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Use of bisphosphonates in experimental bone tuberculous osteitis: CT imaging

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Abstract

Introduction The complex treatment of osteoarticular tuberculosis is based on combination of anti-tuberculosis therapy, complete (radical) removal of involved bone and restoration of the supporting and motor function of the affected musculoskeletal segment. Inhibited activity of osteoclasts involved in osteoresorption as one of the mechanisms of reparative osteoregeneration can be involved in regulation of bone formation after radical reconstructive surgery. **The objective** was to explore CT signs of osteoregeneration due to multimodal treatment of experimental tuberculous osteitis with use of bisphosphonates as targeted inhibitors of osteoclasts. **Material and methods** An experimental study was carried out on 21 mature male Chinchilla rabbits. The first stage included bone tuberculosis simulated in the medial condyle of the right femur using invasive local infection with M. tuberculosis strains H37Rv, a virulent reference laboratory strain. Pathological focus was resected and bone graft used at the second stage. Animals receiving anti-tuberculosis therapy (ATT), ATT and bisphosphonates (BP) and BP only were divided into three groups at the third stage. Animals were sacrificed at 3 and 6 months of surgical treatment at the fourth stage. Autopsy implantation zone, bone of the contralateral condyle and intact femur were quantitatively and qualitatively assessed using micro-CT imaging. **Results** Positive dynamics in bone restoration was seen in the groups. ATT group showed complete lysis of the implant with bone cavities identified and no bone restoration in half of the cases seen at 6 months. Rabbits treated with BP demonstrated absence of complete lysis of the implant and CT signs of ingrowth of bone trabeculae. CT signs of maximum osteoregeneration were noted in the group of isolated BP therapy. **Discussion** The use of bisphosphonates can prevent lysis of grafts preserving the osteoconductive properties and facilitating formation of new bone. **Conclusion** Targeted osteoclast inhibitors can be safety and efficaciously used in the complex treatment of focal infectious skeletal lesions and be recommended as a potential component of pathogenetic therapy in the postoperative treatment of infectious (tuberculous) skeletal lesions.

Keywords: bone tuberculosis, bisphosphonate, ostitis, osteomyelitis, osteoregeneration, computed tomography

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INTRODUCTION

Osteoarticular tuberculosis is a specific disease of bones and joints, etiologically associated with local contamination of the skeleton with bacteria of the Mycobacterium tuberculosis complex (M. tuberculosis, M. bovis, M. africanum, M. microti, M. pinnipedii and M. caprae) as a result of hematogenous infection. A local focal lesion known as tuberculous osteitis is one of the forms of osteoarticular tuberculosis which is characteristic primarily for children. The historically established concentration of such patients treated in the Russian Federation at the Clinic for Pediatric Surgery and Orthopedics, St. Petersburg Research Institute of Physiology, allowed for assessment of the long-term postoperative catamnesis in a large cohort of patients identifying a number of problems including those that do not have a universal solution. One of them includes anatomical and orthopaedic consequences of tuberculous osteitis which evolves and develops during child's growth after recupertion of the infection caused by post-destructive scar cavities in the absence of a complete restoration of bone tissue and can result in repeated operations, disability and social maladjustment. That is why a complete restoration of bone tissue without residual cavities can be considered as the best option for postoperative bone regeneration.

The complex treatment of osteoarticular tuberculosis is based on radical resection of lesions within healthy tissues. A large volume of post-resection cavities, in the lower limbs in particular, requires bone grafting with implants of the appropriate size including allogeneic biological, biocomposite and / or non-biological grafts. Bone grafting is aimed at complete post-resection bone restoration avoiding a residual cavity filled with scar tissue. The graft is subjected to biological processes of adaptation due to initial infectious process and may undergo accelerated lysis and lose the desired

properties. Large bone defects require more time for repair, immobilization and limited axial loading on the affected segment with a normally adapted implantation that can result in worse conditions for revascularization and osteogenesis and slow osteoregeneration.

