

## Research Article

<https://doi.org/10.23934/2223-9022-2025-14-2-360-370>

## Reconstruction of Cranial Vault With Autologous Graft in Combination With Allogeneic Bone-Plastic Material and Platelet Lysate in Delayed Cranioplasty

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**INTRODUCTION** In patients with cranial vault defects autologous cranioplasty with the bone flap is often accompanied by flap lysis. Bio-conductive factors, including additional biological structures and drugs, can be used to stimulate osteogenesis and integration of the bone flap.

**THE AIM OF THE STUDY** To evaluate the safety of using a modified autologous cranial graft combined with bone-plastic material (BPM) and autologous platelets' preparations, as well as the dynamics of cranial vault reconstruction in patients with delayed cranioplasty.

**MATERIAL AND METHODS** 7 patients, aged from 32 to 69 years, with diagnoses involving delayed cranioplasty were examined and treated. To increase the bioconductive properties, the bone flap was perforated, saturated with 0,8% allogeneic solution of human type 1 collagen, lyophilized, sterilized with ionizing radiation and stored at 20–22°C. The diastasis region was filled with allogeneic BPM, consisted of 0,8% human type 1 collagen and bone chips. Autologous platelet lysate was used to stimulate the revitalization of the cranial graft. The assessment of reparative processes was performed by multispiral computed tomography (CT) and three-phase osteoscintigraphy after 6, 12 and 18–24 months.

**RESULTS** In all the patients, the postoperative period was uneventful. Edges of the bone flap maintained their stability during all time of observation. In 6 of 7 patients the appearance of bone bridges was observed after 6 months, which led to formation of consolidation sites within 12–24 months. The X-ray density of the bone flap decreased slightly after 12 months without pronounced signs of flap lysis. In the postoperative period, steady decrease in the size of diastasis was noted from 2,0 (2,0; 3,1) mm to 0,9 (0,8; 1,5) mm after 18–24 months. Three-phase scintigraphy showed intensification of blood flow in the bone flap after 6–12 months, which was normalized by 12–24 months. Thus, active repair and regeneration of bone tissue in the area of diastasis began after 6–12 months and lasted for 18–24 months, followed by stabilization of the bone flap.

**CONCLUSION** The use of autologous cranial graft in combination with allogeneic bone-plastic material and platelet preparations was safe for delayed cranioplasty. Within 24 months after surgery, high preservation of the bone flap is observed, followed by gradual regeneration and consolidation of the bone in the diastasis area, which could be observed by CT and three-phase scintigraphy.

**Keywords:** autologous bone flap, bone-plastic material, diastasis, bone regeneration, computed tomography, scintigraphy

**For citation** Ofitserov AA, Borovkova NV, Makarov MS, Kudryashova NE, Leshchinskaya OV, Mironov AS, et al. Reconstruction of Cranial Vault With Autologous Graft in Combination With Allogeneic Bone-Plastic Material and Platelet Lysate in Delayed Cranioplasty. *Russian Sklifosovsky Journal of Emergency Medical Care*. 2025;14(2):360–370. <https://doi.org/10.23934/2223-9022-2025-14-2-360-370> (in Russ.)

**Conflict of interest** Authors declare no conflicts of interests

**Acknowledgments, sponsorship** The study has no sponsorship

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Russian Sklifosovsky Journal of Emergency Medical Care. 2025;14(2):360–370.

<https://doi.org/10.23934/2223-9022-2025-14-2-360-370>

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BPM — bone-plastic material  
 CT — computed tomography  
 DM — dura mater  
 IV — intravenous

PRP — platelet-rich plasma  
 RAI — relative accumulation index  
 RP — radiopharmaceutical  
 SPECT — single-photon emission computed tomography

## INTRODUCTION

Reconstructive plastic surgeries, in particular cranioplasty, have become firmly established in modern neurosurgical practice. At the same time, the presence of a defect in the bones of the cranial vault is the cause of complications, and leads to a significant decrease in the quality of life [1]. In such cases, cranioplasty is highly relevant. Despite many years of experience in cranioplasty practices, there is no generally accepted and unambiguous algorithm for choosing the material and timing of the surgical intervention. Of the artificial materials, titanium plates and meshes are primarily used. Depending on the type of alloy, different types of metal prostheses have their own specific complications. The use of metal structures in cranioplasty not only complicates the operation itself, but also creates the preconditions for the occurrence of additional

complications, such as rejection, heating, deformation, or the appearance of interference during functional diagnostic examination procedures [2–4].

