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The protocol for the examination and selection of potential living donors of bone tissue for the production of allografts

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ABSTRACT

The responsible stage of choosing the source of bone allografts is the process of examination and selection of the donor, which includes moral and ethical, legal and medical aspects. The selection of potential donors should be carried out with the participation of a qualified doctor, with a thorough collection of anamnesis, social conditions and a general medical examination. Up to 48 % of all potential donors are rejected to collect bone in the pre-operative period; up to 22 % of the received tissues are not suitable for transplantation according to the results of further examinations.

***PURPOSE** – to develop a protocol for the examination of potential living donors of bone tissue based on anamnestic, clinical, laboratory and instrumental examinations to ensure the selection of high-quality bone material for the production of bone grafts.*

***MATERIALS AND METHODS.** From 01.01.2016 to 01.12.2021, 640 patients for hip replacement were involved in the selection of bone tissue donors. The following selection steps were applied: obtaining informed consent, filling in a questionnaire about the possibility of donation, express-tests for infections and X-ray morphometric examination based on the results of radiography of the hip joints in direct projection.*

***RESULTS.** Based on the results of the examination of potential donors, it was established that the number of patients who were excluded from further research due to refusal to donate was 3 %, due to contraindications according to the results of filling out the questionnaire – 15 %, according to X-ray morphometric criteria – 51 %, according to the results of laboratory examination – 2 %.*

***CONCLUSIONS.** A protocol of examination of bone tissue donors for the production of scaffolds has been developed. It was established that about 30 % of all examined potential donors are suitable for donation.*

***KEY WORDS:** regenerative technologies; bone plastic surgery; scaffolds; bone allografts*

The replacement of bone tissue defects is a significant problem in traumatology and orthopedics as well as in regenerative medicine. Deficiency of bone tissue occurs as a result of injuries, surgical interventions for neoplasms and tumor-like diseases, inflammatory processes, revision endoprosthetics, congenital malformations, etc.

The global bone graft market was valued at USD 2.91 billion in 2021 and it is expected to grow by an average of 6.2 % each year from 2022 to 2030. One of the key factors contributing to this is the increasing incidence of bone and joint diseases. According to a Center for Disease Control and Prevention (CDC) report, the global risk of hip fracture is expected to increase by 240 % among women and 310 % among men. These forecasts are associated with the influence of such factors as osteoporosis, the increase in the frequency of systemic diseases, the use of glucocorticoids, etc. [1].

Almost two million bone plastic surgeries are performed annually in the world [2]. More than half a million bone graft surgeries are performed in the United States each year, making bone grafting the second most common tissue graft after blood transfusion [3]. Improvements in the methods of manufacturing bone allografts over the past decades have created an alternative to autologous bone transplantation as the only method of replacing bone tissue, due to the ease and availability of its application, virtually unlimited volume, the possibility of combined use with antibacterial drugs, other regenerative technologies, etc., safety and the absence of risks and complications associated with the collection of autologous bone [4-6].

The responsible stage of choosing the source of allografts is the process of examination and selection of the donor, which includes moral and ethical, legal and medical aspects. Legal aspects of transplantation

in Ukraine are regulated by the Law of Ukraine "On the Transplantation of Anatomical Materials to Humans" [7], which provides for the possibility of receiving and using biological materials from living donors. Mostly, these are patients undergoing total hip arthroplasty.

One of the biggest threats when performing allotransplantation remains the possibility of transmitting infectious diseases. In order to exclude donors who may pose a potential threat, standards to maximize the quality and safety of the obtained bone scaffolds were developed by leading European specialists [8-9].

Current operating standards of tissue banks provide for strict control at all stages – the selection of a potential donor, the quarantine of bone tissues and the process of their treatment. In addition, the possibility of further control of all used allogeneic tissue samples is provided.

The selection of potential living donors is an important stage and should be carried out with the participation of a qualified doctor, with a thorough collection of anamnesis of infectious diseases, social conditions and a general examination. In the UK, up to 48 % of all potential donors are rejected even in the pre-operative period; up to 22 % of the obtained bone tissues do not undergo further examinations [10-11].

