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Structural changes in the distal articular end of the femur in experimental modeling of osteomyelitis

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Abstract

Features of structural changes in the joint components (synovial membrane, articular cartilage, subchondral bone) under the conditions of adjacent limb segment osteomyelitis are poorly understood and require thorough histological studies. **Purpose** Comparative assessment of the structural reorganization of the main components of the distal articular end of the femur in experimental modeling of osteomyelitis. **Material and methods** Objects: distal metaphyses of the femur of intact rats (n = 5) and experimental ones (n = 16) in the conditions of modeled osteomyelitis of the femur. The culture of *S. aureus* was injected into the medullary canal in the experimental animals (n = 8) while saline was injected in the control group (n = 8). The animals were taken out of the experiment on the 21st day. Methods: histological, morphometric, and statistical methods were used. **Results** In the control group, the articular cartilage, subchondral bone plate, and subchondral zone retained their normal structure. Synovitis was not revealed. The values of the morphometric parameters were comparable with the intact norm. In the experimental group, bone microsequesters, osteoclastic resorption of the subchondral bone plate, inflammatory infiltration with the content of plasma cells and neutrophils were detected in the subchondral zone. Histological changes in the articular cartilage according to the classification of the International Society OARSI (2006) corresponded to grades 1 to 3 and were accompanied by synovitis. There was a significant ($p < 0.05$) decrease in the thickness of non-calcified cartilage, a significant twofold decrease in the thickness of the subchondral bone plate, while the values of the thickness of the calcified cartilage exceeded those in the control group and the intact norm. **Conclusion** Under the conditions of an experimental model of chronic osteomyelitis of the femur, the revealed structural changes in the subchondral zone contribute to the progression of the destruction of the subchondral bone plate, articular cartilage and synovitis. This model of chronic osteomyelitis can be used to experimentally study various therapeutic strategies aimed at modifying subchondral bone remodeling and relieving synovitis.

Keywords: osteomyelitis, experimental model, histomorphometry, subchondral bone, articular cartilage, synovium

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INTRODUCTION

Osteomyelitis or purulent and necrotic lesion of bone tissue is a serious medical and social problem. The data of domestic and foreign literature show a constant increase in the incidence of chronic osteomyelitis after injuries and orthopedic operations [1-4]. After open fractures, osteomyelitis develops in 3-25 % of cases, after surgical interventions for closed fractures – from 1 to 8 %. Recurrence occurs in 20-35 %. People of working age constitute 78 % of patients with chronic osteomyelitis [5, 6].

Treatment of osteomyelitis may end with the amputation of a limb segment and disability. The consequences of osteomyelitis are anatomical and functional defects of the limbs. And therefore, bone infection osteologists face a difficult task of not only to restore the bone and its shape, but also limb functions [5, 7].

Pathological studies of joints in osteomyelitis of the adjacent limb segment are few [8, 9, 10].

To date, the question remains disputable whether to leave the articular cartilage during amputation and use it as a "barrier" to stop the spread of infection to the adjacent bone [11, 12]. Based on the data of only clinical material, it is not possible to form a detailed idea of the nature of histostructural changes in the articular cartilage in osteomyelitis of the adjacent limb segment. This knowledge is necessary for the development of an adequate pathogenetically substantiated rehabilitation program aimed at improving the quality of life of patients with osteomyelitis.

Experimental models are important for studying the pathogenesis and for developing treatments of many diseases. Features of structural changes in the main components of the joints (synovial membrane, articular cartilage, subchondral bone) in osteomyelitis are poorly understood and require detailed histological studies. Experimental studies in this direction are obvious and expedient.

Purpose: comparative assessment of the structural reorganization of the main components of the distal articular end of the femur in an experimental model of osteomyelitis.

MATERIALS AND METHODS

The distal metaphysis of the femur was studied in 16 experimental Wistar rats aged 11-12 months and weighed 350-430 g (experimental group, n = 8; control group, n = 8), in which chronic osteomyelitis was modeled in the conditions of external metal osteosynthesis. Clinical and morphological study confirmed the adequacy of the obtained model [13, 14].

