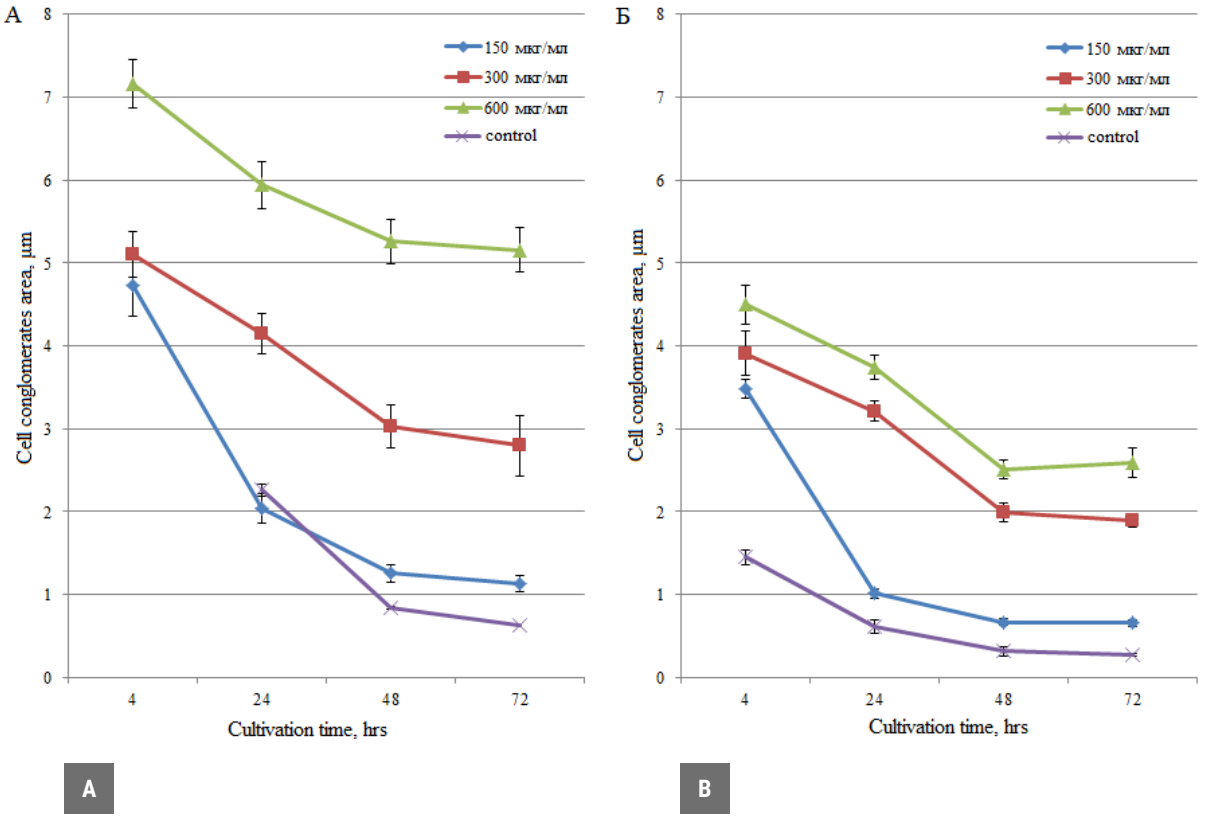

Fig. 3. The size of cell conglomerates with adding different concentrations of collagen. A – uncoated polystyrene. B – coated with SIEL 159-330. Mean ± 95 % conf. interval.

SIEL 159–330 makes it possible to improve the practice of obtaining cellular spheroids by the hanging drop method. Accelerated aggregation of cells and fibers of the extracellular matrix, which we observe in the first hours of cultivation of drops on SIEL 159–330, allows us to reduce the time of experiments, both with the addition of substances that accelerate the formation of spheroids (MC and collagen), and in their absence. In addition, the tested silicone coating also reduces the material costs of conducting studies in which collagen is used by reducing the variability in the size of the formed cellular conglomerates.

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Timofeeva S. V. – writing the text;
Shamova T. V. – revision of the text;
Mezhevova I. V. – conducting experiments;
Gnennaya N. V. – conducting experiments;
Novikova I. A. – scientific guidance.
All authors made an equivalent contribution to the preparation of the publication.

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CLINICAL CASE REPORT

STEREOTACTIC RADIOSURGERY FOR BRAIN METASTASES IN A CHILD WITH EXTRA-SKELETAL EWING'S SARCOMA

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ABSTRACT

Ewing's sarcoma is the second most common oncological disease of bones and soft tissues in children and adolescents, which is characterized by rapid growth and early metastasis. Brain metastases (BMs) occur in 10–12 % of cases and constitute a factor in the unfavorable prognosis of the disease. The possibilities of surgical treatment are often limited by the localization of a metastatic tumor, and the vast majority of chemotherapy drugs don't penetrate the blood-brain barrier, therefore radiation therapy, particularly stereotactic radiosurgery, the principle of which is a single high dose (15–24 Gy) of ionizing radiation to the pathological focus, is the most important method of treatment. High accuracy of tumor irradiation is ensured by rigid immobilization of the patient (using stereotactic frames or individual three-layer thermoplastic masks) in combination with positioning of the patient and control of his position by orthogonal X-rays. According to various authors, the use of stereotactic radiosurgery provides local control over BMs in 90 % of patients, regardless of the histological type of the primary focus, age and gender of the patient. The article describes a clinical case of successful radiosurgical treatment of a child suffering from extra-skeletal Ewing's sarcoma, in which following the complex treatment, progression of the disease, represented by multiple metastatic brain damage was revealed; the cumulative volume of metastatic foci was 2.3 cm^3 and due to the proximity of the brain stem, as well as in order to avoid exceeding the tolerant load on healthy brain tissues, the total focal dose did not exceed 16 Gy. A complete response to therapy in the form of regression of all foci was noted six months after the treatment. To date, insufficient data has been published on the use of stereotactic radiosurgery in pediatric oncology, but in the available literature, the authors demonstrate the effectiveness and safety of treatment. Further research is needed to study the effect of the radiosurgical method on the children.

Keywords:
stereotactic radiosurgery, Ewing's sarcoma, brain metastases

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КЛИНИЧЕСКОЕ НАБЛЮДЕНИЕ

ОПЫТ ПРИМЕНЕНИЯ МЕТОДИКИ СТЕРЕОТАКСИЧЕСКОЙ РАДИОХИРУРГИИ ПРИ ЛЕЧЕНИИ МЕТАСТАТИЧЕСКОГО ПОРАЖЕНИЯ ГОЛОВНОГО МОЗГА У РЕБЕНКА С ВНЕСКЕЛЕТНОЙ САРКОМОЙ ЮИНГА

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РЕЗЮМЕ

Саркома Юинга – второе по распространенности онкологическое заболевание костей и мягких тканей у детей и подростков, которое характеризуется быстрым ростом и ранним метастазированием. Метастатическое поражение головного мозга (МПГМ) встречается в 10–12 % случаев и является фактором неблагоприятного прогноза заболевания. Возможности хирургического лечения зачастую ограничены локализацией метастатической опухоли, а подавляющее большинство химиопрепаратов не проникает через гематоэнцефалический барьер, поэтому важнейшим методом лечения является лучевая терапия, в частности, стереотаксическая радиохирургия, принципом которой является однократное подведение высокой дозы (15–24 Гр) ионизирующего излучения к патологическому очагу. Высокая точность облучения опухоли обеспечивается жесткой иммобилизацией пациента (использование стереотаксических рам или индивидуальных трехслойных термопластических масок) в сочетании с позиционированием пациента и контролем его положения по ортогональным рентгеновским снимкам. По данным различных авторов применение методики стереотаксической радиохирургии обеспечивает локальный контроль над МПГМ у 90 % пациентов вне зависимости от гистологического типа первичного очага, возраста и пола пациента. В статье описан клинический случай успешного радиохирургического лечения ребенка, страдающего внескелетной саркомой Юинга, у которого после комплексного лечения было выявлено прогрессирование заболевания – множественное метастатическое поражение головного мозга; кумулятивный объем метастатических очагов составил 2,3 см^3, а ввиду близкого расположения ствола мозга, а также во избежании превышения толерантной нагрузки на здоровые ткани головного мозга суммарная очаговая доза не превысила 16 Гр. Полный ответ на терапию в виде регресса всех очагов отмечен через полгода после проведенного лечения. К моменту исследования период безрецидивного наблюдения составил 9 мес. На сегодняшний день опубликовано крайне мало данных о применении методики стереотаксической радиохирургии в детской онкологии, но в имеющейся литературе авторы демонстрируют эффективность и безопасность лечения. Необходимы дальнейшие исследования по изучению влияния радиохирургического метода на организм ребенка.

Ключевые слова:
стереотаксическая радиохирургия, саркома Юинга, метастатическое поражение головного мозга

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RELEVANCE

Ewing's sarcoma ranks second among bone and soft tissue tumors of childhood and adolescence. The disease is characterized by an extremely rapid and aggressive course; distant metastases are detected in a third of patients by the time of diagnosis [1; 2]. The lungs (40–85 %), bones (30–70 %), lymph nodes (10–35 %), as well as the brain are most often affected, where metastases develop in 10–12 % of cases. The localization and size of the metastatic focus are of paramount importance for the prognosis of the disease [3]. Metastatic brain injury (MPGM) is an unfavorable prognosis factor with a median survival of 51 days [4]. The possibilities of surgical treatment are often limited by the localization of a metastatic tumor, and the vast majority of chemotherapy drugs do not penetrate the blood-brain barrier, therefore radiation therapy is the most important method of treatment.

Stereotactic radiosurgery (SRS) is a modern method of radiation therapy based on a single application of a high dose (15–24 Gy) of ionizing radiation to a pathological focus, while radiation therapy in the mode of classical fractionation provides a single focal dose of 1.8–2.2 Gy for several fractions, depending on the prescribed total focal dose. High accuracy of tumor irradiation is ensured by rigid immobilization of the patient (using stereotactic frames or individual three-layer thermoplastic masks) in combination with positioning of the patient and control of his position by orthogonal X-rays. According to various authors [4–6] local control over intracranial neoplasms of secondary genesis can be achieved in 90 % of patients, regardless of the histological type of the primary focus, age and gender of the patient.

To date, very little data has been published on the use of the SRS technique in pediatric oncology, but in the available literature, the authors demonstrate the effectiveness and safety of treatment. In the study carried out by Napieralska et al. [7] radiosurgical treatment at a dose of 6 to 15 Gy for relapse of medulloblastoma or ependymoma was received by 14 patients, including four children aged 3 to 10 years. The authors state the development of local post-radiation cerebral edema in all patients, which was not clinically manifested and was determined only on MRI images. With further dispensary observation for two years, no other early or late post-radiation injuries were detected, and the edema was eliminated by the administration of systemic glucocorticosteroids within a week. Nicolato et al. [8] studied the irradiation of arteriovenous malformations with high doses (from 9 to 26.4 Gy) in 100 children aged 3 to 18 years. Bleeding was observed in 9 % of patients and was due to the peculiarity of the pathology, no other complications were reported.

The purpose of the study: to demonstrate the clinical effectiveness of the stereotactic radiosurgery technique in children with metastatic brain damage.

Clinical case description

Patient B., 3 months old, was directed by a pediatrician to the National Medical Research Center for Oncology of the Russian Ministry of Health (Rostov-on-Don) for examination and determination of treatment tactics for education in the field of soft tissues of the back, which, according to parents, is marked from birth. Ultrasound of the soft tissues of the back from 09/27/2017 – in the soft tissues of the back on the left, a hypoechoic formation is located with smooth, clear contours, heterogeneous structure, with dimensions of 1.4 × 2.7 cm. CT of the thoracic, abdominal cavity, pelvic organs from 10/13/2017. – the lung tissue is without foci, there is no fluid in the pleural cavities, the pleura is not changed, the liver is diffusely heterogeneous, the spleen, the pancreas is not changed, there is no ascites. Adrenal glands, kidneys without pathology, retroperitoneal l / n are not enlarged, intra-thoracic l /n are not enlarged. In the ribs, sternum, pelvic bones without destruction. On the left, a paravertebral multi-nodular soft tissue formation of 4.5 × 3.7 × 3.0 cm with an inhomogeneous structure, calcinates, spreads through 6.7 intercostals into the soft tissues of the back. 16.10.2017 in the conditions of drug-induced sleep, in order to verify the tumor process, the patient underwent a core biopsy of the tumor. Histological analysis No. 70830/17 dated 16.10.2017 – the morphological picture is most characteristic of Ewing's sarcoma, it is necessary to differentiate with neuroblastoma, rhabdomyosarcoma, lymphoma. IHC from paraffin block No. 70830/17 dated 10/23/2017 – morphological picture and immunophenotype of tumor cells are most characteristic of extra-skeletal Ewing's sarcoma. Based on the above data, a clinical diagnosis was made: Ewing's sarcoma with paravertebral growth at the level of 6, 7, 8 intercostal space on the left, with growth in the soft tissues of the back, T3N0M0, stage III. Clinical group 2.