The **PURPOSE** of our study was to develop a protocol of examination of potential living donors of bone tissue based on anamnestic, clinical, laboratory and instrumental examination methods to ensure the selection of high-quality bone material for the production of bone grafts.

MATERIALS AND METHODS

640 patients hospitalized for hip replacement at the State Institute of Traumatology and Orthopedics NAMS of Ukraine and Kyiv City Clinical Hospital No. 12 from 01.01.2016 to 30.11.2022 were involved in the selection of bone tissue donors. The average age of the participants at the time of inclusion in the study was 52.5 years (from 38 to 84 years). Female patients predominated: 370 women and 270 men, respectively. Patients with the following diagnoses were included: dysplastic coxarthrosis – 90 patients, coxarthrosis (other) – 330 patients, femoral neck fracture - 150 patients, femoral head avascular necrosis – 70 patients (**Table 1**).

Table 1. Distribution of the total number of patients involved in the study by nosology and sex.

Diagnosis	Men	Women	Total
Primary coxarthrosis	160	170	330
Dysplastic coxarthrosis	30	60	90
Femoral neck fracture	60	90	150
Femoral head avascular necrosis	20	50	70
Total	**270**	**370**	**640**

The donor examination was carried out by orthopedic doctor who has a transplant coordinator certificate.

The first stage for further production of a bone allograft from the femoral head is obtaining informed consent from a potential donor for bone tissue collection. All potential living donors were asked to sign consent. Without informed consent, further stages regarding the donor are inappropriate. After obtaining consent for donation, the next stage is obtaining clinical data on the possibility of a person being a bone tissue donor.

Step 2 of living donor selection is the filling in a questionnaire on the possibility of donation, developed by our group [12].

Bone tissue donor questionnaire

Name, Surname _____
Date of birth _____
Hospital, department _____
Case-record No. _____
Diagnosis _____

Dear donor!

Please provide information about your health by answering "yes" or "no" to the following questions:

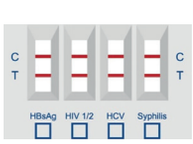
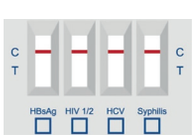
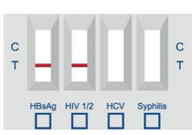
Questions		
1. Have you had hepatitis B or hepatitis C in the past or are you currently suffering from hepatitis? Have you ever been tested positive for hepatitis B or hepatitis C?	Yes	No
2. Have you had hepatitis A or hepatitis E during the last 2 months? Are you currently suffering from hepatitis A or E? Do you currently have or have previously had other liver diseases of unknown origin (chronic hepatitis, liver cirrhosis)	Yes	No
3. Are you HIV positive (regardless of whether symptoms of the disease appeared or not)?	Yes	No
4. Do you have an increased risk of infection with hepatitis B or C, HIV caused by your lifestyle? (are you in the group of risk of infection with diseases transmitted through biological fluids; patients with hemophilia are also in the group of high risk of infection)	Yes	No
5. Have you had contacts during the last 12 months with persons from the risk groups of infection with diseases transmitted through biological fluids?	Yes	No
6. Have you suffered from infectious diseases (for example, coronavirus infection, malaria, toxoplasmosis, tropical infectious diseases) that required long-term treatment, including intensive care?	Yes	No
7. Have you had or are you being treated for a serious infectious disease (for example, osteomyelitis, toxoplasmosis, tropical infectious diseases, diseases caused by protozoa)?	Yes	No
8. Have you had laboratory confirmed salmonellosis more than once in your life?	Yes	No
9. Do you have or have you been treated for tuberculosis? (You can become a donor if at least 2 years since the recovery)	Yes	No
10. Have you or your relatives been diagnosed with Creutzfeldt-Jakob disease? Do you suffer from neurological diseases of unknown origin?	Yes	No
11. Have you undergone treatment or are you suffering from cancer?	Yes	No
12. Have you had diabetes since childhood or insulin-dependent diabetes for more than 10 years?	Yes	No
13. Have you undergone hemodialysis or plasmapheresis in the last 6 months?	Yes	No
14. Do you suffer from severe neurological diseases (for example: meningitis, encephalitis, Alzheimer's disease, polyneuritis)?	Yes	No
15. Do you suffer from systemic autoimmune diseases that may have a negative effect on donor tissue (for example: rheumatoid arthritis, sarcoidosis, polychondritis, ankylosing spondylitis, Reiter's syndrome, rheumatism, lupus erythematosus, Graves' disease or myasthenia gravis?	Yes	No
16. Have you had any major surgery or endoscopy (biopsy) of the gastrointestinal tract, bronchi using flexible instruments during the last 6 months? Have you had minor surgery or tooth extraction in the last two weeks?	Yes	No
17. Have you had a blood transfusion within the last 6 months?	Yes	No
18. Have you ever been treated with human-derived pituitary hormones (such as growth hormones) or had surgery involving human or animal-derived implants (dura mater, heart valves, or cornea)?	Yes	No

