ORIGINAL ARTICLE

BIOMECHANICAL FACTORS IN RESIDUAL LIMB FORMATION AFTER AMPUTATION

DOI: 10.36740/WLek202107106

Viktor I. Shevchuk[1], Yurii O. Bezsmertnyi[1], Halyna V. Bezsmertna[1], Tetyana V. Dovgalyuk[1], Yankai Jiang[2]
[1]RESEARCH INSTITUTE OF REHABILITATION OF NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSIA, UKRAINE
[2]THE SECOND HOSPITAL OF SHANDONG UNIVERSITY, JI'NAN, CHINA

ABSTRACT
The aim: To study the influence of biomechanical factors on the character of morphological disorders in the process of reparative regeneration at the end of the residual limb after amputation.
Materials and methods: 10 series of experiments on 144 rabbits were conducted. We used myodesis with normal, insufficient and excessive muscle tension, their electrical stimulation, tight and leaky closure of the bone marrow canal. Terms of observation 1, 3, 6 months. The method of research – histological with the filling of vessels with ink-gelatin mixture.
Results: Dense closure of the meduallary cavity and uniform muscle tension during plasty in the first three series of experiments allow to obtain a cylindrical residual limb with preservation of the cortical diaphyseal plate, formation of the bone closing plate, normalization of intraosseous microcirculation, completion of the reparative process. In the majority of observations of the IV-X series there was a reparative regeneration disorder connected with the incorrect tension of muscles and the absence of normalization of intraosseous circulation, the reparative process was not observed to be complete, which led to the pathological reorganization of bone tissue with the formation of stumps of various shapes.
Conclusions: Uneven muscle tension and lack of closure of the intramedually canal except for microcirculation disorders leads to increased periosteum bone formation, formation of periosteum cartilage exostases, clavate stumps, resorption and fractures of the cortical diaphyseal plate with curvature and stump axis disorders, formation of a conical stump.

KEY WORDS: amputation, reparative regeneration, intraosseous microcirculation, pathological bone restructuring

Wiad Lek. 2021;74(7):1581-1586

INTRODUCTION
Amputation of the limb is a maiming intervention, the incorrect execution of which makes a prosthetic restoration impossible. The intersection of muscles, nerves, vessels and bone makes it much more difficult to form a rational relationship between these anatomical formations to create a prosthetic residual limb that can be used.

At present, despite the widespread use of fascio- and myoplastic methods of amputation in amputation surgery, there is a significant variability of healing and residual limbs formation outcomes, and the percentage of residual limbs suitable for prosthetics remains high [1-4].

There is a large number of researches which present the technique of operations [3-9], the description of diseases and defects of the stump [10-18] and the technique of re-amputations [19]. From our point of view, however, these works do not highlight many serious issues of residual limb formation. Thus, the authors do not pay attention to the optimal tension of the muscles stitched up during the amputation, the significance of the ostium of the open meduallary cavity, the state of the crossed intraosseous main vessels. In this connection it is necessary to study the dependence between the reparative regeneration processes in the residual limb and reactive changes in it, and the nature of plastic measures and peculiarities of relations between residual limb tissues. It is necessary to understand a character as well as the morphological parameters that are the most appropriate and rational for the reparative processes and residual limb formation. Understanding the regularities of the flow of reparative and form building processes, favorable and unfavorable factors affecting the outcome of amputations can serve as a basis for the development of more advanced methods for the residual limb plasty.

THE AIM
The aim to study the influence of biomechanical factors on the nature of morphological disorders in the process of reparative regeneration at the end of the residual limb after amputation.