In the period from November 2017 to November 2018, the patient underwent 6 courses of neoadjuvant chemotherapy according to the EURO Ewing 2008 protocol. On 04/02/2018, surgical intervention was performed in the following volume: removal of a residual soft tissue tumor of the middle third of the back on the left, histological conclusion No. 30576–81/18 of 04/06/2018 – Ewing's sarcoma with pronounced dystrophic changes in tumor cells, extensive foci of fibrosis, with areas of angiomatosis. The formation is removed within healthy tissues. Remote radiation therapy was performed on a Novalis Tx, Varian linear accelerator, according to the planned plan: from 2 arches using the technology of volume-modulated arc therapy (VMAT) to the area of primary tumor spread against the background of drug-induced sleep, from 1.8 G to

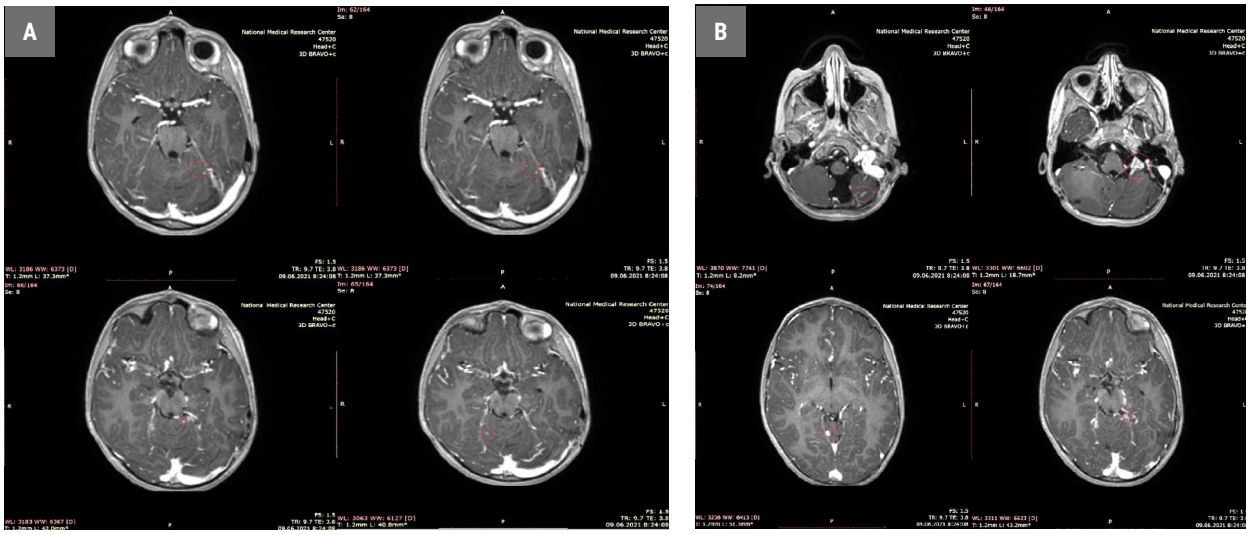

Fig. 1. Revealed metastatic foci on MRI of the brain from 06/09/2022.

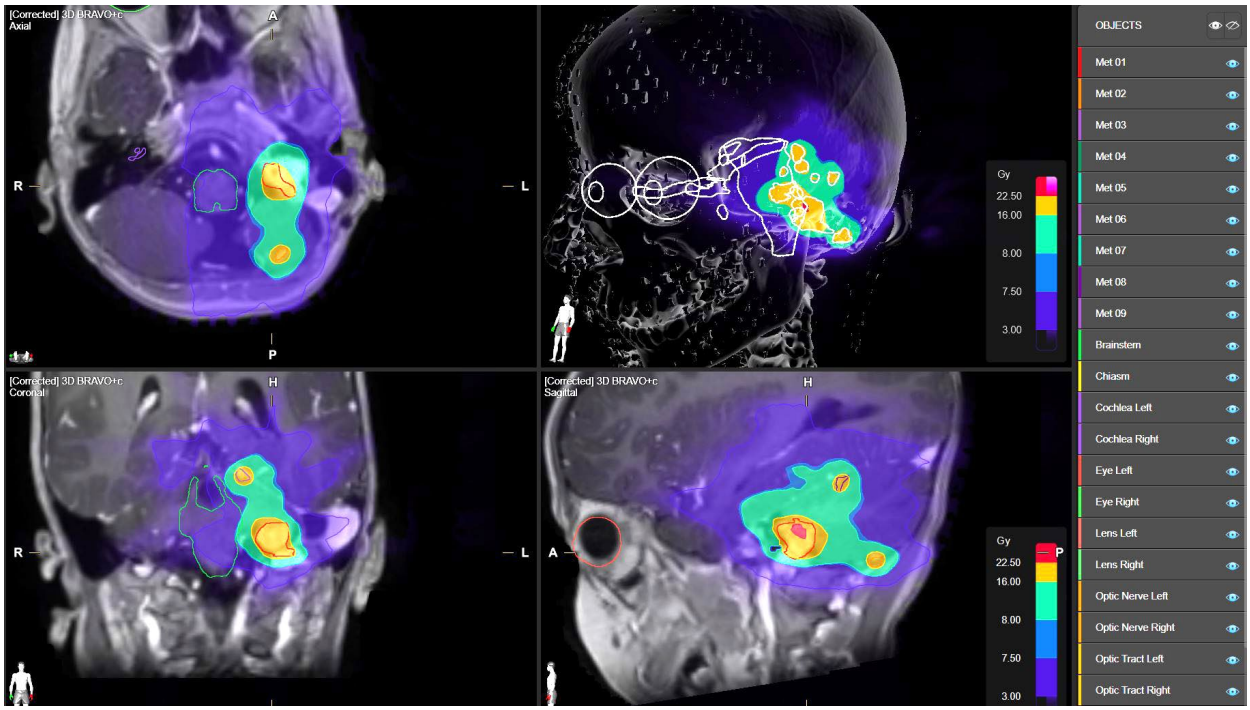

Fig 3. Stereotactic radiosurgery plan.

SOD 40 G. The treatment was carried out against the background of drug-induced sleep. Subsequently, the patient received 8 courses of adjuvant chemotherapy according to the above protocol.

03/21/2020, according to his parents, he fell off the sofa in a dream, screamed a lot. On 03/22/2020, the parents noted the unsteadiness of the gait, the tilt of the head to the left. A CT scan of the brain was

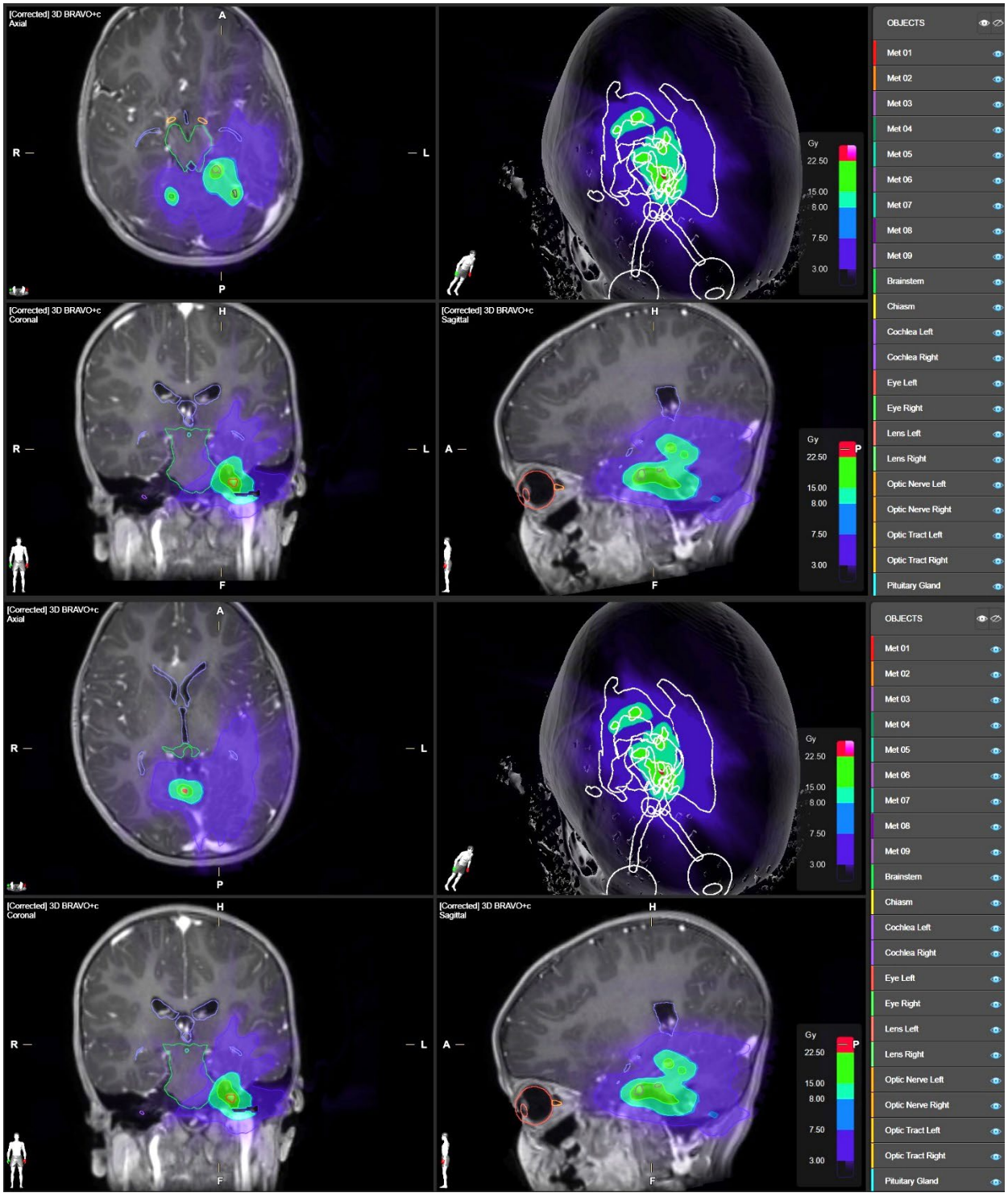

Fig. 4. Stereotactic radiosurgery plan.

performed, conclusion: the formation of up to 16 mm is determined on the right in the hemisphere of the cerebellum. In the left hemisphere of the cerebellum, a 33 × 38 × 30 mm volumetric formation with a cystic component of 32 × 22 × 30 mm with calcinates is parasaggitally determined, squeezing and deforming the 4th ventricle, the median structures are not displaced. On 03/27/2020, an MRI of the brain was performed: the median structures were shifted to the right at the level of the PH up to 8.3 mm. In the right hemisphere of the cerebellum, there is a cystic-solid formation with moderate vasogenic edema along the periphery, with a total size of up to 28 × 42 mm. The formation of a cystic solid structure of the worm, the left hemisphere, the middle leg of the cerebellum, the left parts of the Varolian bridge with a slight edema on the periphery, dimensions 55 × 51 mm. Compression of the IV ventricle, the plumbing of the brain, the trunk, the legs of the cerebellum, the left leg of the brain. Displacement of the left amygdala of the cerebellum below the BZO level by 6.4 mm. After intravenous amplification, an active heterogeneous accumulation of contrast is determined by a solid component of tumors: on the right, the size is 18 × 17 × 13 mm, on the left, 29 × 26 × 33 mm. The presence of tumor contact with the walls of the left sigmoid venous sinus, without intraluminal invasion. The lateral ventricles of the brain are asymmetric D < S, moderately dilated, without periventricular edema. III ventricle up to 5.5 mm. Conclusion: MR is a picture of the same type of cystic solid formations in the right hemisphere of the cerebellum, in the left parts of the cerebellum, brain stem. Differentiate metastasis and hemangiblastoma. Compression of the plumbing of the brain and the IV ventricle with compensated internal hydrocephalus. Lateral dislocation of median structures at the RF level, initial manifestations of descending axial wedging. On 03/27/2020, an operation was performed – removal of metastatic tumors of the cerebellum. GA, IHC No. 32105/20, 800/20: Morphological picture and immunophenotype of tumor cells, taking into account clinical data, are characteristic of Ewing sarcoma metastasis, Ki 67–60 %.

From 04/15/2020 to 09/21/2020, 6 anti-relapse courses of polychemotherapy were conducted (irinotecan, temozolomide.) In the future, he was under dynamic observation, MRI of the brain 1 time in 3 months, CT scan of the chest, abdominal cavity, pelvis 1 time in 3 months.

On the next control MRI of the brain with contrast enhancement from 06/09/2021, multiple metastatic foci of the following sizes and localizations were found: 7 × 4 mm, 2 × 2 mm and 2 × 1 mm in the left hemisphere of the cerebellum, 13 × 11 mm in the left bridge-cerebellar corner, 5 × 6 mm and 2 × 2 in the cranial part of the cerebellar worm, 5 × 4 mm and 3 × 3 mm in the left hemisphere of the cerebellum at

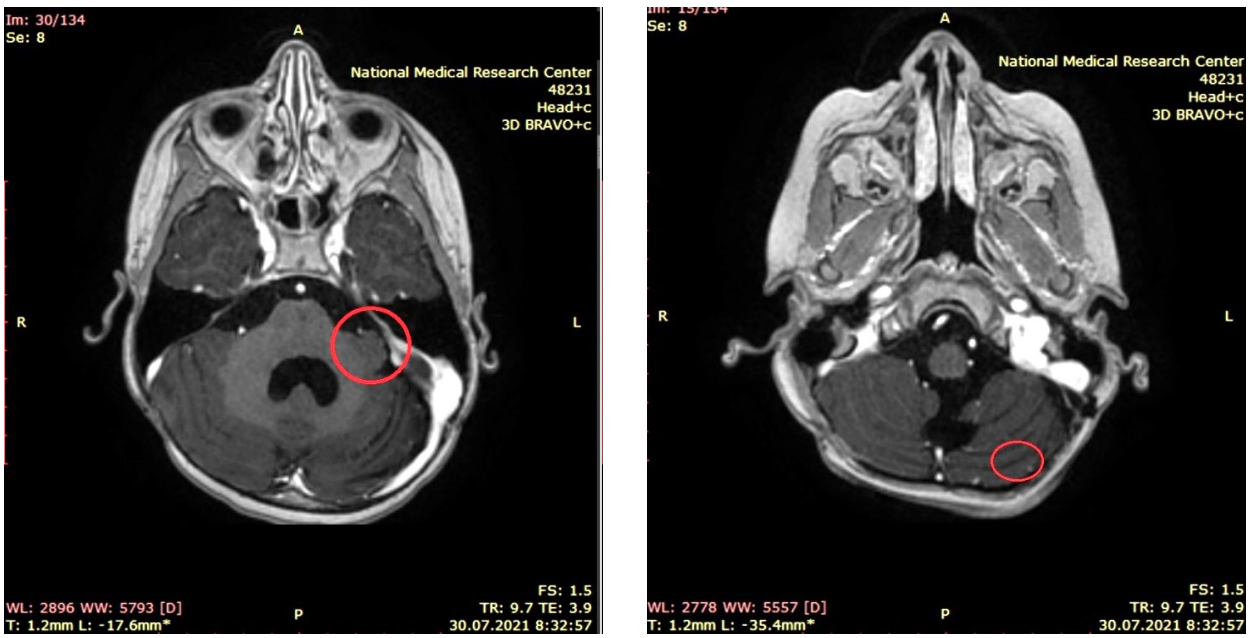

Fig. 5. Regression of metastatic foci a month after treatment, control MRI of the brain from 02/28/2022.

the level of the quadrilaterals, 3 × 2 mm in the right hemisphere of the cerebellum (Fig. 1). A consultation of doctors of the National Medical Research Centre for Oncology recommended a session of stereotactic radiosurgery. The patient was hospitalized in the radiotherapy department of the National Medical Research Centre for Oncology, Rostov-on-Don.

