

УДК 617.57/.58-089.844:616.717/.718-008.9-079:577.1](045)

DOI: <http://dx.doi.org/10.15674/0030-59872025320-25>

Dynamics of biochemical markers of bone metabolism in patients with segmental bone defects treated with the Masquelet technique

S. Magomedov, Yu. V. Polyachenko, A. V. Kalashnikov, Yu. M. Litun, L. V. Polishchuk

SI «National Institute of Traumatology and Orthopedics of the NAMS of Ukraine», Kyiv

Segmental bone defects represent a complex clinical challenge in reconstructive orthopedics, etiologically associated with high-energy trauma, oncological resections, and osteomyelitic processes. The two-stage Masquelet technique with induced membrane formation demonstrates high efficacy in reconstructing critical-size bone defects, however, the molecular-biochemical mechanisms of reparative osteogenesis during its application remain insufficiently investigated. Objective. To evaluate the dynamics of biochemical markers of bone metabolism in patients with segmental defects of long tubular bones during treatment using the induced membrane (Masquelet) technique in order to determine the metabolic characteristics of osteogenesis and to optimize bone regeneration. Methods. The study included 85 patients aged 18 to 65 years. The experimental group consisted of 44 patients with segmental bone defects, while the control group comprised 41 patients with low-energy fractures. Biochemical markers such as alkaline phosphatase, osteocalcin, β -CrossLaps, calcium, phosphorus, and parathyroid hormone were analyzed. Results. Before treatment, significant abnormalities in bone metabolism markers were observed in the experimental group: elevated alkaline phosphatase (175.45 ± 46.2) U/L, osteocalcin (53.70 ± 12.4) ng/mL, β -CrossLaps (0.949 ± 0.271) ng/mL. Following treatment, 75–92 % of patients demonstrated normalization of biochemical parameters, indicating stimulation of reparative osteogenesis. Conclusions. The Masquelet technique effectively stimulates bone regeneration in segmental defects, as confirmed by the normalization of biochemical markers. Comprehensive biochemical monitoring can serve as a valuable tool for assessing treatment efficacy.

Сегментарні дефекти кісток є доволі складною проблемою реконструктивної ортопедії, етіологічно пов'язаної з високоенергетичними травмами, онкологічними й остеомієлітичними процесами. Двохетапна методика Masquelet з формуванням індукційної мембрани демонструє високу ефективність у відновленні критичних кісткових дефектів, проте молекулярно-біохімічні механізми репаративного остеогенезу в разі її застосування залишаються недостатньо дослідженими. Мета. Вивчити динаміку біохімічних маркерів кісткового метаболізму в пацієнтів із сегментарними дефектами довгих трубчастих кісток у процесі лікування з використанням методики індукованої мембрани (Masquelet) для визначення метаболічних особливостей остеогенезу й оптимізації остеореєнєрації. Методи. Обстежено 85 пацієнтів віком від 18 до 65 років. Дослідну групу склали 44 особи з сегментарними дефектами кісток, пролікованих за двохетапною методикою Masquelet. Контрольну групу (41 пацієнт) становили хворі з низькоенергетичними переломами. Дослідження включало визначення маркерів кісткового метаболізму (лужна фосфатаза, остеокальцин, β -CrossLaps), показників мінерального обміну (кальцій, фосфор) та регуляторний фактор (паратгормон). Результати. До лікування в пацієнтів із сегментарними дефектами виявлено підвищення рівнів лужної фосфатази ($175,45 \pm 46,2$) мккат/л, остеокальцину ($53,70 \pm 12,4$) нг/мл та β -CrossLaps ($0,949 \pm 0,271$) нг/мл. Після лікування в 75–92 % випадків спостерігалася нормалізація показників біохімічного ремоделювання кісткової тканини, що свідчить про стимуляцію репаративного остеогенезу. Висновки. Методика індукційної мембрани сприяє стимуляції репаративного остеогенезу, що підтверджується нормалізацією біохімічних маркерів. Комплексне оцінювання біохімічних показників є важливим інструментом моніторингу ефективності лікування. Ключові слова. Сегментарний дефект, індукційна мембрана, остеогенез, біохімічні маркери, кістковий метаболізм.

