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СОДЕРЖАНИЕ

CONTENTS

South Russian Journal of Cancer 2020, v.1, №3, p. 6-17
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ORIGINAL ARTICLE

DEVELOPMENT OF POSTCASTRATION SYNDROME AND CORRECTIVE EFFECT OF XENON IN EXPONENTIAL DOSE REGIMEN IN YOUNG PATIENTS WITH GYNECOLOGICAL CANCERS

O.I.Kit, N.N.Popova, A.I.Shikhlyarova*, E.M.Frantsiyants, T.I.Moiseenko, A.P.Menshenina, G.V.Zhukova, T.P.Protasova, Yu.Yu.Arapova

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ABSTRACT

Purpose of the study. Investigation of possible optimization of treatment in patients with breast cancer and cervical cancer with low-dose xenon therapy.

Patients and methods. The study included 156 patients with pT1B2N0M0 cervical cancer (CC) and pT2N1M0 breast cancer (BC) of the reproductive age (29–45 years) after radical treatment, including forced surgical castration in hormone-positive breast cancer with concomitant gynecological pathology. Since the formation of pathological syndromes, 1 cycle (5 sessions) of low-dose xenon inhalation therapy (XT) was performed, with an algorithm for xenon dose calculation and exposure according to the exponential pattern of decreasing concentration and increasing exposure, with an individual approach. Together with general clinical and laboratory examinations, we used international scales for assessing the severity of the patient condition by the Kupperman menopausal index (MMI), ESAS, quality of life (MOS-SF-36), in a modification of the Russian International Center, pain (VAS); the types of general adaptive reactions were identified by the method of L.Kh.Garkavi.

Results. Important advantages of a new method associated with a rapid regression of pathological psychosomatic symptoms were revealed after XT. MMI values (p<0.05) decreased, 96.8% of patients reported no pain at all on activity, manifestations of neurovegetative disorders significantly decreased (p=0.02–0.04), and the coefficient of antistress reactions to stress increased, which was congruent with the data on improving the quality of life.

Conclusion. High efficiency of the technology demonstrated possible prevention of surgical menopause development and clinical manifestations of postcastration syndrome in order to improve the quality of life and social rehabilitation of young patients with gynecological cancers.

Keywords:
cervical cancer, breast cancer, postcastration syndrome, xenon therapy, exponential dose regimen, adaptive reactions

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ОРИГИНАЛЬНАЯ СТАТЬЯ

РАЗВИТИЕ ПОСТКАСТРАЦИОННОГО СИНДРОМА И КОРРИГИРУЮЩЕЕ ДЕЙСТВИЕ КСЕНОНА В ЭКСПОНЕНЦИАЛЬНОМ ДОЗОВОМ РЕЖИМЕ У ПАЦИЕНТОК МОЛОДОГО ВОЗРАСТА С ОНКОПАТОЛОГИЕЙ РЕПРОДУКТИВНЫХ ОРГАНОВ

О.И.Кит, Н.Н.Попова, А.И.Шихлярова*, Е.М.Франциянц, Т.И.Моисеенко, А.П.Меньшенина, Г.В.Жукова, Т.П.Протасова, Ю.Ю.Арапова

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РЕЗЮМЕ

Цель исследования. Исследовать возможность оптимизации лечения больных РМЖ и РШМ при помощи низкодозной ксенонотерапии.

Пациенты и методы. В исследование включено 156 онкологических больных раком шейки матки (РШМ) pT1B2N0M0 и раком молочной железы (РМЖ) pT2N1M0 репродуктивного возраста (29–45 лет) после радикального лечения, включая вынужденную хирургическую кастрацию при гормонпозитивном РМЖ с сопутствующей гинекологической патологией. С момента формирования патологических синдромов применяли 1 курс (5 процедур) ингаляционной низкодозной ксенонотерапии (КсТ), включающей алгоритм расчетных доз ксенона и экспозиции воздействия в соответствии с экспоненциальной закономерностью снижения концентрации и увеличения экспозиции с учетом персонализированного подхода. Наряду с общеклиническими и лабораторными исследованиями использовали международные шкалы оценки степени тяжести состояния по менопаузальному индексу (ММИ) Kupperman, ESAS, качества жизни (MOS-SF-36), в модификации Российского Межнационального Центра, боли (ВАШ); идентифицировали тип общих адаптационных реакций по Л.Х.Гаркави.

Результаты. После проведения КсТ были установлены важные преимущества нового метода, связанные с быстрым регрессом патологической психосоматической симптоматики. Значительно снизились показатели ММИ (p<0,05), 96,8% пациенток отметили полное купирование болевых ощущений при нагрузке, достоверно снизились проявления нейровегетативных нарушений (p=0,02–0,04), увеличилось соотношение антистрессорных реакций к стрессу, что совпадало с данными повышения качества жизни.

Заключение. Высокая эффективность используемой технологии демонстрирует возможность предупреждения развития хирургической менопаузы с клиническим проявлением посткастрационного синдрома для улучшения качества жизни и социальной реабилитации пациенток молодого возраста с онкопатологией репродуктивных органов.

Ключевые слова:
рак шейки матки, рак молочной железы, посткастрационный синдром, ксенонотерапия, экспоненциальный режим дозирования, адаптационные реакции

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In the modern structure of oncological diseases in women, about 40% are occupied by tumors of the reproductive system [1, 2], among which the absolute leader is breast cancer (BC) — 20.9%, the second place is cervical cancer (CC) with a frequency of 5.9% [3, 4]. These problems become particularly relevant due to the increase in the number of young women of reproductive age among cancer patients who found themselves after surgical castration in the epicenter of their own systemic disorders of neuroendocrine regulation, inversion of metabolic processes and psychological status [5–8].

First of all, such events are associated with the carcinolytic effects of the tumor on the state of the internal environment. Secondly, after radical treatment of the tumor and disabling ovarian function the trigger mechanism changes. Precisely, the leading pathogenetic factor becomes hypoestrogenia, which forms an equally complex metabolic picture of the post-castration syndrome long before the onset of the natural menopausal period. The frequency of occurrence of this symptom complex is from 50 to 100% among operated women [9]. For young women, ovarian removal is an irreversible loss of reproductive function and, unlike natural menopause, it is always stressful when the body is neither physiologically nor psychologically prepared for the onset of premature menopause [10, 11]. In other words, in contrast to the natural processes of extinction of reproductive function, one-time total shutdown of ovarian function is accompanied by acute neuro-hormonal and metabolic restructuring of the body [12]. The scenario of breast cancer, which has a catastrophic acceleration of spread among young women, is also developing, affecting the most important systemic regulatory and Executive mechanisms of neuro-hormonal status [13, 14]. This requires constant improvement and development of new treatment approaches, including the use of technologies of restorative medicine [15–19]. Replacement therapy with natural estrogens is used as an alternative correction of estrogendeficiency states. At the same time, neurovegetative manifestations are leveled, but the risk of developing cardiovascular and metabolic-endocrine disorders remains [20–22].

Existing treatment and rehabilitation programs with the use of plasmapheresis, relaxation, auto-training, phytotherapy, healing fitness massage, as well as the appointment of anti-anxiety agents from the group of high-potential benzodiazepine tranquilizers, including tricyclic and tetracyclic antidepressants and neuroleptics, do not always have a positive result in achieving full normalization of the disturbed neurovegetative and psychoemotional status.

Taking into account the problem of choosing an adequate accompanying therapy for relieving the complex of side effects, our attention was drawn to the medical experience of using an inert gas — xenon, which is considered to be a highly promising factor in correcting functional disorders in various pathologies, including cancer. It is known that xenon does not have teratogenic and mutagenic properties, and its clinical effect is characterized by nootropic, antidepressant, antihypoxic, immunostimulating, and anti-inflammatory effects [23–25]. Moreover, during the rehabilitation period, it is recommended to use sub-narcotic doses of this biologically active factor.

It would seem that given the antistress effect of xenon, no special testing of individual sensitivity and dose selection is required. However, such a standardized approach for patients with hormone-positive BC and CC at an early stage after surgical treatment is not quite appropriate. This is due to the development of an inadequate response against the background of the formation of post-mastectomy and postovariectomy syndromes or their simultaneous course, which was noted in practice in the form of high reactivity and violent vegetative manifestations after subnarcotic doses of xenon therapy, refusal of medical care.

Due to these circumstances, in order to create an adequate dose regime, we took as a basis the theory of General non-specific adaptive reactions of the body, which contains ideas about a multilevel periodic response system, principles and technologies of activation therapy, as a scientifically based approach to managing the processes of increasing the body's resistance [26].

The purpose of the study: to investigate the possibility of optimizing the treatment of patients with BC and CC using low-dose xenon therapy.

PATIENTS AND METHODS

The criteria for inclusion in the formation of study groups were reproductive age (up to 45 years), diagnosis — locally advanced BC and CC, comprehensive treatment in the FGBU "RNIOI" of the Ministry of health of the Russian Federation in 2016–2018, the stage of surgical treatment. All research protocols were prepared in accordance with the ethical standards of the Declaration of Helsinki (1964, as amended in 2013) and approved by the ethics committee of the FGBU "RNIOI" the Ministry of health.

In the first main group included 60 women with locally developed CC, hospitalized in the oncological Department of the FGBU "RNIOI" of the Ministry of health for comprehensive treatment, including a modified hysterectomy with appendages (classification by Piver III), which in the early postoperative period developed signs of post-castration syndrome. The degree of prevalence of the process according to the TNM classification corresponded to $pT_{1b}N_0M_0$ (n=14), $pT_{2a}N_0M_0$, according to histological analysis — squamous cell cancer of various degrees of differentiation. The second main group consisted of 60 patients of reproductive age hospitalized in the Department of tumors of the skin, soft tissue and breast cancer No. 1 FGBU "RNIOI" of the Ministry of health in order to perform surgical treatment in the form of radical mastectomy by Madden. The degree of prevalence of the process according to the TNM classification corresponded to T2N0M0 st II, histological analysis data — infiltrating carcinoma. The third main group included 36 patients with hormone-positive (luminal) subtypes of breast cancer and such concomitant genital pathologies as uterine fibroids (23 people, 64.3%), endometriosis (9 people, 25.1%), ovarian cyst (4 people, 11.2%). Previously, these patients received combined treatment (surgery in the volume of Madden mastectomy and hormone therapy), and then — surgical treatment in the volume of laparoscopic extirpation of the uterus with appendages. The operation was preceded by obtaining consent from the patients to complete the fertility function. The degree of prevalence of the process according to the classification of TNM corresponded to pt2n3m0 st III, according to histological analysis was diagnosed: infiltrating carcinoma.

Specifically for this study, based on the principles of activation therapy, we developed a new exponential dose algorithm for xenon inhalation therapy, aimed at modeling the harmonious adaptive response of the body by gradually increasing small doses of xenon exponentially with a coefficient of 0.7 and reciprocally reducing the time of each procedure. This excluded the possibility of overdose and provided a soft, but productive recovery of indicators of the functional state of cancer patients after surgical castration for locally advanced BC and hormone-positive CC. We used medical xenon-Xemed, dosage form-compressed gas, produced by AKELA-N LLC, Russia, Moscow region, Khimki, no. 99/363/4, registration certificate LS-000121–240810. Instructions for the use of xenon (Pharmacopoeia article 42–2891–97 of 8.10. 1999 and Pharmacopoeia article of the enterprise no. 42–0523–5109–04. Hazard class-4 according to GOST 12.1007). Order of the Ministry of health of the Russian Federation No. 363 of 10.10. 1999. application of xenon in medical practice. Technology has included the implementation of 5 sessions of xenotherapy (KsT), starting from the third day after surgical castration, carried out every other day in the morning. In the first procedure, the dose algorithm started with the supply of xenon concentration of 12–14% in the volume of the inhaled xenon-oxygen mixture for 20 minutes. Then, during the following procedures, the xenon concentration and exposure value changed reciprocally: the 2nd procedure-14–16%, 17min, the 3rd — 16–18%, 15min, the 4th-18–20%, 12 min., the 5th — 20–22%, 10 min. In other words, the exponential mode of gradual increase in small doses of xenon was accompanied by the reverse mode-an exponential decrease in exposure.

The criteria for the effectiveness of the method were considered to be clinical manifestations in the form of surface sleep, calm breathing and a decrease in heart rate relative to the initial data. The severity of the post-castration symptom complex was assessed based on the results of the modified menopausal index (MMI) Kupperman (1959), modified by E.V. Uvarova (1983), estimated in points [27]. At the stages of treatment, various types of adaptive reactions (signaling criteria for blood morphological composition) were identified by Garkavi.

The adequacy of analgesia was determined based on subjective indicators of questionnaires with an assessment of the degree of pain intensity on a graphical visual-analog scale (VAS):

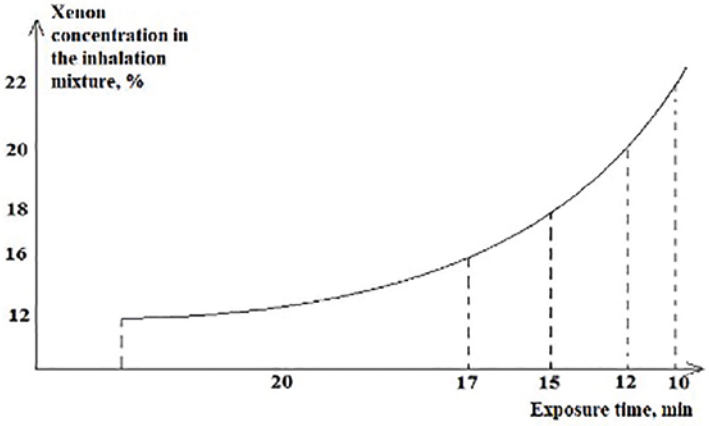

Fig. 1. Calculated exponential curve of the phase algorithm of xenon therapy, reflecting an increase in the concentration of xenon in the xenon-oxygen mixture (vertically), coupled with a decrease in exposure (horizontally).

Table 1. Structure of clinical manifestations of post-castration syndrome in patients with CC, depending on the treatment, with or without xenon therapy, %

Indicators	After surgery , *n*=60	Main group with KcT, *n*=32	Control group , without XT, *n*=28
	%	%	%
Increase in blood pressure	62.7	56.2	71.4
Headache	56.1	56.2	71.4
Dizziness	56.1	50.0	64.2
Sweating	72.6	37.5*	78.5
Hot flushes	59.4	43.7*	78.5
Arrhythmia	33.9	37.5	35.7
Sleep disorders	59.4	25.0*	100.0
Apathy	100.0	18.7*	85.6
Increased fatigability	72.6	12.5*	78.5
Reduced performance	56.1	12.5*	85.6
Anxiety	66.6	25.0*	71.4
Decreased appetite	13.2	12.5	14.2
Psycho-emotional complaints	85.8	18.7*	78.5
Pain in muscles	49.5	12.5	21.4
Violation of carbohydrate metabolism	13.2	12.5	14.2

Note: * – statistically significantly different from the values in the control group, $p<0.05$

0 points — no pain, 10 points — maximum pain. Patients determined their pain after 6 hours, 24 hours, and on the third day after surgery [28]. Postoperative analgesia was considered satisfactory if the level of pain intensity was not higher than 4 points according to VAS. The obtained data were processed using standard computer techniques (Statistica, 8. Microsoft Excel). When comparing the groups, the student's parametric T — test and the nonparametric Mann — Whitney U-test were used. the critical significance level of p was assumed to be 0.05.

RESEARCH RESULTS AND DISCUSSION

The dynamics of the main multi-time menopausal neurovegetative and emotional disorders in patients with a diagnosis of CC, due to the development of post-castration syndrome, and the state of symptoms after standard treatment and after the use of CT are presented in the table 1.

Analysis of clinical data showed that in the early postoperative period, all patients were characterized by a predominance of psychoemotional disorders: apathy in 100%, psychoemotional complaints in 85.8%, increased fatigue in 72.6%, anxiety in 66.6%, sleep disorders in 59.4%. Neurovegetative symptoms also included quite pronounced manifestations: high blood pressure, sweating and hot flashes were observed in patients in 65–72%, headache and dizziness-56.3%, which corresponded to well-known literature sources.Comparative analysis of groups after XT showed the advantage of a new method associated with rapid regression of pathological symptoms: such vegetative manifestations as sweating and hot flashes decreased by 2 times; anxiety by 2.8 times; sleep disorders by 4 times; psychoemotional complaints decreased by 4.2 times; decreased performance by 6.8 times; increased fatigue decreased by 6.2 times.

Table 2. Structure of clinical manifestations of post-castration syndrome in patients diagnosed with BC and forced castration with or without xenon therapy, %

Indicators	After the surgery , *n*=36	Main group with XT, *n*=19	Control group without XT, *n*=17
	%	%	%
Increase in blood pressure	61.6	36.8*	75.4
Headache	78.4	36.8*	52.2
Dizziness	78.4	26.3*	52.2
Sweating	67.2	26.3*	75.4
Hot flushes	67.2	26.3*	75.4
Arrhythmia	42.0	13.2*	29.0
Sleep disorders	100.0	36.8*	87.0
Apathy	86.8	26.3*	75.4
Increased fatigability	25.2	13.2*	75.4
Reduced performance	100.0*	36.8*	58.8
Anxiety	95.2*	26.3*	58.8
Decreased appetite	25.2	36.8	34.8
Psycho-emotional complaints	100.0	26.3*	87.0
Violation of carbohydrate metabolism	25.2	26.3	29.0

Note: * – statistically significantly different from the values in the control group, $p<0.05$

As for patients with hormone-positive (luminal) subtypes of BC, the multivariance and severity of clinical symptoms of post-castration changes after forced surgical castration are poorly studied and insufficiently reflected in the literature. In our study, we analyzed the most actively manifested clinical signs of post-castration syndrome and their reduction using XT. This data is presented in the table 2.

Analysis of the results showed that the use of personalized exponential programming technology for low-dose xenotherapy in patients with hormone-positive BC after total ovarian removal significantly differs from the results of patients with a similar diagnosis and treatment without xenotherapy. This is reflected in the positive dynamics and regression of pathological symptoms on the part of neurovegetative disorders. First of all, we managed to normalize the level of blood pressure, reduce the frequency of arrhythmia episodes, headache and dizziness by 2 times, vegetative crises by 3 times, reduce the frequency of anxiety by 2.5 times, sleep disorders by 2.3 times, apathy by 3 times, psychoemotional complaints by 3.5 times, increased fatigue by 6 times.

The score coefficient allowed us to determine the frequency of detection and severity of MMI in patients who underwent forced castration, data on which are shown in table 3.

As can be seen from table 3, the level of MMI, which characterizes a mild degree of psychoemotional and somatic disorders in patients diagnosed with BC after forced castration, was found with the lowest frequency and level of score compared to those in patients with moderate and severe MMI. The data show that during this period, the vast majority of patients had moderate and, especially, severe MMI. A different situation appeared when comparing the indicators obtained after the XT with the group without the use of xenon. It was noted that in the main group, the frequency of moderate and severe MMI decreased significantly (by 4.8 and 2.4 times, respectively) and the level of score significantly decreased.

Table 3. Determining the severity of post-castration syndrome in patients diagnosed with breast cancer and forced castration using xenon therapy

The severity of MMI	After the surgery , *n*=36		Main group with XT, *n*=19		Control group without XT, *n*=17	
	%	Points	%	Points	%	Points
Mild degree	5.6%	22.6±3.5	73.6%	19.3±2.8	11.8%	24.4±2.9
Middle degree	19.6%	41.2±3.1	12.2%	37.2±2.0*	58.5%	44.2±3.4
Severe	72.8%	61.3±4.2	12.2%	54±3.6	29.5%	66.1±6.1

Note: * – statistically significantly different from the values in the control group, $p<0.05$

Table 4. Relief of pain in the postoperative period in CC patients during xenon therapy

Indicators	After the surgery, *n*=60	Main group with XT, *n*=32	Control group without XT, *n*=28
	%	%	%
Pain in rest	59.2	12.6*	31.5
Pain in minor activity	70.4	19.2*	45.5

Note: * – statistically significantly different from the values in the control group, $p<0.05$

This coefficient clearly demonstrated the effectiveness of the xenotherapy technology used in the conditions of a simultaneous course of post-mastectomy and post-castration syndrome in young patients diagnosed with hormone-positive BC.

We took into account that in the complex of combined treatment of cancer patients, the surgical component is one of the main ones. Postoperative pain is an unavoidable symptom of the operated patient due to surgical tissue damage, the presence of drains and, in some cases, the development of postoperative complications. However, postoperative pain is usually underestimated and can lead to cardiorespiratory, thromboembolic, and other complications with poor quality of life [29]. Cancer patients, more than others, need protection from operational aggression associated with radicalism, the removal of regional lymphatic collectors, with the actualization of the issue of effective methods for relieving pain in the early postoperative period. To assess the severity of subjective manifestations of pain syndrome, the intensity of pain was assessed at rest and with moderate physical activity on the 3rd and 9th days after surgery. In the main group of patients, it was suggested to determine their pain before and after a session of xenon therapy.

On the example of determining the severity of pain in patients with a diagnosis of CC, it was found that on the 3rd day after surgery, before the start of xenotherapy, the average level of pain in all patients studied was 3 points (range 2–4). At the same time, 59.2% of patients registered pain in the area of the postoperative wound at rest and 70.4% of patients — pain with a slight load (when moving in bed).

At the end of the course of xenon therapy, no more than 12.6% of patients diagnosed with CC complained of pain, while in the control group, the relative number of such patients was 44.8% (table 4).

When analyzing the results of the analgesic effect of xenon in patients with luminal subtypes of BC in the early post-castration period, was noted that after the first procedure with xenon, almost all patients - 96.8% - noted complete relief of pain symptoms at rest and a significant reduction in pain during exercise. The pronounced analgesic effect was maintained for 10-12 hours. That's noteworthy, that migraine pain is reduced in 4 cases, without the use of additional medications, even after a single procedure with xenon–oxygen mixture.

To determine the adaptive status, we used an algorithm that included, first, an individual assessment of the type of AP at different stages of the study and the formation of a General group structure of AP, second, calculating the quantitative share and cluster formation of each reaction in the group, and finally, calculating the ratio of the total cluster of antistress reactions to that for stress (K=AC/S). In fact, this coefficient represented the relative digital equivalent of the dominant adaptive state and allowed us to objectify the effectiveness of the xenon therapy used.

When studying the structure of AP in a group of patients with CC in the early postoperative period, deep changes in the reactivity of the body of a pathological nature were noted. In the vast majority of patients, a General non-specific stress reaction in acute form was identified. The frequency of detection of this reaction increased by more than 2 times relative to the background (before the operation). On the contrary, the frequency of development of an integral reaction of calm activation of the physiological type decreased by almost 4 times, and increased activation was not detected in any case. During the recovery postoperative period, the situation did not change dramatically under the standard regime of accompanying therapy, namely, the stress reaction prevailed, which acquired a chronic form.

The use of programmed modes of low-dose xenon therapy in the early postoperative period in patients with CC contributed to the formation of the AP structure. The dominant link was antistress type reactions, the detection rate of which was close to 80%. Of these, the predominant type was the training response, characterized by the severity of anabolic processes, the development

of protective inhibition in the CNS, the functional activity of the endocrine and thymic-lymphatic system within the lower half of the norm, and a gradual increase in non-specific, including antitumor resistance. In the interpretation of L.H. Garkavi, M.A. Ukolova, E.B. Kvakina, the normotype of this reaction is characterized by an increase in the anti-inflammatory potential, which is of great importance for accelerating the healing and restoration of protective systems.

Speaking about the change in the integral indicator of adaptive status, represented by K=AC/S, it can be seen that its initial values decreased by 8.7 times after surgery, which indicated a natural shift in the ratio towards the pathological type of AR-stress. During recovery therapy without xenon, the value of K=AC/S increased only 1.6 times compared to the previous level. In contrast, the dynamics of the AR/C ratio increased significantly after the use of programmed modes of low-dose xenon therapy, exceeding the K values after surgery by 17.5 times. This indicator demonstrated the high significance of the biotropic effect of xenon for regulating the adaptive status of the body and, consequently, the possibility of regression of post-castration syndrome in women of reproductive age after radical oncogynecological operations. These changes in the AP ratio at the stages of treatment of CC patients can be illustrated by a graphical curve (continuous line) reflecting the course of adaptive processes as a result of an individualized approach using the developed modes of low doses of xenon (fig. 2).