Operations were performed under general anesthesia (Rometar 2 % – 1-2 mg/kg, Bioveta, Czech Republic; Zoletil 100 – 10-15 mg/kg, Virbac Sante Animale, France). A surgical approach was made to the anterior surface of the femur, and trepanation of the medullary canal was performed at the level of the third trochanter of the femur. *S. aureus* culture (museum strain ATCC 29213) in a volume of 50 µl and concentration of microorganisms 108 ml/1 was intramedullarily injected in the rats of the experimental group. Saline was injected into the medullary canal of control group rats. Then, in all animals, the end of an L-shaped 1.0-mm curved wire was inserted into the medullary canal to a depth of 7 mm. The surgical wound was sutured in layers with interrupted sutures Vicryl 4-0 (ETHICON). Next, at the level of the lower third of the femur, a 0.6-mm wire was cantilevered. Both wires were interconnected with a reinforced self-hardening plastic "Protacryl-M". Animals were euthanized on the 21st day of the experiment by decapitation with preliminary anesthesia (Rometar 2 % (1-2 mg/kg), Zoletil 100 solution (10-15 mg/kg)).

The study was carried out in accordance with SanPiN 3.3686-21, GOST 33216-2014; GOST 33215-2014, the European Convention for the Protection of Vertebrate Animals, Directive 2010/63 EU of the European Parliament and the Council of the European Union for the Protection of Animals, approved by the ethics committee of the institution.

For histological study, osteochondral fragments of the distal articular end of the femur with adjacent soft tissues were fixed in 10 % neutral formalin solution then washed in running water and partially decalcified in a mixture of equal volumes of hydrochloric and formic acid solutions, dehydrated in ethyl alcohol, embedded in paraffin. Histological preparations with a thickness of 5-7 µm were made on a Reichert microtome (Austria), stained with Masson's three-color method, hematoxylin and eosin. Light-optical study and digitization were performed using an AxioScope.A1 microscope equipped with an AxioCam digital camera and Zen blue software (CarlZeissMicroImaging GmbH, Germany). Synovitis grade was assessed according to V. Krenn et al. (2006) [15]. The following parameters were studied: the thickness (µm) of subchondral bone plate ($hs_{ubch.b.pl}$), uncalcified ($h_{uncal.cr}$) and calcified ($h_{cal.cr}$) cartilage. The distal articular end of the femur of 5 intact rats was examined for normal parameters.

Data analysis was performed using descriptive statistics. For some samples, the hypothesis of normality was rejected; quantitative data are presented as medians and quartiles (Me (p25-p75)). Hypotheses about differences between the compared groups were tested using the Wilcoxon test; differences were considered significant at $p < 0.05$ (AtteStat program, version 9.3.1).

RESULTS

In intact animals, the articular cartilage, subchondral bone plate, and subchondral zone retained a normal structure (Fig. 1a); the synovial membrane had no signs of inflammation (0-1 point, Krenn et al. (2006) [15]); synoviocytes of the integumentary layer were located in 1-2 layers; the collagen elastic layer was characterized by moderate cellularity (Fig. 1b).

The articular cartilage in the control group had a normal structure similar to the intact animals; the articular surface was without signs of fibrillation, the zonal structure was preserved, the basophilic line had clear contours, and was continuous throughout (Fig. 2). In the subchondral zone, the intertrabecular spaces were filled with adipose and hematopoietic bone marrow (Fig. 2b).

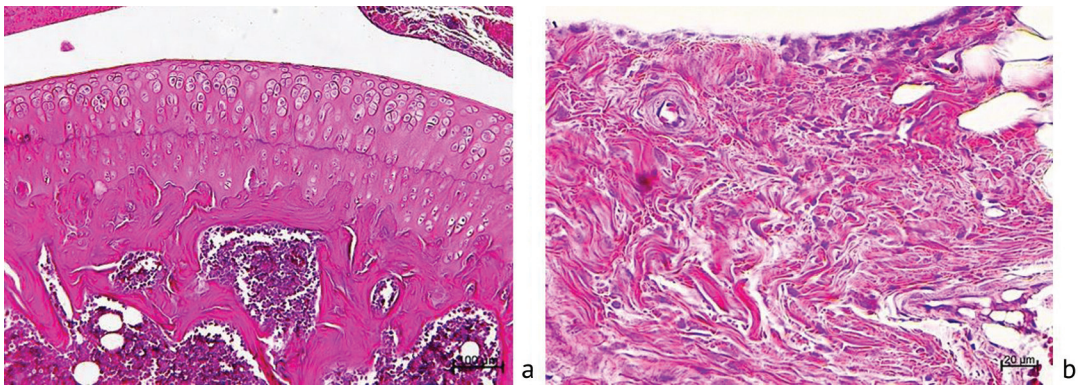

Fig. 1 Osteocartilaginous fragments of the femoral condyle (*a*) and synovium (*b*) of intact animals. Paraffin section, stained with hematoxylin and eosin. Magnification ×100 (a), ×400 (b)