On 06/15/2021, preliminary topometric preparation was carried out: in the conditions of drug–induced sleep, an individual fixing device was made – a three-layer thermoplastic mask for stereotactic radiation therapy, X-ray contrast tags were installed, the isocenter was determined using the LAP Laser laser navigation system, topometric tomography was performed on a Siemens Somatom computer tomograph, the effective dose for the study was 3.7 m^3v. Preliminary topometry data was processed at the Singo Via virtual simulation station. Segmentation was performed using the Elements (BrainLab) software. The calculation and formation of a treatment plan (3D planning) (Fig. 3, 4) for the Novalis Tx linear accelerator (Varian, USA) were carried out.

On 06/17/2021, in the conditions of drug-induced sleep, a SRS session was conducted on a Novalis Tx linear accelerator, Varian on the identified metastatic foci, the total volume of which was 2.3 cm^3, using the SRS technique with an irradiation energy of 6 MeV and the following target coating parameters: V_{100} % ≥ 95 %, D_{max} ≤ 150 % (Fig. 3). Due to the close location of the brain stem, as well as to avoid exceeding the tolerant load on healthy brain tissues (V_{12Gy} ≤ 10 cm_3,) the prescribed focal dose was 16 Gy. Positioning was performed using the Exactrac system (BrainLab). The period after irradiation took place against the background of standard decongestant therapy. No radiation reactions were observed, the treatment was carried out satisfactorily.

A month after the SRS session with a control MRI of the brain from 07/30/2021, according to the criteria of RANO-BM, stabilization of the process in the brain is noted: regression of the metastatic focus in the left area of the cerebellum and the focus in the left bridge-cerebellar corner (Fig. 5). The total volume of foci decreased by 23 % and amounted to 1.77 cm^3 (previously 2.3 cm^3). A complete response to therapy in the form of regression of all previously determined metastatic foci was noted six months later with a control MRI of the brain from 12/01/2021.

Currently, supportive chemotherapy is being performed. Dynamic examination is carried out. There is no data for progression.

DISCUSSION

Modern methods of treatment can prolong the life of patients in difficult clinical situations. Despite multiple metastatic brain damage, with the help of radiosurgical treatment, it was possible to achieve complete regression of foci without reducing the quality of life of the child. By the time of the study, no early symptoms (nausea, vomiting, headache, fever, post-radiation dermatitis) were detected or late (delayed growth of the skull bones) post-radiation injuries. The period of relapse-free follow-up is 9 months.

CONCLUSION

The technique of stereotactic radiosurgery in pediatric oncology can prove itself as a safe and effective non-invasive method of treatment. Further research is needed to study the effect of the radiosurgical method on the child's body.

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CLINICAL CASE REPORT

EFFECTIVE USE OF EXTRACORPOREAL MEMBRANE OXYGENATION IN SURGICAL TREATMENT OF KIDNEY CANCER PATIENT WITH TUMOR THROMBOSIS

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ABSTRACT

The presented clinical case demonstrates the potential of medical technologies with organ replacement in the treatment for kidney cancer (KC) with a tumor thrombus located in the retrohepatic inferior vena cava (IVC) complicated by thromboembolism of the medium and small branches of the pulmonary artery (PATE). The treatment outcomes in such patients are usually poor due to a great number of complications and high mortality. The literature data estimates operative mortality rate of 13 %, and the frequency of early postoperative complications reaches 60 %. Standard approaches to anesthesia in case of thromboembolism and the threat of massive PATE are ineffective. In this clinical case, we chose the therapeutic tactics with extracorporeal membrane oxygenation (ECMO) in the intra- and early postoperative period in order to avoid or minimize life-threatening complications in the KC patient with a tumor thrombus in IVC and PATE. The effective use of ECMO minimized the risk of fatal complications during nephrectomy with thrombectomy for a malignant kidney tumor in the patient with pulmonary embolism and subcompensated disorders of the oxygen transport function of the lungs. The main objective of medical care for this patient involved both the radical treatment of kidney cancer and the elimination of a potential cause of thromboembolism of the pulmonary artery branches, fragments of hematogenous and tumor microthrombi. The total duration of ECMO was 30 hours. No significant complications in organs and systems were recorded during the surgery and in the early postoperative period. The development and implementation of new technologies, including devices for oxygen blood saturation and carbon dioxide elimination, undoubtedly gives a chance for a cure for cancer patients with decompensated organ and functional capabilities.

Keywords:
kidney cancer, tumor thrombus, extracorporeal membrane oxygenation, pulmonary embolism, nephrectomy with thrombectomy, blood gas transport function

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КЛИНИЧЕСКОЕ НАБЛЮДЕНИЕ

ЭФФЕКТИВНОЕ ПРИМЕНЕНИЕ ЭКСТРАКОРПОРАЛЬНОЙ МЕМБРАННОЙ ОКСИГЕНАЦИИ В ХИРУРГИЧЕСКОМ ЛЕЧЕНИИ БОЛЬНОЙ РАКОМ ПОЧКИ С ОПУХОЛЕВЫМ ТРОМБОЗОМ

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РЕЗЮМЕ

Представленный клинический случай демонстрирует возможности медицинских технологий с органозамещением в лечении рака почки (РП) с опухолевым тромбом расположенным в ретропеченочном отделе нижней полой вены (НПВ), осложненного тромбоэмболией средних и мелких ветвей легочной артерии. Опыт лечения данной группы пациентов является крайне неудовлетворительным из-за большого количества осложнений и высокой летальности. По имеющимся данным литературы, операционная летальность составляет около 13 %, а частота ранних послеоперационных осложнений достигает 60 %. Стандартные подходы анестезии в случае состоявшейся тромбоэмболии и угрозы развития массивной тромбоэмболии легочной артерии (ТЭЛА) являются малоэффективными. Для возможности избежать или минимизировать жизнеугрожающие осложнения, в данном клиническом случае больной РП с опухолевым тромбом в НПВ и проявлением ТЭЛА нами была выбрана тактика терапии с применением экстракорпоральной мембранной оксигенации (ЭКМО) в интра- и раннем послеоперационном периоде. Эффективное применение ЭКМО позволило минимизировать риск развития фатальных осложнений при выполнении нефрэктомии с тромбэктомией по поводу злокачественного новообразования почки у пациентки с ТЭЛА и субкомпенсированными нарушениями кислородно-транспортной функции легких. Основной задачей медицинской помощи данной пациентке являлось не только радикальное лечение рака почки, но и ликвидация потенциального источника тромбоэмболии ветвей легочной артерии в виде фрагментов гематогенных и опухолевых микротромбов. Общая продолжительность ЭКМО составила 30 часов. За время проведения хирургического вмешательства и в раннем послеоперационном периоде значимых осложнений со стороны органов и систем зафиксировано не было. Разработка и внедрение новых технологий, в том числе устройств для насыщения крови кислородом и элиминации углекислого газа, несомненно дает шанс на излечение онкологическим пациентам с декомпенсированными органными и функциональными возможностями.

Ключевые слова:
рак почки, опухолевый тромб, экстракорпоральная мембранная оксигенация, тромбоэмболия легочной артерии, нефрэктомия с тромбэктомией, газотранспортная функция крови

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RELEVANCE

In the structure of oncological morbidity, kidney cancer (RP) is one of the leading localizations and occupies the 10th place [1]. According to the literature, a feature of the clinical course of renal cell carcinoma is venous invasion with the penetration of the tumor into the lumen of the renal vein, which is fixed in 25–30 % of cases. Invasion of the inferior vena cava (IVC), up to the right atrium, is registered in 4–10 % of patients, the spread of a tumor thrombus into the right chambers of the heart is diagnosed in 1 % of patients [2].

The interaction of various branches of science and medicine contributes to the introduction of new technologies in modern oncology. It is important to solve the problems of early diagnosis and pathogenesis of tumors, including the study of the mechanisms of normal functioning of the kidneys and their pathological changes in tumor processes [3]. Of course, the application of multimodal principles of antitumor therapy is of priority importance, but the only effective method in the treatment of patients with KCcomplicated venous thrombosis is surgical. At the same time, until recently, patients with diagnosed tumor invasion of IVC were considered incurable. The treatment experience of this group of patients was extremely unsatisfactory due to the large number of complications and high mortality [2; 4]. At the same time, back in 1972, Skinner D. G. et al. It was determined that KC with widespread thrombosis in conditions of total tumor removal is potentially curable. Moreover, the survival rate of patients after surgery – nephrectomy with thrombectomy depends on the prevalence of the malignant process [5]. There is an opinion that the degree of venous invasion and localization of a tumor thrombus also affect the survival of patients who underwent combined surgery. Thus, according to Shiff B. (2021), the 5-year survival rate of patients with non-metastatic KC and retrohepatic thrombi reaches 70 %, and in patients with suprahepatic tumor thrombosis – 55 % [6].

It should be noted that today the choice of treatment tactics for this category of patients remains a very difficult problem. According to various data, the operational mortality is about 13 %, and the frequency of early postoperative complications reaches 60 %. Venous thromboembolic complications (3.8 %), pneumonia (3.6 %) and acute renal failure (3.1 %) predominate in patients after nephrectomy with removal of a tumor thrombus [2]. Research by Borza T. it was shown that the number of early postoperative complications depends on the extent of the thrombus, so at the level of thrombus 0 (renal vein), complications develop in 8.7 % of cases; at level I (mouth of the renal vein) – in 15.3 % of cases; at level II (subhepatic department of IVC) – in 14.2 %, level III (retrohepatic department IVC) – in 17.8 %, IV (intra-atrial) – in 30.0 % of cases ($p < 0.001$) [7]. To minimize complications and hospital mortality, Marshall V. et al. in 1970, cardiopulmonary bypass was used for the first time for surgical removal of a tumor thrombus of III and IV grade of spread, which remains relevant today [2]. The main advantage of this medical technology is the prevention of the development of thromboembolic complications. At the same time, it should be borne in mind that cardiopulmonary bypass requires additional traumatic and complex manipulations, which can lead to an increase in the risk of perioperative complications, including fatal ones [4].

The improvement of medical technologies makes it possible to expand the criteria of surgical and functional operability of oncological patients with decompensated concomitant pathology, as well as with a widespread tumor process. Nevertheless, the complicated course of cancer causes additional difficulties in the treatment of patients with KC. Despite the possibilities of modern diagnostic technologies, more than 40 % of patients with KC are detected in common stages, or there is a metastatic lesion. The absence of obvious clinical signs contributes to the latent course of the process, and the onset of the disease, in such cases, may be bleeding, pulmonary embolism (PE), acute cardiovascular insufficiency due to a decrease in the ejection fraction of the right ventricle and /or blocking of the heart valves [2].

The development of surgical technologies and the possibilities of anesthesiological support, including substitution therapy, cardiopulmonary bypass, hypothermia and other techniques allow optimizing the treatment of patients with KC with vascular invasion [4]. An important point is the fact that thromboembolism has taken place at the diagnostic stage, which undoubtedly limits the possibility of radical treatment. Moreover, the choice of tactics of specialized care in this case is extremely difficult. Often, patients with KC with signs of PE are doomed to a fatal outcome. Standard anesthesia approaches in the case of thromboembolism and the threat of massive PE are ineffective. In order to avoid or min-

imize life-threatening complications, in this clinical case of a KC patient with a tumor thrombus in the IVC and a manifestation of PE, we chose a therapy tactic using extracorporeal membrane oxygenation (ECMO).

Extracorporeal membrane oxygenation is a method of temporarily maintaining the function of the heart and/or lungs when they are damaged using hardware extracorporeal circulation combined with an oxygenator to saturate the blood with oxygen and eliminate carbon dioxide. The basis of the ECMO method is the use of a special polymer membrane that allows oxygenating venous blood circulating through the extracorporeal circuit. At the present stage of development of medical technologies, ECMO is effectively used in the practice of treating primary pulmonary etiology, as well as extrapulmonary pathology complicated by decompensated respiratory insufficiency [8; 9]. At the same time, there is a danger of patients developing pathological conditions caused by the ECMO procedure itself. In addition to potential complications associated with invasive technical features, it is possible to develop unpredictable and uncontrollable life–threatening conditions that are associated with forced heparinesis during the procedure – these are massive bleeding, intracranial hemorrhages, as well as limb ischemia, set thrombosis, neurological complications (convulsive syndrome, ischemic stroke) [10]. Due to these circumstances, before performing ECMO, it is necessary to evaluate the criteria for the reversibility of the functional state and the prognosis of the disease as a whole [11].