Osteoregeneration can be controlled with the use of bisphosphonates (BP), selective inhibitors of osteolysis that would inhibit differentiation of osteoclasts from precursors and improve anti-inflammatory activity. The properties of BP can be employed for the treatment of systemic genetic diseases (primary osteoporosis) and secondary osteoporosis (for example, in cerebral palsy) and in focal bone lesions including bone cysts, metastases of lytic tumors, non-bacterial osteomyelitis. There is a single report on the use of BP in adult patients with tuberculous osteitis that can be associated with side effects of drugs. Safe and efficacious use of BP in children with osteogenesis imperfecta, cerebral palsy and HMD are reported in series with a high level of evidence with the absence of such complications as osteonecrosis in children. Evidence of the effective use of bisphosphonates in the treatment of tuberculous osteitis can be practical, primarily for the pediatric population. The more important is the experimental study of the effectiveness of the treatment.

The objective was to explore CT signs of osteoregeneration due to multimodal treatment of experimental tuberculous osteitis with use of bisphosphonates as targeted inhibitors of osteoclasts.

MATERIAL AND METHODS

Laboratory animals

The study was performed with 21 mature male Chinchilla rabbits with an initial body weight of 3456.2 ± 321.6 g obtained from the Federal State Unitary Enterprise "Rappolovo Laboratory Animal Nursery" National Research Center "Kurchatov Institute" (Leningrad Region) according to the order of the Ministry of Health of the Russian Federation of April 1, 2016 No. 200n "On approval of the rules of good clinical practice", GOST 33216-2014, GOST R 33044-2014. Euthanasia of animals was performed in accordance with GOST 33215-2014 and the principles set out in the European Commission Recommendations on euthanasia.

Simulation of tuberculous osteitis

Bone tuberculosis was simulated using the method previously developed for simulating tuberculous osteitis in the medial condyle of the lapine right femur. The simulation was produced using a virulent reference laboratory M. tuberculosis H37Rv (1×10^6 CFU) strain from the collection of the Federal State Budgetary Institution "Scientific Center for Expertise of Medicinal Products" Ministry of Health of Russia. The strain was sensitive to anti-tuberculosis drugs. Confirmation of infection and tuberculosis process was based on local clinical manifestations of the inflammatory process and the cellular immune response to specific M. tuberculosis antigens with an intradermal test with the recombinant tuberculosis allergen (Diaskintest). A positive clinical and immunological result was registered in all infected animals 4 weeks after infection. The effective model of infection was confirmed at 4.5 and 7.5 months by examining the autopsy material of 3 rabbits (group 1, "infection control"). With a positive result of Diaskintest, specific chemotherapy received by the remaining animals according to the known sensitivity of the strain of mycobacteria used included isoniazid – 10 mg/kg, ethambutol and pyrazinamide 20 mg/kg orally according to the accepted standards for anti-tuberculosis care. Against the background of ATT, the surgery performed for the remaining animals receiving ATT after 6 weeks of infection included resection of pathological foci and bone grafting. The post-resection cavity was filled with granules of the Osteoset®2DBM Pellets implant with osteoconductive and osteoinductive properties due to the porous structure of calcium sulfate, the presence of bone morphogenic proteins BMP-2, BMP-4 and growth factors IGF-1, TGF-β1 (Wright medical, USA). The material was registered in the Russian Federation as a plastic material and was optimal in terms of standardization and quality characteristics for osteoinduction at the time of the experiment.

The biological material taken during the operation was examined by PCR to detect M. tuberculosis complex DNA (DNA was found in 17 out of 18 samples (94.4 %)).

In the future, the animals were divided into 3 groups depending on the treatment option:

group 2: 4 animals received only ATT postoperatively in the dosages that had been used preoperatively;

group 3: 7 animals received a single injection of BF (Pamidronate Medak 1 mg/kg intravenously slowly) 10 days after the operation and continued ATT;

group 4: 7 animals received a single injection of BF on day 10 after the operation in the absence of postoperative and subsequent ATT.

Adverse reactions to Pamidronate Medak were not recorded in the animals.

Animals of groups 2-4 were euthanized at 3 and 6 months by injecting Zoletil (VirbakSA, France) into the lateral vein of the ear at a dose of 100 mg/kg and autopsy material sampled. The right (operated) femur was sampled at 3 months and the right and left femurs were sampled at 6 months. The autopsied material was fixed in 10 % neutral formalin solution.