For comparison, we analyzed the structure of AR in BC patients with post-mastectomy syndrome, whose complex treatment included radical breast surgery. An important point in the group of patients with BC was the comparison of the structure of AR 9 days after surgery, when the recovery period passed without the use of low-dose xenon therapy. According to the data obtained, no cases of chronic stress were observed in the group of patients without xenon, while the structure of antistress reactions was completely restored, which was close to the original one. The difference was that in the initial state, the reaction of increased activation dominated, and in the subsequent recovery period, it developed in 1.4 times fewer cases. At the same time, the largest cluster was the reaction of training, which represented a symptom complex that leads not to exhaustion, but to the accumulation of reserves with small energy expenditures and moderate functional activity of regulatory systems whose activity is balanced.

When studying the structure of AR in BC patients with post-mastectomy syndrome, the situation was almost identical after low-dose xenon

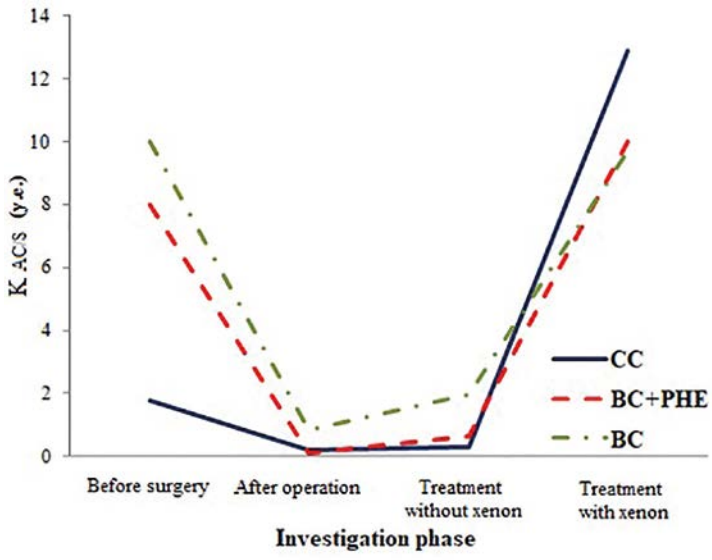

Fig. 2. Dynamics of the ratio of antistress reactions and stress in young patients with reproductive oncopathology in the development of post-castration syndrome and xenotherapy.

therapy. The structure of AR consisted only of clusters of antistress reactions with twice the frequency of development of the training reaction over calm and increased activation, the representation of each of which was the same. In this case, it seemed appropriate to compare the level of values of K=AC/S when using xenon therapy and without it in BC, which showed equally stable indicators of the dominance of antistress reactions. This parallel led to the conclusion that there is no need to include this technology of rehabilitation and rehabilitation therapy in this group of patients.

On the contrary, against the background of these results with BC, the situation was clearly manifested, which indicated the mandatory inclusion of xenotherapy in hormone-positive subtypes, when surgical treatment is not limited to mastectomy, but in the presence of a pronounced pathology of the genitals is accompanied by surgical castration. Thus, when considering the as/S ratio in the period after 9 days after ovarian removal in BC patients without xenon, the value of K=AC/S was 12 times less than the initial value, although it exceeded the postoperative level of values (fig. 2).

In the same period of time after the end of the developed programmed mode of low-dose xenon therapy after surgical castration, it was demonstrated that it was possible to achieve the highest K=AC/S, exceeding that without the use of xenon by 15.2 times. It was this co-ordinal difference that emphasized the need for effective use of such a biologically active factor as xenon, which has a regulatory effect on the integral adaptive mechanisms of the body as a whole.

It should be noted that the inclusion of mechanisms that limit or completely suppress the development of stress under the influence of adequate functional load primarily affects the higher regulatory centers of the CNS, in particular the hypothalamic-pituitary regulation of the thyroid and sex glands, the adrenal cortex, inter-system immune-hormonal relations, the processes of metabolism, proliferation, and apoptosis. In other words, small launcher reason, the role of which in this case executes the xenon, is able to launch the cascade of complex functional transformations on the level of regulatory and Executive systems of the body, helping maintain the structure of physiological reactions through the processes of self-organization. Due to the possibility of developing various discrete States, the organism, as a complex open nonlinear system, makes phase transitions to the most favorable state at the moment. When a stress reaction is protected by damage and high energy consumption, and with physiological loads, such a response is biologically impractical and even a small impact (xenon) against the background of a large one (antitumor treatment) will cause an adequate ONAR of a physiological type of another level of reactivity, which was confirmed by us. However, it should be taken into account that the severity of the stress effect will differ when performing BC surgical treatment followed by surgical castration or limited only to radical breast surgery.

CONCLUSION

Thus, the research allowed us to justify the effectiveness of using a personalized approach to xenon therapy by developing a programmable exponential algorithm for dosing the xenon-oxygen mixture. As a result, a pronounced regulatory effect was obtained, which consists in restoring the adaptive status against the background of removal of the reproductive system organs in women of childbearing age. This effect demonstrates its importance for justifying the prevention of the development of surgical menopause with the clinical manifestation of post-castration syndrome in the form of functional psychoemotional and neurohumoral disorders in order to improve the quality of life and social rehabilitation.

Authors contribution:

Kit O.I. – determination of a relevant direction to study, general management of the study.

Popova N.N. – study performing, processing and analysis of results, manuscript writing.

Shikhlyarova A.I. – formulation of the purpose of the study, establishment of the study design and exposure algorithm, analysis of results, manuscript writing.

Frantsiyants E.M. – analysis of results.

Moiseenko T.I. – statement of specific clinical objectives of the study, monitoring of patients.

Menshenina A.P. – direct formation of patient groups and clinical support of the study.

Zhukova G.V. – analysis of results, manuscript writing.

Protasova T.P. – assessment of the adaptive status of patients.

Arapova Yu.Yu. – assessment of parameters of the psychosomatic status of patients.

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ORIGINAL ARTICLE

USE OF SKIN-FASCIAL FLAPS ON PERFORATING VESSELS IN THE SURGICAL TREATMENT OF SKIN MELANOMA

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ABSTRACT

Purpose of the study. Improving the results of surgical treatment of melanoma of the skin of the extremities by using skin-fascial flaps on perforating vessels.

Patients and methods. In 42 patients with limb skin melanoma T1–3N0M0, the closure of a skin defect was performed by islet flaps on perforating vessels. Perforating vessels of the donor zone were detected with an assessment of the blood supply of the flaps in the pre- and postoperative period using ultrasound and marking of perforants with adjustment of the preliminary marking of the flaps.

Results. Permanent perforating vessels with a diameter of more than 1 mm were used. After excision of the tumor, on the opposite sides of the wound defect, taking into account the location of the perforating vessels, flaps were taken, with further mobilization by excision of the fiber and muscle fascia, they were separated from the underlying tissues while maintaining the integrity of the supply vessels. The circulatory state of the selected flaps was determined by skin color and capillary response to digital pressure. The flaps were displaced to the center, covered the area of the defect and sutured with single sutures, the edges of the donor wound were mobilized, sutured with single sutures until light tension appeared and sutured into the remaining wound defect. In the postoperative period, the determination of the parameters of the blood flow of perforating vessels showed the absence of hemodynamically significant violations of the blood flow during the movement of the flap. Transient ischemia of one of the oncoming flaps after surgery developed in 11.9%, marginal necrosis of the distal flap — in 7.1% of cases. A normotrophic scar was formed, with a width of not more than 0.3 cm, which aesthetically satisfied 92.8% of patients. Assessment of two-year relapse-free survival showed a complete absence of local relapses.

Conclusion. The flaps vascularized by perforating vessels have high viability, are identical in color and texture to the skin of the recipient area, and the close proximity to the receiving area contributes to minimal deformation of the donor area, which increases the radicality of the operation, reduces the incidence of postoperative complications and improves aesthetic and functional results.

Keywords:
limb skin melanoma, islet skin-fascial flaps, perforating vessels, ultrasound, blood flow intensity of perforating vessels

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ОРИГИНАЛЬНАЯ СТАТЬЯ

ИСПОЛЬЗОВАНИЕ КОЖНО-ФАСЦИАЛЬНЫХ ЛОСКУТОВ НА ПЕРФОРАНТНЫХ СОСУДАХ В ХИРУРГИЧЕСКОМ ЛЕЧЕНИИ МЕЛАНОМЫ КОЖИ

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РЕЗЮМЕ

Цель исследования. Улучшение результатов хирургического лечения меланомы кожи конечностей с использованием кожно-фасциальных лоскутов на перфорантных сосудах.
Пациенты и методы. 42 больных меланомой кожи конечностей T1–3N0M0. Закрытие кожного дефекта выполнялось островковыми лоскутами на перфорантных сосудах. Детекцию перфорантных сосудов донорской зоны с оценкой кровоснабжения лоскутов осуществляли в пред- и послеоперационном периоде с помощью УЗИ и производили их маркировку с последующей корректировкой предварительной конфигурации лоскутов.
Результаты. Использовались постоянные перфорантные сосуды диаметром более 1 мм. После иссечения опухоли на противоположных сторонах раневого дефекта, учитывая расположение перфорантных сосудов, выкраивали лоскуты по разметке, рассекая кожу, жировую клетчатку и фасцию подлежащей мышцы, отделяли лоскут от мышечной ткани, сохраняя при этом целостность перфорантных сосудов. Состояние кровообращения лоскутов определяли по цвету кожи и капиллярного ответа на пальцевую компрессию. Лоскуты смещали к центру, укрывали область дефекта и сшивали между собой, края донорской раны мобилизовывали, ушивали одиночными швами до края перемещенных лоскутов. В раннем послеоперационном периоде исследование параметров кровотока в перфорантных сосудах выявило отсутствие гемодинамически значимых нарушений. Преходящая ишемия одного из встречных лоскутов после операции развилась в 11,9% наблюдений, краевой некроз дистального лоскута — в 7,1% случаев. Формировался нормотрофический рубец, шириной не более 0,3 см, который эстетически удовлетворял 92,8% пациентов. Двухлетняя безрецидивная выживаемость показала отсутствие местных рецидивов.
Заключение. Лоскуты на перфорантных сосудах высоко жизнеспособны, сопоставимы по текстуре и цвету коже реципиентной области, а близость лоскутов к воспринимающему ложу минимально деформирует донорскую зону, что повышает радикальность операции, снижает число послеоперационных осложнений и повышает эстетические и функциональные результаты.

Ключевые слова:
меланома кожи конечностей, островковые кожно-фасциальные лоскуты, перфорантные сосуды, ультразвуковое исследование, интенсивность кровотока перфорантных сосудов

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Информация о финансировании: финансирование данной работы не проводилось.
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ABSTRACT

The incidence of cutaneous melanoma is increasing worldwide on average 2 times every 15 years [1], and the frequency of deaths is up to 80% of cases among all forms of malignant skin tumors [2, 3]. Surgical method is the main in treatment of the disease, but replacement of the skin defect often causes difficulties due to lack of local tissues or unfavorable for the healing of autodermatoplasty, in connection with the peculiarities of the recipient bed and the deficit area of the surrounding skin [4]. These problem areas are the upper and lower extremities, and the localization of skin melanoma in this area occurs in 73.4% of cases.

One of the main tasks in plastic closure of defects is to preserve the viability of displaced tissue fragments with their primary engraftment in the receiving bed, which depends on an accurate assessment of blood circulation in the displaced tissues [5, 6]. for closing soft tissue defects of the extremities, perforant flaps are successfully used, since the formation of such

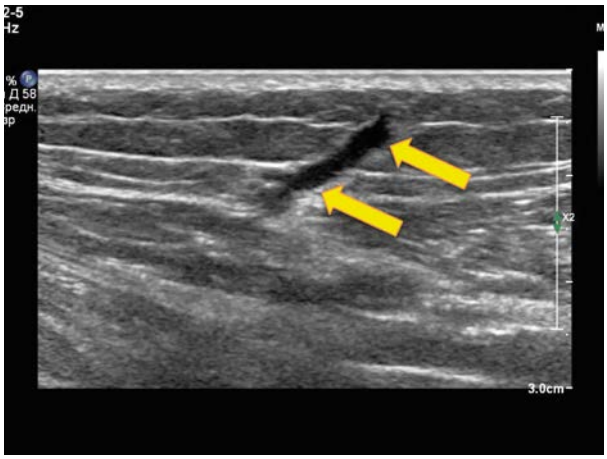

Fig. 1. image of the perforant vessel (indicated by arrows) at the broadband range of the linear sensor 18-4 MHz in the mode of high-contrast seroscale sonography.

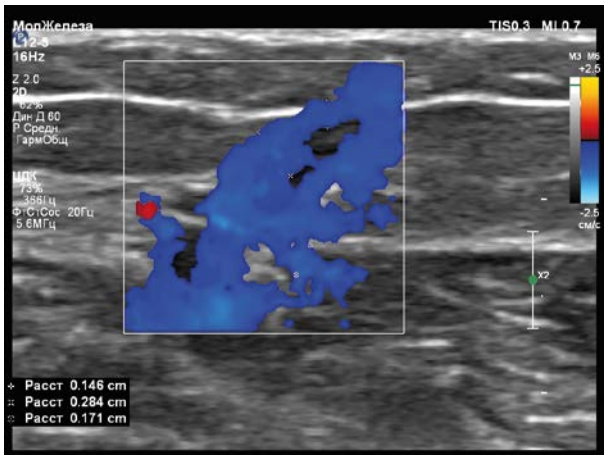

Fig. 2. Branched network of perforant veins in magnification mode with color Doppler mapping of blood flow, measurement of tributary diameters (D=0.1 cm, D=0.2 cm, D=0.3 cm).

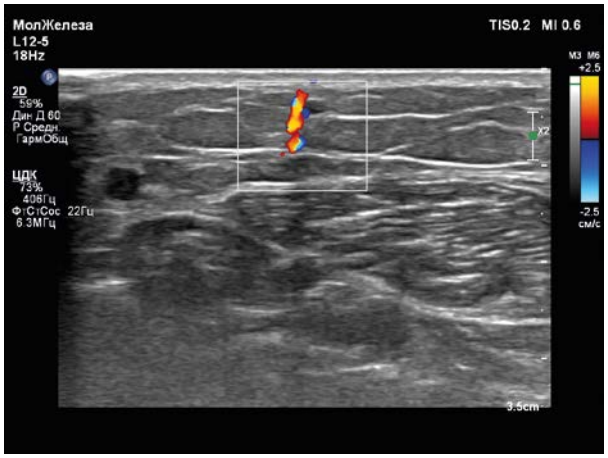

Fig. 3. Perforant artery in color Doppler mapping mode.

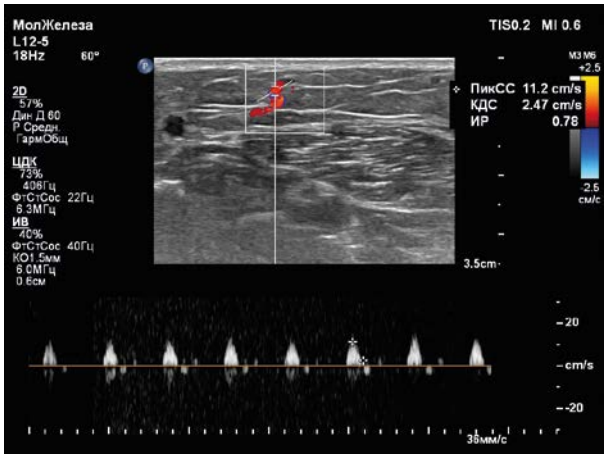

Fig. 4. Dopplerometry-determination of maximum velocity parameters in the perforant artery (MAS-11.2 cm / s).

flaps is not associated with significant trauma of the donor zone, which allows for the plasticization of skin defects with tissue complexes from the same anatomical area. These flaps are identical in color, texture and texture to the lost tissues, therefore, they contribute to a favorable course of the postoperative period and obtain good aesthetic results [7]. The use of perforant flaps allows not to affect the main arteries, which is important for possible subsequent microsurgical reconstruction using this zone as a recipient [8].

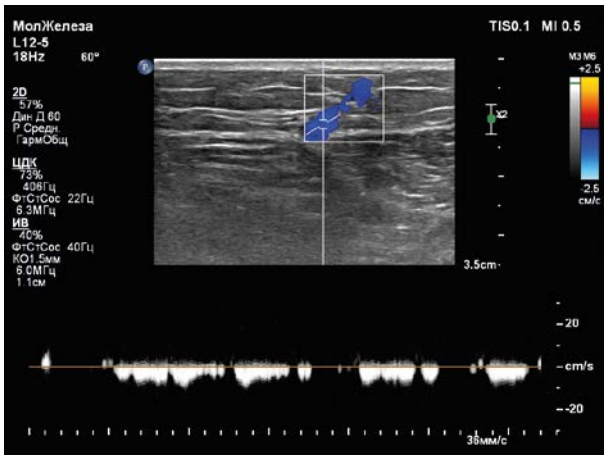

Fig. 5. identification of the perforant vein for determining the parameters of the maximum venous blood flow rate (MVS 9.0 cm / s).

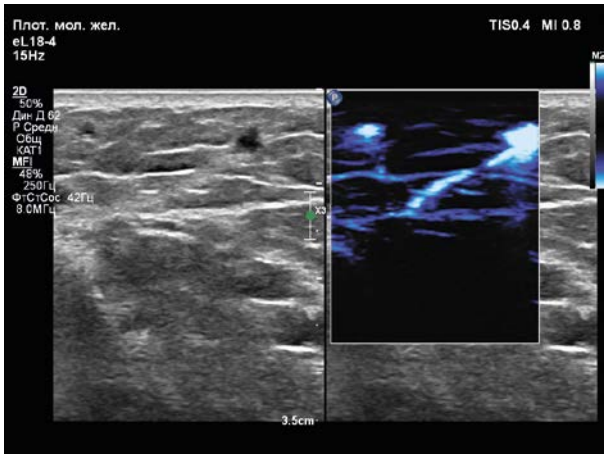

Fig. 6. Image of the microvascular network of perforant vessels in the energy Doppler mode at a broadband frequency range of 4-18 MHz when selecting appropriate vessels for moving the flap.

When the skin melanoma is located on the extremities, it is possible to use a method for replacing extensive wound defects with insular skin-fascial flaps fed through perforant vessels. Flaps of triangular or Crescent shape, there are skin-fat, with dissection at the level of subcutaneous fat (epifascial) or skin-fascial, with the release of muscle fascia (subfascial). These flaps are supplied with blood by perforant musculoskeletal vessels originating from segmental vessels that penetrate through the fascia into the subcutaneous tissue. In the flap, nutrition is provided by vascular anastomoses between the dermal-subdermal plexus and the superficial capillary plexus of the papillary layer. Knowledge of the topography of the vessels feeding the flap affects the duration of the operation, its trauma, the possibility of successful isolation of the tissue complex, as well as the course and duration of the postoperative period [5].

The purpose of the study: To improve the results of surgical treatment of melanoma of the skin of the extremities by plasticizing the soft tissue defect with flaps based on perforant vessels.

PATIENTS AND METHODS

The work was performed at the Department of reconstructive plastic surgery and Oncology

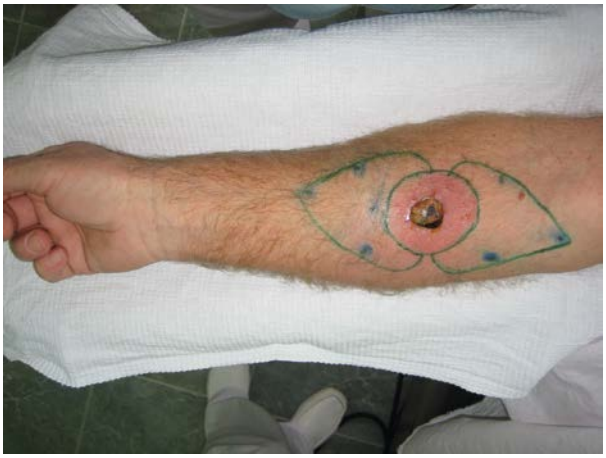

Fig. 7. Perforant vessels are marked with dots on the marking of future flaps.

of the FGBU "NMRC Oncology" of the Ministry of health of Russia in 2015–2020. The study was approved by the local independent ethics Committee of the Rostov cancer research Institute of the Russian Ministry of health IN 2015. All patients signed informed voluntary consent to the use of biological material for scientific purposes.

The study included 48 patients with histologically confirmed skin melanoma of the extremities T1–3N0M0. All patients underwent a surgical stage of treatment with a wide excision of the primary focus and subsequent closure of the skin defect with islet flaps on the perforant vessels. The criteria for inclusion in the study were: the size of the tumor is not more than 25% of the perimeter of the limb at the level of the lesion, the presence of perforant vessels of acceptable diameter in the area of interest (at least 1 mm according to ultrasound scanning). The majority of patients were women-26 (54.2%), men — 22 (47.8%). The average age of patients was 51.7±8.7 years.

Nodular morphological form was found in most cases and accounted for 81.3% (39 out of 48 patients), surface-spreading form was observed in 18.7% (9 out of 48 patients). According to the localization of the neoplasm, the patients were distributed as follows: shoulder-8 (16.7%) patients, forearm — 13 (27.1%), hip — 5 (10.4%), lower leg — 22 (45.8%) patients. Stage I of the process was diagnosed in 8(16.7%) patients, stage II — in 17 (35.4%), stage III — in 23 (47.9%) patients.

When planning the operation, the assessment of the future wound defect was carried out on the basis of the principles of oncosurgery, taking into account the size, shape, depth of tumor invasion and tissue condition, taking into account the operations, radiation therapy and other factors.

The detection of perforant vessels, vessel diameter, and blood flow intensity (MAS — maximum arterial speed and MVS-maximum venous speed) was performed in the pre — and postoperative period (on day 5–7) on the epiq 5 ultrasound device (Phillips) with broadband multi-frequency sensors with a frequency range of 4–18 MHz in the modes of energy, color, (EDM and CDM) and spectral Doppler mapping. The blood flow rate was classified as low — up to 5 cm/s, average–5.1–10.0 cm/s, high — from 10.1 cm/s or more). The type of blood flow was also differentiated (arterial or venous)) (fig. 1–6).

Table 1. The distribution of perforant vessels in diameter

The diameter of perforant vessels, mm	The number of perforant arteries		The number of perforant veins	
	Abs.	%	Abs.	%
1.0-1.5	120	51.7	92	41.1
1.6-2.0	82	35.3	88	39.3
2.1-3.0	30	13.0	44	19.6
The total:	232	100	224	100

The range of the maximum blood speed, cm/s		Average value of the maximum arterial speed, cm / s		The range of maximum venous speed, cm/s		Average value of maximum venous speed, cm / s	
Before the surgery	After the surgery	Before the surgery	After the surger	Before the surgery	After the surger	Before the surgery	After the surger
5-25	5-18	14.2±5.3*	12.1±3.2*	5-12	5-10	9.1±1.3**	7.3±1.2**

Note: *-* * $p>0.05$ (the differences are not valid)

Before the surgery, perforants were detected on the skin with their marking using ultrasound examination, if necessary, with subsequent correction of pre-marked flaps (fig. 7). At the same time, it was necessary to include at least one arterial and one venous perfor-mant vessels in each flap.

If necessary, the shape and location of the flaps changed depending on the topography of the vessels.

RESEARCH RESULTS AND DISCUSSION

During the study, it was found that the surface-spreading type of skin melanoma growth in 100% of the observations had the avascular type of ultrasound image, and the nodular forms — the arterio-arterial type of blood flow (100%) of high and medium intensity (95%). For nodal forms, the characteristic feature was a branched vascular network with multiple vessels of different diameters, the presence of pathological anastomoses and pseudopulse, which are quite typical for neoangiogenesis processes, with a MAS range of 5.3–47.8 cm/s [9, 10].

By ultrasound scanning have been identified peculiarities of topographic anatomy of perforating vessels of the extremities. Most often, non-permanent perforant vessels were detected in the areas that were later used by us during the closure of the wound defect. Thus, the proportion of non-permanent arterial perforant vessels in the upper extremities was: on the shoulder — 60.0% (24 out of 40 perforant arteries), on the forearm — 64.1% (41 out of 64). The assessment of the topographic anatomy of the perforated vessels of the lower extremities also showed the prevalence of non-permanent arteries: on the hip, their share was 56.0% (14 out of 25 detected vessels), and on the lower leg, 80.9% (85 out of 105 detected vessels). It was shown that the most constant perforating vessels was on the front of the thigh — from the pool of hip and knee arteries; on the back of the thigh from the popliteal artery and the medial artery, the envelope of the femur and on the lateral surface of the femur — from the deep femoral artery. The results of this part of the study we can conclude that pre-Doppler monitoring of blood vessels of the donor area when planning for reconstructive plastic surgery is mandatory, because of different localization of pathological formations, as well as a pronounced variability of the vascular topography of the limbs compared to the torso. Earlier, ultrasound scanning in CDM and EDM modes revealed different types of vascularization and blood flow intensity of the pathological foci themselves.