Keywords. Segmental defect, induced membrane, osteogenesis, biochemical markers, bone metabolism

Introduction

Replacement of segmental bone defects is currently an extremely relevant issue in orthopedics and traumatology. Well-known techniques, such as vascularized fibular autograft or the Ilizarov distraction osteosynthesis method are common. Although they are quite effective, they also have certain drawbacks. In particular, they are technically complex, require highly qualified specialists and modern material and technical support, as well as a large number of complications in the postoperative period [1, 2].

To minimize the number of complications and unsatisfactory results, in 1986 A. Masquelet proposed a two-stage technique, which consists in the implantation at the first stage of polymethyl methacrylate (PMMA) (bone cement) saturated with an antibiotic, which is responsible for the formation of a pseudosynovial membrane. The second stage after the formation of the induced membrane within a period of 6 to 8 weeks implied removal of the spacer and filling the cavity with a spongy autograft. The properties of the membrane are defined by its mechanobiological characteristics: mechanically, it prevents the invasion of fibrous tissue into the recipient site while facilitating the restoration of the damaged segment's anatomy; biologically, it stimulates tissue regeneration, leading to the formation of an induction membrane that revascularizes the bone graft and prevents its resorption [3, 4].

This technique allows to reconstruct significant diaphyseal defects, even if the recipient area has been irradiated or infected, provided that a pre-formed shell is formed to protect and revascularize the bone graft [5].

Stimulation of the osteogenic potential of autografts using growth factors and osteoinductive cells remains an area of active research [6, 7]. Current studies are still in their early stages and require answers to many unresolved questions.

Ultimately, further investigation into the metabolic properties of induction membranes holds great promise for uncovering new mechanisms of bone tissue biology and regeneration. This could significantly improve the quality of care for many patients. The technique of using the induced membrane is unique and has the potential to shift the paradigm for implanting foreign bodies into the human body. Despite being used in practice for over 30 years, scientific and practical studies on its properties are just beginning.

Objective: to assess the time course of biochemical markers of bone metabolism in patients with seg-

mental bone defects during treatment using the induced membrane technique (Masquelet) in order to determine the metabolic features of osteogenesis and optimize osteoregeneration.

Material and methods

The study was conducted on the basis of the Biochemistry Laboratory of the State Institution "Institute of Traumatology and Orthopedics of the National Academy of Medical Sciences of Ukraine". The research was carried out in accordance with the terms of the Declaration of Helsinki with the approval of the Bioethics Committee (Protocol No. 3 dated 26.04.2025). All patients involved in this study signed a voluntary informed consent to participate.

The study involved examination of 85 subjects aged 18 to 65 years (mean age (42.3 ± 8.7) years).

The experimental group included 44 patients with segmental bone defects, mostly lower limbs, in which the two-stage Masquelet method was used. The control group included 41 individuals hospitalized for intra-articular injuries and low-energy closed fractures of the lower limbs. The groups were identical in terms of age and sex.

The clinical observation was based on the quantitative determination of bone tissue remodeling markers by the method of parallel biochromatic and monochromatic adsorption measurement (Automatic electrochemiluminescent analyzer Cobas E 411 and biochemical analyzer Cobas E 311 using Roche Diagnostics test systems).

Laboratory studies included the determination of the following indicators: markers of bone formation (alkaline phosphatase (ALP), osteocalcin), indicators of resorption (β -CrossLaps) and mineral metabolism (total calcium, phosphorus), regulatory factors (parathormone), additional enzymes (creatine phosphokinase (CPK), lactate dehydrogenase (LDH)). Blood sampling was performed in the morning on an empty stomach before the start of treatment and after its completion. The patients' blood serum was analyzed after being separated using an ELMI Centrifuge CM-6MT.

For statistical analysis of values, a descriptive method was used with the calculation of the mean (M), the mean standard error (m). The probability of the difference was assessed depending on the type of data cut by parametric (Student's t-test for unrelated samples) and non-parametric (Pearson's χ^2 , Mann-Whitney U-test) methods. The relationship between the parameters studied was revealed by calculating the Spearman correlation coefficient. All data are presented as the mean and standard

error of the mean. The difference was considered significant at $p < 0.05$. Calculations were performed in the Statistica 12 software.