Diagnostic imaging

Imaging examinations were performed using a Skyscan 1172 microtomograph (Bruker) with a pixel size of 13 and 27 μm, a 0.5 mm Al filter, U = 100 kV, I = 100 μA, rotation angle 0.4 degrees, shooting 180 degrees, averaging over 4 shots. The sample (femur) was placed in a polyethylene tube with formalin and fixed with filter paper at scanning. Reconstruction was performed using the NRecon program (Bruker), the processing of reconstructed images was carried out using the CTAn, DataVeiwer (Bruker), RadiAnt DICOM Viewer (Medixant, Poland) programs.

A quantitative and qualitative assessment of the implantation zone, the contralateral condyle of the right femur, and the medial condyle of the left femur was performed according to the following parameters:

– optical density was evaluated in Hounsfield units in the RadiAnt DICOM Viewer medical imaging data viewer over an area of 0.001 cm2;

– radiation signs of osteoregeneration were assessed in accordance with a conditional qualitative-quantitative scale rated from 0 to 2, where '0' with the sign being absent, '1' being moderately pronounced, '2' being pronounced.

Five signs characterizing regeneration were evaluated: preservation of visualization and the implant with ingrowth bone trabeculae, restored structure of the cortical end and epiphyseal plates, structure of the bone beams (10 was the maximum possible score) and 2 signs of osteolysis including bone cavities and microsequesters (four as the maximum score).

Statistical analysis

The sample size was not previously calculated. The statistical analysis was performed using the STATISTICA software package, version 10.0 (StatSoft Inc., USA). The quantitative parameters were described using the median (25^{th}; 75^{th} percentile). Qualitative variables were evaluated in absolute numbers and shares (%). Prior to calculations, quantitative parameters were evaluated for compliance with the normal distribution with the Kolmogorov-Smirnov test.

RESULTS

Computed tomography scans of 21 right femurs and 11 left intact femurs were obtained. Non-operated left femurs demonstrated the maximum number and structural patterns of bone trabeculae in the femoral condyles with a gradual decrease towards the central bone and proximal to the metaphysis. The metaphyseal plate was visualized being the border to the medullary canal. Rarefied trabecular structure in the central bone took the form of cavities in some preparations that could have been mistaken for destruction analyzing right (infected) femurs (Fig. 1). The average density based on 10 measurements over an area of 0.001 cm^2 was 96.3 (86.7; 105.9) HU in the cortical layer, 31.2 (19.5; 42.9) HU and 33.3 (26.3; 40.3) HU in the medial and lateral condyles, respectively. The variability of the radiation data was identified in the experimental groups (Fig. 2).

Measurements of the density in the experimental groups using the RadiAnt DICOM Viewer program showed implant density values ranging from 27 to 1117 HU, with a significant increase in the parameters within groups in the dynamics between 3 and 6 months of observation, and in groups - from group 2 to group 4 with maximum values in the latter (289.3 HU at 3 months, 929.7 HU at 6 months) (Fig. 3).

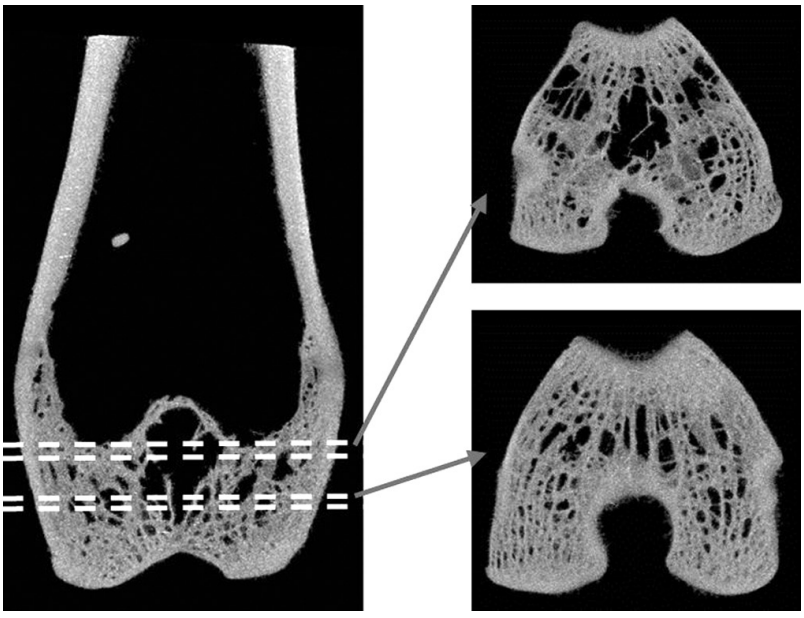


Fig. 1 Variability in the structure of the distal epiphysis of the femur, CT reconstruction with a slice thickness of 1 mm