19. Do you take any immunosuppressive drugs?	Yes	No
20. Have you had a tattoo or other piercing or acupuncture of the skin during the last 6 months outside of a hospital?	Yes	No
21. Have you been injured by needles contaminated with blood during the last 6 months?	Yes	No
22. Have you been vaccinated against rabies or received animal serum within the last 12 months?	Yes	No
23. Have you been vaccinated with so-called "live vaccines" during the last 4 weeks (measles, mumps, rubella, poliomyelitis, typhus, cholera, yellow fever, tuberculosis)? Have you been vaccinated against hepatitis B in the last 3 weeks?	Yes	No
24. Have you had an infectious disease caused by bacteria, viruses or fungi in the last 4 weeks?	Yes	No
25. Have you suffered from diseases accompanied by fever or diarrhea of unknown origin during the last 4 weeks?	Yes	No
26. Have you had contact with persons suffering from infectious diseases (for example, rubella, mumps, measles) during the past 6 months?	Yes	No
27. If you have ever been a blood donor, have you been refused a donation?	Yes	No
28. Have you been injured after contact with stray animals during the last 3 months?	Yes	No
29. Have you had intensive contact with toxic substances during the last 2 years? If Yes, explain please.	Yes	No
30. Did you participate in the liquidation of the accident at the Chernobyl nuclear power plant? Do you belong to the 1-4 categories of victims of the accident at the Chornobyl nuclear power plant? Do you live in the Chornobyl Exclusion Zone due to the accident at the nuclear power plant? Have you had a prolonged contact with ionizing radiation for another reason?	Yes	No

Step 3 – the analysis of X-ray morphometry of the potential living donor – we determined the volume of the femoral head suitable for processing and subsequent manufacture of the graft in patients-potential donors who passed the previous stages of selection. Patients were selected for the next stage if more than 2/3 of the femoral head was not damaged by a pathological process according to X-ray images. If more than 1/3 of the femoral head was injured the potential donor was excluded from further selection.

Step 4 – the serological examination of potential donor's blood for HIV infection, hepatitis B and C, syphilis was used in all patients who passed the previous stages of selection. The chromatographic immunoassay-based rapid test "Profitest" (New Vision Diagnostics, China) was used. The results were interpreted according to the standard criteria (**Table 2**):

Table 2. Interpretation of the results of chromatographic immunoassay-based testing of potential donors for the infections.

Positive		Two colored bands appear within 15 minutes, the test result is positive and valid. The test result can be read as soon as a clear colored band appears in the test area (T).
Negative		If there is no colored band in the test zone and a colored band appears in the control zone (C), the test result is negative and valid.
Invalid		The test result is considered invalid if a colored band is not formed in the control zone. It is necessary to repeat the test using a new test kit.