Results

The analysis of the initial indicators in the study group revealed significant deviations from the reference values. An increased level of alkaline phosphatase ($> 129 \mu\text{kat/l}$) was observed in 23 % of patients with an average value of $(175.45 \pm 46.2) \mu\text{kat/l}$ in the group with an increased level. Deviations from the norm of osteocalcin were detected in 55 % of patients, of which $> 22.0 \text{ ng/ml}$ in 45 % (up to $(53.70 \pm 12.4) \text{ ng/ml}$). Increased β -CrossLaps levels were recorded in 42 % of cases (mean value $(0.949 \pm 0.271) \text{ ng/ml}$), with the highest rates observed in patients with disease duration of more than 2 years. Calcium-phosphorus metabolism disorders were detected in 35 % of cases, including hyperphosphatemia in 18 and hypocalcemia in 12 % (Fig. 1).

After the treatment, positive dynamics were recorded: normalization of alkaline phosphatase levels in 85 % of patients (the average value decreased to $(102.3 \pm 28.6) \mu\text{kat/l}$), restoration of osteocalcin levels to reference values in 75 %. The β -CrossLaps level decreased by an average of 35 % (to $(0.621 \pm 0.248) \text{ ng/ml}$) with complete normalization of the indicator in 82 % of people. Indicators of mineral metabolism normalized in most patients: calcium levels in 92 %, phosphorus in 88 % of cases.

The indicators of the control group were characterized by stability and were within the reference values: alkaline phosphatase — $(103.4 \pm 25.6) \mu\text{kat/l}$, calcium — $(2.52 \pm 0.28) \text{ mmol/l}$, phosphorus — $(1.28 \pm 0.38) \text{ mmol/l}$, β -CrossLaps — $(0.621 \pm 0.248) \text{ ng/ml}$, osteocalcin — $(29.8 \pm 11.7) \text{ ng/ml}$, parathormone — $(28.9 \pm 11.8) \text{ pg/ml}$ (Fig. 2).

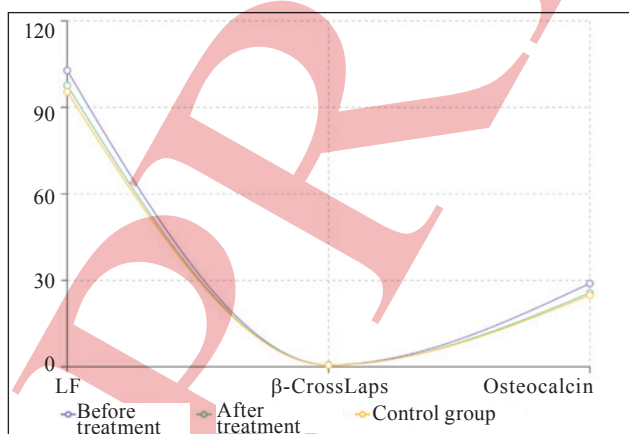


Fig. 1. Changes in the main markers of bone metabolism

Discussion

The analysis of the obtained results demonstrates complex changes in biochemical markers in patients with segmental defects of bone tissue. Particular attention is drawn to changes in markers of bone formation and resorption, which showed characteristic changes. Alkaline phosphatase levels were elevated ($(102.5 \pm 31.2) \mu\text{kat/l}$) with significant gender differences ($(105.3 \pm 29.8) \mu\text{kat/l}$ in men vs. $(97.7 \pm 24.6) \mu\text{kat/l}$ in women, $p < 0.05$). Osteocalcin levels were also elevated ($(27.8 \pm 10.7) \text{ ng/ml}$), indicating osteoblast activation.

A similar pattern was found in the study by N. S. Rathwa et al., who associated it with compensatory activation of bone formation processes [8, 9]. The β -CrossLaps resorption marker demonstrated an elevated level ($(0.64 \pm 0.23) \text{ ng/ml}$) with significant individual variability ($\text{CV} = 35.7\%$).

In the publications of F. Perut et al. and S. Wei et al. also noted the high variability of this indicator and its importance for studying the intensity of bone resorption [10, 11]. The data on mineral metabolism were the most stable. The calcium level ($2.55 \pm 0.23) \text{ mmol/l}$ was characterized by the lowest variability ($\text{CV} = 8.9\%$), which indicates the preservation of homeostasis mechanisms.

M. Kumar et al. emphasize the importance of maintaining a stable calcium level for the processes of reparative osteogenesis [12]. Of particular note is the increased level of parathormone ($28.2 \pm 13.7) \text{ pg/ml}$ with high variability ($\text{CV} = 456\%$) and right-sided asymmetry of distribution.