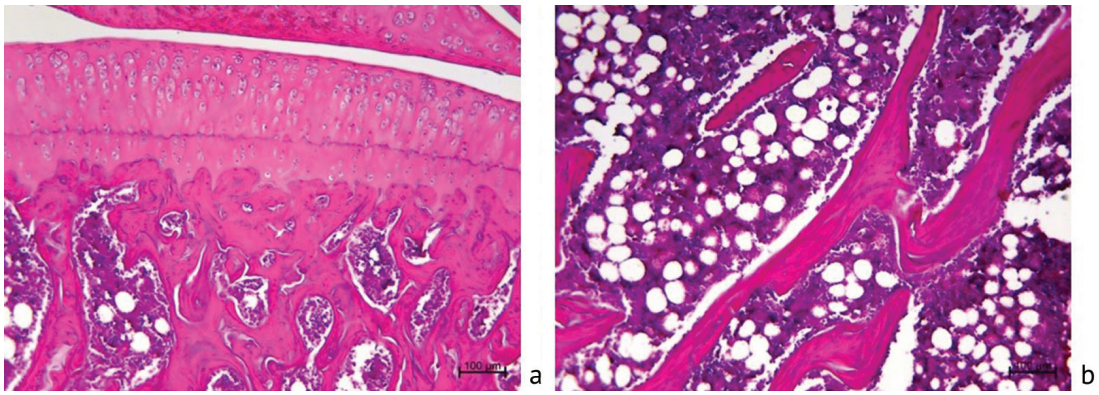

Fig. 2 Osteo-cartilaginous fragments of the femoral condyle of the control group: *a* articular cartilage and subchondral zone of the femoral condyle; *b* fatty and hematopoietic bone marrow in the intertrabecular spaces. Paraffin section, stained with hematoxylin and eosin. Magnification ×100

In the experimental group, the articular cartilage was thinned; there were many empty lacunae, cell-free fields, especially in the deep zone; occurrence of polynomial isogenic groups of cells was more frequent in the intermediate zone, up to 6-8 chondrocytes per group (Fig. 3a). Signs of the advance of the mineralization front into the uncalcified cartilage were recorded; the basophilic line was stratified and it was not determined in some areas; the calcified cartilage exceeded the uncalcified one in thickness. The subchondral bone plate was absent for a great extent, and active osteoclasts resorbing the remnants of the bone plate were found (Fig. 3b).

In the subchondral zone, there were signs of osteonecrosis, disruption of trabecular architectonics, their rarefaction and thinning (Fig. 4a), autolysis, splitting along the adhesion lines (Fig. 4b), bone microsequesters, inflammatory infiltrate with a high content of plasma cells and neutrophils from 3 to 5 in the field of view (Fig. 4c). Reconversion (hypercellularity) of the bone marrow and a decrease in the proportion of adipose marrow were observed (Fig. 4d).

The synovial membrane in the control group had no signs of inflammation and edema. The synoviocytes of the integumentary layer were located in one layer, less often in 2 layers (Fig. 5a). Evaluation by V. Krenn et al. (2006) [15] from 0 to 1 point indicated absence of synovitis. In the experimental group, synovial villi were not expressed, the synoviocytes of the integumentary layer were located in 2 to 4 layers (Fig. 5b), accumulations of plasma cells were noted, and the occurance of microvessels was increased. In the collagen elastic layer, there was an inflammatory infiltrate with a content of neutrophils, more than 5 in the field of view (Fig. 5c). In the deep layers of the joint capsule, a dense inflammatory infiltrate of the perivascular type was detected (Fig. 5d). Semi-quantitative assessment according to V. Krenn et al. (2006) [15] from 4 to 6 points [5(4-5)] corresponded to a high grade synovitis.

Histomorphometrically, there were no significant differences in the morphometric parameters in the control group compared to the intact norm. In the experimental group, all morphometric parameters significantly differed relative to the intact norm and the control group. A decrease in the thickness of uncalcified cartilage and a significant (2-fold) decrease in the thickness of the subchondral bone plate were noted, while the values of the thickness of calcified cartilage exceeded those in the control group and the intact norm (Table 1).