Allotransplantation of bone tissue is relatively common in reconstructive traumatology and orthopedics, but it is not widely used in neurosurgery for cranioplasty [5]. Cranioplasty with an autologous transplant is an economically advantageous, and the most physiological method for closing trepanation defects. The autograft is physiological, biocompatible, non-toxic, with low thermal and electrical conductivity, and also has an osteoconductive effect. However, in some cases, the use of autotransplants in reconstructive cranial surgery is accompanied by lysis of the transplant, which leads to the need for repeated cranioplasty [6–8]. This complication may be caused by a decrease in

the osteoconductive and osteoinductive properties of the transplant during its preservation and storage.

In this regard, the issue of developing methods for additionally “enhancing” the properties of the autograft during delayed cranioplasty remains relevant, one of which is saturation with substances that promote cell migration, proliferation and differentiation. One of the most well-known and accessible materials used for this purpose is human collagen type 1. It is known that it ensures cell migration to the bone defect area, stimulates vascular growth, and promotes adhesion of immobilizing structures and implants. However, collagen itself does not have osteoinductive properties. Regenerative processes can be stimulated directly in the area of the bone defect using various growth factors: platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor (TGF-1), insulin-like growth factor (IGF-1), vascular endothelial growth factors (VEGF). All of the above factors are contained in granules (secretory vesicles) of biologically complete platelets [9].

Thus, the development of methods for preparing and preserving the autotransplant, its modification in order to improve osteoreparative properties is particularly relevant, which will allow the cranioplasty method to reach a qualitatively new level. At the same time, there is reason to believe that saturation of the autograft with collagen and platelets will significantly accelerate the regeneration of the bone defect.

**The aim** of the work was to evaluate the safety of using a modified autologous cranial graft in combination with bone-plastic material (BPM) and drugs based on autologous platelets, as well as the dynamics of cranial vault restoration in patients with delayed cranioplasty.

#### MATERIAL AND METHODS

At the Department of Neurosurgery of the N.V. Sklifosovsky Research Institute for Emergency Medicine, 7 patients with post-trepanation defects of the cranial vault were examined and surgically treated from 2019 to 2024 after decompressive trepanations performed for aneurysms and arteriovenous malformations of the brain. The age group was from 32 to 69 years (mean age  $50.9 \pm 15.8$

years), 3 men and 4 women, who underwent delayed cranial vault bone grafting with an autologous transplant. The patients expressed their consent to participate in the study both orally and in writing according to the established form. All risks, as well as alternative treatment methods, were discussed with patients and their relatives in person. The work was carried out with the approval of the local committee on biomedical ethics (extract from Protocol No. 3-19 dated 19.08.2019), and with the support of a Grant (Moscow Center for Innovative Technologies in Healthcare, agreement No. 1603-22/23.). In the pre- and postoperative period, all the patients were assessed for the condition of the skin around the postoperative suture, the presence of hyperemia, edema and purulent discharge.

#### AUTOLOGOUS CRANIAL VAULT BONE GRAFT

The bone fragment explanted during surgery was hermetically packed sequentially into two sterile specialized polyethylene bags, and sent to a specialized tissue preservation and transplant production department, where it was placed in a refrigeration chamber at  $-40^{\circ}\text{C}$  until the decision was made on performing delayed cranioplasty.

After the decision was made to perform autocranioplasty, the frozen skull flap was mechanically cleared of soft tissues using a scalpel, scissors, and tweezers. To remove bone marrow and fat, as well as for subsequent saturation of the transplant with liquid type 1 collagen and intraoperative saturation of the transplant with platelet-rich plasma (PRP), holes were made in the cortical layers using a drill (Proxxon Micromot Super Set No 28 475, Aesculapius) and a 4 mm diameter drill bit. The holes were made in a checkerboard pattern with a density of 3-5 holes per 6 cm<sup>2</sup> depending on the thickness of the bone layers, but in such a way that the holes on the outer and inner cortical layers did not coincide. This was done to ensure that collagen would better penetrate the spongy layer of the transplant and be retained in it. Some of the holes (3–5) were made through for fixation to the dura mater and ensuring drainage function. The sawdust obtained during drilling (from 3 holes) was collected in a gauze napkin for further seeding for sterility.