It should be noted that the choice of tactics for the treatment of a patient with KCwith clinical manifestations of PE and subcompensated lung function disorders was based on the prevention of the following complications: cardiorespiratory, hemorrhagic, water-electrolyte, neurological, etc. We took into account the initial violations of the oxygen transport function of the lungs, as well as the high risk of intraoperative thromboembolic complications with fatal outcome. When detailing the upcoming therapy of a patient with KC with PE, we comprehensively reviewed the principles, technologies and possible complications of ECMO. They chose to use the veno-venous ECMO (VV/ECMO) technique. It is known that VV/ECMO provides balanced oxygen enrichment of the blood of patients with hypoxic respiratory insufficiency, and is also a method of reserving a situation with a high risk of death, with the underlying disease that caused respiratory failure. During the procedure, oxygenated and decarboxylated blood, passing through the membranes, is injected into the right atrium, and then released into the pulmonary circulation by the preserved cardiac function of the patient [8].

In this clinical case, the main task of highly qualified medical care was not only to rid the patient of a malignant disease, but also the simultaneous elimination of a potentially dangerous source of PE, represented by fragments of hematogenous and tumor microthrombs.

The purpose of the study was to demonstrate a clinical case of the use of extracorporeal membrane oxygenation during nephrectomy with thrombectomy for malignant neoplasm of the kidney in a patient with PE and initial disorders of oxygen transport function of the lungs.

Clinical case

Patient D., 63 years old, applied at her place of residence in September 2019, complaining of a persistent cough, weakness, shortness of breath with little physical exertion, an increase in body temperature to 38 °C. For five days, she was treated at the Central District Hospital for lower-lobe pneumonia on the left, where a further examination revealed the formation of the right kidney. She independently applied to the National Medical Research Center for Oncology ("NMRC for Oncology"), where the diagnosis – cancer of the right kidney was confirmed by magnetic resonance imaging, and signs of thromboembolism of small and medium branches of the pulmonary artery, significant pulmonary hypertension were revealed. The patient was hospitalized in the oncourology department for surgical treatment of KC.

Initial data of computed tomography of the chest, abdominal cavity and pelvis with intravenous contrast:

– there are areas of pneumosclerosis in the lungs on both sides, in the lumen of the inferior lobar artery on the left and its segmental branches, filling defects in the arteries of the lingual segments, narrowing their lumen to 0.3 cm; intra–thoracic lymph nodes – aortic windows and preaortic up to 1.4 cm;

– tumor of the upper and middle segments of the right kidney 12.6 × 7.6 × 10.8 cm with germination of the pelvis, kidney collar, right adrenal gland, tumor thrombus in the right renal vein up to 7.2 cm with spread in the IVC for 5.9 cm; massive extrarenal

component, inseparable from the right lobe of the liver (germination of the S1 segment of the liver for up to 2.5 see).

Laboratory parameters at the time of hospitalization: 1. General blood test: hemoglobin 100 g/l, erythrocytes 3.1 × 10^{12}/l, color index 0.89, hematocrit 31 %; 2. General urine analysis: specific gravity 1016, protein, sugar, acetone-not detected, leukocytes – 3–4 in the field of vision, erythrocytes – 3–4 in the field of vision; 3. Biochemical blood analysis: glucose 7.86 mmol, amylase 48.6 units/L, ASTL 28.6 units / L, ALTL 27.6 units /L, creatinine 95.3 mmol/L, urea 8.59 mmol/L, total protein 59.6 g/l, bilirubin 16.5 mmol/l; 4. Acid-base state: PCO_2 – 47 mm Hg, PO_2 – 79 mm Hg. pH – 7.32, deficiency or excess of bases (BE) – 5 mmol/L, bicarbonate (HCO_3) – 32.1 mmol/L, SO_2 – 90 %, Na^+ – 145.0 mmol/L, K^+ – 3.8 mmol/L, Cl^- – 104.0 mmol/L, Ca^{2+} – 1.08 mmol/L (laboratory signs subcompensated respiratory acidosis).

Electrocardiogram: heart rate (HR) – 112 per minute, sinus tachysystole, decreased recovery processes in the myocardium of the posterior parts of the left ventricle.

Consultation of a pulmonologist: community-acquired left-sided polysegmental pneumonia; respiratory insufficiency of the 2nd degree, there is no data for infectious pathology of the lower respiratory tract. Therapist consultation: myocardiodystrophy, arterial hypertension stage 3, risk 2, chronic heart failure stage II, functional class 2, risk IV.

Comparison of ultrasound and computed tomography data made it possible to accurately determine the topography of tumor formation and thrombus. Thus, the cranial border of the thrombus was located in the retrohepatic section of the IVC (gradeIII thrombosis), and the extrarenal component of the tumor had signs of germination into the right adrenal gland and partially the liver capsule.

Based on the above, a clinical diagnosis was established: cancer of the right kidney $T_4N_xM_0$ (tumor thrombus IVC, grade III) clinical group 2. Complication: thromboembolism of the left pulmonary artery (tumor genesis), anemia of mixed genesis. Concomitant diagnosis: myocardiodystrophy; stage II chronic heart failure, functional class 2, risk IV.

Taking into account the ineffectiveness of conservative methods of treatment of the complicated course of KC, as well as the initial subcompensated violations of the oxygen transport function of the lungs and the risk of fatal complications, a consultation of specialists of anesthesiologists, resuscitators and oncologists determined a plan for surgical treatment of the patient using the BB/ECMO technique in the intra- and early postoperative period. In addition, we have not ruled out the possibility of emergency connection of veno-arterio-venous ECMO (VAV/ECMO) in case of massive PE and total heart failure.

Intraoperative monitoring included: continuous monitoring of the electrocardiogram and hemodynamic parameters, monitoring of neuromuscular conduction and pulse oximetry (SpO_2). The depth of anesthesia was monitored using a bispectral index of brain activity. Data of functional indicators before surgery: blood pressure (BP) – 168/109 mm Hg, heart rate – 110 per minute, respiratory rate (BH) – 22 per minute. Initial indicators of blood gas composition: partial oxygen tension of arterial blood (pO_2) – 89 mm Hg; partial voltage of arterial carbon dioxide (pCO_2) – 46 mm Hg; pH – 7.32; BE – 3.4; HCO_3 – 24.0 mmol/l, $SatO_2$ – 89 %. These indicators demonstrate a subcompensated state of the patient's blood gas transport function. Anesthetic provision corresponded to the generally accepted principles of multicomponent anesthesia: introductory anesthesia – induction: propofol (3 mg/kg), rocuronium bromide (1 mg/kg), fentanyl (4 mcg/kg).; maintenance of anesthesia – metered administration of propofol (3–4 mg/kg), anesthesia – fentanyl 100–150 mcg/kg/min. After tracheal intubation, the patient was transferred to artificial lung ventilation in volume control mode (Drager Infinity C 700, Germany). Infusion therapy was carried out according to the standard procedure with the dosed administration of a balanced crystalloid solution (4.5 ml/kg*h).

Before the operation, the right femoral vein (draining cannula 21 Fr) and the right internal jugular vein (return cannula 19 Fr) were punctured and catheterized under ultrasound navigation, and the left femoral artery was cannulated for emergency connection of the VAV/ECMO. Then, the VAV/ECMO procedure was started by the Cardiohelp device (Maquet, USA) with the specified parameters: pump capacity (PC) 3200, oxygenated blood volume (V) – 3.4 l/min, oxygen flow (Flow O_2) 4.5 l/min, while the intake pressure (Rven) – 67 mm Hg. art., ΔP (pressure difference before and after the oxygenator) 22 mm Hg, SvO_2 – 60 %. Anticoagulation with heparin 350 U/h was carried out under the control of active blood clotting time (ABC), activated partial thromboplastin time (APTT), prothrombin time (Automatic clotting timer – "ACT Plus", USA).

The indicators of the coagulation system in dynamics made it possible to rationally dose the level of heparin in the patient's blood when using the procedure for organ protection. Optimal heparinization was displayed on the automatic timer graph taking into account time intervals and indicating the required amount of protamine sulfate to neutralize heparin. Constant monitoring of blood clotting indicators significantly reduces complications of a hemorrhagic nature and, accordingly, minimizes the number of repeated surgical interventions for developed bleeding. Ventilation parameters after the start of the BB/ECMO were changed compared to the stage before the start of the oxygenation procedure. Ventilation mode: respiratory volume 4.5–5.0 ml/kg, the level of constant positive pressure in the respiratory tract 8–10 cm H_2O, fractional oxygen content in the inhaled mixture 60 % [12]. The assessment and correction of the functional state of the patient was carried out taking into account the data of her oxygen status (pO_2, pCO_2, $SatO_2$) and the dynamics of indicators of the acid-base state (ABS) of arterial blood.

After organoprotective preparation, the patient underwent a combined operation in the volume: median laparotomy, radical nephradenalectomy on the right with liver resection, cavatrombectomy. Surgical intervention was performed taking into account the prevalence of the malignant process and the anatomical topography of the tumor thrombus. A feature of the combined operation was a phased vascular mobilization: before the removal of the thrombus, tourniquets were placed on the hepatoduodenal ligament, contralateral renal vein, on the IVC at the level of the lower border of the thrombus and above the tip of the thrombus; after cavatomy and removal of the tumor thrombus from the IVC, the walls of the vein were revised and the cavatomic wound was sutured. The vascular stage of the operation was 1 hour and 25 minutes. To prevent post-hemorrhagic syndrome and in connection with intraoperative blood loss at the stage of thrombus removal and liver resection, a Cell-Saver type device (Haemonetics, USA) was used. This blood-saving tactic contributed to the effective correction of hypovolemia while maintaining a balanced oxygen-transport function of the blood. The volume of reinfusion was 900 ml, which made it possible to dispense with additional blood transfusion in the intraoperative period.

At the stage of removal of a tumor thrombus, the patient had a decrease in blood pressure to 68/52 mm Hg with an episode of compensatory tachysystole – heart rate 124 per minute. Emergency connection of vasopressive therapy (dosed administration of dobutamine – 3 mcg/kg/min) allowed to normalize hemodynamic parameters in a short time. Prior to the completion of the operation, the average blood pressure was maintained at the level of 65–75 mm Hg. No other serious cardio-respiratory disorders were recorded before the end of the operation. The study of the gas state of arterial blood at the stage of cavatomy and thrombus removal had no obvious negative dynamics: pCO_2 – 43 mm Hg, pO_2 – 110 mm Hg, pH – 7.41, BE – 7.1 mmol/l, HCO_3 – 31.2 mmol/l, $SatO_2$ – 96 %. The total time of anesthesia was 4 hours and 50 minutes.

After the operation, the patient, in the conditions of drug sedation, continued BB / ECMO with the same parameters. Against the background of compensated respiratory and hemodynamic parameters, an hour after the completion of the operation, cardiac arrhythmias were registered in the patient in the form of episodes of polyphocus atrial tachycardia, which were stopped within 30 minutes by the introduction of beta-blockers. These symptoms did not exclude myocardial ischemia, thromboembolism, impaired perfusion-transport function of blood, electrolyte disorders, etc. After additional diagnostic studies, life-threatening pathological conditions were excluded, the ECMO procedure was continued for another 12 hours. The parameters of BB/ECMO in the first hour in the intensive care unit were: RPM 2655 rpm, V – 3.2 l/min, Flow O_2 – 3 l/min. To complete the procedure, the centrifuge pump speed was gradually reduced by 10–15 % by analyzing respiratory status data and indicators of the coagulation and anticoagulation systems (D-dimer, APTT, fibrinogen, activated coagulation time, antithrombin activity). During thromboelastometry (Rotem, Germany), no serious deviations from the norm were recorded. Hemodynamics with a tendency to hypotension persisted. The extended artificial ventilator (Hamilton G5, Switzerland) had a pneumoprotective purpose and was aimed at adequate maintenance of gas exchange, which was ensured by conducting auxiliary ventilation in pressure support mode with a low respiratory volume of 3.5 ml/kg. Respiratory deficiency was not recorded, which was confirmed by the data of the gas composition of arterial blood. The level of sedation, from 0 to 2 on the Richmond Agitation-Sedation Scale (RASS), which corresponded to light sedation,

was maintained by dosed administration of a highly selective agonist of α2-adrenergic receptors – dexmedetomidine 0.6 mcg/kg/h. Muscle relaxants were not used. With stable gas exchange rates, 4 hours after the completion of the operation, the patient was transferred to an auxiliary (spont) ventilation mode with parameters: FiO_2 – 43 %, PEEP – 9 cm H_2O, PS – 15 cm H_2O, f – 16–26 in min., Vt – 480–540 ml.

Objective data: consciousness (outside sedation) is clear, according to the Glasgow scale of 15 points; the tongue is moist; the abdomen is soft, symmetrical, moderately painful, with palpation in the area of the postoperative wound, sluggish peristalsis is heard, there is no stool; adequate diuresis, corresponds to parenteral fluid administration. Under the control of gas exchange indicators, the degree of respiratory support gradually decreased. The patient is extubated. Auscultation: vesicular respiration in the lungs, weakened in the lower parts, more on the left, moist, wired wheezing, decreasing after coughing; the heart tones are rhythmic, muted. During the next day of the patient's stay in the intensive care unit, no significant complications from organs and systems were recorded. The total duration of the BB/ECMO was 30 hours. According to the data of an automatic timer with extended blood clotting indicators (APTT, fibrinogen, activated clotting time, antithrombin activity), we were guided in choosing the optimal amount of protamine sulfate to neutralize heparin. After the procedure was completed, the patient was prescribed the introduction of unfractionated heparin in a standard dosage.

An hour after the end of ECMO, the patient's functional parameters corresponded to the norm: pCO_2 – 40 mm Hg, pO_2 – 118 mm Hg, pH – 7.40, BE – 5.4 mmol/L, HCO_3 – 26.2 mmol/L, $SatO_2$ – 96 %, BP – 114/52 mm Hg, according to cardiac monitoring – sinus rhythm, heart rate 89 per minute, pulse at the periphery of satisfactory tension and filling, pulse deficit was 10 %.