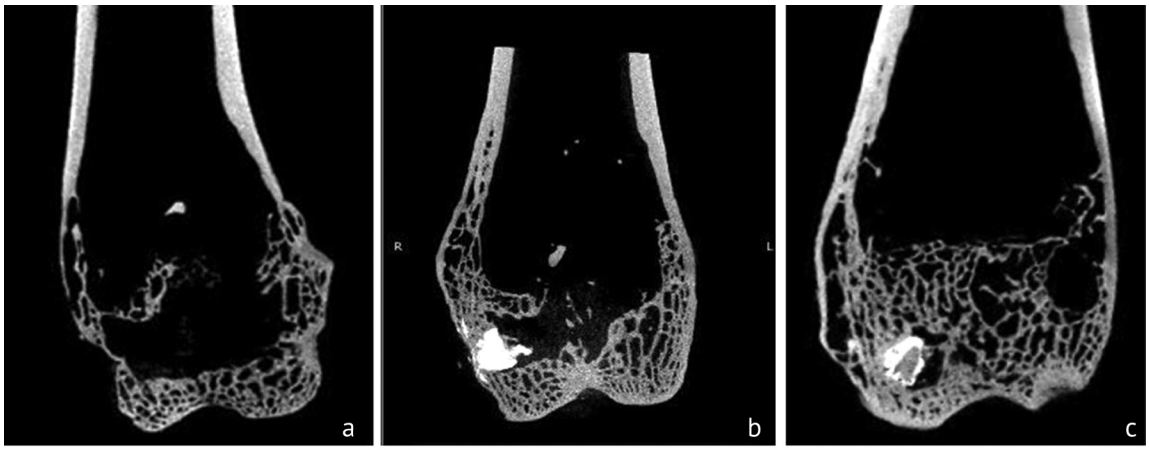


Fig. 2 CT, MPR in the frontal plane of the femur showing variability of implant location and bone structure, CT images of representative preparations of right femurs, autopsied at 6 months of surgical treatment. (a) the implant not identified, a bone cavity, partial epiphyseal destruction visualized; (b) implant in the medial condyle, the epiphyseal plate being partially preserved, (c) implant in the medial condyle with in-growing bone trabeculae, the epiphyseal plate being preserved, no bone cavities visualized

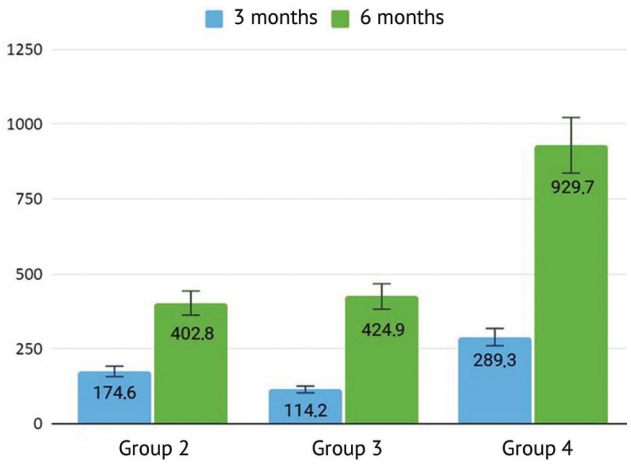


Fig. 3 Density of the implant (HU) over the area of 0.001 cm^2 (sliced at 1 mm), Me

The results of a conditionally quantitative assessment of the qualitative parameters of osteoregeneration/ osteolysis (Table 1) allow to conclude:

– integral signs of osteoregeneration increase between 3 and 6 months (Fig. 2) in the groups (2-4) with the minimum values in group 2 (ATCT) at both terms. The maximum values of the parameters were seen in group 3 (ATCT + BF) at 3 months and in group 4 (BF) at 6 months. A decrease in osteolysis scores was observed in groups 2 and 4 in dynamics;