We apply this rapid test, because it has high sensitivity and specificity: for hepatitis B (HBsAg) – sensitivity 99.5 %, specificity 100 %; for hepatitis C – sensitivity 100 %, specificity 99 %; for HIV – sensitivity and specificity 100 %; for syphilis – sensitivity 100 %, specificity — 99.9 %. It is permissible to use other chromatographic immunoassay-based tests, provided they have the appropriate sensitivity and specificity. If there is a positive testing result for at least one of the infections, the patient cannot be a donor.

RESULTS AND DISCUSSION

The results of the questionnaire of potential donors of bone tissue.

The survey was conducted in patients who were indicated for hip arthroplasty due to coxarthrosis (primary and dysplastic), femoral neck fracture, and aseptic necrosis. Among all 640 surveyed potential donors of femoral head, 18 patients refused to donate at the stage of obtaining informed consent. That is, the percentage of refusals was 2.8 %.

According to the questionnaire, potential donors were excluded from patients with systemic and endocrine diseases that negatively affect the state of donor tissue [13-16], patients from risk groups for HIV infection, hepatitis B and C, and syphilis, and persons who have been in contact with toxic substances for a long time, as well as those who lived in the Chornobyl Exclusion Zone for a long time or were liquidators of the accident at the Chornobyl Nuclear Power Plant, since long-term contact with ionizing radiation is also a contraindication to donation [16-18].

In case of at least one "yes" answer, the patient was excluded from potential donors. We received positive answers from 96 respondents, which was 15 % of the total number of potential donors. That is, 546 patients were admitted to the next stage.

The results of X-ray morphometric examination.

In order to assess bone tissue defects, all patients underwent radiography of the hip joints in a direct projection in the pre-operative period. At the same time, such indicators as the shape of the femoral head, its size, the presence of cysts, areas of aseptic necrosis, and defects were evaluated.

The presence of cysts and zones of aseptic necrosis, even of a small size, was considered a risk factor for further selection of donors, since in the presence of subchondral cysts and aseptic necrosis, it is necessary to remove the injured tissue to the zone of unchanged trabecular bone. However, according to radiography, the volume of the changed tissue does not always correspond to the actual defect, which may require the removal of a larger volume of bone tissue (**Fig. 1**).

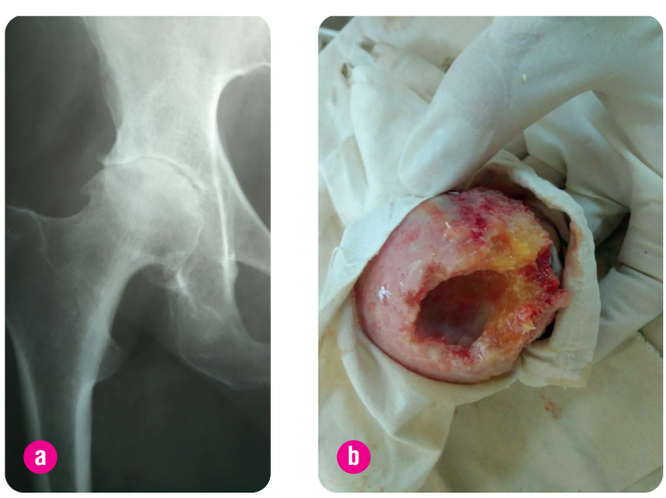


Fig. 1. Correlation of the image of the femoral head cyst according to radiography (a) with the actual size of the bone tissue lesion after surgical treatment (b).

Thus, patients with a cystic reconstruction or with aseptic necrosis of the femoral head, who have a lesion of more than 1/3 of its volume according to radiographs, are an exclusion criterion for potential donation, due to the fact that when processing such a femoral head more than 50 % of bone samples are lost for further plastic surgery. In such cases, a situation arises when the actual volume of bone tissue obtained after treatment will be insufficient for further use as a scaffold.

There are some examples of X-ray morphometric examination of potential donors of bone tissue, when patients were excluded from further selection based on their results. In each of the cases, the exclusion criterion is the presence of lesion by a pathological process of 50 % or more of the total volume of the femoral head (**Fig. 2-4**).