The bone fragment was placed in a desiccator with a 3% hydrogen peroxide solution for 30 minutes. A negative pressure of 910 mbar was created in a desiccator using a standard Atmos C 361 surgical aspirator (Germany). The bone fragment was then rinsed in 0.9% sodium chloride solution; this process was repeated 3–4 times for 6–8 hours. The graft was then immersed again in 3% hydrogen peroxide solution, and left in a refrigerated chamber on a shaker for 15–20 hours. After deproteinization, the bone fragment was left on a shaker in a 95% alcohol solution for 24 hours to dehydrate. After that, delipidization was performed by double exposure of the bone fragment to a mixture of 95% ethyl alcohol and diethyl ether in a 1:1 ratio for 24 hours. At the final stage of chemical modification, the bone fragment was left in pure ether for 24 hours, after which it was passively dried in a fume hood at room temperature. To enhance the bioconductive properties, the bone fragment was additionally saturated with 0.6–0.8% allogeneic solution of human type 1 collagen obtained by acid extraction from tendons. The collagen-saturated bone fragment was frozen to  $-40^{\circ}\text{C}$  and lyophilized in a chamber with a rarefied atmosphere.

The lyophilized bone fragment was packed in double polypropylene bags, labeled and subjected to radiation treatment in a GU-200M gamma unit with a surface dose of  $20 \pm 5$  kGy and with inversion (Fig. 1).



Fig. 1. Autologous cranial vault transplant, collagen-saturated and lyophilized

#### ALLOGENIC BONE-PLASTIC MATERIAL

Allogenic bone-plastic material (BPM) was manufactured using our own method (10). For the production of BPM, solutions of human type 1 collagen isolated from tissue donor tendons by acid

extraction in 0.01 M acetic acid and bone crumbs were used. Bone crumbs with a granule size of 315–630  $\mu\text{m}$  were mixed with a 0.7–0.8% collagen solution, aiming to achieve uniform distribution and the absence of conglomerates. Then the finished mixture was placed in square plastic forms measuring  $10 \times 10$  cm, frozen, and lyophilized in a chamber with a rarefied atmosphere. The finished BPM samples were sterilized with ionizing radiation of 25 kGy.

#### CRANIOPLASTY WITH AN AUTOLOGOUS TRANSPLANT

Cranioplasty using an autologous fragment of cranial vault bones was performed 4–6 months after decompressive trepanation. In all the patients, the skin flap sank, transmitted the pulsation of the brain, and the skin scar was solvent.

Cranioplasty was performed with the patient lying on his back. The head was fixed with the three-pronged Mayfield head clamp, raised relative to the body by  $10\text{--}15^{\circ}$ , and turned away from the vertical depending on the location of the defect (Fig. 2).

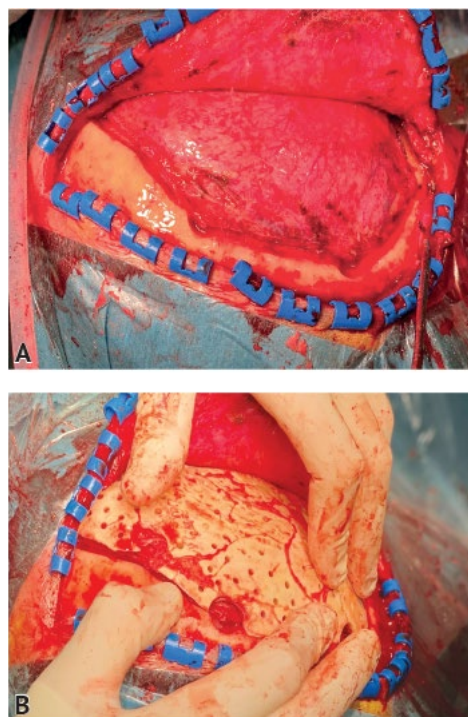


Fig. 2. Installation of bone autograft. A — cranial vault defect, B — graft in the defect area