The planned treatment corresponded to generally accepted norms and standards. On the 4th day of the postoperative period, the patient was transferred to the specialized department in a satisfactory condition to continue treatment. The final severity of surgical complications according to the Clavien–Dindo classification corresponded to the second degree (anemia, increased blood creatinine levels, an episode of cardiac arrhythmia). Patient D., on the 17th day, was discharged for planned restorative treatment and, later, was registered at the dispensary at the place of residence. During the next examination of the patient in the consultative and diagnostic department of the "NMRC for Oncology" in 2021, no signs of disease progression were revealed.

DISCUSSION

A characteristic feature of KC is the spread of the tumor into the lumen of the venous vessels. So, after damage to the intra-organ network, the tumor grows into the lumen of the renal vein, and then into the lumen of the IVC. A common neoplastic process, in rare cases, reaches the cavity of the right atrium with prolapse into it. In fact, vascular invasion worsens the survival of patients with KC, however, radical surgery with thrombectomy gives a chance for recovery [2].

The risk of an unfavorable outcome (development of complications and sudden death) in the intra- and postoperative period depends on the initial somatic status of the patient, concomitant organ dysfunction, as well as the features of the combined operation. Successful nephrectomy with thrombectomy in patients with KCis possible only in conditions of coordinated work of anesthesiological and surgical teams of specialists. The technical specifics of the operation consists in observing certain principles: circular mobilization of IVC s above and below the thrombus, ligation of collaterals, as well as compression of the hepatoduodenal ligament and contralateral renal vein, removal of the thrombus and reconstruction of the IVC s with preservation of the venous bed [4].

The task of the anesthesiologist is to ensure that the operation is performed with the maximum possible compensation for the functional state of the patient, including gas exchange. During the stage of kidney mobilization and IVCs, there is a risk of thrombus fragmentation with the development of a massive pulmonary embolism and sudden death during surgery. Undoubtedly, the use of cardiopulmonary bypass during surgery reduces the likelihood of thromboembolic complications. However, the facts accumulated to date indicate a number of complications of a hemorrhagic nature and technical difficulties of carrying out this bypass technique [4]. The development of new technologies and optimization of devices used in artificial blood circulation undoubtedly contributes to improving the immediate results of treatment of patients, including those with oncological pathology. The initial disturbances of the oxygen transport function of the lungs and the high risk of

massive intraoperative thromboembolic complications with the development of a fatal outcome were the basis for the use of the ECMO procedure. The ECMO technique consists in partially or completely providing the systemic blood flow with adequate blood oxygenation and carbon dioxide elimination using a centrifuge pump [8].

The presented clinical case demonstrates the effective use of modern technologies in the radical treatment of KC complicated by thromboembolism of medium and small branches of the pulmonary artery. The main goal facing the anesthesiological team during the combined operation was to prevent the development of critical hypoxemia and hypercapnia, life-threatening cardiac complications with the control of the dynamics of the coagulation and anticoagulation systems, as well as the prevention of pain syndrome and acute renal failure.

CONCLUSION

The use of new medical technologies in anesthesiology and resuscitation gives a chance to cure cancer patients with decompensated organ and functional capabilities. Thus, the effective use of extracorporeal membrane oxygenation made it possible to minimize the risk of fatal complications when performing nephrectomy with thrombectomy for malignant neoplasm of the kidney in a patient with PE and initial disorders of oxygen transport function of the lungs.

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REVIEW

EVOLUTION OF DRUG THERAPY FOR CLASSICAL HODGKIN LYMPHOMA

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ABSTRACT

Hodgkin's lymphoma is a malignant disease of the lymphatic system. Hodgkin's lymphoma was first described by Dr. Thomas Hodgkin in 1832 and later named "Hodgkin's disease" by Samuel Wilkes. Hodgkin's lymphoma accounts for about 24 % of all lymphomas. Hodgkin's lymphoma is classified as classical and nodular lymphoid-predominant (Nodular type of lymphoid-predominant Hodgkin's lymphoma). Classical Hodgkin's lymphoma includes the following histologic variants: nodular sclerosis variant (types I and II), mixed cell variant, classic lymphocyte-rich variant, and rare lymphoid depletion variant. Epidemiological and serological studies showed the involvement of the Epstein-Barr virus into Hodgkin's lymphoma etiology, since its genome was found in the study of the biopsy material samples from patients with Hodgkin's lymphoma. A relationship with the human immunodeficiency virus (HIV) was revealed as well, and patients infected with HIV have a significantly increased risk of developing Hodgkin's lymphoma compared to healthy people. An in-depth study of the Hodgkin's lymphoma pathophysiology revealed new therapeutic targets in the treatment of this disease. All these discoveries changed the understanding of the Hodgkin's lymphoma pathogenesis, and were important for the development of new methods of treatment. The history of therapy begins on the cusp of the 19th and 20th centuries. Over the past four decades, achievements in radiation therapy and combined chemotherapy have significantly improved overall survival of patients with Hodgkin's lymphoma. Currently, more than 80 % of patients under 60 years old with first diagnosed Hodgkin's lymphoma can be cured from this disease after first-line chemotherapy.

Keywords:
Hodgkin lymphoma, HIV, Epstein-Barr virus, IHC, remission, progression, chemotherapy resistance, overall survival

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ОБЗОР

ЭВОЛЮЦИЯ ЛЕКАРСТВЕННОГО ЛЕЧЕНИЯ КЛАССИЧЕСКОЙ ЛИМФОМЫ ХОДЖКИНА

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РЕЗЮМЕ

Лимфома Ходжкина – злокачественное заболевание лимфатической системы. Лимфома Ходжкина была впервые описана доктором Томасом Ходжкином в 1832 г., а позже названа «болезнью Ходжкина» Сэмюэлем Уилксом. Лимфома Ходжкина составляет около 24 % среди всех лимфом. Лимфома Ходжкина классифицируют как классическую и нодулярную с лимфоидным преобладанием (нодулярный тип лимфоидного преобладания лимфомы Ходжкина). Классическая лимфома Ходжкина включает следующие гистологические варианты: вариант с нодулярным склерозом (I и II типа), смешанно-клеточный вариант, классический вариант с большим количеством лимфоцитов и редко встречающийся вариант с лимфоидным истощением. Эпидемиологические и серологические исследования выявили причастность вируса Эпштейна-Барр к этиологии лимфомы Ходжкина: геном вируса Эпштейна-Барр был обнаружен при исследовании образцов биопсийного материала пациентов с лимфомой Ходжкина. Также выявлена связь с вирусом иммунодефицита человека (ВИЧ), заключающаяся в том, что пациенты, инфицированные ВИЧ, имеют значительно повышенный риск развития лимфомы Ходжкина по сравнению со здоровыми людьми. Углубленное изучение патофизиологии лимфомы Ходжкина позволило найти новые терапевтические мишени в лечении данного заболевания. Все эти открытия принесли изменения в понимании патогенеза данной патологии, и имеют важное значение в разработках новых методов лечения. История терапии начинается на рубеже XIX и XX вв. За последние четыре десятилетия достижения в лучевой терапии и использование комбинированной химиотерапии, значительно повысили уровень общей выживаемости пациентов с лимфомой Ходжкина. В настоящее время более 80 % пациентов моложе 60 лет с впервые диагностированной лимфомой Ходжкина могут быть излечены от данного заболевания после проведения первой линии химиотерапии.

Ключевые слова:
лимфома Ходжкина, ВИЧ, вирус Эпштейна-Барр, ИГХ, ремиссия, прогрессирование, резистентность к химиотерапии, общая выживаемость

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RELEVANCE

Hodgkin's lymphoma (HL) is a B-cell malignant lymphoproliferative disease. Hodgkin's lymphoma was first published in 1832 in the journal Medical Chirurgical Transactions under the title "On some morbidappearance of the absorbent glands and spleen", thanks to the persistent work of the British physician Thomas Hodgkin (Thomas Hodgkin, 1778–1866) [1]. However, the etiology of this lymphoma remained unclear for a long time. Only in the last 20 years has the B-cell nature of Hodgkin and Reed-Sternberg pathognomonic cells been revealed, as well as several recurrent genetic lesions. It was noted that there is a relationship between Epstein-Barr virus infection and the incidence of Hodgkin's lymphoma. Reed-Sternberg cells in classical Hodgkin's lymphoma have some characteristics that are atypical for lymphoid tumor cells, and extensive inflammatory infiltrate prevails in the microenvironment of Hodgkin's lymphoma. Specific markers PD-1 and CD30 were also found to express on Reed-Sternberg cells [2]. This allowed us to take a broader look at the pathophysiology of Hodgkin's lymphoma and find new therapeutic targets in the treatment of this disease. All these discoveries have brought changes in the understanding of the pathogenesis of this pathology, and is important in the development of new treatment methods.

Epidemiology

HL is about 24 % among all lymphomas. The incidence of Hodgkin's lymphoma in Russia is 2.1 per 100,000 population per year (3149 newly diagnosed patients), mortality is 0.74. In the structure of malignant neoplasms – LX is 0.51 %. The disease is more common in men than in women. People of any age suffer from Hodgkin's lymphoma, but the peak incidence occurs at the age of 20–35 years. The incidence has not changed much over the past two decades [3]. In the revised classification of tumors of hematopoietic and lymphoid tissues WHO 2017 there are: classical HL (cHL) (according to ICD-10 C81.1 – C81.9) and nodular with lymphoid predominance of HL (NLPHL) (according to ICD-10 – C81.0 Nodular type of lymphoid predominance of Hodgkin's lymphoma). Classical HL includes the following histological variants: a variant with nodular sclerosis (type I and II), a mixed-cell variant, a classic variant with a large number of lymphocytes and a rare variant with lymphoid depletion (morphological and immunohistochemical diagnostics of various variants of HL). In addition to determining the type of HL and the histological variant of cHL, the stage of the disease should be determined for each patient, and the risk group for cHL should also be determined [4].

Evolution of therapy

The history of therapy begins at the turn of the XIX and XX centuries. then for X-ray and gamma radiation were applied to the foci. In the 1940s, chloromethine, a derivative of mustard gas, began to be used as cytostatics.

Then they began to use: vinblastine, cyclophosphamide, vincristine, methotrexate, procarbazine, chlorambucil in monotherapy and in various combinations, as well as in combination with radiation therapy. However, all these options did not give a significant therapeutic effect. Until 1964, at the US National Cancer Institute in Bethesda, V. De Vita proposed using the MOPP scheme a combination of chloromethine, vincristine, procarbazine in combination with glucocorticosteroids, which led to a cure in more than 50 % of cases. When using this scheme, 77 % – 4-year overall survival (OS) were obtained [5]. In 1973 the protocol of combined chemotherapy according to the ABVD scheme included adriamycin, bleomycin, vinblastine and dacarbazine proved to be more effective in comparison with the MOPP scheme, the overall survival after 5 years was 82 % [6]. This therapy remained the standard of treatment until 1998, when the Stanford V protocol was approved, which included combined chemotherapy with doxorubicin, vincristine, mechloretamine, vinblastine, bleomycin, etoposide and prednisone [7]. This 12-week chemotherapy regimen was followed by consolidating radiotherapy to the primary lesion. The Stanford Protocol did not give better results in comparison with ABVD chemotherapy in relation to OS, but this treatment really increased the effectiveness of treatment [8]. Five years later, the German Hodgkin's Lymphoma Group presented the BEACOPP scheme, consisting of a combination of bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine and prednisone, a scheme that was later improved by increasing the doses of drugs. This allowed the majority of patients with advanced stages to have a high percentage of a complete cure of the disease. When comparing ABVD with escalated BEACOPP, the latter had a significant advantage in terms of

better progression-free survival (PFS) and allowed to achieve a 5-year overall survival in more than 94 % of patients, but it is often accompanied by hematological toxicity of III–IV degree [9]. At the same time, in patients with early stages of the disease, the ABVD +/- radiation therapy chemotherapy regimen remains the gold standard of treatment, which allows achieving an 8-year overall survival rate of about 95 %, but the emergence of new drug therapy options in the form of targeted drugs reduces the number and intensity of adverse events.

In the last few years, various targeted drugs for the treatment of HL have been investigated, of which the most promising results have been shown by the anti-CD30-antibody conjugate- brentuximab vedotin – is an antibody-drug substance conjugate (ADSC), which delivers an antitumor substance to CD30-positive tumor cells, which leads to their apoptosis and death [10]. Brentuximab vedotin monotherapy has been investigated as an induction therapy and as a consolidating treatment after high-dose chemotherapy and autologous stem cell transplantation. In a one-cent study conducted at Memorial Sloan Kettering Cancer Center in New York, 45 patients with recurrent HL received weekly infusions of brentuximab vedotin at a dose of 1.2 mg/kg over two 4-week cycles. Patients with a PET-negative result switched to further therapy and subsequent stem cell transplantation, while patients with a PET-positive result continued treatment with two cycles of CT according to the ICE scheme (ifosfamide, carboplatin, etoposide). The two-year event-free survival of these patients was 92 % and, thus, is comparable to the two-year event-free survival of patients with a PET-negative result after ICE chemotherapy (91 %) [11]. Taking into account the results of a multicenter study, brentuximab vedotin before high-dose chemotherapy and transplantation shows good results in about 1/3 of patients with HL with relapse of the disease after 1-line treatment. A study was conducted to evaluate the effectiveness of brentuximab vedotin in combination with chemotherapy for newly diagnosed HL. A multicenter study from the USA included 30 patients with an early unfavorable stage of the disease [12]. The study treatment consisted of four courses of brentuximab vedotin (1.2 mg/kg every two weeks) in combination with doxorubicin, vinblastine and dacarbazine, followed by radiation therapy in the affected areas with a dose of 30 Gy. Intermediate PET was performed after 2 and 4 CT cycles. Metabolic remission after 2 and 4 cycles of treatment was observed in 93 % of patients. Due to its high efficacy, this drug is included in clinical recommendations [12].