O. W. Omogbai et al. associate this with the activation of compensatory mechanisms of regulation of calcium-phosphorus metabolism [13]. Enzymatic activity also underwent characteristic changes. CPK level was increased ($15.3 \pm 4.3) \text{ U/L}$ with significantly higher values in the female group ($p < 0.05$). LDH

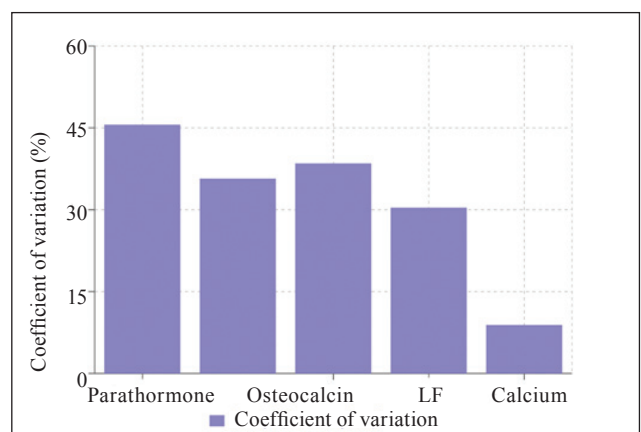


Fig. 2. Coefficients of variation of biochemical parameters

activity (197.4 ± 47.6) U/L showed a normal distribution of data.

J. A. Nicholson et al. proved similar changes and their significance for assessing the metabolic activity of bone tissue. The identified correlations between markers (LF/osteocalcin $r = 0.72$, β -CrossLaps/LF $r = 0.68$) confirm the relationship between the processes of formation and resorption [14].

The results indicate that a comprehensive assessment of biochemical markers can be used to monitor treatment effectiveness. At the same time, it is necessary to take into account the high individual variability of some indicators and their gender characteristics. When analyzing the pathogenic mechanisms of reparative osteogenesis, it is important to note the role of various growth factors and cytokines. An increased level of osteocalcin (27.8 ± 10.7) ng/ml together with high activity of LF (102.5 ± 31.2) μ kat/l may reflect the activation of the BMP2-dependent osteogenesis pathway.

S. Martin-Iglesias et al. demonstrated that such changes correlate with BMP2 expression and osteoblast activation [15, 16]. The relationship between markers of bone resorption and formation deserves special attention. An increased level of β -CrossLaps (0.64 ± 0.23) ng/ml with a simultaneous increase in osteocalcin indicates a disruption of the balance of remodeling processes. N. Patel et al. showed that such a combination of changes is characteristic of delayed consolidation [17].

The role of inflammatory factors is also significant. The high variability of parathyroid hormone (CV = 45.6 %) and the right-sided asymmetry of its distribution may reflect the activation of pro-inflammatory mechanisms. H. ElHawary et al. noted similar changes in case of impaired fracture consolidation [18]. The stability of mineral metabolism indicators (calcium CV = 8.9 %) with significant variability of other markers may indicate the preservation of systemic regulatory mechanisms under conditions of local disorders of osteogenesis.

V. Fischer et al. emphasize the importance of maintaining mineral homeostasis for successful consolidation. Gender differences in the levels of LF (105.3 ± 29.8) μ kat/l in men versus (97.7 ± 24.6) in women) and CPK may be associated not only with hormonal characteristics, but also with different intensity of mechanical load [19]. The identified changes also suggest the participation of VEGF-dependent mechanisms in the disruption of reparative osteogenesis. K. Hu et al. noted that the activation of angiogenesis is a critical factor for successful consolidation [20]. Therefore, a comprehensive analysis

of biochemical markers opens up new opportunities for understanding the pathogenesis of reparative osteogenesis and optimizing treatment tactics.

Conclusions

The study showed that before treatment, patients had significant bone metabolism disorders, which were manifested by deviations of biochemical markers from reference values.

Patients with segmental bone defects were found to have an increase in the levels of markers of both formation (LF — (102.5 ± 31.2) μ kat/l, osteocalcin — (27.8 ± 10.7) ng/ml) and resorption (β -CrossLaps — (0.64 ± 0.23) ng/ml), which indicated a disruption in the balance of remodeling processes.

Most of the studied indicators were within the reference values but were characterized by significant individual variability. Statistically significant gender differences were found for LF indicators (105.3 ± 29.8) μ kat/l in men and (97.7 ± 24.6) in women, $p < 0.05$) and CPK, which should be considered when interpreting the results.