The skin incision area was infiltrated by a 0.5–1% lidocaine solution with adrenaline at a dilution of 1:100,000. Before access was created, the autologous graft was placed in sterile distilled water for 40 minutes for rehydration. The skin incision was made along the postoperative scar with its partial excision. Hemostatic clamps or Raney skin clips were applied to the edges of the skin incision. A cutaneous-aponeurotic flap was formed with preservation of feeding vessels and nerve trunks. The flap was turned back to place a gauze roll underneath it, and then it was fixed to the operating table above the patient using spring hooks. The brain tissue was assessed to exclude its prolapse (Fig. 2A). The edges of the bone defect were treated with a bur or a curved raspatory until "blood dew" appeared. The graft was placed into the defect (Fig. 2B), and the BPM was placed into the diastasis between the graft and the bed, replacing all bone voids; on average, about 40 cm<sup>3</sup> of BPM was required. The cranial graft was fixed to the skull bones along the perimeter using titanium mini-plates and microscrews. To prevent the formation of postoperative epidural hematomas, the dura mater (DM) was sutured to the transplant in the center and along the periphery. After careful hemostasis, the BPM was saturated with 4 ml of autologous platelet-rich plasma. The aponeurosis and skin were sutured with continuous or interrupted sutures. It is worth noting that due to additional nibbling of bone tissue with a Luer bone nibbler after the skull vault fragment was cut out with a craniotome (during the first operation), the autograft made from it could be somewhat smaller than the defect itself.

#### INTRAOPERATIVE USE OF BONE-PLASTIC MATERIAL

After installation and partial fixation of the transplant in the defect area, the volume of lost bone tissue was assessed. Immediately before use, the BPM was placed in distilled water for 30 minutes. Using tweezers and a spatula, the required volume of BPM was tightly placed into the milling holes and along the perimeter of the transplant, which allowed almost complete filling of all the formed bone voids. The bone flap was finally fixed with titanium plates on microscrews, and the skin flap was fixed with a continuous suture.

#### Preparation of autologous platelet lysate

One day before cranioplasty, 40 ml of venous blood was collected from the cubital vein of the patients while maintaining sterility, and placed in vacuum tubes with ethylenediaminetetraacetic acid (EDTA) anticoagulant. Then, using a centrifuge (Beckman Coulter, Allegra X-15R, USA), the blood was separated into its components by centrifugation at 300 g for 5 minutes, maintaining its sterility, and transferred to new centrifuge tubes (Falcon type). To concentrate platelets, the Falcon tubes were centrifuged again at 700 g for 17 minutes to obtain a platelet pellet and supernatant in the form of platelet-poor plasma. Three-quarters of the platelet-poor plasma volume was removed from the tube, and the platelet sediment was resuspended in the remaining volume. As a result, 4 ml of PRP with a cell concentration of 1000–1600×10<sup>9</sup>/l were obtained from 40 ml of blood. To obtain autologous platelet lysate, PRP was frozen at -40°C for cell cryodestruction and thawed immediately before use. The platelet lysate was slowly thawed at 2–4°C, and then the samples were centrifuged at 3000 g to remove cell fragments. The prepared autologous platelet lysate was a cell-free fraction with platelet components. Using a syringe, platelet lysate was applied intraoperatively to the autologous graft after its placement, and to the BPM before its introduction into the diastasis (Fig. 3).



Fig. 3. Injection saturation of the autograft with autogenous platelet lysate. The diastasis between the bone fragment and the patient's own bone is filled with BPM (shown by arrows)



### Methods of diagnostic radiology

The work used methods of computed tomography (CT) and scintigraphy. Computer tomographic examinations in the early postoperative period were performed on an Aquilion CXL CT scanner (Toshiba Medical System Corporation, Japan). On a hybrid scanning system (Discovery 670 NM/CT, GE, USA), combined (hybrid) examinations were performed – CT and scintigraphy/ single-photon emission computed tomography, SPECT – to assess the structure and blood supply of the bone flap. During hybrid examinations, scintigraphic and CT data were processed on standard CT (AW VolumeShare 5) and gamma camera (Xeleris) workstations using a standard software package.