A study was conducted, which included patients with classical HL with a relapse or refractory course of the disease, in which the effect of lenalidomide was evaluated (inhibits the proliferation of certain malignant hematopoietic cells, enhances immunity mediated by T-lymphocytes and natural killer cells (NK cells), increases the number of NK (T cells), suppresses angiogenesis, blocking migration and the adhesion of endothelial cells and the formation of microvessels, increases the production of fetal hemoglobin CD34+ by hematopoietic stem cells and inhibits the production of proinflammatory cytokines) at doses of 25 mg/day for 1–21 days with cycles of 28 days. Patients received the drug before the progression or occurrence of unacceptable toxicity. 38 patients who received an average of 4 courses of previous chemotherapy courses were studied, of which the percentage of refractory patients was (55 %) and 87 % of them who had previously received high-dose chemotherapy. The overall response rate was 19 %. Among the phenomena of undesirable toxicity: hematological toxicity of 3–4 degrees: neutropenia (47 %), anemia (29 %) and thrombopenia (18 %). All this indicates the presence of the desired effect of lenalidomide therapy in relation to HL, which needs to be studied further [13]. At the moment, it is not included in the clinical recommendations.

A study was conducted on the use of a targeted drug mTOR inhibitor (everolimus is an inhibitor of the transmission of proliferative impulse in cells. The blockade of this signal leads to the arrest of cell division at the G1 stage of the cell cycle), with the use of everolimus at a dose of 10 mg/day in 19 patients with refractory or recurrent HL, while 84 % of them had autoHSCT transplantation, the overall response was 47 %, 8 patients had a partial response, and only one patient had a complete response. The average time to disease progression was 7.2 months [14]. In another study, 37 patients on the same treatment received an overall response of 35 %. Stabilization of the process was observed in 27 %, survival without progression was 7.2 months. Treatment was accompanied by hematological toxicity of 3–4 degrees: thrombocytopenia – 38 %, fatigue – 43 %, neutropenia – 8 % and anemia – 8 %. As in the case of other previously described studies, it is reported that there is a good

response to treatment in patients who have received treatment. This allows us to think that everolimus may have a clinically significant role in recurrent or refractory HL. In addition, there is evidence of its possible synergism with other RAF inhibitors [15]. It is not included in the clinical recommendations.

A study was also conducted to study the use of nivolumab in patients with HL. Checkpoint drug therapy is a new word in the treatment of cancer patients, nivolumab is a human monoclonal antibody that blocks the interaction between the programmed death receptor (PD-1) and its ligands. The study obtained encouraging results – 87 % of patients managed to achieve a complete response to treatment, and this treatment has an acceptable toxicity profile. This study included patients who failed to undergo chemotherapy followed by bone marrow transplantation and therapy with brentuximab vedotin [16]. Based on the results obtained, several studies were launched using anti-PD-1 drugs. In 2020, the results of a 5-year follow-up of a multicomponent study called CheckMate 205 were published. The study included 243 patients with HL who had a relapse of the disease after 1 line of CT with subsequent autotransplantation of stem cells and treatment with brentuximab vedotin. The average duration of treatment was 14 months. The overall response rate was 71 %, and the full response rate was 21 %. The median PFS was 15 months. The frequency of OV after 2 and 5 years was 87 % and 71 %, and the frequency of PFS was 37 % and 18 %, respectively. This 5-year CheckMate 205 analysis demonstrated favorable survival and confirmed the high efficacy and safety of nivolumab [17]. After the studies conducted with the use of immune checkpoint drugs, the European Medical Agency approved nivolumab and pembrolizumab in a single mode for the treatment of patients with HL, but in the Russian Federation, to date, only nivolumab has been registered. Anti-PD-1 have shown significant activity in patients with recurrent/refractory HL, and they also have an acceptable toxicity profile with side effects that are usually treatable [18].

The results of a study using the drug anti-CD30 CAR by T cells in adult patients with HL were published, currently this type of treatment is actively used only in pediatric hematology. Anti-CD30 is a promising target for immunotherapy in Hodgkin's lymphoma. The use of autologous anti-CD30 CAR T-cells in Hodgkin's lymphoma was studied in 41 patients in two different centers, the complete response rate was 59 %. Unfortunately, after 1 year, PFS was only 36 %, and the one-year overall survival rate was 94 % [19]. This method of treatment allows patients with a resistant course of the disease to achieve a positive effect; however, CAR T-cell therapy is associated with extremely severe adverse events in the form of cytokine release syndrome, which must be taken into account when planning this type of treatment.

CONCLUSION

Taking into account the interest of the world community in studying this group of lymphoproliferative diseases, this review gives an idea of modern changes in the process of diagnosis and treatment tactics in this cohort of patients. Despite the fact that patients suffering from Hodgkin's lymphoma have the possibility of a complete cure from the disease, at the moment a large percentage of refractory or recurrent course remains after first-line therapy and even after subsequent transplantation of autologous stem cells. Relapse of the disease after high-dose chemotherapy and autologous stem cell transplantation remains the main cause of death in patients with recurrent or resistant Hodgkin's lymphomas. But the new treatment options that have appeared make it possible to improve progression-free survival and overall survival. Many of them are undergoing clinical trials and demonstrating efficacy, but it is too early to say whether all of these drugs will contribute to increased survival more than the standard treatments we use for these patients. Nevertheless, long-term observation and further research are necessary for final conclusions.

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Kapuza E. A. – collecting the material;
Kamaeva I. A. – scientific text editing;
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REVIEW

D2, D3 LYMPH NODE DISSECTION IMPORTANCE IN COLON CANCER SURGERY

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ABSTRACT

Colorectal cancer (CRC) is a relevant issue of modern oncology and ranks the third place among most common malignancies. Every year, more than 1 million new cases of CRC are diagnosed worldwide, with approximately the same frequency of prevalence among the male and female population. Colon cancer (CC) amounts for more than half of all cases of CRC, and it's incidence and mortality remain rather high.

Surgery remains the main method of CRC treatment, and determining the extent of surgery and lymph node dissection remains an urgent problem.

For the first time in Japan, a classification of groups of lymph nodes (l.n.) was proposed depending on the level of lymph outflow and location in relation to the main vessels. According to the numbering of l.n. groups by the Japanese Society for Cancer of the Colon and Rectum (JSCCR), all lymph nodes are numbered with three digits.

As a rule, lymphogenic metastasis occurs in one direction, bilateral spread is possible if the tumor is located at the same distance from two feeding vessels. With tumors of the right-sided localization, all groups of l.n. located along the branches of the superior mesenteric artery are removed, and with tumors of the left half of the colon, all l.n. located along the trunk of the inferior mesenteric artery are removed. The presence of affected l.n. is important for assessing the prognosis and further determining the need for adjuvant therapy. Some literature data demonstrate good results of surgical interventions performed in accordance with the concept of embryonic planes and complete mesocolonic excision. D3 lymph node dissection is not performed in daily practice in some European countries and North America, unlike a number of Eastern countries. However, the level of vessel ligation remains the subject of scientific discussion.

The purpose of this review was to analyze the available literature on the problem of choosing the level of lymph node dissection in CC surgery.

Keywords:
colorectal cancer, colon cancer, surgical treatment D2/D3 lymph node dissection

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ОБЗОР

РОЛЬ D2, D3 ЛИМФОДИССЕКЦИЙ В ХИРУРГИИ РАКА ОБОДОЧНОЙ КИШКИ

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РЕЗЮМЕ

Колоректальный рак (КРР) является актуальной проблемой современной онкологии и занимает третье место в структуре общей онкологической заболеваемости. Ежегодно во всем мире диагностируются более 1 млн. новых случаев заболевания КРР, приблизительно с одинаковой частотой распространенности среди мужского и женского населения. Более половины всех случаев КРР приходятся на рак ободочной кишки (РОК), заболеваемость и смертность от которого остается на достаточно высоком уровне, несмотря на современные диагностические возможности и принципы терапии. Основным методом лечения пациентов с опухолями ободочной кишки остается хирургический, а определение объема оперативного вмешательства и уровня лимфодиссекции остается актуальной проблемой современной онкопроктологии. Впервые в Японии была предложена классификация групп лимфатических узлов (л.у.) в зависимости от уровня лимфооттока и расположения по отношению к магистральным сосудам. Согласно нумерации групп л.у. по Japanese Society for Cancer of the Colon and Rectum (JSCCR) все лимфатические узлы пронумерованы трехзначными цифрами. Как правило, лимфогенное метастазирование происходит в одном направлении, билатеральное распространение возможно в случае, если опухоль расположена на одинаковом расстоянии от двух питающих сосудов. При опухолях правосторонней локализации удаляются все группы л.у., располагающиеся вдоль ветвей верхней брыжеечной артерии, а при опухолях левой половины ободочной кишки – все л.у., находящиеся вдоль ствола нижней брыжеечной артерии. Наличие пораженных л.у. важно для оценки прогноза и дальнейшего определения необходимости проведения адъювантной терапии. В литературе имеется данные, которые свидетельствуют о хороших результатах оперативных вмешательств, которые выполнены с использованием принципов эмбрионально-ориентированной хирургии и тотальной мезоколонэктомии. В некоторых европейских странах и Северной Америке D3 лимфодиссекция (расширенная лимфодиссекция) не выполняется в повседневной практике, в отличие от ряда восточных стран. Однако, уровень перевязки сосудов остается предметом научных споров.

Цель данного обзора: провести анализ имеющейся литературы, посвященной проблеме выбора уровня лимфодиссекции в хирургии РОК.

Ключевые слова:
колоректальный рак, рак ободочной кишки, хирургическое лечение, D2/D3 лимфодиссекция

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RELEVANCE

Colorectal cancer (CRC) is an urgent problem of modern oncology and ranks third in the structure of general oncological morbidity both in Russia and in foreign countries [1; 2]. More than half of all cases of CRC occur in colon cancer (CC), the morbidity and mortality from which remains at a fairly high level [3]. The main method of treatment of CRC remains surgical, and determining the volume of surgery and the level of lymph dissection remains an urgent problem of oncosurgery.

There are data in the literature that indicate good results of surgical interventions performed using the principles of embryonic-oriented surgery and total mesocolonectomy (complete mesocolon excision, CME). In some European countries and North America, D3 lymph dissection is not performed in everyday practice, unlike in a number of Eastern countries. However, the level of vascular ligation remains a subject of scientific debate [4].

The purpose of the study: to analyze the available literature on the issue of choosing the level of lymphodissection in CC surgery.

Japanese classification of lymph node groups

For the first time in Japan (1977), a classification of groups of lymph nodes was proposed depending on the level of lymph outflow and location in relation to the main vessels [5; 6]. According to the numbering of the l.n. groups by the Japanese Society for Cancer of the Colon and Rectum (JSCCR), all lymph nodes are numbered with three digits [6] (Fig. 1).

L.ns. are divided into 3 levels:

- N1 – paracolitic,
- N2 – mesocoliic,
- N3 – central l.n.

Usually, lymphogenic metastasis occurs in one direction, bilateral spread is possible if the tumor is located at the same distance from two feeding vessels. With tumors of the right-sided localization, all groups of LV located along the branches of the upper mesenteric artery are removed, and with tumors of the left half of the colon, all l.n. located along the trunk of the lower mesenteric artery are removed [9].

D2, D3 lymphodissection in colon cancer surgery: meta-analysis

The presence of affected l.ns. is important for assessing the prognosis and further determining the need for adjuvant therapy.

The aim of the Tsai H. L. et al. study was to determine whether the number of removed l.ns. can affect the prognosis of the clinical course of patients who underwent surgery for CRC. The authors noted that the amount of tumor invasion and the number of removed lymph nodes are independent prognostic factors for the development of postoperative relapse ($p < 0.05$). The 5th overall survival of patients who had 18 or more lymph nodes examined was significantly higher than those who had less than 18 nodes examined ($p = 0.015$). The results of this work show that the removal and examination of at least 18 HP can be taken into account for more reliable and correct postoperative staging [10].

According to Willaert W. et al. the described method of total mesocolonectomy (complete mesocolon excision, CME) with complete removal of l.n. should be subjected to a prospective randomized study. However, there is undoubtedly a proven relationship between the number of removed l.ns. and survival in CRC [11].

The question of whether extended lymphadenectomy in CC leads to an increase in postoperative complications or improves survival is still controversial. In some European countries and North America, D3 lymph dissection is not performed in everyday practice, unlike in a number of Eastern countries. However, the level of vascular ligation remains a subject of scientific debate. There are data in the literature that indicate good results of surgical interventions that are performed using the principles of embryonic-oriented surgery and CME.

In a randomized RELARC phase 3 trial, the hypothesis is considered that survival after D2 lymphodissection is better than after CME. The primary endpoint is 3-year relapse-free survival. To date, researchers have presented the results of comparing the following criteria: intra- and postoperative complications within 30 days after surgery (according to the Clavien-Dindo classification), mortality (death from any cause within 30 days after surgery) and the frequency of metastasis to the central l.ns. only in the CME group. The frequency of postoperative surgical complications was 20 % (97 out of 495 patients) in the CME group compared with 22 % (109 out of 500 patients) in the D2 group ($p = 0.39$). The frequency of Clavien-Dindo grade I–II complications was the same between the groups and amounted to 18 %, and grade III–IV complications They were significantly less common in the CME group than in the D2 group (1 % and 3 %, respec-

tively, $p = 0.022$). There were no fatal outcomes in the compared groups. In the structure of intraoperative complications, vascular damage was significantly more common in the CME group than in the D2 group (15 (3 %) vs. 6 (1 %), $p = 0.045$). Metastases in the central l.n. were detected in 13 (3 %) of 394 patients, but none of the patients had their isolated metastatic lesion. Thus, the authors concluded that CME can increase the risk of intraoperative vascular damage, but in general it seems safe and feasible for experienced surgeons [12].