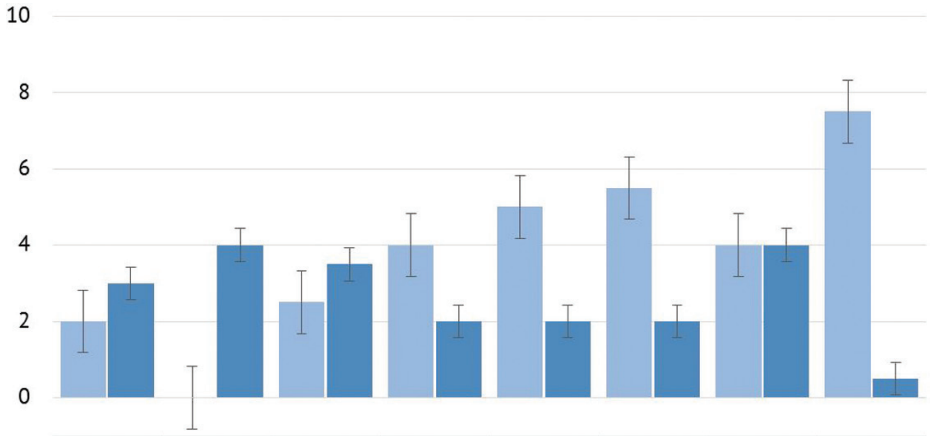
– the maximum values of osteolysis were noted at 3 months in group 4 with the maximum values of osteoregeneration (7.5 points) and minimal values of osteolysis (0.5 points) seen at 6 months in the group.

In terms, the dynamics of bone restructuring is graphically shown in Figure 4.

Table 1

Radiation signs of osteoregeneration and osteolysis

	Points 0-2, Me							
	Group 1		Group 2		Group 3		Group 4	
	3 mo	6 mo	3 mo	6 mo	3 mo	6 mo	3 mo	6 mo
Signs of osteoregeneration								
Implant visualized in the epiphysis			0.5	0	2	1	2	2
Implant in-grown with bone trabeculae			0.5	0	1	1	0	1
Bone trabeculae structure	1	0	0.5	1	1	1.5	0	1.5
Epiphyseal structure	1	0	0.5	1.5	0	1	2	1.5
Cortical endplate formed at the site of trepanation	0	0	0.5	1.5	1	1	0	1.5
Total	2	0	2.5	4	5	5.5	4	7.5
Signs of osteolysis								
Bone cavities	2	2	2	1	1	1	2	0.5
Microsequesters	1	2	1.5	1	1	1	2	0
Total	3	4	3.5	2	2	2	4	0.5



	3 months	6 months	3 months	6 months	3 months	6 months	3 months	6 months
	Group 1		Group 2		Group 3		Group 4	
Osteoregeneration	2	0	2.5	4	5	5.5	4	7.5
Osteolysis	3	4	3.5	2	2	2	4	0.5

Fig. 4 Dynamics in radiation signs of osteoregeneration and osteolysis, scores, Me, intergral score

DISCUSSION

Restoration of bone structure in patients, primarily in children who underwent surgical treatment of osteoarticular tuberculosis, remains challenging. On the one hand, stimulation of osteoregeneration facilitates restoration of the anatomical structure of the bone and ensures the restoration of the motor activity of the child. On the other hand, bone restoration of epimetaphyseal lesions of pediatric long bones allows for the normalization of the bone structure to be considered as a factor potentiating normal bone growth to prevent secondary orthopedic consequences of limb deformities and shortenings. Normalization of the bone structure in post-resection cavity can indirectly simplify the follow-up of the children at a long term when an asymptomatic focus (residual anatomical changes without functional impairment according to the classification of extrapulmonary tuberculosis) is radiologically interpreted as "chronic residual" by orthopaedic surgeons that would require additional imaging examinations, consultations, considerations of repeated operations. The use of modern materials with desired osteoplastic properties is practical to control bone restoration during radical surgical reconstructions. However, any plastic material is subject to lysis because of infectious-inflammatory process with the risk to lose the characteristics. Postoperative use of BF with antiresorptive and anti-inflammatory properties can be quite effective for the condition, as demonstrated by the experiment. The safe use of BF in the integral treatment of tuberculous osteitis in rabbits in dosages similar to those used in children was demonstrated in the series. The analysis of intact (non-infected left) femurs showed architectonic features of the lapine femoral epiphysis and were considered in the evaluation of experimental samples.