Isolated CT of the skull was performed in the early postoperative period - within the first week after cranioplasty. Then, after 6, 12, and 18–24 months, hybrid examinations (CT and scintigraphy/SPECT) were performed to assess bone tissue consolidation, bone flap condition, and BPM distribution. CT examinations in the postoperative period were performed without contrast with a slice thickness of 0.25–1 mm. CT images were viewed in a "bone window" with WL (Window Level) 1000 and WW (Window Width) 3000. When performing CT in the area of the cranial vault defect, the bone flap edge, the degree of consolidation of the bone defect and implanted flap edges, the presence of bone bridges in the diastasis area, and the presence of osteolysis in the bone flap were assessed. To quantify the bone flap and healthy bone, the X-ray density of the bone flap substance and the density of the contralateral skull in Hounsfield units (Hu) were measured by constructing a region of interest (ROI) in the form of a circle, including the entire thickness of the bone, and capturing both compact plates in symmetrical areas – in the implanted bone flap and the native bone of the contralateral area, but without capturing the soft tissue areas and cerebrospinal fluid spaces. To assess the integrity of the autologous bone flap in the regions of interest, the density coefficient (K1) was calculated,  $K1 = Dt : D_{native} \times 100$ , where Dt is the mean X-ray density of the transplanted flap, D<sub>native</sub> is the mean X-ray density of the native bone in the contralateral area.

Three-phase bone scintigraphy of soft tissues and skull bones was performed dynamically (after 6, 12, and 18–24 months) with <sup>99m</sup>Tc-Pirfotech osteotropic radiopharmaceutical (RP) (intravenous (IV) 500 MBq; radiation load 2.5 mSv). In bone tissue, <sup>99m</sup>Tc-Pyrfotech binds to hydroxyapatite crystals and immature collagen. The level of RP accumulation is determined by the metabolic activity of bone tissue, intensity of blood flow in it, and sympathetic innervation affecting the tone of the arteries. The first phase is the hemodynamic one (immediately after intravenous (IV) administration of the RP, dynamic recording for 1 minute at 1 frame/sec); the second phase is the tissue one (10 min after IV administration of the RP, static image in the anterior and posterior projections, 300 sec/frame); the third phase is the bone one (2–3 hours after IV administration of the RP, static image in the anterior and posterior projections, 300 sec/frame). Planar scintigraphic images were supplemented by the volumetric tomographic mode of SPECT. The recording was performed in 60 projections, in a 128×128 pixel matrix, with the detector rotated by 360°; the exposure time per projection was 30 s. The SPECT data were compared with CT images. When analyzing scintigraphic images and SPECT data, the nature of RP accumulation in the defect area, and in the symmetrical area of healthy bone was visually assessed. The ratio of RP inclusion in pathological and intact bone tissue was assessed using the relative accumulation index (RAI) and the formula:

$$RAI = Ng / Np,$$

where Ng is the mean pulse count in the area of the autologous cranial vault bone graft, determined in the region of interest; Np is the mean pulse count in the bone tissue of the contralateral area of the skull.

### Statistical data processing

Statistical processing of the obtained data was performed using analysis of variance, the Microsoft Excel 2023 software package, and R programs (R version 4.2.2 (2022-10-31 ucrt) – "Innocent and Trusting" Copyright (C) 2022 The R Foundation for Statistical Computing Platform: x86\_64-w64-mingw32/x64 (64-bit). The median, 1st and 3rd quartiles, arithmetic mean, and standard deviation were calculated. The distribution was tested for

normality using the Shapiro-Wilk test. To assess the differences, the Friedman test for related samples with their non-normal distribution was used. Differences in values were considered statistically significant at a significance level of more than 95%.

## RESULTS

After cranioplasty, no complications such as suture hyperemia, purulent discharge, or graft instability upon palpation were observed in any patient in the postoperative period. In the early postoperative period, the patients underwent isolated CT to assess adequate distribution of the BPM and exclude postoperative complications. Fourteen days after surgery, the patients were discharged under the observation of a neurologist and surgeon, with subsequent control examinations after 6 months, 12 months, and 18–24 months. At these periods, control CT and scintigraphy examinations were performed to assess the regeneration of the transplants. At all observation periods, stability of the bone flap was noted, even when signs of osteolysis appeared in it in the form of local marginal areas or in the form of a diffuse decrease in flap density and widening of perforation holes. The configuration of the bone flap and metal fixators did not undergo significant changes. In 6 out of 7 patients, after 6 months, we observed the first signs of forming bone “bridges” (Fig. 4) which increased in size after 12–24 months, and formed

longer areas of consolidation of the flap and healthy bone (table, Fig. 5).