A number of papers have been published indicating a significant decrease in the frequency of local relapses and an increase in the overall 5-year survival after surgical intervention using the CME technique.

So in the work of Bertelsen C. A. et al. It was proved that 4-year relapse-free survival was 85.8 % (95 % CI 81.4–90.1) after CME and 75.9 % (72.2–79.7) after surgery without using the CME technique ($p = 0.0010$), and Cox regression showed that CME was a significant independent prognostic factor for higher relapse-free survival for patients. Thus, according to the authors, CME can improve the results of treatment of patients with CRC [13].

A prospective study by Galizia G. et. al. showed that the number of removed l.n. and the length of vascular ligation were significantly better in the CME group ($p < 0.01$). A larger number of affected l.n. were removed, which allowed for an adequate selection of adjuvant treatment. The authors proved that CME with CVL is a safe and effective surgical approach for right colon cancer, reduces local recurrences and improves survival, especially in patients with N+ [14]. However, no increase in the frequency of postoperative complications was found in these studies.

In a retrospective study by K. Kotake et al. The advantages of D2 and D3 lymph node dissection in CC surgery were studied. In all groups of patients, there was a statistically significant difference in overall survival between patients who underwent dissection of lymph nodes D3 and D2 ($p = 0.00003$). It was found that dissection of D3 lymph nodes in pT3 and pT4 colon cancer is associated with a significant advantage in improving patient survival, which can serve as a basis for dissection of D3 lymph nodes in radical surgery of pT3 and pT4 colon cancer [15].

The aim of the Hwang D. Y. et al. study was the assessment of the safety and oncological results of

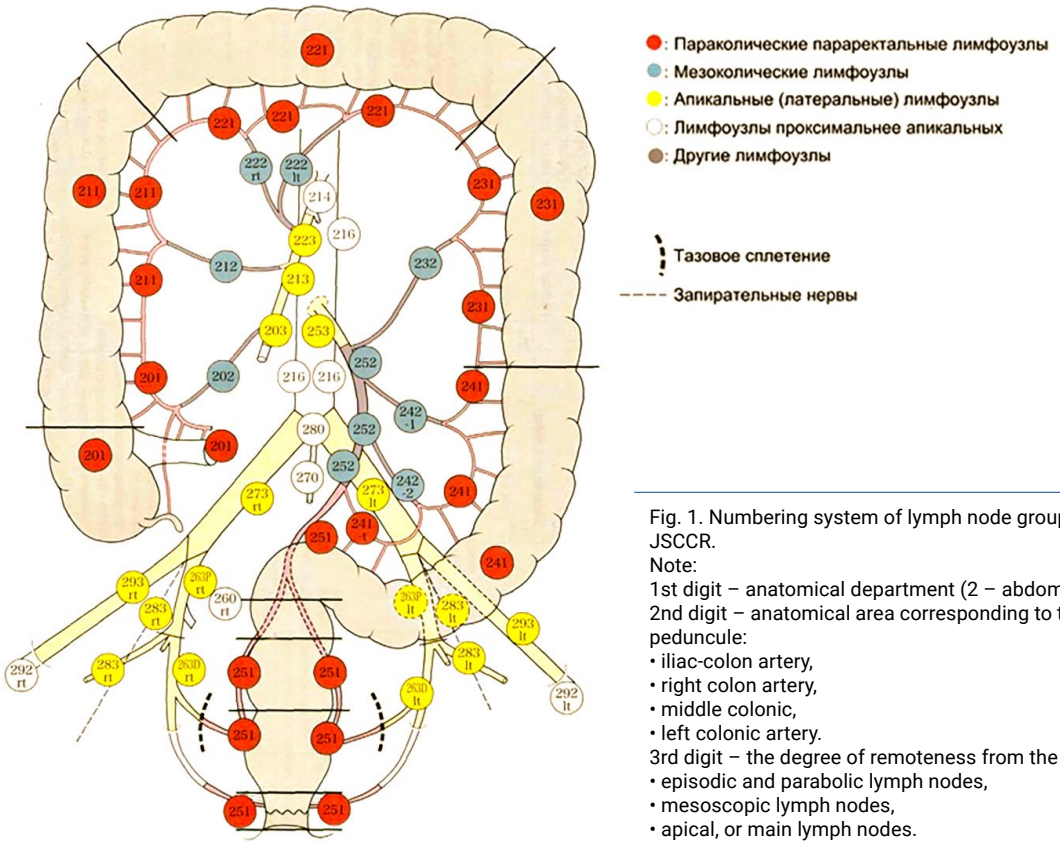

Fig. 1. Numbering system of lymph node groups according to JSCCR.
Note:
1st digit – anatomical department (2 – abdominal cavity, pelvis).
2nd digit – anatomical area corresponding to the lymphovascular peduncule:
• iliac-colon artery,
• right colon artery,
• middle colonic,
• left colonic artery.
3rd digit – the degree of remoteness from the intestine:
• episodic and parabolic lymph nodes,
• mesoscopic lymph nodes,
• apical, or main lymph nodes.

laparoscopic CME with D3 in cancer of the right colon in elderly patients. Patients who underwent right-sided hemicolectomy were divided into groups A (age ≥ 70 years, *n* = 80) and B (age < 70 years, *n* = 127). Short-term and long-term results were analyzed. It was found that the overall and relapse-free survival rates were the same between the compared groups, and laparoscopic CME with D3 lymphodissection is a safe and feasible surgical option for cancer of the right half of the colon in the elderly [16].

In our country, an international multicenter randomized COLD Trial was launched in 2017, the main purpose of which is to evaluate and compare the overall 5-year survival after D2- and D3-lymphodissection performed in CC [4]. Patients with resectable CC were randomized for D2 or D3 dissection in a 1:1 ratio.

The data obtained were analyzed to assess the safety of D3 lymph dissection

In the work of Karachun A. et al. the results for the first 100 patients are presented. There were no fatalities. The 30-day postoperative morbidity rate was 47 % in group D2 and 48 % in group D3 with a RR1.04 (95 % CI from 0.68 to 1.58) (p = 0.867). There were two anastomosis failures (5 %) in group D2 and none in group D3. Postoperative recovery, the frequency of complications and repeated hospitalizations did not differ between the groups. N-positive status was more common in group D3 (46 % vs. 26 % in group D2) with RR 1.81 (95 % CI from 1.01 to 3.24) (p = 0.044). The authors concluded that D3 lymph node dissection is possible and may be associated with a better staging of the process [17].

CONCLUSION

After the carried out analysis of the available up to date literature, the following conclusions can be drawn. On one hand, the relationship between the number of removed HP and a more accurate determination of the N status has been proven, which is important for assessing the clinical prognosis and further determining the need for adjuvant therapy. On the other hand, it is indisputable that D3 lymph dissection can increase the risk of intraoperative vascular damage. Thus, due to the lack of a convincing evidence base, it is difficult to talk about the advantages and long-term results of performing D2 and D3 lymphodissections in CC surgery. The recruitment of patients in some large studies continues to this day, and the publication of the results of others is expected in the near future.

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REVIEW

MODERN ASPECTS IN ANESTHESIA OF SMALL LABORATORY ANIMALS

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ABSTRACT

Experiments with small laboratory animals are required for better understanding of a disease flow, for studying the mechanisms of it's development and for the development of new therapeutic strategies. A significant part of experimental studies involve anesthesia. In this regard, the choice of the optimal anesthesia protocol is an important point of research, since an inadequate depth of anesthesia or the influence of undesirable factors can lead to death; the type, duration, and goals of the procedure should be also taken into account.

We have aimed to find out what has changed in anesthesia of laboratory animals lately, which drugs are currently relevant and what is the reason for their popularity.

Anesthesia of mice is challenging for several reasons: animal size, metabolic rate, and high risk of hypothermia and hypoglycemia. In addition, anesthetics affect physiological parameters and therefore even more affect the results of experiments. At the moment, there is a large list of drugs used in laboratory animals. Since they are divided into groups depending on the routes of administration, we selected the following drugs from a number of articles: injectable anesthetics (medetomidine, dexmedetomidine, zoletil-100, ketamine, xyla, propofol) and inhalation anesthetics (isoflurane, sevoflurane). Advantages and disadvantages of the drugs and their combinations were studied and described.

An analysis of the literature showed that injection anesthesia is considered the main method of anesthesia for experimental animals and is relatively well tolerated by animals; it also does not require additional bulky equipment and additional staff qualifications, there are antagonists for a number of drugs, and is also affordable.

In the majority of studies inhalation anesthesia was used in long-term complex manipulations/operations, since it is more manageable, agents require minimal metabolism, and in some cases do not require additional sedation.

Keywords:
anesthesia, analgesia, inhalation anesthesia, injection anesthesia, laboratory animals, antagonist, sedation

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ОБЗОР

СОВРЕМЕННЫЕ АСПЕКТЫ В ОБЛАСТИ АНЕСТЕЗИИ МЕЛКИХ ЛАБОРАТОРНЫХ ЖИВОТНЫХ

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РЕЗЮМЕ

Эксперименты с использованием лабораторных животных необходимы для лучшего понимания течения той или иной патологии, изучения механизмов ее развития и разработки новых терапевтических стратегий. Значительная часть экспериментальных исследований требует выполнения процедуры анестезии. В связи с этим выбор оптимального протокола по анестезии является важным пунктом исследований, так как недостаточная глубина наркоза и влияние нежелательных факторов на организм объекта могут привести к летальному исходу.

Для нас представляло интерес выяснить, что поменялось в области анестезии лабораторных животных за последнее время, какие препараты актуальны на данный момент и в чем причина их популярности.

Анестезия мышей является сложной задачей по нескольким причинам: размер животного, скорость метаболизма и высокий риск гипотермии и гипогликемии. Кроме того, анестетики действуют на физиологические параметры, что еще больше влияет на результаты экспериментов. На данный момент существует большой перечень препаратов, применяемых на лабораторных животных. Поскольку они подразделяются на группы в зависимости от путей введения, из ряда статей мы отобрали следующие препарата: инъекционные – «Медетомидин», «Дексмедетомидин», «Золетил-100», «Кетамин», «Ксила», «Пропофол» и ингаляционные – «Изофлуран», «Севофлуран». Изучили и описали преимущества и недостатки препаратов и их сочетаний.

Согласно анализу литературных источников, инъекционная анестезия считается основным методом наркоза для экспериментальных животных и сравнительно хорошо переносится животными, так же не требует дополнительной громоздкой аппаратуры, дополнительной квалификации сотрудников, на ряд препаратов существуют антагонисты, имеет доступную стоимость.

При длительных сложных манипуляциях/операциях в большинстве литературы использовали ингаляционный наркоз, так как он более управляем, агенты требуют минимального метаболизма, в ряде случаев не требует дополнительной седации.

Ключевые слова:
анестезия, анальгезия, ингаляционный наркоз, инъекционный наркоз, лабораторные животные, антагонист, седация

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RELEVANCE

A significant aspect for understanding the patterns of disease development and the development of new antitumor drugs are studies performed on laboratory animals [1]. The goal of the scientific direction for the creation of effective tumor models is the fullest reproduction in the body of experimental animals of human oncogenesis and related systemic changes, which leads researchers to a deeper study of anesthesiology [2].

Currently, various versions of anesthesia protocols are widely used in practice, depending on the tasks and the type of laboratory animals used: white mice, white rats, various types of hamsters, guinea pigs, rabbits, etc. [3]. One of the most frequently used types of experimental animals are mice [3; 4].

The provision of anesthesia and anesthesia for these animals is an important practice in the laboratory, which is necessary for performing invasive procedures, achieving prolonged immobility, for sensitive imaging techniques and pain relief [4; 5]. Thus, the safe and effective use of anesthesia is an important aspect of improving experimental methods, taking into account the specific effect of the selected agents on the physiological parameters that are relevant to the results of the study. For these reasons, the choice of an appropriate anesthetic procedure is an important factor in experimental studies and should correspond to the type and duration of the procedure, as well as their purpose [3].

Laboratory mice have specific physiological and anatomical features that affect the effect of sedatives. Due to their small size and metabolism, the excretion of drugs from the body occurs very quickly. These features bring their own nuances to the procedure of introduction into anesthesia [6].

Also, when choosing anesthesia, the following factors should be taken into account: the weight and age of the animal, the model of the disease being investigated and the type of procedure [7].

At the moment, there is a large list of anesthetics used on laboratory animals. They are grouped depending on the routes of administration: inhalation – based on the introduction of drugs into the body in the form of steam or gas through the respiratory tract ("Isoflurane", "Sevoflurane", etc.) and non–inhalation – based on the introduction of anesthetics parenterally ("Propofol", "Xyla", "Medetomidine", "Zoletil" etc.) [8; 9].

It was interesting to find out what has changed in the field of anesthesia of laboratory animals, in particular mice, which drugs are relevant at the moment and what is the reason for their frequent use.

In this regard, the purpose of our review is to analyze and systematize the data obtained from scientific articles on anesthetics.

Literature search methods

We conducted a systematic search in the PubMed database, using the keywords "anesthesia, laboratory, mice, inhalation, injection" and e-Library: "anesthesia, laboratory mice, experiment". For 10 years, 4180 results were found by search queries in the PubMed database, by keywords, and 2812 articles were found in the e-Library.

The exclusion criteria were:

1. studies in which there was no name of the anesthetic;
2. studies in which laboratory animals were not mice;
3. studies that did not describe the condition of animals when anesthetics were administered, and there were no data on the condition of animals after manipulations under the influence of anesthetics
4. studies for which there was only a summary in the public domain;
5. articles that can only be accessed on a paid basis;
6. studies that did not describe side effects on the animal's body.