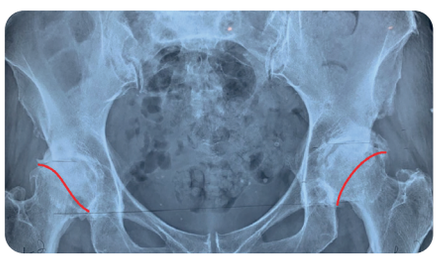


Fig. 2. The X-ray image of a patient with hip osteoarthritis with morphometric exclusion criteria for bone tissue donation (the border between the normal and injured area of the femoral head is marked with a red line).

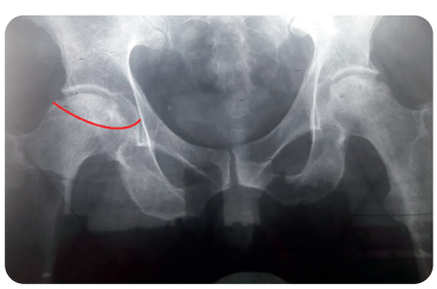


Fig. 3. The X-ray image of a patient with right-sided aseptic necrosis of the femoral head with morphometric exclusion criteria for bone tissue donation (the border between the normal and injured area of the femoral head is marked with a red line)

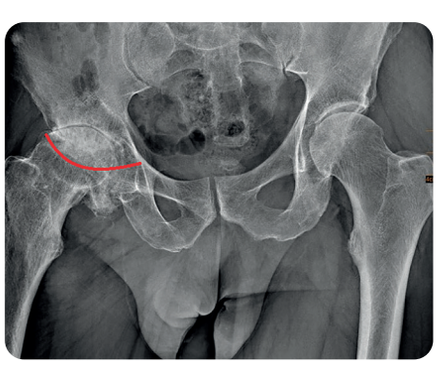


Fig. 4. The X-ray image of a patient with right-sided hip osteoarthritis with morphometric exclusion criteria for bone tissue donation (the red line marks the border between the normal and injured area of the femoral head)

According to the results of X-ray morphometric selection, 327 patients were excluded from further examination, which was 51 % of the initial cohort and almost 60 % of those potential donors of bone tissue who were evaluated by X-rays.

Results of the selection of bone tissue donors based on laboratory testing.

One of the biggest threats when using allogeneic bone scaffolds remains the possibility of transmitting infectious diseases. At the same time, not all donors are fully aware of the presence of negative factors that can affect the quality of the received material (suffered and concomitant diseases) or they may deliberately hide them.

In the course of the survey, 199 potential bone tissue donors, who were selected based on the results of the first three stages, were given a rapid tests to detect hepatitis B, hepatitis C, HIV, and syphilis, and the following results were obtained: in 187 patients, the test was negative, in 12 patients – positive, which was 2 % of the initial group and 6 % of those potential donors who were screened for the presence of infections. Among the positive results, the distribution was as follows: HIV – 1 case, hepatitis B – 7 cases, hepatitis C – 4 cases. All these patients denied the presence of these infections in the anamnesis. To clarify the diagnosis, they were referred for a PCR study in the postoperative period.

Thus, the protocol of bone tissue donor selection proposed by us can be presented as follows (**Fig. 5**):

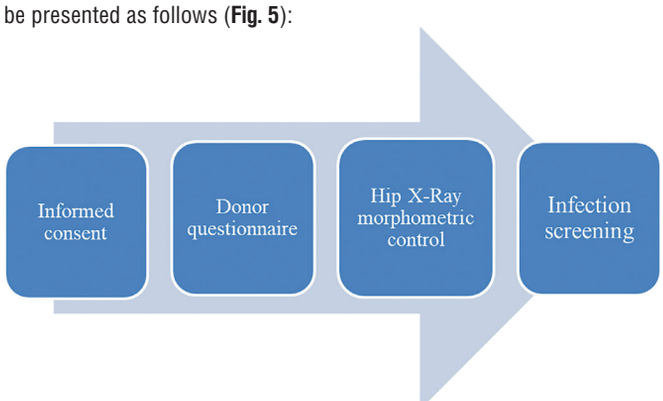


Fig. 5. Scheme of selection of potential bone tissue donors.