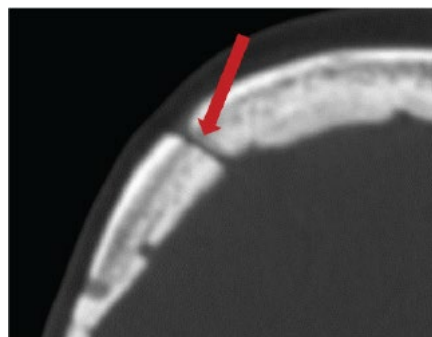


Fig. 4. Initial signs of consolidation along the edge of the bone defect after 6 months. The arrow indicates the forming bone tissue along the edge of the bone cut (CT, axial section)

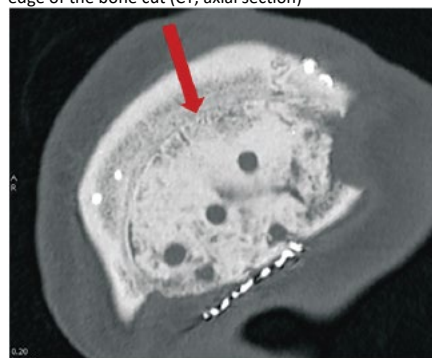


Fig. 5. The area of formed consolidation between the edge of the bone defect and the bone autograft (marked with the red arrow) with replacement of the diastasis by newly formed bone tissue 24 months after surgery

Table

**Evaluation of bone flap edges at different stages of postoperative examination**

| Post-operative examination period | Bone flap margin assessment, number of patients |                    |       | Bone regeneration at the edge of a bone flap, number of patients                |                               |
|-----------------------------------|-------------------------------------------------|--------------------|-------|---------------------------------------------------------------------------------|-------------------------------|
|                                   | "fresh" cut                                     | signs of sclerosis | lysis | Individual bone bridges/newly formed bone tissue along the edge of the bone cut | Areas of formed consolidation |
| Early postoperative period (n=7)  | 7                                               | 0                  | 0     | 0                                                                               | 0                             |
| 6 months (n=7)                    | 0                                               | 7                  | 1     | 6                                                                               | 1                             |
| 12 months (n=6)                   | 0                                               | 6                  | 4     | 4                                                                               | 6                             |
| 18–24 months (n=3)                | 0                                               | 3                  | 3     | 0                                                                               | 3                             |

After 6 months, all patients showed signs of sclerosis along the perimeter of the bone defect; signs of osteolysis were detected in 1 patient. After 12 months, signs of local marginal osteolysis were noted in 4 patients, after 18–24 months — in 3 patients, which may be associated with flap reconstruction and its integration into healthy bone. In the postoperative period, we noted a steady decrease in the diastasis size at the level of measurements along the edges of the bone defect and autograft: after surgery, the average size of the diastasis was 2.0 (2.0; 3.1) mm; after 6 months - 1.8 (1.4; 2.0) mm; after 12 months - 1.0 (0.9; 1.3) mm; after 18–24 months - 0.9 (0.8; 1.5) mm ( $p < 0.05$ ), which also confirms the presence of reparative and regenerative processes. After 24 months, 3 patients showed formed consolidation of the autologous flap and healthy bone with almost complete closure of the diastasis along the line of contact between the flap and the edge of the defect (Fig. 5).

It should be noted that over a period of 6–24 months, a number of patients showed a combination of signs of consolidation of varying intensity, and signs of local marginal osteolysis at different areas of contact between the bone defect edge and the autograft (Table 1). The zones of formed consolidation were more often observed in the anterior-superior part of the bone defect, which may be associated with a closer adjacency of the bone autograft to the edge of the defect. Zones of marginal osteolysis were most often detected in the posterosuperior or posterior part of the defect (in the temporal region).