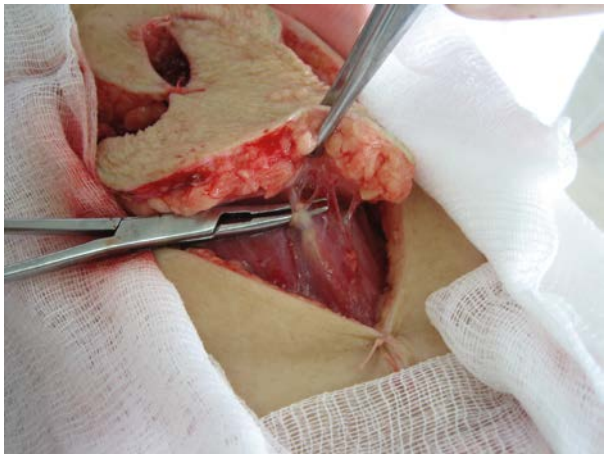

Fig. 8. Skin-fascial flaps are mobilized and fixed with a bridging suture. In the venous clip shows a perforating vessel.

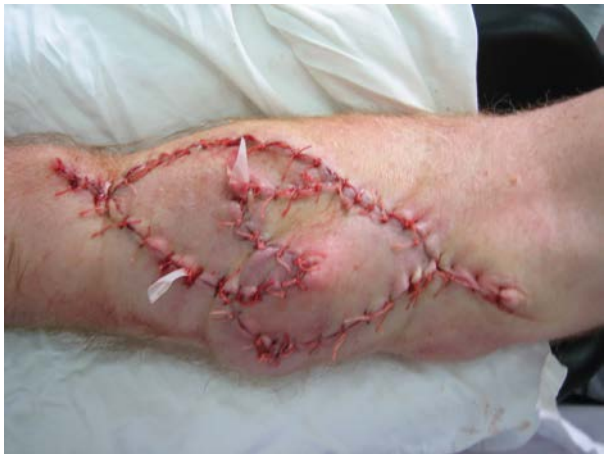

Fig. 9. The appearance of the wound after the surgery. The flaps are displaced along the length and width in the direction of the tissue defect and are sewn together.

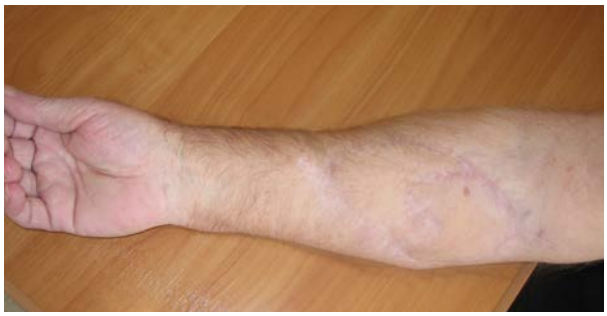

Fig. 10. Aesthetic and functional result of plastic closure of the defect with counter-perforant flaps after 6 months.

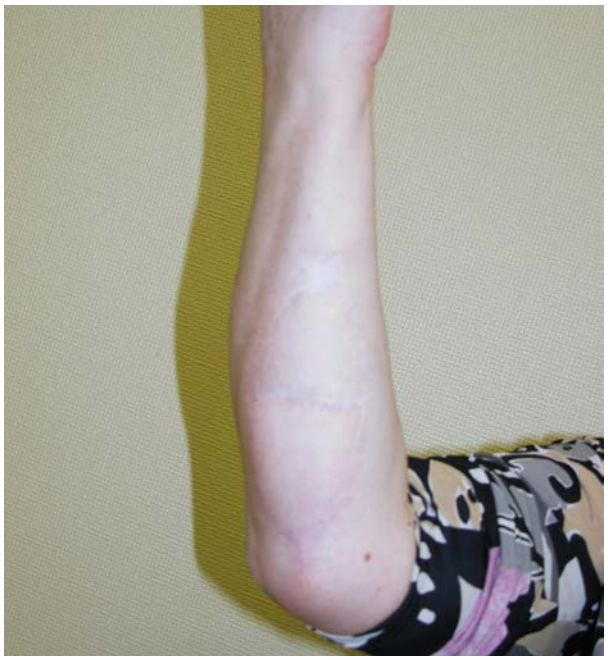

Fig. 11. Aesthetic and functional result of plastic closure of the defect of the medial surface of the forearm with counter-perforant flaps after 8 months.

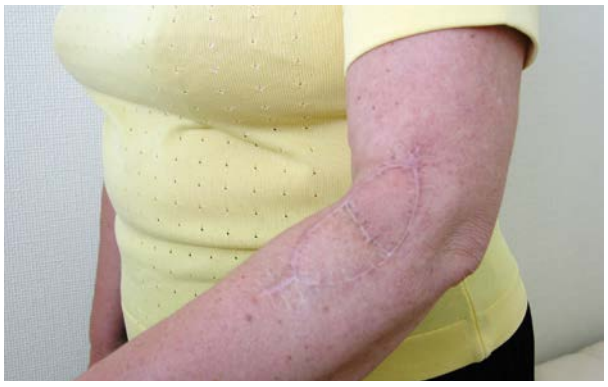

Fig. 12. Aesthetic and functional result of plastic closure of the defect of the lateral surface of the forearm with counter-perforant flaps after 6 months.

When evaluating the size of perforant vessels, it was shown that their internal diameter was from 1 to 3 mm, with a predominance of the range of 1.0–1.5 mm for both arterial and venous vessels (table 1).

Then, under ultrasound control, the identified perforators were marked on the skin, the shape and location of the flaps were corrected, taking into account the vascular architectonics of the donor zone. Under local anesthesia, the pathological focus was excised, retreating to the necessary distance, ensuring the radical nature of the surgical intervention. The most rational, in our opinion, is a circular incision around the pathological focus, since it allows you to remove an equal amount of skin around the tumor from all sides without unnecessary resection of intact surrounding tissues.

On the opposite sides of the defect, triangular or horseshoe-shaped flaps were cut out, taking into account the localization of the perforant vessels. Additional flap mobilization was performed by cutting through the muscle fascia, then the skin-fascial flap was bluntly separated from the underlying tissues while preserving the integrity of the feeding vessels (fig. 8).

It should be noted that hemostasis on the flap was performed exclusively in the bipolar mode, since coagulation of the bleeding vessel on the mobilized flap in the monopolar mode is fraught with coagulation of the perforant vessels, as the only current-conducting bridges. Perfusion of flaps isolated on vascular legs was assessed using two main clinical symptoms: skin color and the rate of capillary response to compression of the flap with a finger. Then the flaps were moved to each other, closing the area of the defect directly or with a lateral offset and sewn together. The skin edges of the donor area mobilized, the distal wound was sutured "on" until a slight tension, then the flaps are sutured by single stitches in the wound defect in the receptor zone. Wounds were drained by rubber graduates in order to prevent compression of the vascular pedicle by the wound separable (fig. 9). the Sutures were removed after 12–16 days.

Ultrasound examination of the blood flow rate in the perforant vessels before surgery found that the linear blood flow rate in the perforant arteries varied from 5 to 25 cm/s (on average, 14.2±5.3 cm/s), and the maximum venous velocity was from 5 to 12 cm/s (on average, 9.1±1.3 cm/s) (table 2).

Determination of blood flow parameters of perfor-manent vessels in the early postoperative period showed that the average MAS was 12.1±3.2 cm/s versus 14.2±5.3 cm/s at the preoperative stage, and the average MVS was 7.3±1.2 cm/s versus 9.1±1.3 cm/s, respectively, which can be regarded as a slight (statistically unreliable) decrease in the hemodynamics of tissue fragments after their movement (table 2).

When assessing the condition of the displaced flaps, in some cases, transient ischemia of one or both flaps was detected, which developed in 5 (10.4%), and marginal necrosis of the distal flap — in 3 (6.3%) cases. When evaluating the aesthetic and functional results, it was noted that in most cases an elastic normotrophic scar was formed, up to 0.2 cm wide, which satisfied 91.7% of patients (fig. 10–12). In 4 patients, the formation of long-term non-maturing hypertrophic scars in places of maximum tissue tension was noted.

CONCLUSIONS

With correct ultrasound detection of perforant vessels, skin-fascial flaps retain a high blood supply potential, correspond in texture, texture and color to the skin of the recipient area, and their proximity to the receiving zone contributes to minimal scarring and contour deformation of the donor area. Preoperative ultrasound diagnostics of perforant vessels contributes to the optimal choice of the location and shape of the flap with the inclusion of arterial and venous vessels of sufficient diameter in its composition. The proposed method of surgical treatment of melanoma of the skin of the extremities significantly increases the radicality of the operation due to the possibility of plastic cover of the resulting defect, reduces the frequency of postoperative complications in comparison with traditional methods of wound closure and improves the aesthetic and functional results.

Authors contribution:
Przhedetskiy Yu.V. – research concept and design, operation, scientific editing of the material.
Pozdnyakova V.V. – technical editing of the article material.
Maksimova N.A. – collection, analysis and interpretation of ultrasound data, preparation of illustrations.
Khokhlova O.V. – operating, assisting on operations, text writing.
Zakharova N.A. – materials processing.
Ilchenko M.G. – collection, analysis and interpretation of data, preparation of illustrations.
Przhedetskaya V.Yu. – design of the bibliography, technical design of the article.

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ORIGINAL ARTICLE

MORPHOLOGICAL AND IMMUNOPHENOTYPIC FEATURES OF THE MONOCLONAL POPULATION OF B-LYMPHOCYTES IN CHRONIC LYMPHOCYTIC LEUKEMIA

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ABSTRACT

Purpose of the study. To evaluate the features of morphological and immunophenotypic characteristics of the lymphoid population with different restriction of light chains of immunoglobulins in patients with chronic lymphocytic leukemia (CLL).

Materials and methods. The study included 30 CLL patients aged 47–79 years (20 men and 10 women). All patients underwent a General clinical blood test (SysmexXE 2100, Japan), morphological examination of the bone marrow (BioVision; Micros, Austria), immunophenotyping of bone marrow and peripheral blood by flow cytofluorometry (Navios10/3, Beckman Coulter, USA). B-cell clonality established by detection of restriction of light chains of surface immunoglobulins kappa or lambda. Morphological analysis of lymphocytes that differ in the expression of light chains of surface immunoglobulins: kappa (k) — group I (22 people — 73,3%), lambda (λ) — group II (8 people — 26,7%).

Results. Determination of cell types by values of direct (FSC) and lateral (SSC) light scattering during immunophenotyping of peripheral blood and bone marrow samples showed that in patients of group I (CD19k+/CD5+/CD23+) on the light scattering diagram, the lymphoid population had low parameters: on the FSC scale — from 200 to 400, on the SSC — from 10 to 160 units, which indicates morphological uniformity of cells. In group II (CD19λ+/CD5+/CD23+), on the contrary, on the light scattering sketogram, the lymphoid zone was heterogeneous and stretched: on the FSC scale — from 200 to 1000, on the SSC — from 10 to 400 units, which indicates morphological polymorphism of cells. There were also differences in the expression of the common leukocyte antigen CD45. In group I, the expression is higher: the population of B-lymphocytes in terms of fluorescence intensity is on the dot graph on the CD45 scale in the second half of the third decade and in the fourth decade — to the right, than in group II, in which B-lymphocytes lie in the third decade. The data indicate that the CD19k+/CD5+/CD23+ population is represented by Mature cells, while the Cd19k+/CD5+/CD23+ population is represented by less Mature and / or intermediate forms. Significant morphological differences in lymphocyte populations were also observed in microscopic studies of blood and bone marrow preparations.

Conclusion. The established immunophenotypic and morphological differences in lymphoid populations expressing either kappa — or lambda-light chains of immunoglobulins may be important for identifying risk groups among patients with biologically heterogeneous variants of chronic lymphocytic leukemia.

Keywords:
chronic lymphocytic leukemia, morphological and immunophenotypic features, kappa / lambda light chains of immunoglobulins, flow cytofluorimetry, general leukocyte antigen, CD antigens

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ОРИГИНАЛЬНАЯ СТАТЬЯ

МОРФОЛОГИЧЕСКИЕ И ИММУНОФЕНОТИПИЧЕСКИЕ ОСОБЕННОСТИ МОНОКЛОНАЛЬНОЙ ПОПУЛЯЦИИ В-ЛИМФОЦИТОВ ПРИ ХРОНИЧЕСКОМ ЛИМФОЛЕЙКОЗЕ

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РЕЗЮМЕ

Цель исследования. Оценить особенности морфологических и иммунофенотипических характеристик лимфоидной популяции с различной рестрикцией легких цепей иммуноглобулинов у пациентов с хроническим лимфолейкозом (ХЛЛ).

Материалы и методы. Обследованы 30 больных ХЛЛ (20 мужчин и 10 женщин) в возрасте 47–79 лет. Выполнены общеклинический анализ крови (SysmexXE 2100, Япония), морфологическое исследование костного мозга (BioVision; Micros, Австрия), иммунофенотипирование костного мозга и периферической крови методом проточной цитофлуориметрии (Navios10/3, Beckman Coulter, США). В-клеточная клональность устанавливалась обнаружением рестрикции легких цепей поверхностных иммуноглобулинов kappa или lambda. Проведен морфологический анализ лимфоцитов, различающихся по экспрессии легких цепей поверхностных иммуноглобулинов: kappa (k) — I группа (22 чел. — 73,3%), lambda (λ) — II группа (8 чел. — 26,7%).

Результаты. Определение типов клеток по значениям прямого (FSC) и бокового (SSC) светорассеяния при иммунофенотипировании образцов периферической крови и костного мозга показало, что у больных I группы (CD19k+/CD5+/CD23+) на диаграмме светорассеяния лимфоидная популяция имела низкие показатели параметров: по шкале FSC — от 200 до 400, по SSC — от 10 до 160 единиц, что указывает на морфологическую однородность клеток. Во II группе (CD19λ+/CD5+/CD23+), напротив, на скетограмме светорассеяния лимфоидная зона была неоднородна и растянута: по шкале FSC — от 200 до 1000, по SSC — от 10 до 400 единиц, что свидетельствует о морфологическом полиморфизме клеток. Отмечены различия и в экспрессии общелейкоцитарного антигена CD45. В I группе экспрессия выше: популяция В-лимфоцитов по интенсивности флуоресценции находится на точечном графике по шкале CD45 во второй половине третьей декады и в четвертой декаде — правее, чем во II-й группе, в которой В-лимфоциты лежат в третьей декаде. Данные свидетельствуют, что популяция CD19k+/CD5+/CD23+ представлена зрелыми клетками, а популяция CD19λ+/CD5+/CD23+ — менее зрелыми и/или промежуточными формами. Значительные морфологические различия популяций лимфоцитов отмечены и при микроскопическом исследовании препаратов крови и костного мозга.

Заключение. Установленные иммунофенотипические и морфологические различия лимфоидных популяций, экспрессирующих либо kappa-, либо lambda- легкие цепи иммуноглобулинов, важны для выделения групп риска среди больных с биологически разнородными вариантами хронического лимфолейкоза.

Ключевые слова:
хронический лимфолейкоз, морфологические и иммунофенотипические особенности, каппа/лямбда легкие цепи иммуноглобулинов, проточная цитофлуориметрия, общелейкоцитарный антиген, CD-антигены

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RELEVANCE

Chronic lymphocytic leukemia (CLL) belongs to a group of b-cell tumors from Mature (peripheral) cells and is a tumor of lymphoid tissue characterized by lesions of the bone marrow and lymph nodes [1]. CLL is a common type of B-lymphoproliferative diseases that mainly affects adults over 50 years of age, progresses slowly and often occurs without visible symptoms for a long time. The disease is detected, most often, by accident [2].

CLL patients are characterized by absolute peripheral blood lymphocytosis (more than 5, 0x10^9/l) and bone marrow lymphocytosis (more than 30%) [3]. Based on the cytological characteristics of lymphoid cells in the FAB classification (Bennet J.M., 1989), two morphological variants of B-CLL are distinguished: typical, represented by monotonous small lymphocytes, and mixed-cell, in which the tumor substrate is heterogeneous and consists of cells with different morphological characteristics — typical and atypical lymphocytes, prolymphocytes [4]. It is shown that in cases of mixed-cell variant B-CLL, the clinical condition, susceptibility to therapy and life expectancy of patients have significantly worse characteristics compared to the typical variant of the disease [5].

In modern diagnostics, detection of the immunophenotype of a tumor population is carried out by the method of flow cytofluorometry of blood/bone marrow. Tumor cells in CLL Express antigens-CD19, CD5, CD23, CD20 (weak), CD22(weak), CD43. B-cell clonality is determined by determining the ratio of expression of κ — and λ — (kappa-, lambda-) light chains of immunoglobulins [6, 7]. It is known from the literature that the concentration of free light chains (FLC) of immunoglobulins (Ig) in blood serum can be considered as a new biological marker that allows to divide CLL on this basis into FLC-positive and FLC-negative forms. In the course of a number of studies, the clonal nature of changes in FLC concentrations was noted and it was found that this criterion can be considered as an integral indicator of the mass of the tumor and a factor of the effectiveness of therapy. CLL patients with different forms may have different prognostic risks for the course of the disease [8]. The relationship of the detected changes in serum FLC Ig concentrations in CLL patients with the clinical picture of the disease is presented in a number of papers [9–12]. Interest in these studies remains high.

However, to date, there is no data on comparing the immunophenotypic differences of the tumor pool expressing the κ — and λ-light chains of immunoglobulins with different morphological characteristics of the tumor substrate in B-CLL, which is of undoubted interest.

The purpose of the study: to evaluate the features of morphological and immunophenotypic characteristics of the lymphoid population with different restriction of light chains of immunoglobulins in patients with chronic lymphocytic leukemia (CLL).

MATERIALS AND METHODS

The study included 30 patients with chronic lymphocytic leukemia aged 47–79 years, median 64.9±8.6 l. among them, 20 men (66.7%) and 10 women (33.3%). All patients underwent a General clinical blood test with the calculation of the total leukocyte count (WBC), the parameters of the leukocyte profile — myelocytes, lymphocytes, neutrophils, monocytes, eosinophils, basophils (SysmexXE 2100, Japan), morphological examination of the bone marrow and peripheral blood using the Pappenheim-Kryukov method, which consists in painting smears with May-Grunwald fixative paint and Romanovsky paint and using the software and hardware complex (BioVision; Micros, Austria), immunophenotyping of bone marrow and peripheral blood by multicolored flow cytofluorometry (Navios 10/3, BeckmanCoulter, USA). The study used native bone marrow and peripheral blood cells in a solution of EDTA anticoagulant. The study panel included a combination of monoclonal antibodies: CD45 PB, CD19 ECD, CD20 PC7, CD22 PE, CD23 PE, CD43 APC, CD200 APC, CD5 PC7, CD5 ARS, CD3 PC7, CD4 FITC, CD8 ECD, CD56 PC5, CD38 FITC, kappa FITC, lambda PE, isotypic controls (Beckman Coulter, USA). Marker expression was taken into account if it was detected in 20% of cells

or more. Expressed expression was established when the antigen was detected on more than half of the cells. Expression of B-linearly associated antigens was evaluated in the gate of CD19-positive cells [7, 13]. B-cell clonality was established by detection of restriction of light chains of surface immunoglobulins (kappa or lambda). Monoclonal variants were considered when the ratio of κ: λ was more than 4:1 or less than 1: 2 [14, 15]. The immunophenotype of the B-CLL leukemic clone was characterized by the expression of CD5+ and CD23+ antigens in a population of CD 19-positive lymphoid cells. The number of cells expressing markers was determined as a percentage.

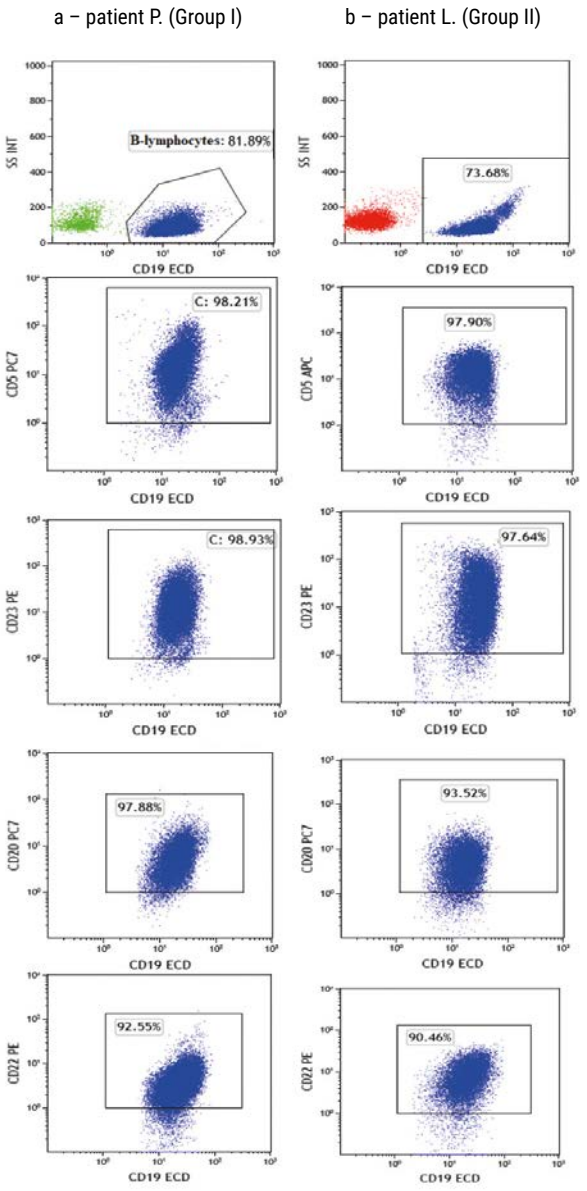

Fig. 1. Results of immunophenotyping of peripheral blood of CLL patients by flow cytometry. Dot graphs of the expression of the main markers analyzed, the blue color indicates the population of pathological B-lymphocytes: a – patient P. (group I), b – patient L. (group II).

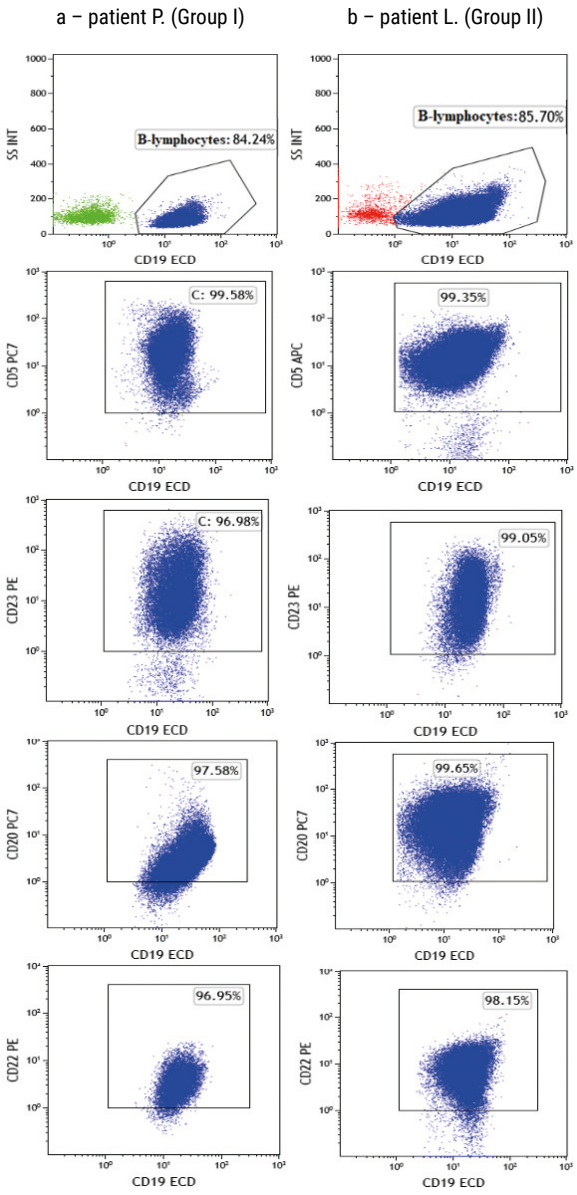

Fig. 2. Results of immunophenotyping of the bone marrow of CLL patients by flow cytometry. Dot graphs of the expression of the main markers analyzed, the blue color indicates the population of pathological B-lymphocytes: a – patient P. (group I), b – patient L. (group II).