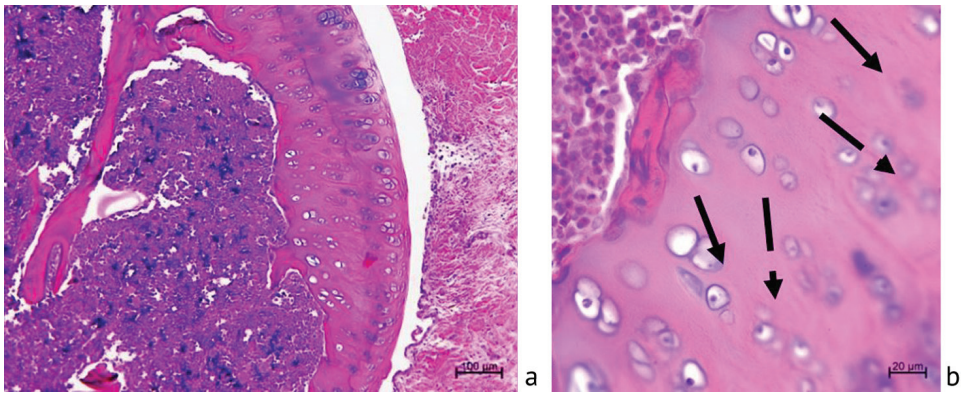

Fig. 3 Osteo-cartilaginous fragments of the knee joint in the experimental group: *a* articular cartilage and subchondral zone of the femoral condyle; *b* preserved fragments of the subchondral bone plate (dashed arrow), active osteoclasts (black arrow). Paraffin section, stained with hematoxylin and eosin. Magnification ×100 (*a*), ×400 (*b*)

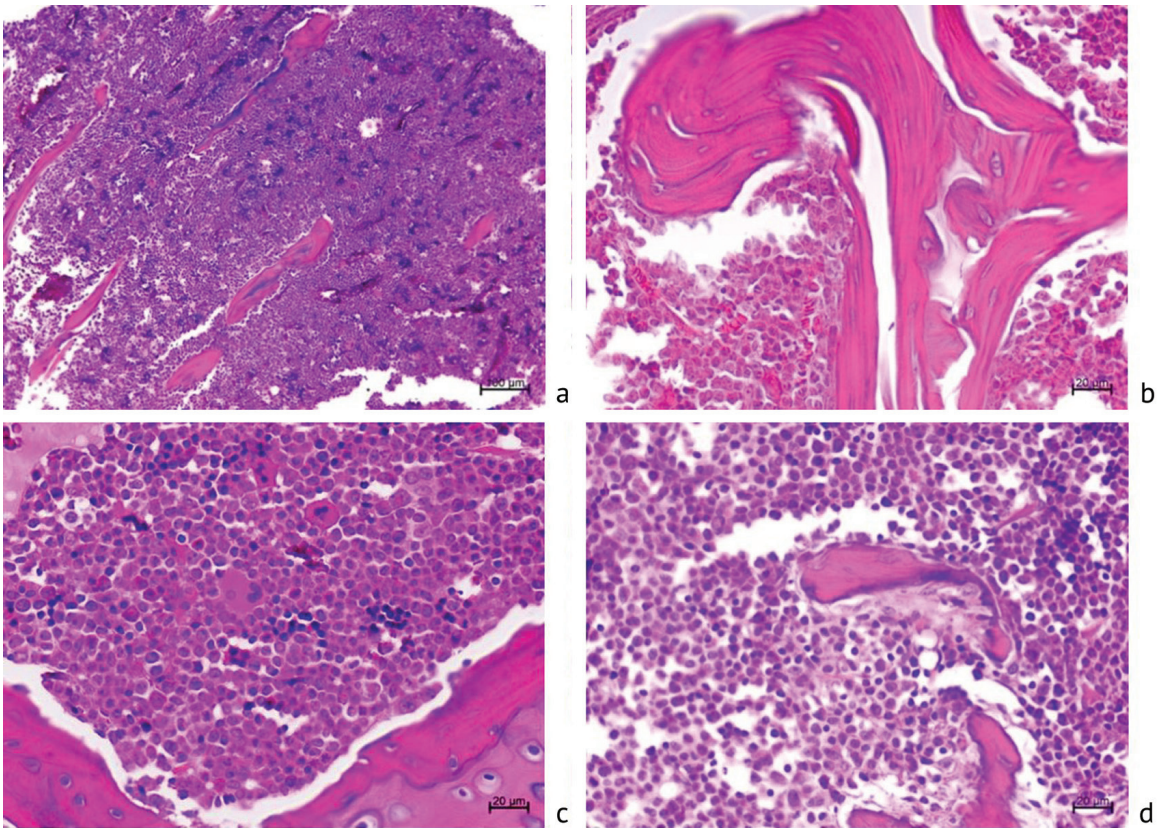

Fig. 4 Subchondral zone of the femoral condyles in the experimental group: *a* rarefaction and thinning of bone trabeculae; *b* autolysis of bone trabeculae, splitting along the gluing lines; *c* bone microsequester; *d* bone marrow reconversion, hypercellular bone marrow. Paraffin section, stained with hematoxylin and eosin. Magnification ×100 (*a*), ×400 (*b, c, d*)