The median values of radiographic density of the bone flap at the measurement level was initially 1260 (1122; 1443) Hu, the density of the contralateral area of healthy bone was 1400 (1260; 1530) Hu, the relative density of K1 was 0.95 (0.89; 0.97), the density of the bone flap was significantly lower than in the contralateral area ( $p < 0.05$ ), but this difference did not exceed 10% on average. After 6 months, these values did not change significantly; after 12 months, the density of the bone flap and K1 slightly decreased, averaging 940 (888; 1233) Hu and 0.91 (0.89; 0.94), respectively ( $p < 0.05$ ). After 18–24 months, the median values of radiographic density of

the bone flap and K1 increased, on average, to 1160 (1063; 1414) Hu and 0.95 (0.89; 0.97), but this difference was not significant ( $p > 0.05$ ). In general, the decrease in the radiographic density of the flap is due to its restructuring during the regeneration process. The absence of a sharp decrease in the radiographic density of the flap over 24 months indicates a high level of preservation of the autologous bone after all preservation procedures. The thickness of the flap immediately after surgery in the areas of the measurements was  $6.3 \pm 1.6$  mm, and did not change significantly after 6 months; after 12 months, a slight decrease in thickness to  $6.0 \pm 1.5$  mm was noted, which continued after 18–24 months ( $5.5 \pm 1.6$  mm).

In the early postoperative period, the BPM was determined in the burr holes and along the perimeter of the bone flap in the diastasis zone in all patients. At later stages of observation, gradual lysis of the BPM was noted, which was confirmed by decreased radiographic density in the BPM location zone; during subsequent examination, areas of formed consolidation with reduction of diastasis were identified at the site of BPM distribution. A longer observation period is required to assess complete consolidation.

### Results of three-phase scintigraphy

Accumulation of  $^{99m}\text{Tc}$ -pyrphotech osteotropic radiopharmaceutical (RP) in hydroxyapatite crystals and immature collagen occurs only in the presence of blood supply to bone tissue. Any bone damage accompanied by increased metabolism and reparation were recorded as areas of increased RP incorporation. All patients demonstrated an enhanced reparation process along the periphery of the bone flap within 6–12 months (RAI from 1.3 to 2.8, which corresponded to an excess of RP inclusion by 30–180% compared to symmetrical intact bone tissue) with a subsequent decrease in RAI by 18–30 months to 1.0, which corresponded to the norm (Fig. 6). In the projection of the bone graft, diffusely uneven RP distribution with sufficient inclusion in bone tissue (RAI from 0.9 to 1.1, which corresponded to normal blood supply and mineralization) was noted throughout the observation period. In two patients, after 18–24 months, a tendency toward a



decrease in RP inclusion in the bone flap was noted (RAI to 0.6–0.3—a decrease of 40–70% from intact bone tissue), which may be associated with the predominance of the osteolysis process during these periods.

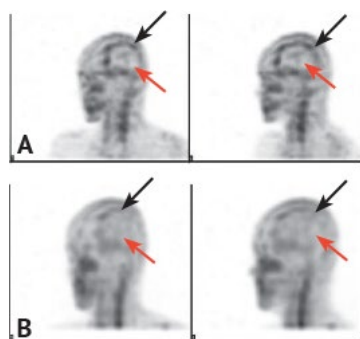


Fig. 6. SPECT of patient D. 6 months (A) and 12 months (B) after autologous cranioplasty. A – significant increase in RPA inclusion at the periphery (RAR=1.9), and a slight increase in the inclusion in the center of the graft (RAR=1.1) against the background of diffusely uneven distribution of RPA. B – SPECT of patient D. 12 months after autologous cranioplasty: a tendency to reduction of RPA inclusion at the periphery (RAR=1.4) against the background of normalization of RPA inclusion in the center of the transplant (RAR=1.0). The edge of the bone flap is shown by the black arrow, the central part of the bone flap is shown by the red arrow