RESEARCH RESULTS AND DISCUSSION

The immunophenotype of bone marrow and peripheral blood lymphocytes was studied in all patients with CLL. Monoclonal B-cell proliferation of lymphocytes with an immunophenotype characteristic of B-CLL/lymphoma from small lymphocytes — CD19+/CD5+/CD23+/CD20+ (weak expression)/CD22+ (weak expression) was detected. When determining clonality by restriction of light chains of surface immunoglobulins-kappa or lambda, it was found that in 22 patients (73.3%), tumor cells Express k-light chains of immunoglobulins — from 87.5% to 100% (group I), in 8 (26.7%) — λ — from 95.9% to 100% (group II) (fig. 1, 2, 3).

CD38 activation antigen is represented variably: in group I from 0.1% to 94.5%, in group II from 0.5% to 69.2%. There were no differences in the expression of other markers. However, immunophenotyping of peripheral blood and bone marrow samples revealed certain differences between these groups of patients. The combination of lateral and direct light scattering allows us to judge the morphology of the cell as a whole and identify different cell populations for further analysis. Direct FSC light scattering gives the researcher information about the cell size. Lateral SSC light scattering allows us to judge the presence of granules in the cell, the nucleus/cytoplasm ratio, and other parameters. For example, using only the two detectors listed above allows for primary analysis of white blood cell populations. Lymphocytes are the smallest cells with a round nucleus, located lower on the SSC axis and to the left on the FSC axis, whereas neutrophils are characterized not only by a larger size, but also by polymorphonuclearity, and therefore they are located higher and to the right in the diagram. Thus, in patients of group I (CD19k+/CD5+/CD23+), the distribution of tumor cells showed morphological uniformity, which was reflected in the low values of light scattering parameters in the diagram: the location to the left on the FSC axis — from 200 to 400 units / lower on the SSC axis — from 10 to 160 units. In group II (CD19λ+/CD5+/CD23+), on the contrary, on the light scattering sketogram, the lymphoid zone is heterogeneous and stretched: the location to the right on the FSC axis is from 200 to 1000 units /higher on the SSC axis is from 10 to 400 units, closer to the monocyte zone, which indicates morphological polymorphism of tumor cells. It was noted that this pattern was typical for both peripheral blood and bone marrow (fig. 4).

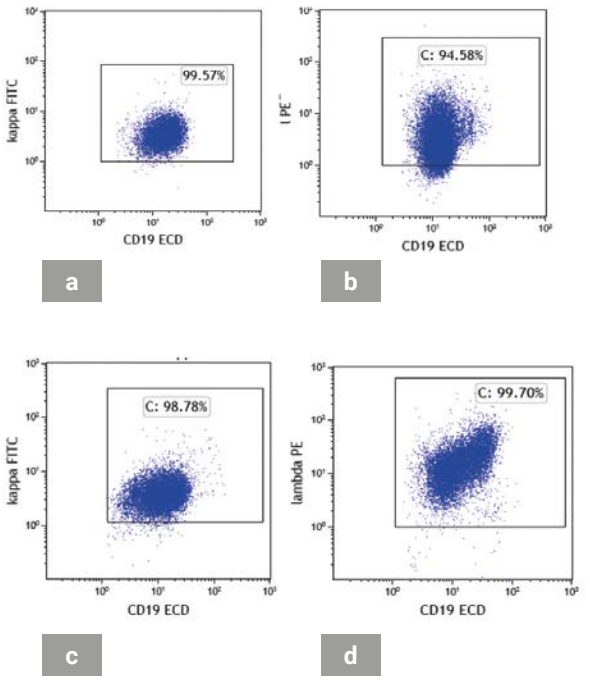

Fig. 3. Histograms of expression of light chains of surface immunoglobulins (kappa/lambda). The population of pathological B-lymphocytes is highlighted in blue: a – peripheral blood of patient P. (group I), b – peripheral blood of patient L. (group II), c – bone marrow of patient P. (group I), d – bone marrow of patient L. (group II).

Also were noted the differences in expression of general leukocyte antigen CD45 in the blood and bone marrow: in the I group, the expression of higher — intensity fluorescence monoclonal population of b-lymphocytes is in a scatter chart on a scale of CD45 in the second half of the third decade and fourth decade — more than in the second. In this group, aberrant B-lymphocytes in terms of fluorescence intensity on the dot graph on the CD45 scale lie in the third decade — to the left than in the I-th (fig. 5).

It is known that the level of CD45 expression increases with the differentiation of hematopoietic cells from immature precursors to Mature forms: on point graphs, cells with minimal ex-

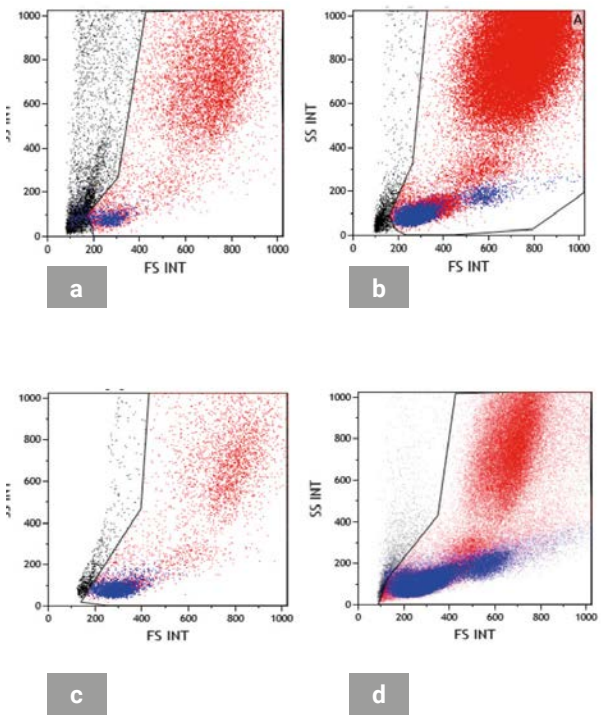

Fig. 4. Results of immunophenotyping of biological material of CLL patients by flow cytometry. Allocation of a lymphocytic gate by parameters of light scattering channels. The population of pathological B-lymphocytes is highlighted in blue: a – peripheral blood of patient P. (group I), b – peripheral blood of patient L. (group II), c – bone marrow of patient P. (group I), d – bone marrow of patient L. (group II).

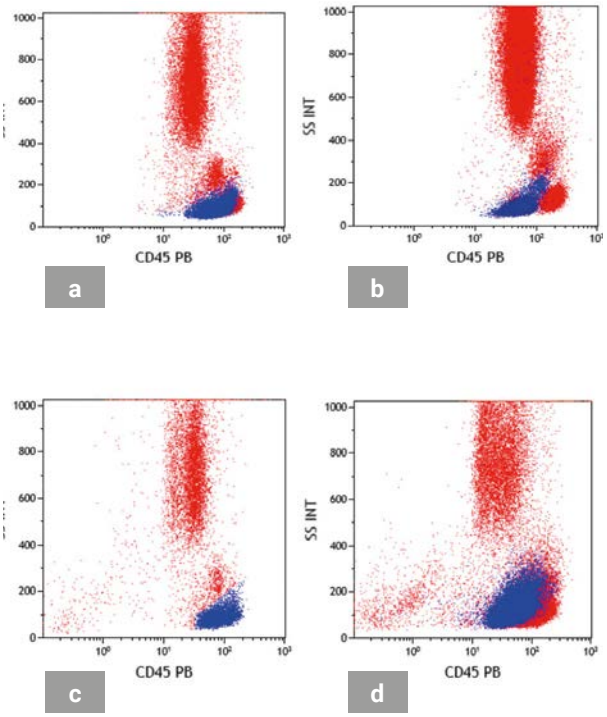

Fig. 5. Results of immunophenotyping of biological material of CLL patients by flow cytometry. Isolation of aberrant b-lymphocyte gate (in blue) by CD45 expression and lateral light scattering (SSC): a – peripheral blood of patient P. (group I), b – peripheral blood of patient L. (group II), c – bone marrow of patient P. (group I), d – bone marrow of patient L. (group II).

Table 1. Indicators of general clinical blood analysis in CLL patients (M±m)

Parameters of the leukocyte profile, %	Groups	
	I *n*=22	II *n*=8
The total number of leukocytes, ×10^9/l	39.96±26.42	35.16±29.30
Myelocytes, %	0.18±0.01	0.50±0.044*
Neutrophils, %	22.05±3.46	15.00±1.41
Eosinophils, %	0.86±0.049	0.42±0.038*
Basophils, %	0.15±0.07	0.67±0.047*
Monocytes, %	2.92±0.31	4.92±0.12*
Prolymphocytes, %	-	8.05±1.73
Lymphocytes, %	72.98±5.76	70.50±7.87

Note: * – differences are statistically significant at p<0.05-0.001

pression (blasts) lie to the left on the scale, myeloid cells occupy an intermediate position, and Mature lymphocytes with the maximum level of expression are located to the right on the scale. [16]. In this regard, it is obvious that in the population of CD19k+/CD5+/CD23+ the tumor clone is represented by Mature cells, and in the population of Cd19k+/CD5+/CD23+ — by less Mature and/or intermediate forms.

The data of the General clinical blood analysis indicate that there are no statistically significant differences in the level of the total number of white blood cells and lymphocytes in patients of CLL groups I and II (table 1). In group I, the WBC was-39.96±26.42×109/l, in group II -- 35.16±29.3 ×109/l and ranged from 10.4 to 113.6 × 109/l and from 14.51 to 85.84 × 109/l, respectively. There is marked lymphocytosis in peripheral blood up to 72.98±5.76% in group I and 70.50±7.87% in group II. However, in group II, in contrast to the I-th, prolymphocytes are determined, making an average of 8.05±1.73% of the total level of white blood cells.

In the bone marrow of CLL patients, pronounced lymphocytosis is observed against the background of suppression of granulocytic and erythroid sprouts of hematopoiesis. The observed changes are more pronounced in group II (table 2). the content of lymphocytes in group I was 62.15±7.47%, in group II — 76.10±8.76% of the total number of myelocaryocytes.

Microscopic examination of blood and bone marrow smears confirmed the differences in the pathological population of lymphoid cells in terms of light scattering parameters established during immunophenotyping. In group I patients (restriction of Kappa light chains of immunoglobulins), lymphoid cells are represented by small cells of the same type with a sparse, often non-visualized cytoplasm. The nuclei have a lumpy chromatin structure, without distinct nucleoli (fig. 6).

In group II patients (restriction of lambda light chains of immunoglobulins) in blood and bone marrow preparations, the size of cells in the lymphoid population varies from small to large, with rounded or folded nuclei, smoothed chromatin structure, 1–2 nuclei, abundant cytoplasm (fig. 7).

So, the degree of severity of lymphoid infiltration of the bone marrow and, as a result, the suppression of granulocytic and erythroid sprouts of hematopoiesis are more pronounced in patients of group II. Attention was drawn to the difference in morphological characteristics of lymphocyte

Table 2. Myelogram indicators in CLL patients (M±m)

Indicators	Groups	
	I *n*=22	II *n*=8
Myelocaryocytes, In 1mcl ×10^9/l	100.68±42.98	104.76±58.82
NBK, %	2.21±0.72	2.98±0.42
Granulocyte germ cells, %	27.63±3.36	14.76±3.92*
Monocytes, %	1.09±0.98	1.00±0.60*
Lymphocytes, %	62.15±7.47	76.10±8.76*
Megakaryocytes, %	0.20±0.06	0.20±0.05
Erythroid germ cells, %	6.87±1.38	4.84±2.88

Note: * – differences are statistically significant at p<0.05-0.001

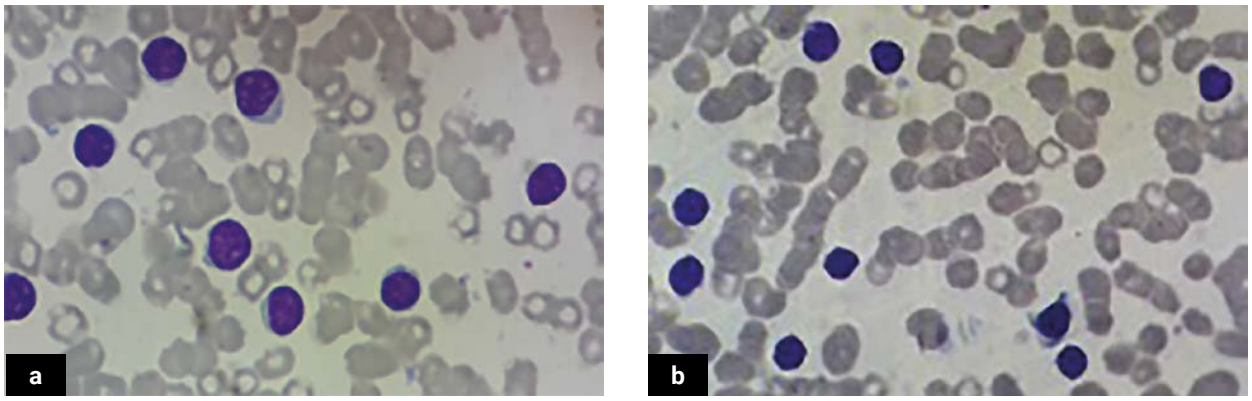

Fig. 6: a – peripheral blood, b – bone marrow. Mature monomorphic lymphocytes with dense nuclei. Painting on Pappenheim-Kryukov ×1000

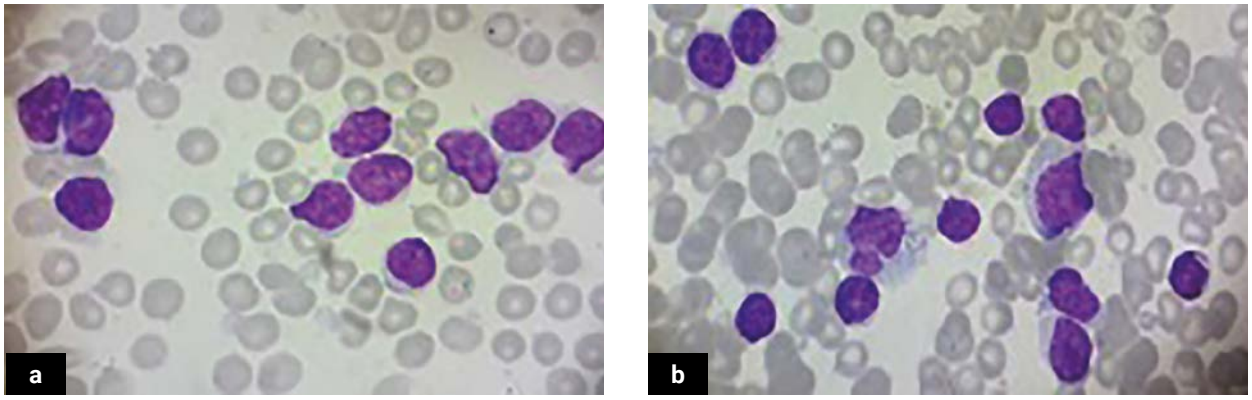

Fig. 7. a – peripheral blood, b – bone marrow. Atypical lymphocytes with a broad rim of cytoplasm, with the nuclei round or folded shape. Painting on Pappenheim-Kryukov ×1000

populations in the blood and bone marrow of the analyzed groups of patients.

Thus, the combination of results of immunophenotyping and microscopic examination of the lymphoid population demonstrated obvious morphological differences between tumor clones with different restriction of light chains of immunoglobulins (kappa or lambda) in patients with CLL, which undoubtedly requires further study.

CONCLUSIONS

The study established immunophenotypic and morphological differences in lymphoid populations expressing either kappa — or lambda-light chains of immunoglobulins. The obtained data are extremely important for identifying risk groups among patients with biologically heterogeneous variants of chronic lymphocytic leukemia (typical and mixed-cell).

Authors contribution:

Guskova N.K. – research design development, morphological research, systematization and analysis of the obtained data, writing the text of the manuscript, consultation.

Selyutina O.N. – performing cytofluorimetric studies, collecting clinical material, systematization and analysis of the obtained data, reviewing publications on the topic of the article, writing the text of the manuscript.

Novikova I.A. – analysis of the received data, consultation.

Maksimov A.I. – analysis of the obtained data, consultation.

Nozdricheva A.S. – collection of clinical material.

Abakumova S.V. – collection of clinical material.

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REVIEW

PRIMARY TUMOR CELL CULTURES: CURRENT METHODS OF OBTAINING AND SUBCULTIVATION

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ABSTRACT

Over the past decades, transplantable cell lines have been an affordable model for studying the biology and effect of chemotherapeutic drugs on tumors. However, numerous studies have shown that these cell lines are not heterogeneous enough and cannot reflect the drug resistance of tumors that occurs in some patients. Primary cell line cultures isolated from solid tumors have become widespread in personalized cancer therapy. This review discusses the basic methods for the preparation and cultivation of primary cell lines. A brief description is given of the methods for the disaggregation of tumor material using enzymatic, chemical and mechanical dissociation. The systems of cultivation of primary cell cultures. The selection of an appropriate dissociation method and cultivation is important to preserve the benefits of primary culture in preclinical studies.

Keywords:
primary cell cultures, cell lines, method of cell dissociation, 2-D culture, 3-D culture, microfluidic platforms, explants

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ОБЗОР

ПЕРВИЧНЫЕ КУЛЬТУРЫ ОПУХОЛЕВЫХ КЛЕТОК: СОВРЕМЕННЫЕ МЕТОДЫ ПОЛУЧЕНИЯ И ПОДДЕРЖАНИЯ *IN VITRO*

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РЕЗЮМЕ

В течение последних десятилетий перевиваемые клеточные линии являлись доступной моделью для изучения биологии и влияния химиотерапевтических препаратов на опухоли. Однако, многочисленные исследования показали, что данные клеточные линии недостаточно гетерогенны и не могут отражать лекарственную резистентность опухолей, возникающую у некоторых пациентов. Культуры первичных клеточных линий, выделенные из солидных опухолей, получили значительное распространение для определения химиочувствительности опухолей к препаратам, применяемым в химиотерапии. В данном обзоре рассматриваются основные методы получения и культивирования первичных клеточных линий. Дается краткая характеристика методикам дезагрегации опухолевого материала при помощи ферментативной, химической и механической диссоциации. Рассмотрены различные системы культивирования первичных клеточных культур. Выбор подходящего метода диссоциации и культивирования имеет важное значение для сохранения преимуществ первичной культуры в доклинических исследованиях.

Ключевые слова:
первичные культуры клеток, клеточные линии, методы диссоциации клеток, 2-D культуры, 3-D культуры, микрофлюидные платформы, эксплантаты

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RELEVANCE

A primary culture is called cell culture at the stage which is immediately after cell isolation from samples and before the first seeding [1]. The primary tumor cell cultures are *ex vivo* populations isolated by surgical resection of tumor tissue fragments [2]. Primary cell lines include both tumor cells and microenvironment cells (fibroblasts, T-cells, vascular endothelial cells) that are relevant in the physiology, structure, and functioning of the tumor [3]. The tumor and it's microenvironment can cause mutual changes in the phenotype and functions that support the continuous process of carcinogenesis. Transferable cell lines derived from a small proportion of tumors, usually very aggressive, are the most common *in vitro* model for research in Oncology. However, such models do not provide a representation of the entire spectrum of tumor subpopulations. Primary cell cultures reflect the high heterogeneity of tumor cells, and represent an important tool for research into the biology of tumors, and new opportunities in personalized medicine in General. While preserving cells with phenotypes similar to those of the original tumor, primary cell lines play an important role in the study of mechanisms of chemoresistance, the search for new drug candidates, which is particularly relevant in preclinical research. The study of interactions between tumor cells and its microenvironment includes the development of optimal models for the study of tumor migration and proliferation [4]. In the era of personalized therapy, researchers need to create more primary tumor lines from patients, which will provide high-quality data for translating *in vitro* results into *in vivo* models and, ultimately, implementation into the clinic. In this review, we will look at the methods currently available for generating and culturing primary tumor cell lines.

TUMOR CELLS ISOLATION METHODS

Isolation and cultivation of tumor cells *in vitro* conditions similar to the microenvironment of the original tumor is a complex task and requires certain methods. Successful isolation of tumor cells with the use of appropriate technologies depends on the method of destruction of the extracellular matrix, which consists of a variety of related factors (connective tissue fibers, glycoproteins and tissue-specific proteins). Additional difficulties in isolating primary cell culture include the presence of tumor material in the samples:

1. cell debris and non tumor cells, that affect the proliferation of tumor cells, often slowing down the proliferative activity of the primary culture;
2. a small number of viable cells due to resection in the necrotic area;
3. fibroblasts, that actively proliferate during the cultivation [5].

The selection of a good method of tumor dissociation material affects the selection of a sufficient number of viable cells and introduction into the primary culture. There are several methods for dissociating material and obtaining primary cell lines from tumors; however, very few methods have been recognized as promising. It is necessary to develop modern techniques adapted to each type of tumor tissue for reproducible generation of primary cell lines from tumors. Currently, such methods as mechanical, chemical and enzymatic disaggregation are used for dissociation of tumor samples [6].

Fermentative Dissociation

The fermentative dissociation is the most usable method for disaggregating tissue and producing a suspension of individual tumor cells, while maintaining their viability and integrity. Usually, proteolytic enzymes are used for dissociation of tumors, including trypsin, papain, elastase, hyaluronidase, collagenase, pronase, and deoxyribonuclease [7]. Some researchers use a mixture of enzymes, such as collagenase/hyaluronidase combinations and a solution of dyspase and DNA-ase to dissociate breast tumor samples [8]. In a study by Volovitz et al. neutral protease (NP) from Clostridium histolyticum, an enzyme not previously used in the field of neuro-

biology, was used for enzymatic dissociation of brain tissues and tumors. Dissociation under the influence of protease allowed to obtain a cell suspension for introduction into the primary culture with significantly higher cell viability, compared to the enzymatic action of collagenase, DNA-ase, and papain [9].

Use of trypsin and accutase in the Skog study et.al on autodermal grafts, it was also shown to produce cells with greater viability after trypsinization, but samples treated with accutase later proliferated better in the primary culture. There was no significant difference between the average intensity of fluorescence of stem cell markers, both after trypsinization and after accutase treatment [10]. For the research of primary cultures of breast cancer Nishikata et.al we used the Explant method and obtained a suspension of cells. For dissociation of a fragment of the tumor used dispute II. The most effective method for obtaining primary culture of breast tumors was considered to be obtaining a suspension of cells after dissociation with a dyspase II [11]. Models for obtaining primary cultures of brain neurons using enzymatic papain dissociation are being actively developed to study cellular and molecular genetic features of brain functioning [12]. Protocols for perfusion of mouse liver fibrous tissue with pronase/collagenase solutions and isolation of mouse liver stellate cells into primary culture are being implemented [13].

Chemical dissociation

Various types of cations maintain the integrity of the cell surface and the intracellular structural matrix [7]. Chemical dissociation is a process in which Ca2 + and Mg2 + cations are washed out of epithelial cells, reducing intercellular interactions. Removal of Ca2 + and Mg2 + is best achieved when exposed to EDTA (ethylenediaminetetraacetic acid) or tetraphenylboron complexes with potassium ions, which are used for dissociation of liver tissues, intestinal crypt cells, and solid breast tumors [14]. Hypertonic solutions of sucrose, maltose, and lactose affect the slit contacts and areas of dense contacts, which causes the presence of cell clusters after enzymatic tissue cleavage [15]. Some researchers perform double perfusion of liver cells with EDTA / collagenase to isolate and culture primary hepatocytes, culturing them with the addition of insulin and glucose [16]. Trojaneck and his colleagues successfully obtained 14 primary melanoma lines using tumor material obtained from 45 patients with melanoma. Tumor samples were affected by EDTA and DTT (dithiotreitol) [17].