Based on the results of the examination, 640 potential bone tissue donors received the following results (**Table 3**):

Table 1. Distribution of the total number of patients involved in the study by nosology and sex.

Selection criteria	Excluded from further examination, patients	%, patients
Informed consent	18	2.8
Questionnaire	96	15
X-ray morphometry	327	51,1
Laboratory testing	12	1.9
Total	**453**	**70.8**

Therefore, according to the results of the examination of potential donors of bone tissue, 187 out of 640 patients were suitable for donation, that is, about 30 % of all examined.

Thus, the method of selecting bone tissue donors for the production of allografts that we have developed includes stages that minimize the risks associated with further transplantation as much as possible. However, it should be remembered that despite all the measures and conducted examinations of infectious risks, it is possible to detect only known

pathogens. Therefore, according to the second stage of selection – filling in the questionnaire, the risk group includes patients with degenerative neurological diseases of unknown origin, Parkinson's disease, multiple sclerosis, encephalitis, Alzheimer's disease, polyneuritis, etc. Tissues of tuberculosis patients (if less than 2 years have passed since recovery), asymptomatic salmonella carriers, patients with a history of sexually transmitted diseases, tropical and protozoan diseases cannot be used. The quality of donor bone tissue is negatively affected by autoimmune diseases and diseases that require long-term use of hormonal and immunosuppressive drugs: rheumatoid arthritis, sarcoidosis, ankylosing spondylitis, Reiter's syndrome, rheumatism, lupus erythematosus, thyroiditis, diabetes, etc., which is also reflected in our questionnaire. These risk factors are reflected as exclusion criteria in other known methods [13-16].

As a separate point, we highlighted patients who had long-term exposure to ionizing radiation. Twenty-four interviewed patients linked their disease directly to the impact of ionizing radiation (accident at the Chornobyl NPP). In addition, 35 patients lived or live in the territory affected by the accident at the Chornobyl disaster, although they did not directly associate their illness with radiation exposure. Ionizing radiation causes not only immediate biological effects, but can also lead to the occurrence of diseases during the entire subsequent life of a person. According to the National Academy of Medical Sciences of Ukraine, there is a significant increase in the frequency of tumors and non-malignant diseases among the victims of the accident at the Chornobyl NPP compared to the general population. The rate of injury to the musculoskeletal system due to exposure to ionizing radiation ranges from 6 to 12.3 % in different age groups and depends on the received dose. In general, according to the results of long-term studies, it was established that the period after the Chornobyl disaster was marked by a significant deterioration in the health of the participants in the liquidation of the accident at the Chornobyl NPP. The percentage of healthy people in the 26 years after the accident at the Chernobyl NPP decreased from 68 % in 1988 to 5.5 % in 2012 [19-20]. Considering the importance of radiological anamnesis for the selection of bone tissue donors, the international version of our questionnaire may contain a slightly modified question regarding the influence of this factor – "Have you had long-term contact with ionizing radiation?".

Our proposed protocol for the selection of living bone tissue donors, which includes patients who underwent hip arthroplasty, in addition to traditional criteria (informed consent, infection test results, donor interview results) [13-16] also takes into account X-ray morphometric criteria determined by a direct X-ray of hip joints, namely the relationship between normal and injured bone tissue. We do not consider it appropriate to donate if the volume of the femoral head is affected by 50 % or more. Thus, according to the results of the evaluation of the X-ray morphometric criterion, more than 50 % of patients were excluded from potential donors in our study.