The highest variability was demonstrated by the levels of parathyroid hormone (CV = 45.6 %) and β -CrossLaps (CV = 35.7 %), the lowest by the concentration of total calcium (CV = 8.9 %). The data distribution for most indicators exhibited right-sided skewness, except for calcium and LDH, which displayed a nearly normal distribution.

Correlations were established between markers of bone formation and resorption ($r = 0.68$ – 0.72 ; $p < 0.01$), which confirms the complex nature of metabolic disorders and the need to monitor a wide range of biochemical markers to determine the effectiveness of treatment.

The results demonstrated high efficiency, as evidenced by the normalization of most biochemical indicators.

Conflict of interest. The authors declare the absence of a conflict of interest.

Prospects for further research. The main limitation of this study is the relatively short observation period, which, at this stage, did not influence the outcomes. However, a comprehensive assessment of biochemical markers remains an effective approach for monitoring bone metabolism disorder treatments and offers promising opportunities for further research.

Information on funding. The authors declare the absence of financial support during the obtaining of the results and writing of this article.

Authors' contribution. Magomedov S. — analysis of the findings, participation in drafting the article; Polyachenko Yu. V. — determination of research directions; Kalashnikov A. V. — analysis of clinical material, drafting of conclusions; Litun Yu. M. — analysis of clinical material, participation in drafting the article; Polishchuk L. V. — processing and conducting biochemical studies.

References

- Gubin, A. V., Borzunov, D. Y., & Malkova, T. A. (2013). The Ilizarov paradigm: Thirty years with the Ilizarov method, current concerns and future research. *International orthopaedics*, 37(8), 1533–1539. <https://doi.org/10.1007/s00264-013-1935-0>
- Mauffrey, C., Barlow, B. T., & Smith, W. (2015). Management of segmental bone defects. *Journal of the American academy of orthopaedic surgeons*, 23(3), 143–153. DOI: 10.5435/JAAOS-D-14-00018R1
- Masquelet, A. C., & Begue, T. (2010). The concept of induced membrane for reconstruction of long bone defects. *Orthopedic clinics of North America*, 41(1), 27–37. <https://doi.org/10.1016/j.ocl.2009.07.011>
- Taylor, B. C., Hancock, J., Zitzke, R., & Castaneda, J. (2015). Treatment of bone loss with the induced membrane technique. *Journal of orthopaedic trauma*, 29(12), 554–557. <https://doi.org/10.1097/bot.0000000000000338>
- Niikura T., Jimbo N., Komatsu M., Oe K., Fukui T., Matsumoto T., Hayashi S., Matsushita T., Sakai Y., Itoh T., Kuroda R. (2021). Histological analysis of induced membranes in patients whose bone defects were treated with the Masquelet technique to identify factors affecting the vascularity of induced membranes. *Journal of orthopaedic surgery and research*, 16(1). <https://doi.org/10.1186/s13018-021-02404-7>
- Liu, S., Hu, C., & Ren, Z. (2017). Bone tissue engineering: Scaffolds with Osteoinductivity for bone regeneration. *BioMed research international*, 2017, 1–1. <https://doi.org/10.1155/2017/1038476>
- Kaspiris, A., Hadjimichael, A. C., Vasiliadis, E. S., Papa-christou, D. J., Giannoudis, P. V., & Panagiotopoulos, E. C. (2022). Therapeutic efficacy and safety of Osteoinductive factors and cellular therapies for long bone fractures and non-unions: A meta-analysis and systematic review. *Journal of clinical medicine*, 11(13), 3901. <https://doi.org/10.3390/jcm11133901>
- Haubruck, P., Heller, R., Apitz, P., Kammerer, A., Alamouti, A., Daniel, V., Schmidmaier, G., & Moghaddam, A. (2018). Evaluation of matrix metalloproteases as early biomarkers for bone regeneration during the applied Masquelet therapy for non-unions. *Injury*, 49(10), 1732–1738. <https://doi.org/10.1016/j.injury.2018.07.015>
- Rathwa, H. S., Verma, T., & Chavali, V. H. (2021). Assessment of union in fractures: Role of serum alkaline Phosphatase and ultrasonography. *Journal of clinical orthopaedics and trauma*, 14, 94–100. <https://doi.org/10.1016/j.jcot.2020.08.004>
- Perut, F., Roncuzzi, L., Gómez-Barrena, E., & Baldini, N. (2024). Association between bone turnover markers and fracture healing in long bone non-union: A systematic review. *Journal of clinical medicine*, 13(8), 2333. <https://doi.org/10.3390/jcm13082333>
- Wei, S., Pan, X., & Wei, J. (2024). Relationship between bone turnover markers and renal disease in elderly patients with type 2 diabetes: A cross-sectional study. *BMC endocrine disorders*, 24(1). <https://doi.org/10.1186/s12902-024-01698-y>
- Kumar, M., Shelke, D., & Shah, S. (2017). Prognostic potential of markers of bone turnover in delayed-healing tibial diaphyseal fractures. *European journal of trauma and emergency surgery*, 45(1), 31–38. <https://doi.org/10.1007/s00068-017-0879-2>
- Omogbai, O. W., & Olaniyan, M. F. (2023). Assessment of selected biomarkers of bone healing and inflammation among subjects with fracture on traditional and conventional treatment methods. *Sokoto journal of medical laboratory science*, 8(3), 46–54. <https://doi.org/10.4314/sokjmls.v8i3.6>
- Nicholson, J., Yapp, L., Keating, J., & Simpson, A. (2021). Monitoring of fracture healing. Update on current and future imaging modalities to predict union. *Injury*, 52, S29–S34. <https://doi.org/10.1016/j.injury.2020.08.016>
- Martin-Iglesias, S., Milian, L., Sancho-Tello, M., Salvador-Clavell, R., Martín de Llano, J. J., Carda, C., & Mata, M. (2022). BMP-2 enhances osteogenic differentiation of human adipose-derived and dental pulp stem cells in 2D and 3D in vitro models. *Stem cells international*, 2022, 1–15. <https://doi.org/10.1155/2022/4910399>
- Kovalchuk, A., Zynych, O., Korpachev, V., Kushnareva, N., Prybyla, O., & Shishkan-Shishova, K. (2021). Osteocalcin: The relationship between bone metabolism and glucose homeostasis in diabetes mellitus. *International journal of endocrinology (Ukraine)*, 17(4), 322–328. <https://doi.org/10.22141/2224-0721.17.4.2021.237347>
- Patel, N., & Ganti, L. (2025). The treatment and monitoring of osteoporosis using bone turnover markers. *Orthopedic reviews*, 17. <https://doi.org/10.52965/001c.127772>
- ElHawary, H., Baradaran, A., Abi-Rafeh, J., Vorstenbosch, J., Xu, L., & Efanov, J. I. (2021). Bone healing and inflammation: Principles of fracture and repair. *Seminars in plastic surgery*, 35(03), 198–203. <https://doi.org/10.1055/s-0041-1732334>
- Fischer, V., Haffner-Luntzer, M., Amling, M., & Ignatius, A. (2018). Calcium and vitamin D in bone fracture healing and post-traumatic bone turnover. *European Cells and Materials*, 35, 365–385. <https://doi.org/10.22203/ecm.v035a25>
- Hu, K., & Olsen, B. R. (2016). The roles of vascular endothelial growth factor in bone repair and regeneration. *Bone*, 91, 30–38. <https://doi.org/10.1016/j.bone.2016.06.013>