Mechanic dissociation

There are several options for mechanical dissociation of tumors: conventional manual homogenization and various automatic dissociators for suspensions of individual cells. Mechanical dissociation of tissue involves shredding a resected tumor sample with scissors or sharp blades, homogenization (using BD Medimachine, Becton Dickinson), filtration through nylon filters or steel mesh filters (with different pore diameters), shaking, re-aspiration through serological pipettes, or any combination of these methods. Usually, tumor samples are first crushed into small pieces (nearly 1–2 mm), and then washed in a tissue-specific medium or salt solutions (Hanks ' solution, Dalbecco's solution) to remove loosely bound cells or non-specific debris by light mixing. In this way, a suspension of individual cells is obtained. Mechanical dissociation is a simple but effective method for removing primary colorectal cancer cell lines obtained from primary tumors with an efficiency of 39.4%, as well as cell lines isolated from corresponding lymph node metastases with an efficiency up to 70% [18]. However, some researchers believe that this type of dissociation of tumor tissue using mechanical methods leads to significant cell death and is not suitable for obtaining tumor cells and introducing them into primary culture [19].

Comparing mechanical and enzymatic dissociation of primary glioblastoma, some researchers prefer enzymatic methods to obtain cells with higher migration activity [20]. Qiu X et.al we have developed a microfluidic device that allows for a softer mechanical cell disaggregation using

a network of channels and "hydrodynamic scalpels" [21]. In the Kar et.al study, the primary ovarian cancer cell lines were successfully obtained both by mechanical tissue dissociation and enzymatically by a Dispase II [22].

The own data

The laboratory of cell technologies National Medical Research Centre for Oncology of the Ministry of Health of Russia studied the possibility of using collagenase from crab hepatopancreas to isolate breast cancer stem cells. After studying the effect of collagenase in three concentrations and a variant without the use of collagenase (using only the mechanical method of disaggregation), we obtained a higher concentration of living cells when applying enzymatic disaggregation [23].

In The next study was used the tumor material obtained from patients with astrocytic tumors. Dissection of the tumor was performed by surgeons of the Department of neuroncology National Medical Research Centre for Oncology of the Ministry of Health of Russia under visual control using the Blue E400 unit of the Opmi Pentero ™ microscope and 5-ALA (5-aminolevulinic acid). The tumor tissue was disaggregated at room temperature on BD Medimachine (Becton Dickinson, USA) in sterile Medicons (Becton Dickinson, USA) with a pore diameter of 50 microns. The study was produced cell lines of low-grade astrocytic tumor material after dissection of the tumor, has also been shown that this method is effective, as it enables selection of a material with viable cells [24].

Isolation of tumor stem cells from brain tumors was performed to obtain primary cell lines. Initially, the noo formation tissue was subjected to mechanical or enzymatic dissociation. Mechanical dissociation of tumor tissue was performed in BD Medimachine (Becton Dickinson) in Hanks solution at room temperature. Enzymatic dissociation was performed using a set of reagents Brain Tumor Dissociation Kit (Miltenyi Biotec) in accordance with the manufacturer's instructions. Based on the results of tests of various combinations of techniques for tissue dissociation: the use of a set or the use of mechanical dissociation, it was found that for brain tumors, the optimal result was achieved when using an enzymatic set. The number of living cells in this case was on average less than in mechanical dissociation, but the proportion of living cells was 2 times higher. In addition, enzymatic treatment of the tissue allowed to obtain a more homogeneous suspension, in which the cells were well separated and did not form conglomerates during subsequent cycles of centrifugation and resuspending.

THE PRIMARY CELL LINES CULTIVATION METHODS

There are several types of cultivation of primary cell lines after dissociation of tumor tissue. The suspension of tumor cells can be cultured in 2D culture (monolayer of cells), 3D culture (spheroids, gels, scaffolds), microfluidic technologies, explants (cultivation of small tumor fragments). After disaggregating the material, the cells are cultured in a nutrient medium, making the necessary tissue-specific additives, fetal bovine serum, amino acids, and antibiotics. Nutrient media are supplemented by various factors detected *in vivo*. In order to maintain the viability and ensure the safety of the genotype and phenotype of tumor cells *in vitro*, mitogenic growth factors are introduced into the nutrient media [25].

The 2D cultures

The 2D cultures are ordinary monolayer cultures, grown under conditions that do not reflect *in vivo* conditions: tissue physiology, tumor microenvironment. The suspension of tumor cells is sown on Petri dishes, culture tablets or vials, conducting passages as the primary culture forms a monolayer. After separation from the tissue and transition to 2D conditions, the morphology of cells changes, as well as the way they divide. Changes in the cell phenotype are the result of 2D culture, which can affect their function, organization of intracellular struc-

tures, cytokine secretion, and cell signaling. Due to violations in interaction with the external environment, cells attached to the plastic surface lose their polarity, which leads to a change in the response of these cells to apoptosis inducers. Another disadvantage of 2D culture is that cells in the monolayer have unlimited access to the environment's ingredients, such as oxygen, nutrients, metabolites, and signaling molecules. For tumor cells *in vivo*, the availability of nutrients, oxygen, etc. is more variable due to the natural architecture of the tumor mass. It is noted that the 2D system changes gene expression and cell biochemistry. Due to many disadvantages of 2D cell line culture systems, it became necessary to search for alternative models. The advantages of 2D crops include ease of operation and their low cost. To grow 2D cultures of tumor cells, a special plastic coated for monolayer cell lines or plastic coated with collagen, D-lysine, or a mixture of various components is used [25].

The 3D cultures

3-D tumor cell culture is currently used in research, both in personalized medicine and in regenerative medicine. This technology most accurately displays the processes occurring in the tumor *in vivo* and recreates the tumor phenotype, which is a valuable tool for studying the biology of the tumor, and will also allow preclinical evaluation of anti-tumor drug candidates on primary cell lines. Currently, the most commonly used model includes small cell aggregates-spheroids, which have been used by oncologists for decades [26]. The use of cell culture spheroids to evaluate the effectiveness of anti-cancer drugs is not a new concept. For almost 50 years, colony formation analysis in soft agar has been the gold standard *in vitro* method used to determine the status of cell transformation, as well as testing new candidate drugs with low throughput [27]. Figure 1 shows an example of spheroids obtained in our own research In the laboratory of cell technologies National Medical Research Centre for Oncology of the Ministry of Health of Russia.

Tumor spheroids are formed using various methods and techniques: the "hanging drop" method; cultivation on plastic with a non-adhesive coating; using scaffolds: hydrogels; using magnetic mixers and bioprinting.

The "hanging drop" method was originally used in Microbiology to study and cultivate bacteria. A suspension of tumor cells is placed on the inside of the Petri dish lid and covered with a Cup containing a phosphate-salt buffer to prevent the droplets from drying out. At the tip of the drop, cells aggregate at the air-liquid interface and then spheroids are formed [28]. This method does not require the use of any substances as a matrix or framework. However, the size of the drops should not be too large — drops with a liquid volume of more than 50 ml will not attach to the Petri plates, because the surface tension of the liquid is overcome by gravity. Replacement of the nutrient medium must be carried out carefully, so as not to damage the resulting spheroid.

An example of the "hanging drop" method Protocol for creating spheroids (permanent lines) is shown in table 1 [29].

Using the "hanging drop" method, spheroids can be maintained in culture for up to several weeks.

Researchers Jeppesen et. al. developed a protocol for obtaining spheroids from colorectal cancer tissue samples (table 2).

From 18 adenocarcinomas, spheroids were successfully created for 15 samples, using this

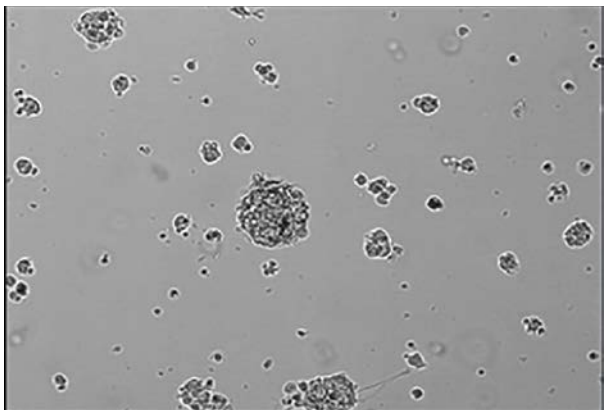

Fig. 1. A culture of spheroids obtained from a glial tumor. Magnification ×100

protocol. We also assessed if the primary culture spheroids of colorectal cancer belong to the histotype of the original tumor. One approach to improving the effectiveness of treatment is to determine the chemosensitivity of tumor cells obtained from the patient's material. Comparison of spheroids with a sample of the original tumor showed that the cells in the culture preserved the histology of the adenocarcinoma and the patterns of expression of cytokeratin 20 and

Table 1. The protocol for the "hanging drop" method.

№ p/p	Steps
Cell suspension making	
1	Cultivate cell lines to form a monolayer. Wash the cells twice with dobs buffer and decant the liquid. Add 2 ml of 0.05% trypsin - 1mm EDTA, incubate at 37 ° C. Control cell detachment. Add 2 ml of the complete nutrient medium to inactivate the trypsin. Resuspend the cells. Transfer to a 15 ml centrifuge tube.
2	Add 40 mcl 10 mg / ml of DNAase, incubate for 5 minutes at room temperature. Shake the test tube and centrifuge at 200 g for 5 minutes.
3	Remove the supernatant, wash the sediment with 1 ml of full nutrient medium. Repeat, then resuspend the cells in 2 ml of full nutrient medium.
4	Count the cells using a hemocytometer or an automatic cell counter. The required cell concentration is 2.5 × 106 in 1 ml.
Formation of "hanging drops"	
5	Add 50 ml of phosphate-salt buffer to the bottom of a 60 mm Petri platelet.
6	Remove and flip the lid off the Petri platelet. With a 20-ml dispenser, place 10-ml drops of the nutrient medium with the cells at the bottom of the lid, so that they do not touch each other. At least 20 drops are placed on one lid.
7	Carefully flip the lid and close the Petri platelets. Incubate at 37°C, 5% CO2, 95% humidity. Daily do microscopy of the platelets, cultivate up to the formation of cell aggregates.
8	After forming of the aggregates, they can be transferred to round-bottomed glass shakers in 3 ml of the complete nutrient medium. Incubate in a shaken water bath at 37 °C, 5% CO2 until spheroids form.

Table 2. The protocol for obtaining spheroids from colorectal cancer tissue samples

№ p/p	Steps
1	Wash the tumor tissue in a phosphate-salt buffer containing antibiotics. Remove fat and necrotic areas with sterile tools (scalpel or scissors).
2	Split the tumor material into 1 ± 2 mm pieces.
3	Add a phosphate-salt buffer containing 1 mg / ml of type II collagenase (Gibco) and antibiotics. Incubate the sample with enzymes for 20 minutes at 37°C.
4	Pass the tissue suspension through several filters in the following sequence: 230 μm filter (Sigma-Aldrich), 100 μm filter (BD Biosciences), 40 μm filter (BD Biosciences) and 30 μm pre-separation filter (MACS, Miltenyi Biotec).
5	Tissue samples,that have not passed through the filter 230 microns, collect and incubate with collagenase (item 2) for 10 minutes at 37°C. Pass it through the filters
6	Collect the tumor fragments with the use of filters of 100.40 and 30 microns and divide them into three fractions according to the size of the filtered cells.
7	Isolated tumor fragments are cultured in a stem cell medium (Thermo Fisher) with the addition of antibiotics (200 u/ml of penicillin, 200 u / ml of streptomycin, 100 u/ml of gentamicin and 2.5 u / ml of amphotericin B) in Petri dishes coated with agarose (Sigma-Aldrich) at 37°C, 5% CO2.

cancer-embryonic antigen. In this paper, chemosensitivity screening using spheroid cultures of five patients showed individual drug response profiles, which is a promising *in vitro* model for use in personalized medicine [30].

Monocultural spheroids obtained in breast cancer research *in vitro* are called mammospheres. There is evidence that the source of metastases are breast cancer cells with a phenotype similar to stem cells. Mammosphere culture is often used to study breast cancer stem cells [31].

Researchers Lombardo and others have developed the protocol for obtaining primary mammospheres from human breast tumor tissue after mastectomy (table 3) [32].

Halfter et al. Have compared the chemosensitivity of spheroids obtained from HER2 — positive breast cancer cell lines with spheroids from 120 fresh tissue samples. Their results showed greater yield efficiency and lower metabolic activity of spheroids obtained from primary cultures compared to spheroids obtained from cell lines [33].

Qureshy-Baig et al. it was reported that primary colorectal cancer spheroids retained their chemoresistance and genetic mutations in relation to the tumor tissue from which they were isolated [34]. In the Weiswald colorectal cancer study et.al. created "colospheres" using the technique of mechanical disaggregation of a sample of tumor tissue with a scalpel and crushing it using a syringe piston. The researchers obtained this model of "colospheres" in 95% of patients.the success of culturing this type of spheroid was associated with the aggressiveness of the tumor [35]. Jaganathan and his colleagues created a cartless 3D model *in vitro* using lines of breast cancer epithelial cells and fibroblasts cultured in a magnetic stirrer incubator in collaboration with NanoshuttlesTM. Fibroblasts were found more on the periphery of three-dimensional structures, while epithelial cells were located in the center. In this model, the authors tried to reproduce the heterogeneity of the tumor environment observed *in vivo*, so they used fibroblasts, thus simulating the extracellular matrix. Treatment of the tumor with doxorubicin resulted in inhibition of the growth of the resulting 3D model [36].

Cultivation in gels

The interaction of the cell with the extracellular matrix (ECM) can modify cellular organization, cell function, and response to therapy. In this regard, there is a need to create a three-dimensional culture model that repeats the role of ECM *in vivo*.

In this context, natural or synthetic hydrogels are used [27] of natural origin (for example, Matri-

Table 3. The protocol for obtaining mammospheres from human breast tumor tissue

№ p/p	Steps
Keep the tumor material in a cold place	
1	Put the sample into 100mm Petri platelets. Remove adipose tissue using sterile tools.
2	Add 2-3 ml of DMEM/F12 and chop the sample into pieces about 1 mm^3 in size with a sterile scalpel.
3	Resuspend tumor samples in 10 ml of DMEM containing proteolytic enzymes (3000 E / ml collagenase and 1000 E / ml hyaluronidase). Incubate at 37°C in a rotary shaker until all tissue fragments dissociate. Complete dissociation takes from 1 to 3 hours. Time of dissociation vary depending on the tissue. (For example, breast adenocarcinoma is usually more difficult to dissociate compared to mucosal carcinoma). Evaluate the degree of dissociation in the hemocytometer every half hour.
4	Precipitate the fragments for 5 minutes, then transfer the supernatant into a 15 ml conical polypropylene tube and centrifuge at 200 g for 10 minutes at room temperature. Remove the capillary fluid and resuspend the cells in 1-5 ml of mammospheric culture medium (it is possible to use specialized culture media for growing tumor stem cells or mesenchymal stem cells).

gel ™, collagen, alginate and fibrin), synthetic (for example, polyethylene glycol or PEG) and some semi-synthetic hydrogels that are a combination of synthetic and natural polymers (for example, hyaluronan, polypeptides) [30]. The cells are inserted into the upper part of the matrix after it has solidified or mixed with a liquid hydrogel. In both methods, tablets for cell culture are pre-coated with hydrogel [37].

Examples of natural scaffolds are Matrigel ™ and collagen. Matrigel ™ is a commercial ECM that includes basal membrane proteins from mouse tumor cells Engelbreth-Holm-Swarm (EHS), such as collagen IV, entactin, laminin perlecan, matrix metalloproteinase-2, and growth factors necessary for polarization, growth regulation, chemotherapeutic resistance, and cell adhesion [38]. Collagen — the most frequent fibrous protein — in the composition of ECM provides strength, regulates cell adhesion, and participates in cell migration and chemotaxis. In three-dimensional cultures, type I collagen is often used, but type II and III collagen can also be used [39]. Like Matrigel ™, collagen varies from batch to batch and has a low stiffness. In addition, natural hydrogels can cause immunogenic reactions [40]. Variability in properties may affect the reproducibility of results and limit the use of such frameworks for drug screening. To overcome the disadvantages of natural hydrogels, synthetic alginate hydrogels have been developed. The use of synthetic hydrogel allows you to control the biochemical and mechanical properties of ECM. There are PEG-based hydrogels, which may include cell adhesion molecules, peptides, or bioactive natural polymers (collagen, fibrin) to enhance cellular activity [41]. Natural and synthetic hydrogels have their limitations for re-determining tumor ECM. Alternatively, semi-synthetic hydrogels can be used. Semi-synthetic hydrogels can provide a controlled environment. Hyaluronan-the main component of natural ECM-is a biocompatible, biodegradable polymer that does not cause immune reactions. It has a high affinity for cell surface receptors involved in cell proliferation, adhesion, migration, and differentiation [42].

Spontaneous formation of spheroids: the method of non-adhesive surface

For this method, pre-coated plates are used, in which the lower surface is hydrophilic, charged neutrally, and covalently bound to the surface of a polystyrene vessel. This coating prevents the cells from adhering to the surface, causing the cells to be suspended and therefore form three-dimensional spheroids. The coating is stable, non-cytotoxic and does not decompose. However, there is a problem associated with the formation of inhomogeneous spheroids [37].

The 3D frame systems

De et.al a new 3D system for *ex vivo* culture of circulating tumor cells (CTCs) from blood samples of breast cancer patients using poly-ε-caprolactone (PCL) scaffolds was presented. It has been shown that this 3D PCL-based frame system can be used to study circulating tumor cells [43].

Since breast cancer cell lines lose their original tumor gene expression profiles when cultured in a monolayer, a model was developed for culturing primary breast cancer cells by decellularization of tumor-associated fibroblasts on three-dimensional polymer scaffolds. The presence of an extracellular matrix derived from tumor fibroblasts deposited on a polycaprolactone scaffold promotes cell attachment and viability, which is associated with higher levels of phosphorylated kinase, which provides cell attachment via integrins. Individual cells of primary breast cancer self-organize into tumor spheroids during long-term cultivation. In this model, the response of tumors received from different patients to chemotherapy drugs differed significantly from sample to sample. The authors suggest using this model as an *ex vivo* platform for culturing primary cell lines to develop effective and personalized chemotherapy regimens [44].

The magnetic levitation method

In this method, cells are grown to 80% confluence, treated with hydrogels containing magnetic iron oxide (MIO), and cultured over-

night [45]. The treated cells are trypsinized and placed in an ultra-low attached plate. Simultaneously, a cover with a neodymium magnet is attached to the top of the plate. Spheroids begin to form within a few hours at the air-liquid phase boundary due to attraction to a magnet. When cells aggregate with each other, they begin to synthesize ECM proteins such as collagen, fibronectin, and laminin. Spheroids can be incubated for several days until they reach the required size for research. This method has many advantages: the growth rate of spheroids is high compared to more common methods; spheroids form their own ECM (there is no need for an artificial framework), spheroids have a size in the mm^2 range (this size better reproduces the necrotic and hypoxic regions found in tumors) and, finally, they do not require a specialized nutrient medium. Disadvantages include the high cost of MIO, as well as it's possible cytotoxicity.

Microfluidic platforms

Microfluidic platforms are devices in which living cells can be cultured and permanently inserted into micrometer-sized chambers. This method allows precise control of the cellular microenvironment, ensuring continuous isolation of growth factors or nutrients [46]. In the simplest system, one microfluidic chamber contains one type of cultured cell. It is also possible to study the interaction between different cell types to recreate the boundaries between different tissues. To do this, micro-channels are connected to each other through porous membranes lined on opposite sides of different cell types (tumor/organ on a chip). The goal is to create an environment in which different types of cells can interact with each other. The organs on the chip allowed us to recreate the entire complex structure and environment, such as skin and hair [47], lungs [48], liver [49] and intestines [50]. This method is convenient for high-performance testing for various drugs, but requires special equipment. Recently, a two-layer microfluidic device has been developed that allows forming, cultivating and testing drugs on 5000 spheroids of tumors of the same size with different geometry of the culture chamber (200x200 m^2 and 300x300 m^2) [51].

Explantates

The method of cell explant culture is suitable for the development of primary tumor cell lines. This technique is the cultivation of small pieces of tumor tissue (2–5 mm in size) in a culture medium. This method significantly facilitates the preservation of the native architecture of tissues and microenvironment, which more fully reflects the interactions in the tumor *in vivo*. However, genetic variation can also occur in the culture being re-grafted and persist in media containing serum. In addition, it is possible to change the cell phenotype due to incorrect orientation of the explant in the culture medium. This method requires sequential subcultivation to produce primary tumor cell lines [6].

By direct explantation, Indian researchers were able to obtain primary epithelial cells of the oral cavity with a yield of up to 90% without microbial expansion in the primary culture [52].

In the research Goldman et.al. suggest the presence of dynamic phenotypic heterogeneity in tumor cells, resulting from the use of chemotherapy, causing resistance to chemotherapy drugs. The authors used explants extracted from the biopsy material of breast cancer patients to analyze the clinical consequences of metabolic reprogramming [53].

Baird et. Al. conducted a study of the STING-ligand (the ligand that stimulates the interferon gene) on an Explant model from patients undergoing resection for head and neck cancer to assess the patient's tumor response to the ligand. Treatment with sting ligands resulted in a statistically significant increase in IFN-α secretion in the Explant [54].

Muff researchers et.al it is believed that the Explant method is suitable for creating primary cell lines of bone and soft tissue sarcoma obtained from a patient, which opens up opportunities for molecular analysis and drug testing for such a heterogeneous group of tumors [55].

Solid tumor culture based on patient-derived explants (PDE) is increasingly being used for preclinical evaluation of new therapeutics and for the detection of biomarkers. Using mass spectrometry, a group of Australian scientists determined the degree of absorption of enzalutamide in 11 explants from prostate tumor samples obtained from 8 patients. At the same time, inhomogeneous intensity of the chemo drug signal was observed in all samples, while a higher area of the drug signal was recorded in the epithelial tissue of the sample with the highest concentration of the drug [56].

The PDE model is also used to study hormone-dependent tumors, such as prostate cancer and breast cancer. PDE cultures obtained from patients with breast or prostate cancer were grown on a gelatin sponge, which is a high-performance and cost-effective method that preserves the natural tissue architecture, microenvironment, and key oncogenic factors [57].

In the Ricciardelli study et.al we used tissue fragments about 5 mm^3 in size of the ovarian tumor after cryopreservation, obtained from patients. It has been shown that this method of cultivating explants using even pre-cryopreserved tissue allows obtaining viable tumor cells with the initial tumor microenvironment for introduction into the primary culture [58].

Karekla et.al have developed a platform for evaluating the response to drugs in non-small cell lung cancer, which will allow conducting preclinical trials of new drug candidates. The researchers propose to use the samples of tumor tissues obtained immediately after surgery. The authors described an optimized model of *ex vivo* explant culture that allows evaluating the response of non-small cell lung cancer to therapy while preserving the tumor microenvironment [59].

CONCLUSION

For decades, the gold standard for preclinical research has been the use of cell lines. However, the long time that cells are maintained in a monolayer, the subcultivation of cell lines used to produce a stable phenotype contributes to the change in the original phenotype of the cell population. Closer collaboration between clinicians and researchers, along with improved laboratory and methodological approaches, has led to the fact that primary cell lines have become a promising model in the field of tumor biology research, as well as opened up wide prospects for the use of these cultures in personalized medicine for preclinical evaluation of chemotherapy drugs. Primary cell lines have the advantages of preserving the original phenotype and features of the tumor, its microenvironment. Obtaining primary cultures is a rather complex process due to the small number of initial tumor cells, as well as the partial loss of cell viability after resection of the tumor and the use of methods of material disaggregation. Many researchers prefer enzymatic methods of dissociation of tumor tissues, since mechanical dissociation is a more "rough" method, while it is possible to obtain the necessary number of viable cells when using two methods simultaneously. The traditional 2D culture systems help to study the morphology and function of tumor cells, while losing important components of the intercellular matrix and intercellular interactions important for cell differentiation and proliferation. The 3D cultivation of primary tumor lines allows you to create cultivation conditions close to those of *in vivo*. The cell culture in Matrigel improves the integration of signaling pathways in cells, increases the expression of biomarkers. Scaffold-based methods for culturing primary cell lines have become important, especially in the past two decades. These methods can potentially overcome some of the limitations of modern three-dimensional cell culture methods, such as uneven cell distribution, inadequate nutrient diffusion, and uncontrolled size of cell aggregates. The use of scaffolds allows obtaining a membrane for attachment, proliferation and migration of tumor cells. Explant culture is a promising method for obtaining primary cell lines for use in personalized medicine and for use in preclinical studies to evaluate the tumor response to new candidate drugs. New methods

and approaches are being developed to isolate and obtain primary cell lines from tumor samples. The choice of the method of the tumor material dissociation and the method of culturing the primary cell line, provides an opportunity to study the biology of the tumor in its various aspects and is an excellent preclinical tool for the study of tumors in the *in vitro* systems.