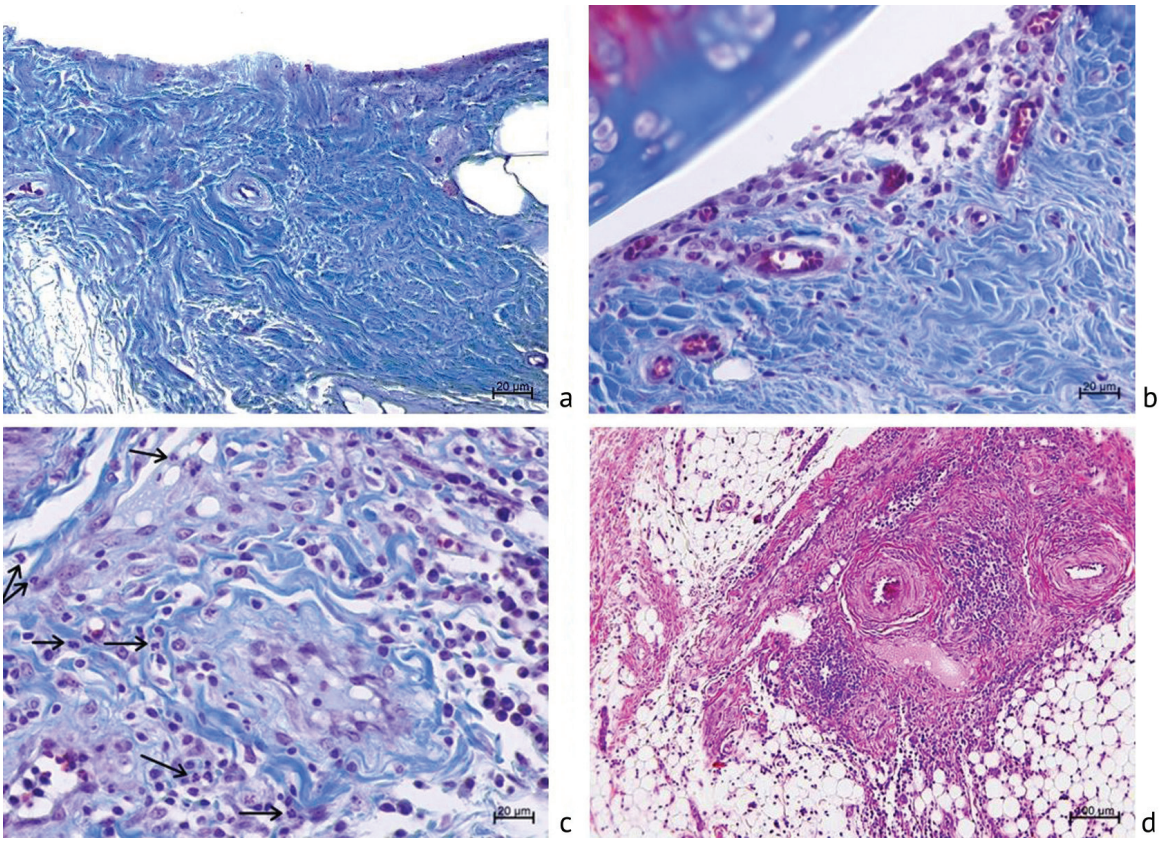

Fig. 5 Fragments of the joint capsule: *a* synovial membrane without signs of inflammation; *b* integumentary layer of the synovial membrane, hypervascularization, increased cell density; *c* neutrophils (arrows) as part of the inflammatory infiltrate; *d* perivascular compacted inflammatory infiltrate in the deep layers of the joint capsule. Control group (*a*); experimental group (*b, c, d*). Paraffin sections, stained with hematoxylin and eosin (*d*), Masson three-color method (*a, b, c*). Magnification ×400 (*a, b, c*), ×100 (*d*)

Table 1

Morphometric parameters of the articular cartilage and subchondral bone of the femoral condyles (Me (p25-p75))

Parameter	Intact norm	Control group	Experimental group	p
$h_{uncal.cr}$	145.77 (135.23-151.54)	140.54 (117.59-147.48)	126.78 (108.79-147/82)	$P_{n-c} = 0.53$; $p_{c-e} = 0.041$; $p_{n-e} = 0.006$
$h_{cal.cr}$	111.38 (98.22-123.19)	92.24 (86.29-100.12)	132.48 (108.45-152.19)	$P_{n-c} = 0.05$; $p_{c-e} = 0.012$; $p_{n-e} = 0.021$
$h_{subch.b.pl}$	100.38 (99.69-105.88)	88.22 (73.58-98.71)	51.68 (50.68-55.39)	$P_{n-c} = 0.14$; $p_{c-e} = 0.012$; $p_{n-e} = 0.012$