Intragroup analysis allowed for identification of the characteristic features of each group:

– the infection effectiveness control group showed greater signs of osteodestruction (bone cavities, sequesters, lack of bone structure) seen in CT scans in dynamics with the maximum severity at 7.5 months of infection. However, there was a positive osteoreparative dynamics of varying degree in all "treated" groups between 3 and 6 months;

– the ATT group demonstrated bone cavities postoperatively at both observation periods with no bone structure seen in 75 % of cases (3 out of 4 samples). The implant was lysed in all animals and was not visualized in the recipient zone at 6 months; signs of restored bone structure were identified in half (2 out of 4) of the samples;

– the implant and/or its fragments could be visualized in 85.7 % of samples (6 out of 7) with in-grown bone trabeculae, on the one hand, and bone cavities identified in 57 % (4 out of 7) cases of the ATT+B group at both periods of observation on the other hand;

– preservation of the implant and/or its fragments was typical for the cases of pronounced in-grown bone trabeculae in the BP group with a minimum basic preoperative period of ATT (Fig. 5). The least pronounced patterns of osteolysis and most pronounced patterns of osteoregeneration were seen in the group at the end of the observation period.

The implant was found in the plasty zone in 85.7-100 % of BP cases (experimental groups 3 and 4) even in the presence of bone cavities being ingrown with bone trabeculae without perifocal resorption.

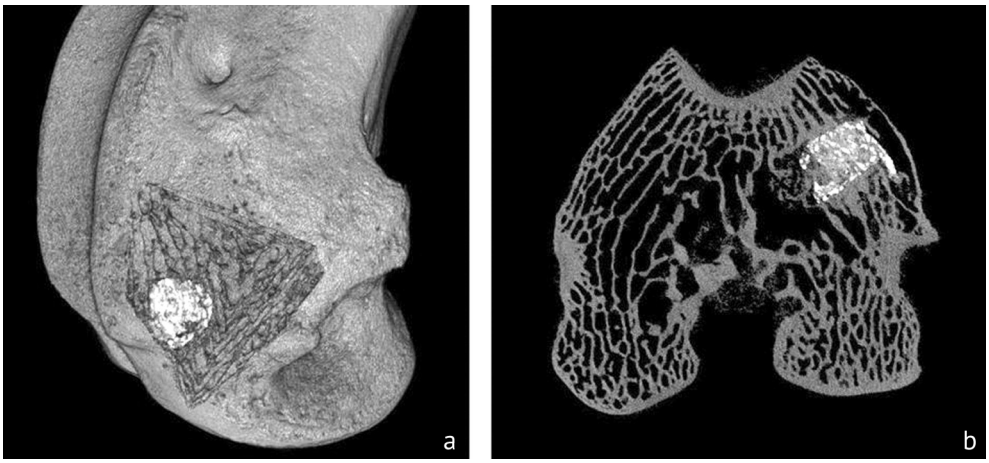


Fig. 5 Implantation zone of the sample group 4 at 6-month follow-up: (a) reconstructed image, (b) CT slice. The implant is visualized in the medial condyle of the right lapine femur of heterogeneous density with inclusions of CaSo4, ingrown bone trabeculae

CONCLUSION

1. CT-signs of osteoregeneration with positive dynamics at 3 to 6 months after the removal of the experimental tuberculous bone focus and replacement with a CaSo4-containing implant were identified whatever the drug treatment was administered.

2. Isolated postoperative anti-tuberculosis therapy of experimental tuberculous osteitis resulted in complete lysis of the CaSo4-containing implant observed in the absence of signs of bone tissue restoration in 50 % of cases at 6 months after surgery.

3. A single administration of Pamidronate Medak at a dosage of 1 mg/kg provided prolonged inhibition of implant lysis with less pronounced signs of resorption seen at 3 and 6 months.

4. CT signs of implant being ingrown with bone trabeculae were observed only in rabbits that received a single injection of Pamidronate Medak that can provide complete restoration of the post-resection cavity in clinical scenario.

Restriction on Reliability of Results:

– the findings were based on a limited number of observations as in any experimental animal study;

– a selective material with high osteoinductive properties was chosen as a plastic material;

– pamidronic acid solution was used as bisphosphonates, the drug is commonly used for children in the Russian Federation and has proven the clinical efficacy in non-infectious inflammatory pathology.

A more complete interpretation of the results can be performed by comparing CT data with morphological and biochemical markers, which was beyond the scope of the publication and is the subject of an ongoing comprehensive study.

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