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КЛИНИЧЕСКОЕ НАБЛЮДЕНИЕ

РЕДКИЕ ФОРМЫ НЕХОДЖКИНСКИХ ЛИМФОМ: ОПЫТ ТЕРАПИИ ПЕРВИЧНЫХ ЛИМФОМ КОСТЕЙ

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РЕЗЮМЕ

К редким локализациям неходжкинских лимфом относят первичную лимфому костей. Эта форма составляет не более 1–2% всех неходжкинских лимфом взрослых. Диагноз первичной лимфомы костей устанавливают в случаях очагового поражения одной или нескольких костей. Кроме того, допускается вовлечение мягких тканей и регионарных лимфоузлов. Критерием исключения служит только поражение костного мозга и вовлечение отдаленных лимфоузлов. Первыми симптомами болезни являются некупируемые боли в костях, нередко сопровождающиеся локальным отеком, формированием опухолевой массы в зоне поражения, изредка присоединяются В-симптомы. Чаще встречается локальное (80%) и реже многофокусное (20%) поражение длинных трубчатых костей в области диафиза и метадиафиза. Диагностика поражения костной ткани при первичном и вторичном ее вовлечении основана на применении всех доступных методов исследования (рентгенография, компьютерная, магнитно-резонансная и позитронно-эмиссионная томография). Дифференциальная диагностика возможна только на основании иммуногистохимического исследования с определением экспрессии общего лейкоцитарного антигена, маркеров В-клеток, Т-клеток, а также клональности по одной из легких цепей иммуноглобулинов к или λ, bcl 2 и bcl 6, ALK, степени пролиферативной активности Ki-67. Оценка эффективности различных методов лечения первичной костной лимфомы осложняется небольшим числом наблюдений и отсутствием единой тактики лечения. В качестве терапии первой линии чаще применяют CHOP-подобные курсы. Персонифицированная терапия включает иммуно-химиотерапию, лучевую терапию и применение хирургических методов лечения — эндопротезирования.

Ключевые слова:
неходжкинская лимфома, кости, диагностика, химиотерапия, эндопротезирование, длительность наблюдения

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CLINICAL CASE REPORTS

RARE FORMS OF NON-HODGKIN LYMPHOMAS: EXPERIENCE IN TREATMENT FOR PRIMARY BONE LYMPHOMAS

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ABSTRACT

Primary bone lymphoma is a rare presentation of non-Hodgkin lymphoma. It accounts for a maximum of 1–2% of all non-Hodgkin lymphomas in adults. Primary bone lymphoma is diagnosed in focal lesions of one or more bones; soft tissue and regional lymph nodes may be involved too. The exclusion criteria are only bone marrow damage and involvement of distant lymph nodes. The first symptoms include intractable bone pain often accompanied by local edema, the formation of a tumor mass in the affected area; B symptoms occasionally join. Local lesions of long tubular bones in the diaphysis and metadiaphysis regions are more common (80%), while multifocal lesions are less frequent (20%). Diagnosis of lesions of the bone tissue in its primary and secondary involvement is based on the use of all available research methods (radiography; computed, magnetic resonance and positron emission tomography). Differential diagnosis requires an immunohistochemical study with determination of the expression of total leukocyte antigen, B-cell and T-cell markers, and clonality in one of immunoglobulin light chains κ or λ, bcl 2 and bcl 6, ALK, proliferative activity of Ki-67. Evaluation of the effectiveness of various treatments for primary bone lymphoma is complicated by a small number of observations and the absence of a uniform treatment strategy. CHOP-like chemotherapy cycles are often used as first-line therapy. Personalized therapy involves immunochemotherapy, radiation therapy and surgical treatment — endoprosthetics.

Keywords:
non-Hodgkin lymphoma, bones, diagnosis, chemotherapy, endoprosthesis, observation period

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RELEVANCE

Rare localities of non-Hodgkin's lymphomas include primary bone lymphoma. This nosological form makes up no more than 2–5% of all primary bone tumors 4–5% of all extranodal lymphoproliferative diseases, and 1–2% of all non-Hodgkin's lymphomas of adults. Until the middle of the last century, bone lymphomas were considered only cases with a local lesion of one bone without involving soft tissues and regional lymph nodes. In modern conditions, the diagnosis of primary bone lymphoma is also established in cases of focal lesions of several bones. In addition, the involvement of soft tissues and regional lymph nodes is allowed. The exclusion criterion is only the bone marrow lesion and involvement of distant lymph nodes. The first symptoms of the disease are non-stop pain in the bones, often accompanied by local edema, the formation of a tumor mass in the affected area, and occasionally b-symptoms are added. Very often, patients are concerned about restriction of limb movement, and pathological fractures are possible. More often, this is a local (80%) and less often multi-focal (20%) lesion of long tubular bones in the area of the diaphysis and metadiaphysis. Bone localization of lymphoma is more common in patients 60–70 years old, it is extremely rare in children younger than 10 years, the ratio of men: women are 1.5:1 [1–5].

Diagnosis of bone tissue damage with primary and secondary involvement is based on the use of all available imaging methods. The data of radiological methods of investigation (radiography, computer — CT, magnetic resonance-MRI and positron emission — PET tomography) for bone lymphomas are variable and non-specific, the initial changes may not go beyond the normal variants of the structure of bone tissue. During radiographic examination, changes can be represented by local lytic foci with uneven edges with areas of sclerotic lesions in the form of small multiple foci along the entire length of the bone, or by a diffusely distributed process with the destruction of cortical tissue and the involvement of adjacent soft tissues. The periosteal reaction occurs in 60% of patients and is characterized by the presence of lamellar or layered areas located along the long axis of the bone, alternating with normal periosteum and serving as an indicator of a poor prognosis. Changes close to different variants of the norm make it difficult to diagnose bone lesions based on review radiographs. In these cases, MRI is a more informative study. In T1-WI mode (WI-weighted image) MRI is better able to detect heterogeneous low-intensity signals that are characteristic of intraosseous changes, fibrosis, and soft tissue lesions, since it can detect areas with a low-intensity signal.T2-WI mode is more informative in the presence of Homo-and heterogeneous changes of high intensity, peritumoral edema, periosteal reaction and reactive changes in the bone marrow. MRI with contrast allows you to detect areas of bone damage with increased accumulation of contrast agent. CT is not the method of choice in PLC diagnostics and can only be used in combination with MRI or PET. However, CT scans can diagnose sequestration and cortical erosion at earlier stages of the disease and in a larger percentage of cases [2, 3, 5–9].

A necessary condition for confirming the diagnosis of bone lymphoma is to perform an open biopsy of the affected area of the bone and/or soft tissue component of the tumor with histological and immunohistochemical examination of the material. Histological examination of surgical / biopsy material usually reveals diffuse proliferation of medium-and large-sized lymphoid cells located in the bone tissue between the trabeculae and fat cells of the bone marrow. Histological variants are different, but in most (60–80%) cases there is diffuse b-large cell lymphoma (DBLCL), of which 10% is the Central-cell variant of DBLCL and b-cell lymphoblastic lymphoma; other more rare variants include follicular lymphoma, lymphoma from the mantle zone cells, ALK+large-cell lymphoma, NK/T-cell lymphoma. Differential diagnosis is possible only on the basis of immunohistochemical studies with the determination of the expression of total leukocyte antigen (CD45 LCA), markers of B cells (CD 19, CD 20, CD 79a, RAX5, MuM1), T cells (CD 3, CD 4, CD 8, CD5), as well as clonality along

one of the light chains of immunoglobulins κ or λ, bcl 2 and bcl 6, ALK, and the degree of proliferative activity of tumor cells (Ki-67). Informative is the cytogenetic study of native material to identify chromosomal rearrangements characteristic of lymphomas — translocations t (8;14) (q24; q32), t(11;14) (q13; q32) and t(14;18) (q32; q21), rearrangements of the bcl 6 gene and detection of hyperexpression of cyclin D1 by polymerase chain reaction [3, 6, 10–12].

Evaluating the effectiveness of various treatment methods for primary bone lymphoma is difficult, because it is based on the analysis of retrospective data over a long period of time. The analysis is complicated to a large extent by a small number of observations and the lack of a unified treatment strategy. As a first-line therapy, SNOR-like courses are more often used. Treatment results depend on the presence of adverse prognosis factors: increased activity of lactate dehydrogenase (LDH) in serum, multiple bone lesions involving soft tissues, presence of B-symptoms, tumor size of 6 cm or more, localization in the spine and pelvic bones, involvement of regional lymph nodes, which reduce the effectiveness of therapy [11]. Data from various research groups agree that in local (IE) stages of bone lymphoma, the 5-year relapse-free survival after radiation or chemoradiation is 35–50 and 90–95%, respectively. In common stages (IIE and IV), only LT is not possible in principle, and with chemotherapy under the SNR program, the 5-year relapsed survival rate is 40–70%. The use of targeted drugs and primary intensification of PCT high dose chemotherapy followed by autologous hematopoietic stem cell transplantation can improve survival rates in patients with risk factors [1, 3, 5, 9, 11–14].

We present clinical cases of primary bone lymphomas.

Description of the clinical case

According to archived data from the FGBU NMRC of Oncology of the Ministry of health of Russia for the period 2009–2019, there were 4 patients with primary bone lymphoma, 3 men and 1 woman, with an average age of 43.5 years (table 1). All patients noted a direct link between the development of the tumor and the previous injury. the average time between the appearance of the first symptoms (in all patients it was local pain in the limb) and verification was 9.25 months (4–16).

In men, the tumor was localized in the left femur, in women, the right humerus was affected, in all patients, there was a multi-focal bone lesion and local involvement of the soft tissues of the limb. B-large-cell NCL was verified in three patients, and b-cell lymphoma from small lymphocytes was initially verified in one patient. later, during additional studies, this tumor was assigned to extranodal follicular lymphoma type 3A (table 1).

The prevalence of the process was determined using CT, MRI, and pet studies in two patients (the method has become widely available in routine practice since 2016), as well as standard clinical examination, including bone marrow trepanobiopsy, was performed in all patients.

R-CHOP/CHOEP schedule (rituximab, cyclophosphamide, vincristine, prednisone, doxorubicin, etoposide) was used as first-line therapy in all patients.after 6 cycles, partial remission was achieved in 3 patients, and these patients received consolidating radiation therapy at a total dose of 40 gray. In 1 patient with adverse prognostic factors B-large cell lymphoma after 6 cycles of R-CHOEP, the progression of the disease with PET-positive infiltration into the lung tissue was determined. The use of second-line therapy (R-GDP 2 cycles, R-MINE 3 cycles) did not bring any effect, the patient had primary refractoriness to chemo-immunotherapy, PET-positive (5 points according to Deauville) tumor infiltration of the bone, soft tissues of the left knee joint, including the patella, and infiltration in the lung regressed (table 1). The patient had a continuously progressive course, which led to a fatal outcome. The patient was followed up for 14 months.

Two patients after the completion of chemoradiation treatment had a successful endoprosthesis with restoration of the functions of the corresponding limb for objective indications. the follow-up period for these patients was 83 and 95 months (table 1).

Table 1. Characteristics of patients with primary bone non-Hodgkin's lymphoma

Patients	A.	Ye.	S.	Sh.
Sex	Male	Male	Male	Male
Age, y.o.	55	29	27	63
Trauma	+	+	+	+
Time from the first symptoms to diagnosis, months	9	8	16	4
Localisation	Left femur, patella	Left femur	Left femur	Right humerus
Type of damage	Multifocal	Multifocal	Multifocal	Multifocal
Soft tissues	+	+	+	+
Local l\n	Inguinal, iliac	Intra-pelvic, popliteal	-	-
B-symptoms	+	+	-	-
LDH	↑↑	↑	N	N
Stage	IVB	IVB	IVA	IVA
International Prognostic Index (IPI)	High intermediate (3)	Lower intermediate (2)	High risk (FLIPI -1)	Lower intermediate (2)
Histology type of tumor	B-large- cellular	Extranodal B-large-cellular	B-cell lymphoma from small lymphocytes. Revision of extranodal 3A type FL	Extranodal diffuse B-large cell
PET for setting the stage	+	+	Not performed	Not performed
First Line Therapy	R-CHOEP 6	R-CHOEP 6	R-CHOP 6	R-CHOP 6
First line therapy response	Progression + easy PET+	PET-full remission	Partial remission	Partial remission
DGT	-	40 Grey	40 Grey	40 Grey
Response time, months	6	10	13	95
Relapse/ refractoriness	refractoriness	-	Relapse +light	-
Second line therapy	R-GDP 2/ R-MINE 3	-	R-B 3 – PD R-GDP 2 - PD	-
Second line therapy response	PD PET+	-	PR	-
Surgical treatment	-	-	Endoprosthesis	Endoprosthesis
Examination duration, months	14	10	83	95
Outcome	Passed away	Alive	Alive	Alive

Note: PET – positron emission tomography; FL – follicular lymphoma; R – GDP-rituximab 375 mg/m^2, gemcitabine 100 mg/m^2, cisplatin 100 mg m^2, dexamethasone 40 mg; R – CHOP/snoer-rituximab 375 mg/m^2, cyclophosphamide 750 mg/m^2, vincristine 1.4 mg/m^2, doxorubicin 50 mg/m^2, prednisone 100 mg/m^2, etoposide 100 mg/m^2; R – mine-rituximab 375 mg/m^2, ifosfamide 1330 mg/m^2, mesna 1330 mg/m^2, etopozod 65 mg/m^2, mitoxantrone 8 mg/m^2; R – b-rituximab 375 mg/m^2, bendamustine 90 mg/m^2

Three patients with primary bone lymphomas are currently alive, with an average follow-up time of 49 months (4–95).

To show the modern possibilities of complex therapy of rare forms of non-Hodgkin's lymphomas using various methods, we present the following clinical case more detailed.

Clinical case 1

Patient Sh., a 63-year-old woman.

The patient noted an acute onset of the disease in December 2010, after a minor bruise, acute pain in the right upper limb appeared, edema, restriction of movement of the hand, "cyanosis of the hand". For a month at the place of residence, the patient was treated for" thrombophlebitis " without effect. In January 2011, radiography of the right upper limb was performed and diffuse changes in the right humerus were detected, and the patient was referred to the NMRC of Oncology with suspicion of "sarcoma". A comprehensive examination in NMRC Oncology revealed an osteodestructive lesion of the distal metaphysis of the right humerus (fig. 1).

The MRI study showed a multi-node formation of inhomogeneous proton density with intra-and extraossal growth with destruction of the cortical layer of the right humerus in the distal metaphysis region and a circular extraossal component with dimensions of 70x40x43 mm. vessels of the main neurovascular bundle of the shoulder without convincing evidence of involvement in the tumor process. Osteoscintigraphy determined a focus of 63 mm, the percentage of pathological hyperfixation of RPF at the level of 70%. Complex examination did not reveal any other pathological foci, lesions of internal organs and bone marrow.

An open tumor biopsy was performed. In the finished histological preparation, a fragment of muscle-fibrous tissue with diffuse infiltration by lymphoid elements. The tumor cells were large with large polymorphic nuclei with uneven angular contours, with coarse chromatin and large centrally or eccentrically located nuclei, the presence of cells with multi-lobed nuclei (fig. 2). immunohistochemical study was performed with antibodies to panCK AE1/AE3, S-100, CD20, CD79a, CD3, CD45LCA, VCL-6, BCL-2, MuM1, Ki — 67–70% (fig.3). Expression of CD20 (membrane reaction) (fig. 4), CD79a (cytoplasmic reaction), MuM1 (nuclear reaction), BCL-6 (nuclear reaction in part of cells) (fig. 5), BCL-2 (cytoplasmic reaction in most cells) was observed in tumor cells. Conclusion of the immunohistochemical study-extranodal diffuse b-large cell lymphoma (CD20+, MuM1+, BCL6+, BCL2+).

The patient had a pathological fracture of the lower third of the right humerus with dislocation of bone fragments and impaired function of the upper limb before starting therapy in March 2011 (fig. 6). on the basis of NMRC Oncology, 6 cycles of R-CHOP polychemotherapy were performed in the Department of oncogematalogy, partial remission was achieved (areas of destruction in the lower third of the humerus were preserved), unfortunately, the function of the arm was not restored. For the purpose of consolidation in the Department of radiotherapy, the patient underwent a course of DGT 40 gy on the area of tumor lesion. There were no adverse events during therapy. An x-ray of the right humerus performed in June 2011 showed a consolidating pathological fracture of the lower third of the right humerus with a rough angular displacement against the background of the bone structure (fig. 7–8).

The callus was weak, the bone edges of the fragments showing signs of sclerosis. Conclusion of the orthopedist — atrophic pseudoarthritis (false joint) of the epimetaphysis of the right humerus. The patient continued to have impaired upper limb function, and in order to improve the quality of life, in November 2013, after confirming remission of the disease, the patient underwent resection of

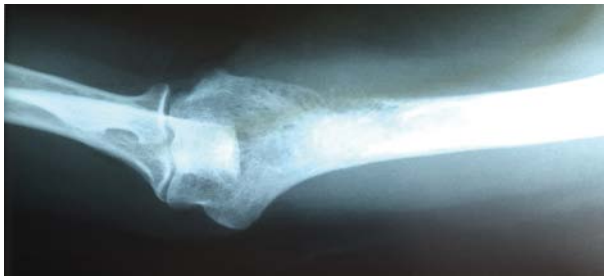

Fig. 1. Patient Sh. An X-ray of the right humerus before getting the treatment.

the lower third of the right humerus (fig. 9) and replacement of the defect with a prosthetic elbow joint PROSPON (fig.10–11).

Histological analysis of the removed bone: the bone marrow is represented by adipose tissue; there are small lymphocytic infiltrates between the bone beams. Healing took place within the standard time frame, and the function of the right upper limb was fully restored. In January 2019, after an injury (falling from the height of her own body with the support of her right arm), the patient again complained of pain in her right arm. Radiography of the right elbow joint in 2 projections showed radiological signs of instability of the leg of the

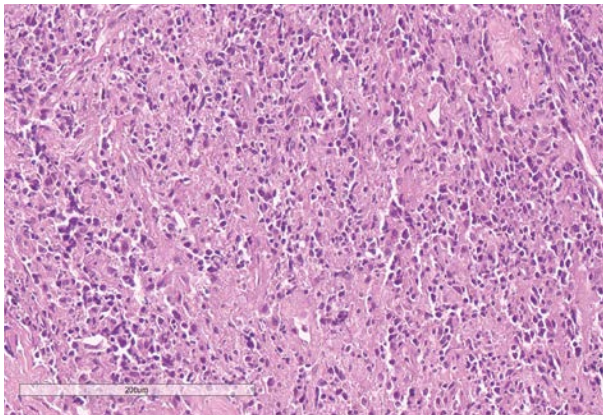

Fig. 2. Patient sh Historiarum bones, hematoxylin x200.

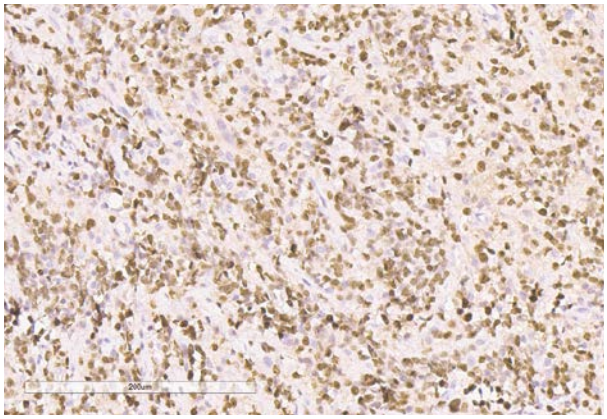

Fig. 3. Patient Sh. The histology slide of bones, Ki67x200 expression.

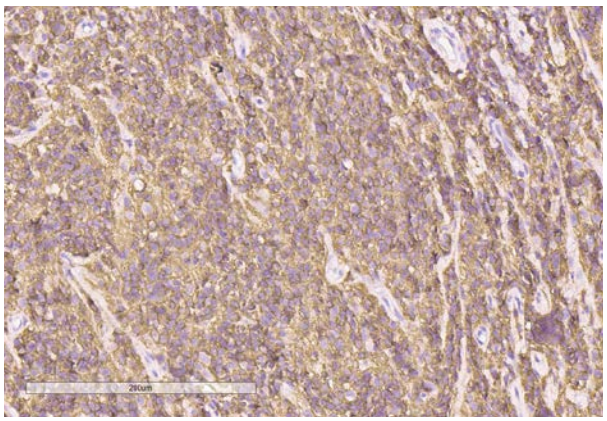

Fig. 4. Patient Sh. The histology slide of bones, CD20x200 expression.

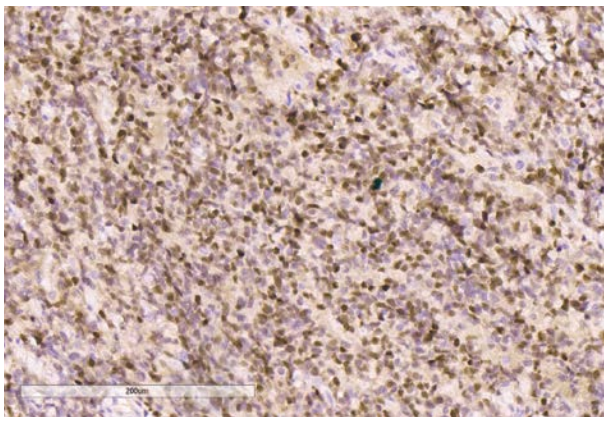

Fig. 5 Patient Sh., The histology slide of bones, BCL6x200 expression.

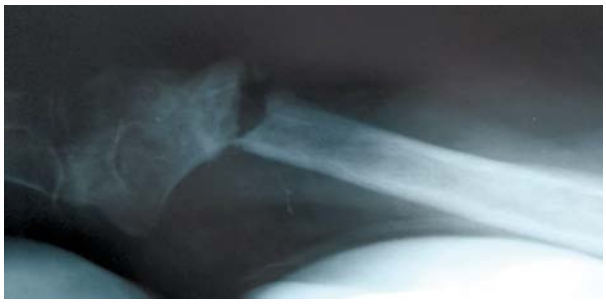

Fig. 6. Patient Sh. An X-ray of the right humerus. Pathological fracture.

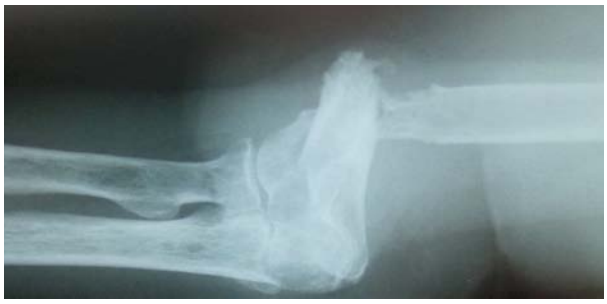

Fig. 7. Patient Sh. An X-ray of the right humerus, after PCT.

endoprosthesis in the ulna. A comprehensive examination confirmed remission of the disease. And in April 2019, the patient underwent a successful re-endoprosthesis of the right elbow joint. Currently, the patient is alive, and the limb function has been fully restored. The total follow-up time for the patient is 95 months.

DISCUSSION

The examined literature data and our own clinical experience confirm that primary bone lymphomas are an extremely rare form of lymphoproliferative diseases. many studies are based on a retrospective analysis of clinical material. We

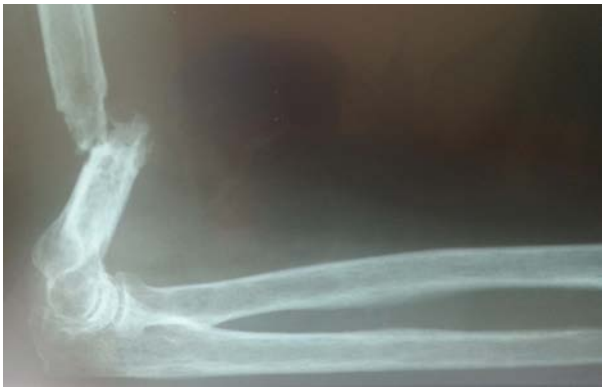

Fig. 8. Patient Sh. An X-ray of the right humerus, after PCT.