Note: Me – median, p25-p75 – percentiles; p_{n-c} – comparison of the norm with the control, p_{n-e} – comparison of the norm with the experiment, p_{c-e} – comparison of the control with the experiment, p – significance levels of differences according to the Wilcoxon test, differences are significant at $p < 0.05$

DISCUSSION

The results of the pathohistological study of the distal articular end of the femur in the experimental group showed the presence of an inflammatory process in the segment of the limb remote from the lesion (the site of injection of *S. aureus*). Foci of destruction in the subchondral zone such as necrotic fragments of bone trabeculae, osteoclastic resorption of the subchondral bone plate, which resulted in its complete and/or partial destruction, inflammatory infiltration with the content of plasma cells and neutrophils are histological signs of chronic osteomyelitis of subacute and acute course [16, 17, 18]. Bone marrow reconversion in the subchondral zone, multi-membered isogenic groups of cells in the articular cartilage indicated the formation of a compensatory reparative reaction.

Histological changes in the articular cartilage corresponded to grades 1 to 3 according to the classification of the International Society for the Study of Osteoarthritis OARSI, 2006 [19], accompanied by a violation of the basophilic line, significant changes in the subchondral bone, and synovitis.

According to Lyons T.J. (2006), stratification of the basophilic line indicates the advancement of the calcification front towards the deep zone of uncalcified cartilage [20]. In our study, a significant increase in the thickness of the calcified cartilage zone and thinning of uncalcified cartilage were histomorphometrically recorded in the experimental group.

It is known that the thinning of the hyaline cartilage is associated with increased destruction of the basophilic line, degradation of the matrix, and accelerated ossification at the border with the subchondral bone plate due to increased penetration of bone resorption products [21].

The role of the subchondral bone in the destruction of the articular cartilage has been confirmed by numerous studies [22, 23, 24].

Pronounced osteoclastic resorption of the subchondral bone plate in the experimental group led to its complete and/or partial destruction.

A number of authors [25, 26, 27, 28] reported that Staphylococcus aureus affects bone tissue remodeling, reduces the intensity of osteoblast proliferation and induces their death due to the produced toxins. On the other hand, Staphylococcus aureus stimulates osteoclastogenesis by enhancing the production of membrane-bound RANK-L, sRANK-L (soluble) and small forms of RANK-L. These factors together lead increased bone resorption without its adequate regeneration [29].

Semi-quantitative assessment of the synovial membrane of the knee joint in the experimental group corresponded in 38 % of cases to mild and in 62 % of cases to a high-grade synovitis. It is known that in the state of synovitis the synovial membrane is the driving force in the destruction of the articular cartilage [30].

In our earlier study of the surgical material from 16 patients, it was found that the intensity of articular cartilage destruction in chronic osteomyelitis of the foot bones depended on the location of the osteomyelitic focus and the phase of the inflammatory process [10].

The hyaline cartilage does not contain vessels, so it is a "barrier" for the spread of the infectious process. Thus, in children by the age of 2, the metaepiphyseal cartilage usually prevents the spread of infection to the epiphysis and to the adjacent joint [31, 32]. Hypertrophied (bubbly) chondrocytes of the deep zone and the zone of calcified cartilage form a chondrohematic "barrier", while the contact of the vessels with the hyaline cartilage is blocked. In pathological conditions and disruption of the continuity of the basophilic line, the vessels may penetrate into the cartilage [33].

CONCLUSION

In the experimental model of chronic osteomyelitis of the femur, the structural changes in the subchondral zone contribute to the progression of the destruction of the subchondral bone plate, articular cartilage and initiation of synovitis. The model of chronic osteomyelitis described by us may be used to experimentally study various therapeutic strategies aimed at modifying subchondral bone remodeling and relieving synovitis.