As a result of the work done, we selected 106 articles on inhalation and 251 on injection anesthesia in laboratory mice and 46 publications were analyzed in detail.

Analysis of research results

Mice are one of the most widely used types of experimental animals. A lot of manipulations are performed on these rodents, which require either a mild sedative effect or deep anesthesia [10]. In the publications we have studied in recent years, the method of "balanced anesthesia" is indicated, which is the introduction of a mixture of sedatives, analgesics and anesthetics to create high-quality anesthesia. It also allows the use of lower doses than if each component was used in a single mode [7; 11]. This practice has the advantage of synergy and avoids undesirable effects observed when using increased

doses of individual components [11]. Depending on the route of administration, anesthetics can be divided into inhalation – based on the introduction of drugs into the body in the form of steam or gas through the respiratory tract ("Isoflurane", "Sevoflurane", etc.) and non–inhalation – based on the introduction of anesthetics parenterally ("Propofol", "Xyla", "Medetomidine", "Zoletil", etc.)

Injection anesthesia

According to the analysis of literature sources, injection anesthesia is considered the main method of anesthesia when conducting experimental procedures in small rodents [12]. This type of anesthesia has its advantages, since it can be used in almost any conditions, does not require additional bulky equipment, and is relatively well tolerated by animals. But there are also disadvantages, these include: poor handling, the possibility of developing cardiac and respiratory complications a few hours after anesthesia [13–15]. Doses of anesthetics for injectable agents depend on the types of animals used, routes of administration, age, gender, stress, body condition, environment, experimental setup, previous drug treatment and the required level of anesthesia. During the initial period of use, it is important to closely monitor the animals and make any adjustments necessary for future use [14].

For the administration of these drugs, one of the methods is used: intravenous, intramuscular, intraperitoneal [15].

The analysis of the literature showed that the most common drugs in the articles are: "Medetomidine", "Dexmedetomidine", "Zoletil-100", "Ketamine", "Xyla", "Propofol" and their combinations. In this review, we have reviewed several of the most common drugs and their combinations.

Drugs:

"Medetomidine"

It stimulates peripheral and central α2-adrenoreceptors, has a selective effect, which reduces the drug load on the animal's body. It is one of the most popular drugs because of its high α2 selectivity against receptors. "Medetomidine" is a sedative and analgesic. Its main side effects include bradycardia, hypotension, respiratory depression, hypothermia and diuresis. To stop the effect of "Medetomidine" on the animal's body, drugs containing such an active substance as atypamezole hydrochloride are used in practice. Due to the antagonistic effect on α2 receptors, the release of norepinephrine is suppressed and, as a result, the effects caused by Medetomidine are stopped [16].

"Dexmedetomidine"

"Dexmedetomidine" has been identified as the active enantiomer, aka the mirror drug "Medetomidine".

"Dexmedetomidine" is an α2-adrenergic receptor agonist similar to "Medetomidine", but it lacks the pharmacologically inactive enantiomer of levomedetomidine. "Dexmedetomidine" has a stronger anesthetic effect than "Medetomidine" and is 40 times stronger than "Xyla" [7; 17]. "Dexmedetomidine" provides excellent muscle relaxation and pain relief during surgical interventions. In addition, the administration of the drug with the active substance – atypamezole hydrochloride, provides rapid elimination of the anesthetic effect and leads to rapid recovery of physiological parameters [17].

"Ketamine"

"Ketamine" causes a state of "dissociative anesthesia", in which deep anesthesia, sedation and muscle rigidity (stage of catalepsy) are observed. It does not depress the central nervous system (CNS), so reflexes remain intact. Side effects are as follows: the eyes remain open, so it is additionally recommended to use eye ointment; the presence of spontaneous movements and muscle tension, which causes an initial increase in blood pressure [18]. Unlike other anesthetics, Ketamine does not inhibit breathing or cardiac output. Also, this drug, used in mono mode, does not provide sufficient anesthesia. But the combination with "Xyla", "Medetomidine" or "Diazepam" creates an effective anesthesia [19].

"Propofol"

The drug is an isopropylphenyl compound available for intravenous administration. Propofol exerts its influence on the central nervous system by modulating gamma-aminobutyric acid (GABA) channels through sites other than barbiturates, steroids or benzodiazepines. It quickly causes loss of consciousness, recovery is faster and more complete with minimal residual effects, has good potential as an anesthesia regimen for functional studies. However, it does not cause analgesia, so when performing painful manipulations, additional analgesic drugs should be used. Cerebral blood flow, perfusion pressure and intracranial pressure decrease after administration of Propofol. It is a powerful respiratory depressant, so apnea often occurs during induction if the drug is not administered slowly [15].

Table 1. Drugs and their combinations for anesthesia

Drug and/or drug combination	Indication	Adverse effects	Method of drug administration	Specific antagonist	References
Medetomidine	Sedation	Bradycardia, hypotension, respiratory depression, hypothermia and diuresis	IM; SC	Atipamezole	[6; 11; 16]
Dexmedetomidine	Sedation	No information was found in the studied articles	IM; SC	Atipamezol	[17; 23]
Ketamine	Anesthesia	Skeletal muscle tone, risk of depression or respiratory arrest.	IM	No information was found in the studied articles	[7; 13; 18]
Propofol	anesthesia	No information was found in the studied articles	IV slowly	No information was found in the studied articles	[13; 14; 19]
Zoletil 100 + Xyla	Sedation + anesthesia	Transient hypertension, prolonged hypotension, a decrease in cardiac output by 30–50 %, diuresis, hypothermia, hyperglycemia, cerebral hypoxia and a decrease in intestinal motility lasting for several hours	IM	No information was found in the studied articles	[15; 20–23]
Alfaxalon + Xyla	Sedation + anesthesia	Hypotension and hypoventilation have a significant effect on the cardiovascular system, manifested by a low pulse rate.	IM	Atipamezol	[24–26]
Ketamine + Xyla	Sedation + anesthesia	Hypotension and hypoventilation have a significant effect on the cardiovascular system, manifested by a low pulse rate.	IM	Atipamezol	[16; 20; 27–29]
Isoflurane	Inhalation anesthesia	Circadian rhythm disorders, as well as hypothermia and hypoglycemia	Inhalation	No information was found in the studied articles	[30; 32–36]
Sevoflurane	Inhalation anesthesia	Circadian rhythm disorders, as well as hypothermia and hypoglycemia	Inhalation	No information was found in the studied articles	[13; 31; 37–39]

"Xyla" + "Zoletil-100"

The drug "Xyla" is used both as a sedative and as a component of balanced combinations of anesthesia. It is believed that the sedative effect of α2-adrenoreceptor agonists is carried out by stimulating the subtypes of α2-adrenoreceptors in the blue spot of the brain stem, which reduces the release of norepinephrine [20]. Although newer drugs "Medetomidine" and "Dexmedetomidine" have a higher specificity to the α2-adrenergic receptor, "Xyla" is still widely used in veterinary medicine [21]. Side effects of this drug are: transient hypertension, prolonged hypotension, a decrease in cardiac output by 30–50 %, diuresis, hypothermia, hyperglycemia, cerebral hypoxia and a decrease in intestinal motility lasting for several hours [22].

The main advantage of α2-adrenergic receptors is the availability of antagonists. Effective relief of xylazine sedation by an antagonist leads to a rapid awakening of the object, as well as to the leveling of most of the previously listed side effects. When deciding on the administration of an antagonist, the researcher responsible for anesthesia should take into account some factors: the antagonist should not be administered until the need for sedative, anxiolytic and analgesic effects of the agonist disappears [23].

To enhance the anesthetic effect, the drug "Xyla" is combined with "Zoletil 100". "Zoletil 100" is used for general anesthesia of animals, it consists of two components: tiletamine and zolazepam. Tiletamine is a general anesthetic of dissociative action, causing a pronounced analgesic effect, but insufficient muscle relaxation. Zolazepam inhibits subcortical areas of the brain, causing anxiolytic and sedative effects, relaxes striated muscles. Zolazepam enhances the effect of tiletamine, and also prevents seizures caused by tiletamine, improves muscle relaxation and accelerates recovery after anesthesia [7].

"Alfaxalon" + " Xyla"

When searching for articles on this drug, we found that "Alfaxalon" was evaluated as an anesthetic in mice.

Alfaxalon is a neuroactive steroid that acts as a GABA agonist. The early version of "Alfaxalon" was insoluble in water, and polyoxyl 35 castor oil was added to it to increase solubility. However, this composition of this drug, which was used as a veterinary anesthetic, was discontinued because the solubilizing agent induced the release of histamine, which contributed to the appearance of anaphylactic reactions [24; 25].

Subsequently, the composition of the drug was changed using 2-hydroxypropyl-β-cyclodextrin as a solubilizing agent, which eliminated these side effects. After the changes, "Alfaxalon" began to gain great popularity in veterinary medicine as a sedative and a component of intravenous general anesthesia in various animal species [24; 26].

One of the studies showed that "Alfaxalon" in combination with "Xyla" can be administered intraperitoneally [25]. The authors found that "Alfaxalon" + "Xyla" provides a longer duration of anesthesia during prolonged manipulations than "Ketamine" + "Xyla". After analyzing this study, the following conclusions can be made: intraperitoneal administration of "Alfaxalon" + "Xyla" provided effective immobilization and anesthesia, which may be suitable for orthopedic operations, imaging or other invasive procedures [25].

"Ketamine" + "Xyla"

Ketamine is used as an additional anesthetic due to its limited ability to provide adequate relaxation of skeletal muscles. In addition, the use of a combination of anesthetics with "Xyla" as an anesthetic in rodents has limitations, including a long induction time and weak local tolerance with intramuscular administration [20; 27].

Analysis of the literature data showed that the combination of "Ketamine" + "Xyla" is one of the most frequently used schemes for anesthesia of mice. Its main disadvantage is a gentle dose – effect curve, which leads to unpredictable consequences. It is noteworthy that comparable dosage regimens of "Ketamine" + "Xyla" in mice can give a variety of results, ranging from surgical anesthesia to death [16; 28].

Combinations of "Ketamine" + "Xyla" can affect the hemodynamics of the brain, causing a decrease in cerebral blood flow and affecting brain oxygenation, which can have mixed effects when visualizing perfusion [28; 29]. In addition, the combination of "Ketamine" with "Xyla" has a significant effect on the cardiovascular system, manifested by a low pulse rate and hypotension [29].

Inhalation anesthesia

This type of anesthesia is based on the introduction of anesthetics into the body in the form of steam or gas through the respiratory tract. The saturation of the body with anesthetics occurs due to their diffusion through the alveoli and depends on the concentration, type of anesthetics, their solubility in blood and tissues, the state of blood circulation and respiratory system [30].

Gas anesthesia has many advantages, including: 1) increased control over the depth of anesthesia; 2) minimization of research variability due to the presence of agents (for example, "Isoflurane") that require minimal metabolism; 3) reduction of cardiopulmonary depression, which leads to increased safety during induction and reduced recovery time [31].

From the proposed spectrum of inhalation anesthesia, we selected the following substances: "Isoflurane" and "Sevoflurane". This is due to the frequency of occurrence of these anesthetics in the literature.

"Isoflurane"

"Isoflurane" is a common inhalation anesthetic in laboratory animal medicine and veterinary practice, although the exact mechanism of its action is complex and not fully understood, it is widely used due to the fact that it is minimally metabolized (< 0.17 %) in the liver and, therefore, less toxic to animal metabolism compared to with injectable anesthetics [32].

"Isoflurane" causes moderate depression of the respiratory and cardiovascular systems, but supports better cardiac function than the combination of "Ketamine" and "Xyla" [33]. However, in addition to these benefits, it has also been found that "Isoflurane" causes reversible object recognition memory deficits, impaired learning function, circadian rhythm disorders, as well as hypothermia and hypoglycemia, which can negatively affect the recovery period. In mice and rats, repeated administration of "Isoflurane" causes a pronounced rejection than a single administration [34–36].

"Sevoflurane"

Sevoflurane was first synthesized in the late 1960s, but was not approved for medical use until 1990 due to concerns about the decomposition of sodium lime and the release of fluorine ions during metabolism, which can also cause nephrotoxicity [37]. After a while, it was proved that none of these problems has clinical significance for humans or animals [38–39]. It is less soluble than Isoflurane, which means that induction and recovery occur even faster. "Sevoflurane" is better tolerated by induction through a facial mask and a camera, since it has low acuity and low irritant effect on the respiratory tract [40]. "Sevoflurane", depending on the dose, causes reversible loss of consciousness and pain sensitivity, suppression of voluntary motor activity, decreased autonomic reflexes, as well as sedation of the respiratory and cardiovascular systems.

CONCLUSION

According to the literature data, the range of topical anesthetics has increased significantly over the past 10 years. Specific antagonists are used for a number of injectable drugs. Analysis of the literature data has shown that inhalation anesthesia is the preferred option for prolonged, complex manipulations/operations, since it is more manageable, agents require minimal metabolism, as a rule, does not require additional sedation with third-party drugs.

But it is also worth considering a number of disadvantages in this method: the cost of equipment, the need for a cleaning system for the removal (withdrawal) of exhaust gases and protection of personnel (oversaturation of employees with gas leads to dizziness, loss of consciousness, tachycardia poisoning, etc.), as well as to work on this equipment requires qualified team and annual maintenance, which carries additional financial costs.

For minimally invasive manipulations, the use of injectable anesthesia is recommended. This type of anesthesia has a number of advantages: the use of the drug and its combination in any conditions, does not require additional cumbersome equipment, staff qualifications, antagonists are used, affordable cost.

There are also disadvantages: poor handling, difficulty in selecting the dosage and administration of the drug due to the small weight of the animal.

All of the above indicates the dynamics of development in the field of anesthesia protocols of laboratory animals.

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