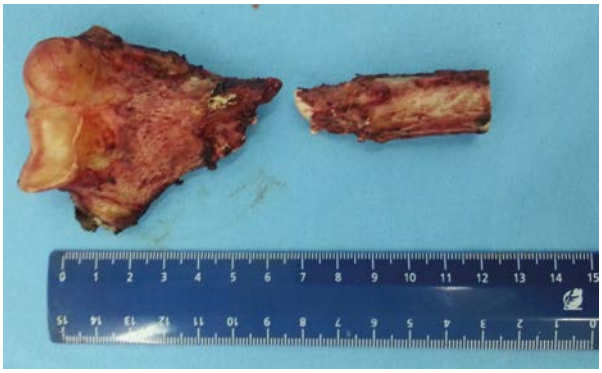

Fig. 9. Patient Sh. Removed gross sample of the right humerus.

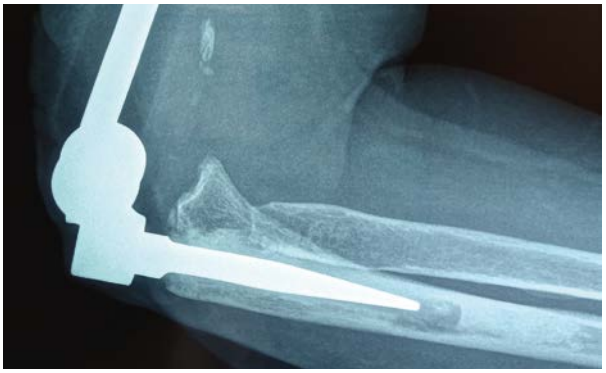

Fig. 10. Patient Sh. An X-ray of the right elbow joint after endoprosthesis (side projection).

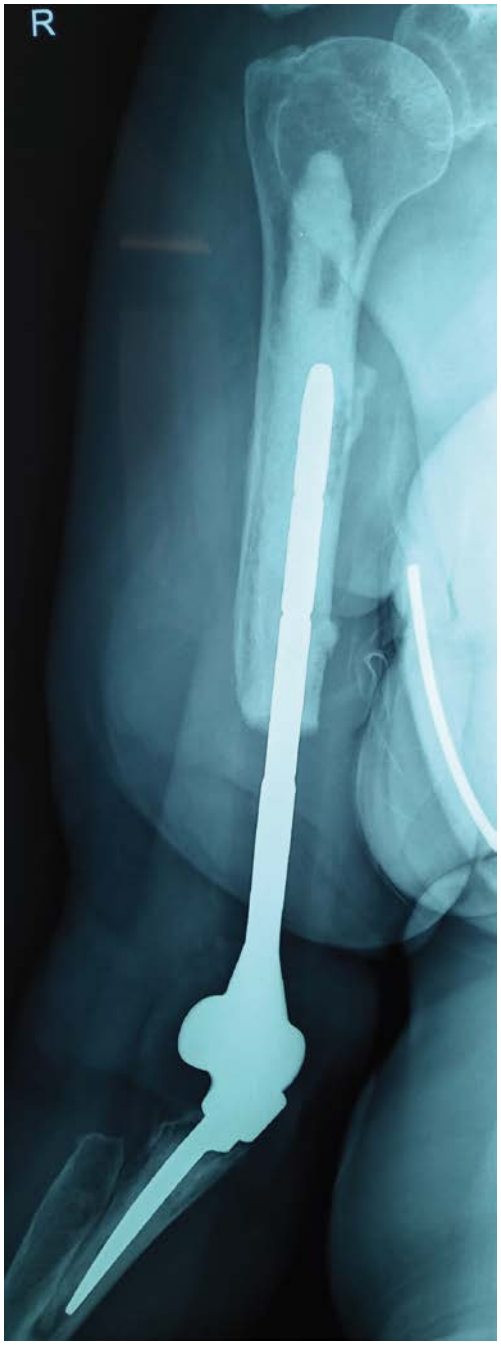

Fig. 11. Patient Sh. X-ray of the right elbow joint after endoprosthesis (front projection).

have seen only 4 cases of primary bone lymphoma in ten years. The General principles of diagnosis of this form of lymphoma are based on the principles of examination of all patients with oncohematological diseases. In determining the treatment tactics for primary bone lymphoma, the prevalence of the process and the immunohistochemical variant of the tumor undoubtedly play a dominant role. A number of researchers performed initial resection of the bone and soft tissue involved in the process, but faced the problem of rapid relapse. We can agree with other researchers that at this stage of disease development, surgical aggression is unnecessary and intervention should be limited to biopsy [2, 11].

Since all lymphomas, including primary bone lymphomas, are still highly sensitive to chemotherapy neoplasms, primary remission can be achieved in more than 80–95% of patients. Many researchers use CHOP-like regimens as basic therapy and achieve a 5-year event-free survival rate of 40–95%, depending on the prevalence of the initial process. This choice may be the main one in groups of patients with local bone damage, without risk factors. Colleagues from FGBU GNC of the Ministry of health of Russia widely use advanced stage lymphoma therapy using the intensive mNHL-BFM-90 program and achieve a 5-year relapse-free survival rate of 92% [5, 12, 13].

In our observation, a patient with an unfavorable subspecies of DBKL and a large spread of the process had a refractory course of the disease. Perhaps in patients with an initially unfavorable prognosis, more aggressive polychemotherapy tactics should be used. Patients with more favorable lymph immunotypes showed a better response to treatment and are still alive with a good quality of life. In our routine practice, we share the position of researchers who use consolidating radiotherapy in patients with primary bone lymphoma [3, 5].

Among the patients we observed, two patients underwent successful endoprosthesis due to existing pathological fractures and impaired function of the corresponding limb, which significantly worsens the quality of life. Our clinical experience allows us to recommend the use of this method of surgical treatment in patients in remission of primary bone lymphoma, as an option that restores the patient's vital needs.

CONCLUSION

Thus, if there is a suspicion of bone lymphoma, the patient should be examined using all available modern research methods to determine the extent of the tumor process, including PET research. Mandatory tumor biopsy with immunohistochemical examination. It should be noted that local bone damage often proceeds relatively favorably. If the limb function is impaired, the quality of life is unsatisfactory and there are clinical indications in the complex of treatment measures, and our experience confirms this, during the period of remission of the disease, surgical methods of therapy are used, in particular endoprosthesis to restore the quality of life of patients. The main method of therapy is polychemotherapy with targeted drugs for patients with advanced stages of bone lymphoma.

Authors contribution:
Lysenko I.B. – research concept and design, scientific editing, text writing.
Barashev A.A. – performing surgery, preparing illustrations, interpreting data, and scientific editing.
Lapteva T.O. – interpretation of data, preparation of illustrations, scientific edition, technical edition.
Nikolaeva N.V. – data collection, scientific edition.
Kapuza E.A. – data collection.
Shatokhina O.N. – data collection.
Pushkareva T.F. – data collection.

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CLINICAL CASE REPORTS

BONE FLAP RESORPTION AFTER CRANIOTOMY IN ELECTIVE NEUROSURGERY (CASE STUDY)

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ABSTRACT

Craniotomy is an integral part of modern elective neurosurgery which involves cutting a free bone flap to provide access to pathological intracranial structures with its reimplantation at the end of surgery.
Bone flap grafting in the trepanation window with various fixation methods in the end of elective neurosurgery in the absence of severe cerebral edema or cancer-induced bone destruction is a standard procedure that restores the skull shape, cerebrospinal fluid dynamics and cerebral perfusion.
According to the literature, the incidence of aseptic inflammation with subsequent resorption of the bone flap after craniotomy in elective neurosurgery is not clearly defined.
An analysis of medical publications in the PUBMED database showed few reports of bone flap resorption after elective craniotomy, and no reports were found after the search in the eLibrary database.
Thus, the number of reports on the bone flap resorption after craniotomy in elective neurosurgery is limited, and the pathophysiology of this process remains unclear.
However, the described complication of craniotomy can lead to the dislocation of a bone flap, the development of a local pain syndrome, a cosmetic defect, and disturbances in cerebrospinal fluid dynamics.
The article describes an example of partial resorption of a bone flap after craniotomy for the removal of meningioma in the middle third of the superior sagittal sinus, which required a number of repeated neurosurgical interventions. The treatment was finished with the removal of a partially resorbed bone flap and implantation of an individual titanium mesh implant.

Keywords:
complications of osteoplastic trepanation, complications of craniotomy, resorption of a cranial bone flap, cranioplasty, postresection defect, accesses in neurosurgery, complications in neurosurgery

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КЛИНИЧЕСКОЕ НАБЛЮДЕНИЕ

РЕЗОРБЦИЯ КОСТНОГО ЛОСКУТА ПОСЛЕ КОСТНОПЛАСТИЧЕСКОЙ КРАНИОТОМИИ В ПЛАНОВОЙ НЕЙРОХИРУРГИИ (СЛУЧАЙ ИЗ ПРАКТИКИ)

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РЕЗЮМЕ

Костно-пластическая краниотомия, предполагающая выпиливание свободного костного лоскута для осуществления доступа к патологическому интракраниальному очагу с его реимплантацией в конце оперативного вмешательства, является неотъемлемой частью плановых операций в современной нейрохирургической практике.

Завершение плановой нейрохирургической операции установкой костного лоскута в трепанационное окно с использованием различных методик фиксации в условиях отсутствия выраженного отека головного мозга или опухолевой деструкции кости является стандартной процедурой и обеспечивает восстановление формы черепа, ликвородинамики и перфузии головного мозга.

По данным литературы, частота развития асептического воспаления с последующей резорбцией костного лоскута после выполнения костно-пластической краниотомии в плановой нейрохирургии четко не определена. Проведенный анализ базы медицинских публикаций PUBMED указывает на единичные сообщения о резорбции костного лоскута после выполнения плановой костно-пластической краниотомии. При анализе в отечественной базе E-Library сообщений о резорбции костного лоскута после плановых костно-пластических краниотомий не обнаружено.

Вследствие ограниченного числа сообщений о резорбции костного лоскута после выполнения костно-пластической краниотомии в плановой нейрохирургии на данный момент остается неясной патофизиология данного процесса.

Тем не менее, представленное осложнение костно-пластической краниотомии может привести к дислокации костного лоскута, развитию локального болевого синдрома, косметическому дефекту, нарушению ликвородинамики. В статье описывается пример частичной резорбции костного лоскута после костно-пластической краниотомии, по поводу удаления менингиомы верхнего сагиттального синуса в средней трети, что в последующем в свою очередь потребовало проведения ряда повторных нейрохирургических вмешательств. Лечение завершилось удалением частично резорбированного костного лоскута с последующей имплантацией индивидуально изготовленного сетчатого титанового имплантата.

Ключевые слова:
осложнения костнопластической трепанациии, осложнения костно-пластической краниотомии, резорбция краниального костного лоскута, краниопластика, пострезекционный дефект, доступы в нейрохирургии, осложнения в нейрохирургии

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According to the literature, the main attention is paid to the analysis of risk factors for resorption of auto bone subjected to preservation, long-term storage in various environments and temperature conditions due to the inability to complete surgery by installing a bone flap (brain edema, decompressive craniectomy). Under these conditions, the risk of resorption of the bone flap can reach 23% (1, 2, 3).

In elective neurosurgery, bone-plastic craniotomy (BPC) involves sawing out a free bone flap to provide access to a pathological focus, followed by its fixation at the end of surgery. When analyzing the PUBMED database of medical publications, there are isolated reports of bone flap resorption after planned BPC (4, 5, 6). When analyzing the national E-Library database, there were no reports of resorption of the bone flap after a planned BPC.

We present a clinical case of partial resorption of the bone flap after planned BPC, which in the future will require several surgical interventions.

CLINICAL CASE

Patient S., born in 1981, has been complaining of headaches and right hemihypesthesia since December 2017. An MRI of the brain in January 2018 revealed a meningioma of the upper sagittal sinus in the middle third on the left side, measuring 41x51x45 mm. (fig. 1). Upon admission, the patient underwent spiral computed tomography (SCT) of the neck, chest, abdominal cavity and pelvis: no pathology was detected.

In January 2018 FGBU RNIOI performed BPC in the parietal region, meningioma removal (Simpson I). Excision of the Dura mater (DM) with tumor tissue was performed. The plastic surgery was made using an artificial DM "Durepair Regeneration Matrix Medtronic". The duration of the operation was 240 minutes. The bone flap is not changed, stowed in the trepanation window is fixed on the perimeter with the help of non-resorbable Medtronic craniofixes.

When performing the control SCT on 1 day after the operation, no hemorrhagic postoperative complications were detected, and no subaponeurotic accumulation of liquor was detected. In the bone mode, the satisfactory position of the bone flap is determined (fig. 2). the Postoperative period is without features. Histological verification — meningotheliomatous meningioma.

After 5 months of the initial surgery, the patient began to complain about the mobility of the bone flap. When examining the area of the postoperative scar without signs of inflammation, palpation determines the instability of the bone flap. The additional examination inflammation markers in the blood are not determined: white blood cell count is normal, relating to stab neutrophile leucocytes is not increased, young forms and myelocytes are defined, the level of CRP is not elevated, the procalcitonin test is negative. During the bacteriological study of blood, the growth of microflora was not obtained. When performing SCT, an epidural accumulation of liquor is detected in the left parietal region. In the bone mode, there is no bone flap disposition. Diastasis is determined along the perimeter of the bone flap (fig. 3). The bone flap was refixed using Medtronic craniofixes. Visually, the bone flap did not differ from the bones of the skull. The presence of mobility of the flap was seen as insufficient fixation with craniofixes. When performing the control SCT, postoperative complications were not detected, and a satisfactory position of the bone flap was determined in the bone mode (fig. 4). The postoperative wound was healed by primary tension, with no signs of inflammation.

Since January 2019, the patient again began to notice a backlash of the bone flap, a feeling of" crunch " when palpating the left parietal area. When performing SCT, areas of resorption of the bone flap are determined (fig. 5). Physical examination again determines the mobility of the bone flap. The skin covering above the flap and the postoperative scar are not changed (fig. 5). markers of inflammation in the blood are not detected.

In February 2019, a bone flap was removed in the left parietal region. Intraoperatively, the mobility of the bone flap and the foci of destruction are determined. A bluish-colored bone flap (fig. 5). During the bacteriological study of the scar tissue surrounding the bone flap and the bone flap, the

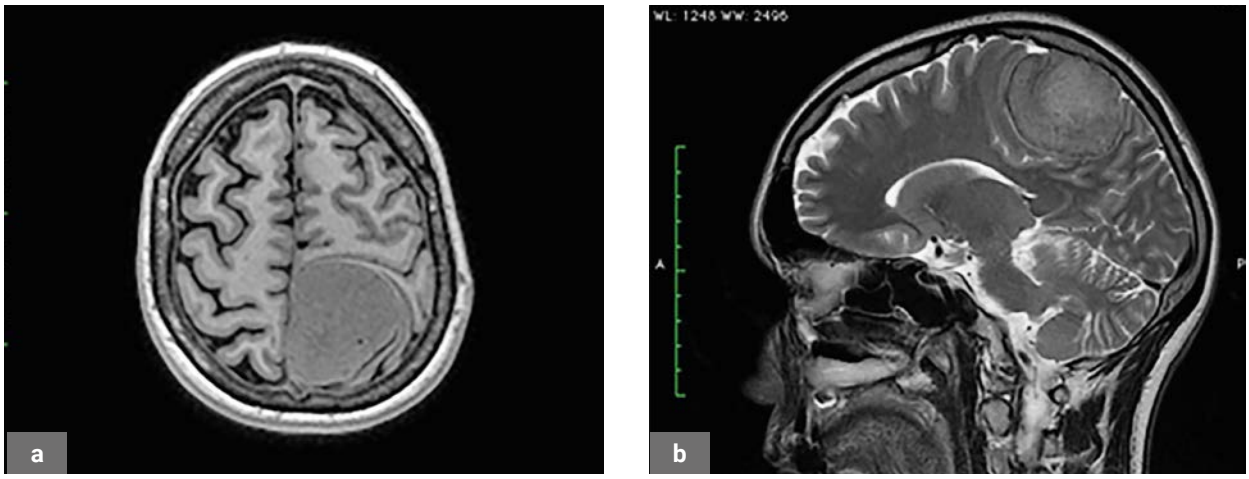

Fig. 1. On preoperative MRI of the brain from 01.2018, falx meningioma of the left parietal lobe is determined: a – axial projection in T1 mode; b – sagittal projection in T2 mode.

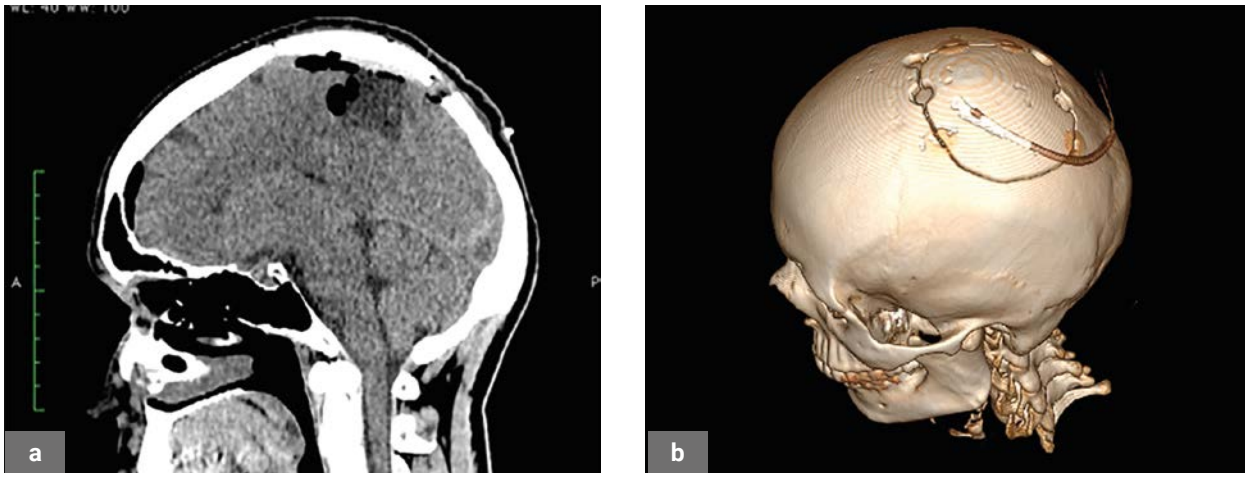

Fig. 2. Postoperative SCT of the brain: a – there are no postoperative hemorrhagic complications; b – during 3D reconstruction in the bone mode, the satisfactory standing of the bone flap is determined.

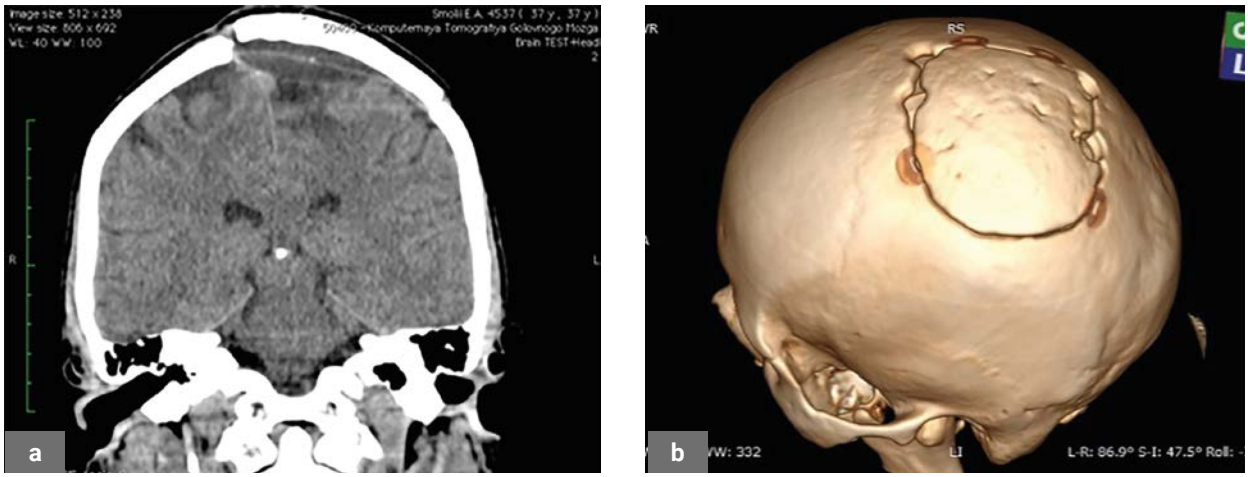

Fig. 3. SCT of the brain 5 months after surgery: a – determined epidural accumulation of liquor in the left parietal region in the projection of the bone flap; b – during 3D reconstruction, partial resorption of the bone flap along the line of the bone cut is determined.

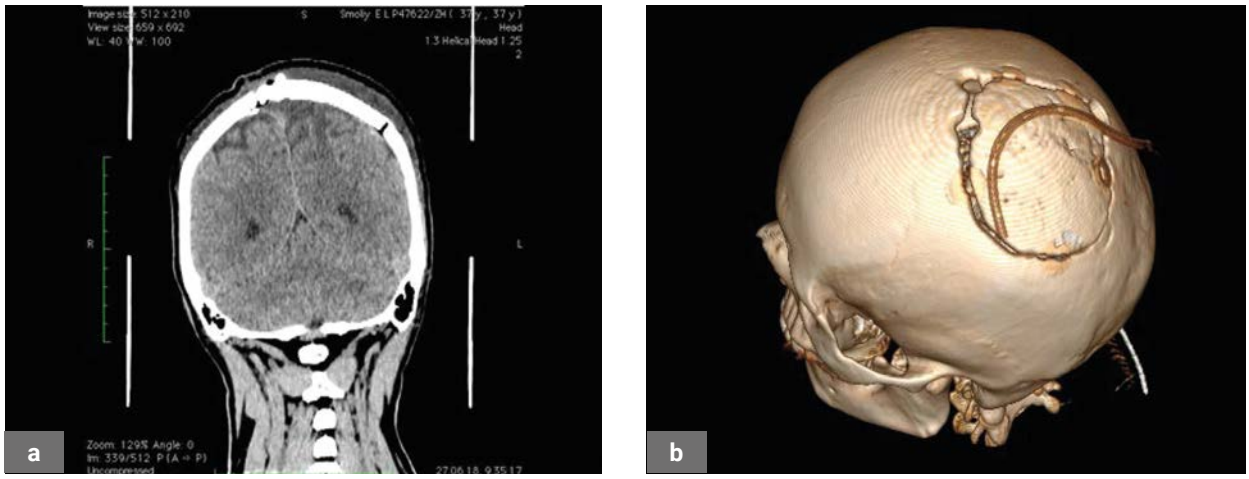

Fig. 4. Postoperative SCT of the brain after the operation of bone flap refixation using Medtronic craniofixes: a – there are no postoperative hemorrhagic complications; b – during 3D reconstruction in the bone mode, the satisfactory standing of the bone flap is determined.