With regard to the next stage – the test for infections, we prefer the rapid test, because it has several advantages over ELISA and PCR. It allows to reduce the pre-operative period and avoid additional manipulations since it is performed directly during surgery. It is economically beneficial in comparison with the mentioned studies, at the same time, it has a fairly high reliability. In addition, in some patients undergoing hip arthroplasty, the femoral head is used as an autologous graft, and often the decision is made by the surgeon directly in operating room. A rapid test is performed only when the surgeon makes a final decision that they will not use the femoral head as an autograft, which has an economic benefit and saves the patient from additional examinations in the preoperative period. Examination for infections in operating room is also performed in other countries [13].

Therefore, if we compare the results of the examination of patients who are potential donors of bone tissue, the exclusion rate obtained by us is generally the most consistent with the results of the study of our Scottish colleagues [15]. At the same time, regarding the structure of exclusion in our study, the largest number of patients are excluded from further selection at the stage of X-ray morphometric examination, which is probably due to the fact that patients in Ukraine apply for arthroplasty in later stages of the disease (hip osteoarthritis, aseptic necrosis) compared to other European countries.

CONCLUSION

1. ***A protocol for the examination of bone tissue donors for the manufacture of scaffolds has been developed, which includes the following selection steps: obtaining informed consent, filling out a questionnaire regarding the possibility of donation, conducting a rapid tests for the infections, and X-ray morphometric examination based on the results of radiography of the hip joints in direct projection.***
2. ***According to the results of the examination of potential bone tissue donors, it was established that the number of patients who were excluded from further research due to refusal to donate was 3 %, due to contraindications according to the results of filling in the questionnaire – 15 %, according to X-ray morphometric criteria – 51 %, according to the results of laboratory examination – 2 %. Thus, the largest number of potential donors is excluded at the stage of evaluation of patients' X-ray images.***
3. ***According to the results of the examination of potential donors of bone tissue, it was established that about 30 % of all examined potential donors are suitable for donation.***

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Методика обстеження та відбору потенційних прижиттєвих донорів кісткової тканини для виготовлення алотрансплантатів

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РЕЗЮМЕ

Відповідальним етапом вибору джерела кісткових алотрансплантатів є процес обстеження та вибору прижиттєвого донора, який включає в себе морально-етичні, правові та медичні аспекти. Відбір потенційних донорів повинен проводитись за участі кваліфікованого лікаря, з ретельним збором анамнезу, соціальних умов та загального обстеження. До 48 % всіх потенційних донорів отримують відмову до забору матеріалу ще в передопераційному періоді, до 22 % отриманих тканин не підходять для застосування за результатами подальших обстежень.

МЕТА. *Розробити методику обстеження потенційних прижиттєвих донорів кісткової тканини на основі анамнестичних, клінічних, лабораторних та інструментальних методів обстеження для забезпечення відбору якісного кісткового матеріалу для виготовлення кісткових трансплантатів.*

МАТЕРІАЛИ ТА МЕТОДИ. *У дослідженні з відбору потенційних донорів кісткової тканини взяли участь 640 пацієнтів з приводу ендопротезування кульшового суглоба за період з 01.01.2016 по 01.12.2021 року. Було застосовано такі етапи відбору: отримання інформованої згоди, заповнення опитувальника щодо можливості донорства, проведення тесту на наявність інфекцій у потенційного донора та рентген-морфометричне обстеження за результатами рентгенографії кульшових суглобів у прямій проекції.*

РЕЗУЛЬТАТИ. *За результатами обстеження потенційних донорів кісткової тканини за розробленим протоколом встановлено, що кількість пацієнтів, які були виключені з подальшого дослідження через відмову від донорства склало 3 %, через протипоказання за результатами заповнення опитувальника – 15 %, за рентген-морфометричними критеріями – 51 %, за результатами лабораторного обстеження – 2 %.*

ВИСНОВКИ. *Розроблено комплексну методику обстеження потенційних прижиттєвих донорів кісткової тканини для виготовлення скафолдів. Встановлено, що придатними для донорства є близько 30 % усіх обстежуваних пацієнтів.*

КЛЮЧОВІ СЛОВА: *регенеративні технології; кісткова пластика; скафолди; алотрансплантація кісткової тканини*