REFERENCES

1. Gubin AV, Kliushin NM. Organizational issues in management of patients with chronic osteomyelitis and the solutions offered by osteology infection clinic. *Genij Ortopedii*. 2019;25(2):140-148. doi: 10.18019/1028-4427-2019-25-2-140-148
2. Diachkova GV, Diachkov KA, Kliushin NM, Larionova TA, Shastov AL. A multifaceted osteomyelitis: radiological diagnosis. *Genij Ortopedii*. 2020;26(3):385-391. doi 10.18019/1028-4427-2020-26-3-385-391
3. Schmitt SK. Osteomyelitis. *Infect Dis Clin North Am*. 2017 Jun;31(2):325-338. doi: 10.1016/j.idc.2017.01.010
4. Walter N, Baertl S, Alt V, Rupp M. What is the burden of osteomyelitis in Germany? An analysis of inpatient data from 2008 through 2018. *BMC Infect Dis*. 2021 Jun 10;21(1):550. doi: 10.1186/s12879-021-06274-6
5. Novomlinskiy VV., Malkina NA., Andreev AA., Glukhov AA., Mikulich EV. Modern aspects of diagnosis and treatment of osteomyelitis. *Modern problems of science and education. Surgery*. 2016;(5). URL: https://science-education.ru/ru/article/view?id=25326 (accessed 15.04.2022). (In Russ.)
6. Sakovich NV, Andreev AA, Mikulich EV, Ostroushko AP, Zvyagin VG. Modern Aspects of Etiology, Diagnostics and Treatment of Osteomyelitis. *Journal of experimental and clinical surgery*. 2018;11(1):70-79. (In Russ.) doi: 10.18499/2070-478X-2018-11-1-70-79
7. Chan JKK, Ferguson JY, Scarborough M, McNally MA, Ramsden AJ. Management of Post-Traumatic Osteomyelitis in the Lower Limb: Current State of the Art. *Indian J Plast Surg*. 2019 Jan;52(1):62-72. doi: 10.1055/s-0039-1687920
8. Berg AJ, Killen MC, Chauhan A, Bhatia C. Osteomyelitis of the patella: ensure a high index of suspicion and beware the negative aspirate. *BMJ Case Rep*. 2014 Oct 15;2014:bcr2014206630. doi: 10.1136/bcr-2014-206630
9. Ellerbrook L, Laks S. Coccidioidomycosis osteomyelitis of the knee in a 23-year-old diabetic patient. *Radiol Case Rep*. 2015 Dec 3;10(1):1034. doi: 10.2484/rcr.v10i1.1034
10. Stupina TA, Migalkin NS, Sudnitsyn AS. Structural reorganization of the cartilage tissue in chronic osteomyelitis of the foot bones. *Genij Ortopedii*. 2019;25(4):523-527. doi: 10.18019/1028-4427-2019-25-4-523-527
11. Li A, Meunier M, Rennekampff HO, Tenenhaus M. Surgical amputation of the digit: an investigation into the technical variations among hand surgeons. *Eplasty*. 2013;13:e12.
12. Rabin SI. Digital Amputations of the Upper Extremity Technique. Available at: http://www.emedicine.medscape.com›article/1238395-technique (accessed 22.05.2022)
13. Ovchinnikov EN, Dyuryagina OV, Stogov MV, Silant'eva TA, Kireeva EA. Osteomyelitis model in rats. *Bulletin of Experimental Biology and Medicine*. 2022;(3):395-399. (In Russ.) doi: 10.47056/0365-9615-2022-173-3-395-399
14. Kubrak NV, Dyuryagina OV, Ovchinnikov EN, Diachkov AN. Clinical and radiological characteristics of osteomyelitis under conditions of external metal osteosynthesis (experimental study). *Modern problems of science and education. Surgery*. 2022;(2). URL https://science-education.ru/ru/article/view?id = 31621 (accessed 15.03.2022). (In Russ.)
15. Krenn V, Morawietz L, Burmester GR, Kinne RW, Mueller-Ladner U, Muller B, Haupl T. Synovitis score: discrimination between chronic low-grade and high-grade synovitis. *Histopathology*. 2006 Oct;49(4):358-364. doi: 10.1111/j.1365-2559.2006.02508.x
16. Tiemann A, Hofmann GO, Krukemeyer MG, Krenn V, Langwald S. Histopathological Osteomyelitis Evaluation Score (HOES) - an innovative approach to histopathological diagnostics and scoring of osteomyelitis. *GMS Interdiscip Plast Reconstr Surg DGPW*. 2014 Oct 20;3:Doc08. doi: 10.3205/iprs000049
17. Sconfienza LM, Signore A, Cassar-Pullicino V, Cataldo MA, Gheysens O, Borens O, Trampuz A, Wörtler K, Petrosillo N, Winkler H, Vanhoenacker FMHM, Jutte PC, Glaudemans AWJM. Diagnosis of peripheral bone and prosthetic joint infections: overview on the consensus documents by the EANM, EBJIS, and ESR (with ESCMID endorsement). *Eur Radiol*. 2019 Dec;29(12):6425-6438. doi: 10.1007/s00330-019-06326-1