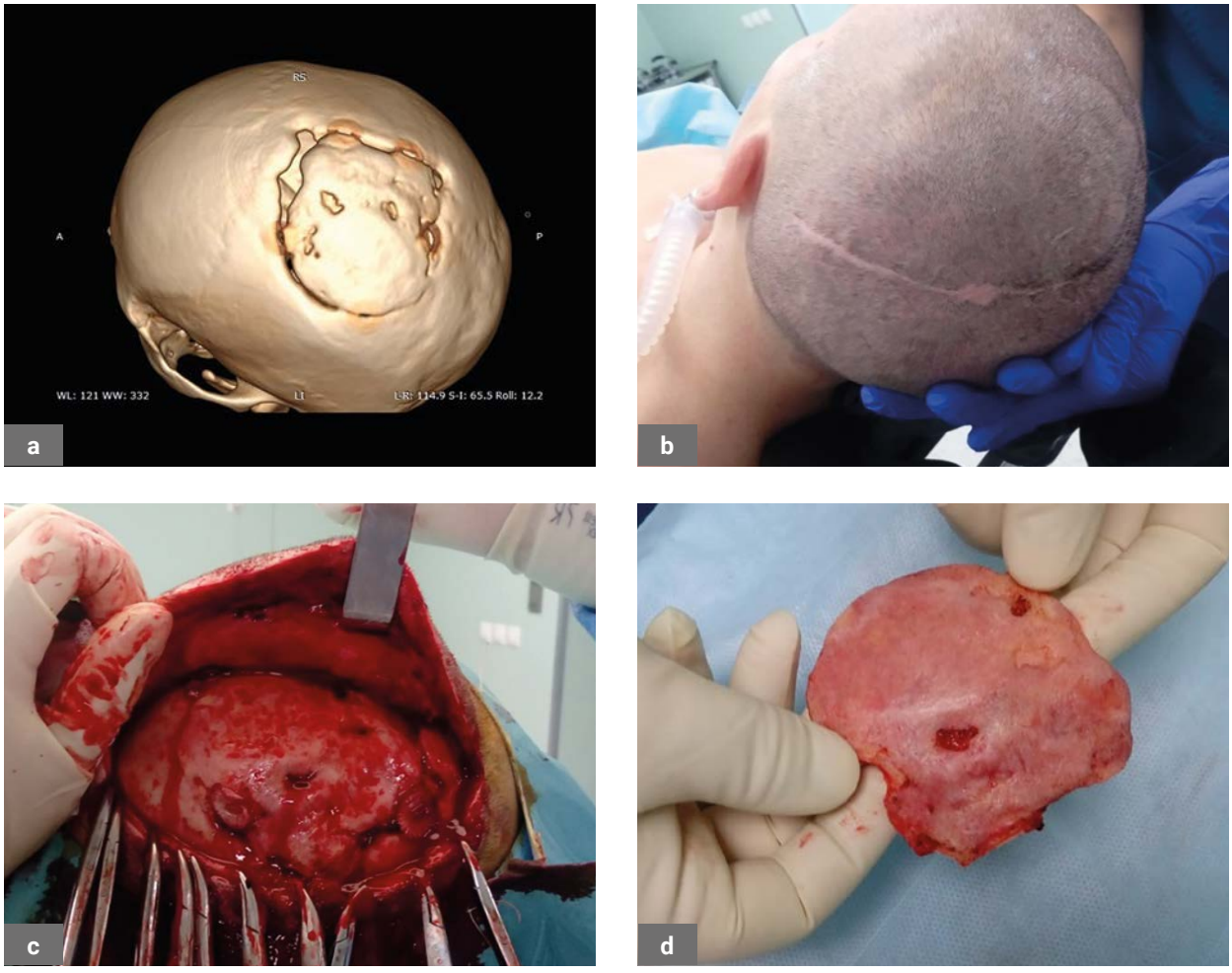

Fig. 5. a – 3D reconstruction in bone mode 6 months after repeated surgery. Bone resorption is determined along the line of the bone cut and in the thickness of the bone flap; b – the area of the postoperative scar and soft tissues of the head are not changed; c – intraoperatively determined mobile cyanotic flap, with multiple foci of resorption; d – removed bone flap.

growth of microflora was not obtained. Histological examination revealed non-specific changes in the bone tissue — pronounced dystrophy, foci of necrosis and small-focal hemorrhages, there are no signs of inflammation. The patient was discharged in a satisfactory condition. The planned step-by-step cranioplasty was completed using a custom-made titanium implant in August 2019.

DISCUSSION

In modern neurosurgery, most of the planned trepanations, in the absence of pronounced edema of the brain with prolapse into the trepanation window or tumor destruction of the bone, are completed with the installation of autostasis in the trepanation window using various fixation methods (7, 8). This procedure is standard and provides restoration of the shape of the skull, liquorodynamics and brain perfusion. Intraoperatively, it does not matter how the bone flap was processed and stored, since its blood supply is completely disrupted: the periosteum is detached, the diploic layer is crossed, and the perforants from the Dura mater are torn (10).

This clinical example shows partial resorption of the bone flap 12 months after performing BPC as planned. From the moment of CPT to the bone flap reimplantation, 2.5 hours passed. The wax was not used for hemostatic purposes. The bone graft was immersed in saline solution. Fixation of the bone flap was carried out using craniofixes company Medtronic.

Due to the limited number of reports of bone flap resorption, the pathophysiology of this process is not clear. A possible explanation for resorption can be observed in the presence of Gorham syndrome, in which progressive osteolysis is observed mainly in the tubular bones. During the examination of this patient, there were no additional foci of resorption or signs of osteoporosis in SCT of the skeleton.

Another likely predictor of resorption may be the use of wax during surgery or subaponeurotic accumulation of liquor in the postoperative period. As mentioned above, BPC destroys all sources of blood circulation in the bone, and the use of wax prevents the restoration of blood circulation after replantation through diploic veins. Subaponeurotic accumulation of liquor in the postoperative period also prevents the formation of scar tissue along the perimeter of the bone flap.

CONCLUSION

Nowadays, it is not possible to clearly determine the predictors of bone flap resorption after performing a planned BPC. The patient must be informed of the possibility of developing such a complication in the late postoperative period, and the neurosurgeon should avoid intraoperative use of wax and seal the Dura mater to prevent accumulation of liquor in the subaponeurotic space in the early postoperative period.

Authors contribution:
Rostorguev E.E. – research concept and design, manuscript writing, material processing, scientific editing.
Kuznetsova N.S. – collection, analysis and interpretation of data, surgical assistance, article preparation.
Yadryshnikova G.N. – collection, analysis and interpretation of data, article preparation.

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CLINICAL CASE REPORTS

BEVACIZUMAB IN MAINTENANCE THERAPY FOR OVARIAN CANCER PATIENTS

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ABSTRACT

Ovarian cancer is one of the most common cancers in women. Growth and extension of the tumor are associated with active neoangiogenesis regulated by vascular endothelial growth factor (VEGF). Bevacizumab decreases VEGF activity and inhibits the tumor growth.

Purpose of the study. The aim of the study was to evaluate results of bevacizumab in maintenance therapy for ovarian cancer.

Materials and methods. 26 patients with ovarian cancer received maintenance therapy with drop infusions of bevacizumab 15 mg/kg once a day for 21 days in 2014–2019 after completing chemotherapy for relapses.

Results. Bevacizumab mainterned partial response or stabilization in 76.9% of patients. The adverse events were mainly of grades 1–2 (in 88.5% of all adverse events) and could be managed by an appropriate medical correction. Hemorrhagic complications caused the cancellation of bevacizumab in one patient.

Conclusions. Bevacizumab in maintenance therapy after completing chemotherapy for ovarian cancer relapses (both platinum-sensitive and platinum-resistant) significantly improves the treatment results. The toxicity profile of bevacizumab in maintenance treatment is acceptable.

Keywords:
ovarian cancer, relapse, bevacizumab, maintenance therapy, progression-free survival, adverse event

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КЛИНИЧЕСКОЕ НАБЛЮДЕНИЕ

ОПЫТ ПРИМЕНЕНИЯ БЕВАЦИЗУМАБА В ПОДДЕРЖИВАЮЩЕЙ ТЕРАПИИ У БОЛЬНЫХ РАКОМ ЯИЧНИКОВ

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РЕЗЮМЕ

Одно из наиболее распространенных онкологических заболеваний среди женского населения — рак яичников. Рост и распространение опухоли связано с активным неоангиогенезом, который регулируется фактором роста эндотелия сосудов (VEGF). Бевацизумаб снижает активность VEGF, что подавляет рост опухоли.

Цель исследования. Оценка результатов применения бевацизумаба в поддерживающей терапии рака яичников.

Материалы и методы. В период с 2014 по 2019 годы 26-ти пациенткам с раком яичников проводилась поддерживающая терапия бевацизумабом 15 мг/кг внутривенно капельно 1 раз в 21 день после завершения курсов химиотерапии по поводу рецидивов заболевания.

Результаты. У 76,9% больных проведение поддерживающей терапии бевацизумабом позволило сохранить частичный ответ опухоли на лечение или стабилизацию. Возникшие нежелательные явления были в основном 1–2 степени (в 88,5% случаев от всех возникших нежелательных явлений) и контролировались назначением соответствующей медикаментозной коррекции. У одной больной возникшие геморрагические осложнения послужили причиной отмены бевацизумаба.

Заключение. Введение бевацизумаба в поддерживающем режиме после завершения химиотерапии рецидивов рака яичников (как платиночувствительных, так и платинорезистентных) позволяет значительно улучшить результаты лечения. Профиль токсичности применения бевацизумаба в поддерживающем режиме приемлем.

Ключевые слова:
рак яичников, рецидив, бевацизумаб, поддерживающая терапия, выживаемость без прогрессирования, нежелательные явления

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ABSTRACT

Ovarian cancer is one of the most common cancers in women. In Russia, there is an increase in the incidence of ovarian cancer. In 2008, the prevalence of this disease per 100,000 population was 59.1, and in 2018–76.2.in 2018, the proportion of patients with stage I–II ovarian cancer was 40.3% of the number of patients with a first-time diagnosis, and the remaining patients initially identified stage III and IV. The mortality rate of ovarian cancer patients within a year of diagnosis was 21.3% in 2018. [1] the Frequency of relapses after primary complex treatment in patients with stage III and IV ovarian cancer reaches 80% [2] in the early stages of the disease and the presence of adverse prognostic factors, the frequency of relapses is also high [3, 4]. The occurrence of ovarian cancer recurrence depends not only on the stage of the process, but also on the adequacy of the primary treatment. The appointment of effective schemes of antitumor drug therapy for ovarian cancer is the most important factor in improving the prognosis of this disease.

Vascular endothelial growth factor (VEGF) is an important regulator of physiological and pathological angiogenesis. It is known that in ovarian tumors, the expression of VEGF is higher than in normal tissue, because due to the rapid growth of the tumor and the increasing demand of cells for oxygen and nutrients, rapid neoangiogenesis is necessary. In the tissue of epithelial ovarian tumors, a sharp increase was detected not only in the absolute level of VEGF-A, but also in its ratio to the receptor-1, which shows the content of free endothelial factor and characterizes angiogenic activity in the tissue [5]. Bevacizumab is a recombinant hyperchymeric monoclonal IgG1 antibody that selectively binds and inhibits the biological activity of vascular endothelial growth factor *in vitro* and *in vivo*. Reduced VEGF expression leads to inhibition of vascular growth, which suppresses tumor growth, thereby affecting long-term results [6]. Major international studies of OCEANS (Ovarian Cancer Study Comparing Efficacy and Safety of Chemotherapy and Anti-Angiogenic Therapy in Platinum-Sensitive Recurrent Disease) and AURELIA (Avastin Use in Platinum-Resistant Epithelial Ovarian Cancer) have shown the effectiveness of anti-angiogenic therapy (bevacizumab) together with platinum and non-platinum combinations for platinum-sensitive and platinum-resistant relapses of ovarian Cancer [7, 8]. In a randomized phase III study, OCEANS compared the effectiveness of treatment of patients with platinum-sensitive recurrent ovarian cancer, primary peritoneal or fallopian tube cancer using gemcitabine+carbopaltin+bevacizumab regimens (main group) and gemcitabine+carboplatin+placebo (control group). The average number of cycles of chemotherapy in both groups is 6 (minimum 1 course, maximum 10 courses). Bevacizumab or placebo was administered intravenously on the first day of each cycle of chemotherapy, and after completing cycles of chemotherapy, continued use of bevacizumab or placebo until the disease progressed or intolerant toxicity appeared. The average number of bevacizumab cycles was 12 (from 1 to 43), placebo — 10 (from 1 to 36). Analysis of the results of this study (median follow-up was 24 months) revealed a 2-fold reduction in the risk of disease progression and a statistically significant increase in the period without disease progression in the group of patients receiving bevacizumab. Thus, the median period without disease progression in the main group was 12.4 months, and in the control group — 8.4 months (RR 0.484; 95% CI 0.388–0.605; log-rank $p<0.0001$). Partial response to treatment was observed in 61.2% of patients in the main group (48.3% in the control group). The response time in the main group was 10.4 months, in the control group — 7.4 months (RR 0.534; 95% CI 0.408–0.698). A randomized phase III trial of AURELIA evaluated the effectiveness of bevacizumab and chemotherapy in patients with platinum-resistant recurrent ovarian cancer. The primary endpoint was progression-free survival. In this study, patients were randomized into two groups, one of which received monohymotherapy (pegylated liposomal doxorubicin, paclitaxel, or topotecan), and the second received monohymotherapy in combination with bevacizumab. In the group of patients

who received monohymotherapy in combination with bevacizumab, the median progression-free survival was 6.7 months (95% CI 5.7–7.9), while in the group with only monohymotherapy — 3.4 months (95% CI 2.2–3.7) [9].

According to the practical recommendations of the Ministry of health of Russia and the Russian society of clinical Oncology, patients with recurrent ovarian cancer are recommended to add bevacizumab to the chemotherapy regimen (7.5 or 15 mg / kg IV once every 3 weeks). After the end of chemotherapy, bevacizumab administration is recommended to continue until progression or unacceptable toxicity [10]. The goal of maintenance therapy is to maintain the patient's clinical status achieved by previous treatment [11]. Thus, extending the time until the subsequent progression of the disease is considered a priority when prescribing maintenance therapy. Maintenance therapy continues for a long time, which is limited to establishing the progression of the disease or the appearance of unacceptable side effects. The first drug shown to be used in a maintenance regimen for ovarian cancer was bevacizumab.

Purpose of the study: generalization of the experience of using bevacizumab in maintenance mode for ovarian cancer.

MATERIALS AND METHODS

From 2014 to 2019, 26 patients with ovarian cancer received supportive therapy with bevacizumab after completing chemotherapy for relapses. At the start of primary treatment for ovarian cancer, the patients were aged 45 to 70 years, with an average age of 51 ± 10 years. The distribution of patients by stages of the disease is shown in table 1.

Serous-papillary carcinoma was determined by morphological examination in 12 patients (46.2%), serous carcinoma — in 5 (19.2%), mucinous carcinoma in 3 patients (11.5%), endometrioid carcinoma in 2 patients (7.7%), serous-mucinous carcinoma in 2 patients (7.7%), undifferentiated carcinoma was detected in 2 patients (7.7%). In 16 patients (61.5%), a low degree of tumor differentiation (G3) was detected, in 2 patients (7.7%), a high degree of tumor differentiation (G1), in the remaining patients, the degree of differentiation was not determined. In most cases (18 people (69.2%), primary treatment of ovarian cancer consisted of performing the surgical stage and then conducting 6 courses of polychemotherapy. In 8 patients (30.8%), the first stage was 3 courses of neoadjuvant polychemotherapy, followed by the surgical stage of treatment and continued polychemotherapy.

Most of the patients (22, 84.6% of) recurrence of the disease in terms of more than 6 months from completion of treatment of the primary tumor (platinum-sensitive relapse), in 4 patients (15,4%) were observed latinomericanas relapse of ovarian cancer. More often, the progression of the disease was manifested by a relapse in the pelvis (53.8%), peritoneal metastases (46.1%) and metastases in distant lymph nodes (38.5%). In most cases, 16 patients (61.5%) had not single metastases, but combined lesions of two or more organs (table 2).

In 10 patients (38.5%), bevacizumab was added to the antitumor drug therapy regimen for the treatment of the first recurrence of ovarian cancer. Four patients from this subgroup had received bevacizumab 15 mg/kg intravenously 1 every 3 weeks in conjunction with doxorubicin 50 mg/m^2 intravenously in the 1st day 21-day course, four

Table 1. The distribution of patients by stage of disease (FIGO)

Ovarian cancer stage by FIGO	Absolute number of patients	Percent (*n*=26)
I	2	7.7%
II	2	7.7%
III	16	61.5%
IV	6	23.1%

of the patient — in combination with paclitaxel 175 mg/m^2 intravenously in the 1st day 21-day cycle and carboplatin AUC 5 intravenously in the 1st day 21-day course, two patients with pegylated liposomal doxorubicin 40 mg/m^2 intravenously in the 1st day, 28-day course. In 10 patients (38.5%), bevacizumab was included in the treatment regimens for the second relapse, which the patients continued to receive even after completing chemotherapy. Of this subgroup of patients in the two bevacizumab 15 mg/kg intravenously 1 every 3 weeks was administered in combination with pegylated liposomal doxorubicin 40 mg/m^2 intravenously in the 1st day of the 28 day course, in two patients with paclitaxel 175 mg/m^2 intravenously in the 1st day 21-day cycle and carboplatin AUC 5 intravenously in the 1st day 21-day course, two patients with doxorubicin 50 mg/m^2 intravenously in the 1st day of the 21-day course two — with gemcitabine 1000 mg/m^2 intravenously in the 1st, 8 days 21-day cycle in combination with carboplatin AUC 4 intravenously in the 1st day 21-day course, two patients with etoposide 100 mg orally in 1 to 5 days 21-day cycle and carboplatin AUC 5 intravenously in the 1st day 21-day course. In the event of a third relapse, bevacizumab at a dose of 15 mg / kg intravenously drip once every 3 weeks in combination with chemotherapy was used in 6 patients (23%). Of this subgroup of patients two patients were injected with doxorubicin 50 mg/m^2 intravenously in the 1st day 21-day course, two pegylated liposomal doxorubicin 40 mg/m^2 intravenously in the 1st day, 28-day course, two — doxorubicin 50 mg/m^2 intravenously in the 1st day 21-day cycle and carboplatin AUC 5 intravenously in the 1st day 21-day course. After completing chemotherapy, the patients continued bevacizumab therapy in a supportive mode. In our group, patients received maintenance therapy with bevacizumab 15 mg / kg intravenously drip once every 3 weeks for periods from 3 to 29 months, on average 10.4 ± 5.4 months. Progression-free survival was calculated using the Kaplan-Meyer method, the objective effect of antitumor drug therapy was evaluated according to the RECIST 1.1 criteria, and statistical data processing was performed in the "Statistica 7.0"program.

RESEARCH RESULTS

Partial response to maintenance therapy with bevacizumab was recorded in 12 patients (46.2%), stabilization of the process — in 8 patients (30.7%), progression was detected in 6 patients (23.1%). Annual non-aggressive survival rate of 77%, the median was not reached.

We did not find data on information on the treatment response when bevacizumab was ad-

Table 2. Localization of the tumor process in the progression of ovarian cancer

Tumor localisation	Absolute number of patients	Percent (n=26)
Relapse in the pelvis	14	53.8%
Metastatic lesion of the peritoneum	12	46.1%
Metastases in the lymph nodes	10	38.5%
Liver metastases	6	23.1%
Metastatic lesion of the mesentery of the intestine	4	15.4%
Metastatic pleural lesion	1	3.8%
Metastases to the lungs	1	3.8%
Bone metastases	1	3.8%
Metastatic lesion of the large omentum	1	3.8%

ministered in a maintenance mode without additional administration of chemotherapy drugs. Therefore, we compared our data with the data from the phase III OCEANS study, which used the treatment regimen of carboplatin + gemcitabine + bevacizumab for the treatment of patients with platinum-sensitive ovarian cancer relapses (and continued administration of bevacizumab after completing cycles of chemotherapy). When comparing the data we received on the response to maintenance therapy with bevacizumab with the data from the phase III OCEANS study, we found similar indicators of partial response — in our group it was 46.2%, in the study — 61.2%.

Our data on progression-free survival do not contradict the results of the OCEANS study, where the time interval without disease progression in patients receiving bevacizumab in combination with chemotherapy was 12.4 months.

Among the adverse events, arterial hypertension was most common — 1–2 degrees in 20 patients (76.9%), 3 degrees in two (7.7%) patients. Grade 2 proteinuria on the background of bevacizumab therapy was observed in 1 patient (3.8%). Hemorrhagic complications were observed in 3 patients (11.5%), in one patient (3.8%), the resulting hemorrhagic complications caused the cancellation of bevacizumab. According to a study of OCEANS when adding bevacizumab to the treatment regimen was noted hypertension > grade 3 in 17% of patients, proteinuria more than 3 degrees at 9%. According to our data, the above adverse events were less frequent than in the phase III study, which may be due to the fact that bevacizumab was used in monotherapy in patients of the described group, whereas in the study it was used in combination with gemcitabine and carboplatin.

Clinical case

Patient E., born in 1953, after a planned visit to the gynecologist, on 10.06.2016, an ultrasound examination of the pelvic organs was performed, which revealed a solid cystic formation 86 x 83 x 69 mm posterior to the uterus and to the right. The level of blood cancer markers was determined: CA-125 was 1840 units / ml, Ne-4 89.34 pmol / l. According to computer tomography, no distant metastases were detected. A puncture biopsy of the ovarian tumor was performed, and a cytological analysis was obtained: «carcinoma». 15.07.2016 performed panhysterectomy, extirpation of the large omentum. A histological analysis was obtained during the morphological study: "low-grade serous-papillary carcinoma with the presence of petrificates, solid structures, infiltrative growth, focal small lymphocytic infiltrates in the fatty tissue of the omentum, ectasia and fullness of blood vessels, in the wall of the fallopian tube, mucosal atrophy, sclerosis of the submucosal layer, in the wall of the cervix, minor dysplasia of the integumentary epithelium, leiomyoma with foci of hyalinosis, hypoplastic endometrium." A clinical diagnosis was established-ovarian cancer St III C (rt3cn0m0), the condition after surgical treatment, clinical group 2. the Patient was given 3 courses of polychemotherapy according to the scheme carboplatin AUC 5 V/V drip on 1 day + docetaxel 75 mg/m^2 V/V drip on 1 day, every 3 weeks. Ultrasound examination of the pelvic organs after 3 courses of polychemotherapy revealed continued growth of the tumor (in the pelvis on the right and closer to the iliac region on the right Hypo-echogenic recurrent substrate up to 28 mm, along the posterior arch Hypo-echogenic recurrent infiltrate up to 45 mm with indistinct contours). In this connection, a change of the chemotherapy line was performed and 6 courses of chemotherapy with gemcitabine 1000 mg / m^2 IV / IV drip were performed on days 1, 8, 15 (28 day course). During the next control ultrasound examination of the abdominal and pelvic organs, the following data were obtained:"in the pelvic cavity, an isoechogenic node of 13 x 20 mm is located, a nodular formation along the back surface of the head of the pancreas (36 x 42 mm)". According to the data of spiral x-ray computed tomography of the abdominal organs, the picture of a volumetric pathological formation of the head of the pancreas (39 x 52 x 30 mm). According to magnetic resonance imaging of the abdominal cavity, the picture is characteristic of a cystic solid tumor

located in the area of the head of the pancreas with extra-organ growth in the area of the duodenum, attached to the right renal leg (58 x 44 x 49 mm). The patient was consulted by an abdominal oncologist, this situation is considered as a progression of ovarian cancer. Due to the progression, a change of the chemotherapy line was performed, since 17.05.2017, 9 courses of chemotherapy were performed with pegylated liposomal doxorubicin 40 mg / m^2 intravenously on 1 day, a 28-day course and bevacizumab 15 mg / kg intravenously once every 3 weeks. Next was made of spiral x-ray computed tomography of the chest, abdomen and pelvis and ultrasonography of the abdomen and pelvis (January 2018), which confirmed the stabilization of disease (epigastric and mesogastric Central and more right close to the head of the pancreas adjacent hypoechoic metastatic infiltration confluent character overall dimensions 74 x 28 mm, with isolated anechoic inclusion in the center, with color Doppler mapping (CDM) is weak, mixed flow, recurrence in the pelvis is not revealed). The level of tumor markers decreased to normal values-CA-125 9 u / ml, Ne-4 99.3 pmol / l. In connection with the stabilization of the disease, since January 2018, bevacizumab has been continued to be administered 15 mg/kg intravenously once every 3 weeks in a maintenance mode. Every 3 months, spiral x-ray computed tomography of the chest, abdominal and pelvic organs and ultrasound examination of the abdominal and pelvic organs are performed, and the levels of tumor markers (CA-125 and Ne-4) are determined, confirming the stabilization of the disease. By the time the article is submitted to the journal, the patient has received bevacizumab for 36 months, including 29 months in the maintenance mode. Somatic status on the ECOG 0 scale, the patient takes an active part in social life, continues to work in the specialty. Among the adverse events on the background of bevacizumab therapy, grade 1–2 proteinuria was detected (it first appeared 9 months after the start of bevacizumab therapy) and grade 2 hypertension (blood pressure is controlled by taking an angiotensin II type 1 receptor antagonist, a loop diuretic and a cardioselective beta1-adrenoblocker).

CONCLUSIONS

Administration of bevacizumab in a maintenance mode after completion of chemotherapy for recurrent ovarian cancer (both platinum-sensitive and platinum-resistant) can significantly improve treatment results. In 76.9% of patients in our group, bevacizumab maintenance therapy allowed maintaining a partial response of the tumor to treatment or stabilization. The toxicity profile of bevacizumab in maintenance mode is acceptable. The adverse events that occurred were mainly 1–2 degrees (in 88.5% of cases of all adverse events that occurred) and were controlled by prescribing appropriate medication correction.

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