## DISCUSSION

According to the results of our study, there was a general positive dynamics of bone tissue in the surgical site, absence of pronounced lysis of the bone flap, which is especially important in the area of the marginal adhesion of the autograft. This pattern was characteristic both for the number of bone bridges formed in the postoperative period by 12 months, with a significant number after 6 months, and for consolidation in general. In the study by Kubon (2024), the results of treatment of 100 patients were analyzed, each of whom had at least three control CT scans (from 6 weeks to 24 months) after delayed cranioplasty with an autograft [8]. All of them showed a decrease in the average density value in HU units for all bone flaps in the first year, and then a slight increase in HU in the second year. In our work, the decrease in the radiographic density of the autologous bone flap after 6–12 months was insignificant. It can be concluded that this process is not associated with flap lysis, but with its reconstruction and partial demineralization, similar to the results obtained previously by other authors,

where successful operations using an autograft of the cranial vault bone were described [11,12]. Reconstruction of the bone flap is necessary for its integration into healthy bone, which we observed during CT examination after 18–24 months, when areas of complete restoration of diastasis could be seen.

Three-phase scintigraphy using isotope-labeled compounds allows for an effective assessment of bone blood flow and the development of reparative or pathological processes. Scintigraphic examination with the osteotropic RP allowed us to evaluate the degree of mineralization, blood flow intensity, and intensity of bone tissue restoration. Bone resorption and osteolysis zones were characterized by defects in RP accumulation. Active bone reparation zones were characterized by increased RP accumulation (hyperfixation areas). The use of the hybrid radiation method of SPECT/CT made it possible to combine the capabilities of the radionuclide and X-ray methods within a single study, and provided comprehensive information on the condition of bone structures in the surgical site and their blood supply. It was shown on experimental animal models with bone defects of various origins that three-phase scintigraphy can be used to identify areas of osteonecrosis, to assess perfusion of bone grafts, and to generally assess bone tissue restoration [13–16]. At the same time, there are no clear data in the literature on the use of three-phase scintigraphy in assessing the safety of autologous cranial vault flaps both in intraoperative trepanations and in delayed cranioplasty. The use of the hybrid radiation method (three-phase scintigraphy + SPECT/CT) allows combining the capabilities of radionuclide and X-ray methods within a single examination, and provides comprehensive information on tissue blood flow. Three-phase scintigraphy demonstrated that after 6–12 months, the bone flap showed increased blood flow with normalization and a general tendency to equalize the indicators by 12–24 months. These results are similar to those obtained previously in other studies: for example, in a retrospective study of 65 patients, 53 of whom underwent emergency decompressive craniectomy and placement of a bone graft in the abdominal wall with subsequent delayed cranioplasty, 92% achieved a satisfactory result after one-stage reconstruction [13]. Three-phase

scintigraphy using technetium-99m methylene diphosphonate is considered to be able to assess the intensity of osteogenesis [15,16], but there are still no clear quantitative criteria for assessing this process. The impossibility of frequent analysis of biopsy material from the flap and diastasis zone significantly complicates the development of methods for assessing osteogenesis based on scintigraphic data. Nevertheless, the perfusion of the cranial transplant and diastasis zones revealed by scintigraphy indicates the development of reparative processes.

### CONCLUSION

The study showed that the use of preserved autologous cranial vault bone graft in combination with allogeneic bone-plastic material and platelet preparations is safe and effective in delayed cranioplasty. For 24 months after the operation, high preservation of the bone flap is observed against the background of gradual regeneration and consolidation of the bone in the diastasis area, which can be noted using computed tomography and three-phase scintigraphy. Active repair and regeneration of bone

tissue in the diastasis area begins after 6–12 months and continues for 18–24 months, while stabilization occurs in the bone flap area. For a more complete assessment of the consolidation processes of the autologous flap, it is necessary to extend the study.

### FINDINGS

1. Collagen-saturated autograft of the cranial vault bone maintains its localization and overall structure for 18–24 months after transplantation.

2. The use of allogeneic bone-plastic material reduces the size of the diastasis between the autograft and healthy bone by 2.0 times after 12 months, and by 2.2 times after 18–24 months ( $p < 0.05$ ), stimulates the formation of bone bridges between the autograft and healthy bone.

3. The intensity of osteotropic radiopharmaceutical inclusion increases by 30–180% compared to symmetrical intact bone tissue after 6–12 months ( $p < 0.05$ ) with subsequent normalization after 18–24 months.

4. Among the 7 patients examined, no cases of autograft destruction with subsequent repeat cranioplasty were identified.

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**Received on 10/12/2024**

**Review completed on 23/01/2025**

**Accepted on 24/03/2025**