18. Sybenga AB, Jupiter DC, Speights VO, Rao A. Diagnosing Osteomyelitis: A Histology Guide for Pathologists. *J Foot Ankle Surg*. 2020 Jan-Feb;59(1):75-85. doi: 10.1053/j.jfas.2019.06.007
19. Pritzker KP, Gay S, Jimenez SA, Ostergaard K, Pelletier JP, Revell PA, Salter D, van den Berg WB. Osteoarthritis cartilage histopathology: grading and staging. *Osteoarthritis Cartilage*. 2006 Jan;14(1):13-29. doi: 10.1016/j.joca.2005.07.014
20. Lyons TJ, McClure SF, Stoddart RW, McClure J. The normal human chondro-osseous junctional region: evidence for contact of uncalcified cartilage with subchondral bone and marrow spaces. *BMC Musculoskelet Disord*. 2006 Jun 20;7:52. doi: 10.1186/1471-2474-7-52
21. Nelin NI, Khomutov VP. [The role of the subchondral bone in osteoarthritis and the possibility of optimizing the repair of osteochondrogenic structures by the electric field of the electret]. *Sovremennaya meditsina* [Modern medicine]. 2021;(2):10-14. (In Russ.)
22. Alexeeva LI, Zaitseva EM. Role of subchondral bone in osteoarthritis. *Rheumatology Science and Practice*. 2009;47(4):41-48. (In Russ.) doi: 10.14412/1995-4484-2009-1149
23. Stupina TA, Stepanov MA, Teplen'kii MP. Role of Subchondral Bone in the Restoration of Articular Cartilage. *Bull Exp Biol Med*. 2015;158(6):820-823. doi: 10.1007/s10517-015-2870-4
24. Zhang L, Kirkwood CL, Sohn J, Lau A, Bayers-Thering M, Bali SK, Rachala S, Marzo JM, Anders MJ, Beier F, Kirkwood KL. Expansion of myeloid-derived suppressor cells contributes to metabolic osteoarthritis through subchondral bone remodeling. *Arthritis Res Ther*. 2021 Nov 16;23(1):287. doi: 10.1186/s13075-021-02663-z
25. Tsiskarashvili AV, Rodionova SS, Mironov SP, Bukhtin KM, Gorbatiuk DS, Taraskin AIu. Metabolic bone tissue disorders in patients with long bone fractures complicated by chronic osteomyelitis. *Genij Ortopedii*. 2019;25(2):149-155. doi: 10.18019/1028-4427-2019-25-2-149-155
26. Claro T, Widaa A, O'Seaghdha M, Miajlovic H, Foster TJ, O'Brien FJ, Kerrigan SW. Staphylococcus aureus protein A binds to osteoblasts and triggers signals that weaken bone in osteomyelitis. *PLoS One*. 2011 Apr 15;6(4):e18748. doi: 10.1371/journal.pone.0018748
27. Kremers HM, Nwojo ME, Ransom JE, Wood-Wentz CM, Melton LJ 3rd, Huddleston PM 3rd. Trends in the epidemiology of osteomyelitis: a population-based study, 1969 to 2009. *J Bone Joint Surg Am*. 2015 May 20;97(10):837-845. doi: 10.2106/JBJS.N.01350
28. Ma X, Han S, Ma J, Chen X, Bai W, Yan W, Wang K. Epidemiology, microbiology and therapeutic consequences of chronic osteomyelitis in northern China: A retrospective analysis of 255 Patients. *Sci Rep*. 2018 Oct 5;8(1):14895. doi: 10.1038/s41598-018-33106-6

29. Josse J, Velard F, Gangloff SC. Staphylococcus aureus vs. Osteoblast: Relationship and Consequences in Osteomyelitis. *Front Cell Infect Microbiol*. 2015 Nov 26;5:85. doi: 10.3389/fcimb.2015.00085
30. Bhattaram P, Chandrasekharan U. The joint synovium: A critical determinant of articular cartilage fate in inflammatory joint diseases. *Semin Cell Dev Biol*. 2017 Feb;62:86-93. doi: 10.1016/j.semcdb.2016.05.009
31. Pöyhiä T, Azouz EM. MR imaging evaluation of subacute and chronic bone abscesses in children. *Pediatr Radiol*. 2000 Nov;30(11):763-768. doi: 10.1007/s002470000318
32. Stephen RF, Benson MK, Nade S. Misconceptions about childhood acute osteomyelitis. *J Child Orthop*. 2012 Oct;6(5):353-6. doi: 10.1007/s11832-012-0435-x
33. Pavlova VN, Pavlov GG, Shostak NA, Slutsky LI Slutskiy LI. *Sustav: morfologiya, klinika, diagnostika, lecheniye* [Joint: morphology, clinic, diagnosis, treatment]. Moscow, 2011. 552 p. (In Russ.)

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