The article has been sent to the editors 27.05.2025	Received after review 19.06.2025	Accepted for printing 03.07.2025
--	-------------------------------------	-------------------------------------

DYNAMICS OF BIOCHEMICAL MARKERS OF BONE METABOLISM IN PATIENTS WITH SEGMENTAL BONE DEFECTS TREATED WITH THE MASQUELET TECHNIQUE

S. Magomedov, Yu. V. Polyachenko, A. V. Kalashnikov, Yu. M. Litun, L. V. Polishchuk

SI «National Institute of Traumatology and Orthopedics of the NAMS of Ukraine», Kyiv

✉ Sadrudin Magomedov, MD, Prof.: alexander@magomedov.kiev.ua; <https://orcid.org/0000-1234-5678-9101>

✉ Yuriy Polyachenko, MD, Prof. in Orthopedics and Traumatology: poliach.yv@gmail.com; <https://orcid.org/00000003-1814-4240>

✉ Andrii Kalashnikov, MD, Prof. in Orthopedics and Traumatology: orgitoua@gmail.com; <https://orcid.org/0000-0001-8092-3451>

✉ Yurii Litun, MD, PhD: litun_yurii@ukr.net; <https://orcid.org/0000-0002-7397-5381>

✉ Larysa Polishchuk: 2501lora@gmail.com; <https://orcid.org/0000-0002-9416-4596>