MATERIALS AND METHODS
10 series of experiments on 144 rabbits were carried out. The myodesis method, that was tested in the clinic was used in the I main series – attaching the truncated muscles

to the end of the bonesaw-line through drilled holes and tightly suturing their ends without taking into account the muscle tension. In series II, the muscles were fixed at a tension of 916-962 μN (optimal). In series III the bonesaw-line was covered with a thin cortical plate tightly fitted to the meduallary cavity. In series IV the muscles were fixed at a tension of 980-1100 μN, in series V – at a tension of 1100-1200 μN, in series VI – at a tension of 650-800 μN, in series VII – the fascioplastic closing of the bonesaw-line was used, in series VIII – muscle plasty with further suturing of antagonist muscles under the bonesaw-line was performed, in series IX in addition to muscle plasty in the postoperative period, the electrostimulation of one of the muscles was carried out applying a current of amplitude 3-12 mA, pulse duration 5-10 ms, modulation frequency 24-36 imp/min, pulse frequency 30-100 Hz, daily 10-15 minutes during 19-22 days, in series X the bonesaw-line was closed with the autograft taken from the removed part, implanting it under <30° into the intramedually canal without ensuring tightness. The observation period was 1, 3, 6 months, in separate series – 3, 6 months. In the postoperative period, all animals underwent daily tonusometry during the month by applying the device to the muscle. The research method –histological, with ink-gelatin mixture vessels filling. Before withdrawing from the experiment, 50000 units of heparin were injected intraarterially on a physiological solution. After 15 minutes the animal was stuffed with a rapid intravenous injection of thiopental sodium and the abdominal aorta was bandaged. Below the ligature, a cannula from the system for intraarterial injection was injected, fixing it in the lumen; the vessels were filled with 10% mascara-gelatin mixture. The limb was extracted and the muscles were removed. A thin layer of bone surrounding tissue was left. A longitudinal frontal slice of the residual limb was made. After decalcification in 15% solution of nitric acid it was poured with cellulose. Cuts of 15-20 microns thick were stained with hematoxylin and eosin and after Van Gieson. Clearing preparations were made at the same time. The thickness of the cuts was 90-100 microns.

The experiments were carried out in accordance with the principles of humane treatment of animals, set out in European Community directives (86 (609) EEC) and the Helsinki Declaration on the Humane Treatment of Animals.

RESULTS

Series I. The experimental group – myodesis. Term of 1 month, 7 observations. The shape of the residual limb end in four observations is preserved cylindrical. Thickness of cortical diaphyseal layer in distal and proximal parts is the same. Bone cortical plate of osteon-beam structure of rather mature bone tissue is formed at the end of the bonesaw-line. In the inter-beam spaces of the residual limb end and proximal parts of the residual limb, the microcirculation network approximately corresponded to that of diaphysis in the norm.

In three observations, there was partial resorption of the cortical diaphyseal plate, and its thickness even far from the end was uneven. The end of the stump was beveled. The bone closing plate consisted of not quite mature bone tissue. Single sinusoids were found near the bonesaw-line.

Term of 3 months, 6 observations. The residual limb retains its cylindrical shape in four observations. The thickness of the cortical diaphyseal layer is preserved throughout. The bone cortical closing plate consists of mature bone tissue. Poured with ink vessels of the microcirculatory network correspond to the norm. The reparative process is complete.

In two observations, while maintaining the cylindrical shape, the residual limb end is rounded due to partial resorption of the cortical diaphyseal plate edge. The endostal regenerate consisted of not quite mature bone tissue. Thin wall vessels of sinusoid type and single cysts were detected in the intramedually canal.

Term of 6 months, 6 observations. In all observations the shape of the residual limb end is cylindrical. The bone closing plate consisted of mature bone tissue. The cortical diaphyseal plate was mostly of uniform thickness. In the proximal and distal parts, the adipose bone marrow with thee microcirculatory network, corresponding to the bone, is normal. The reparative process is complete.

In assessing the formation of the residual limb in this series, the muscle tone required for an optimal flow of the reparative process was determined. It made up 920-960 μN. Providing for such a tone and density of the canal closure allows to obtain a residual limb of cylindrical shape with bone closing plate on the end surface and normal intraosseous microcirculation. Such stumps were obtained in the second and the third series. In other series, the obtained shape of a residual limb sharply differed from these series.

In the stumps of the first three series after amputation with tight closure of the intramedually canal and optimal muscle tension, at the end of the canal, a bone closing plate was formed by 1 month on the basis of a slightly determined endostal bone formation. It started from the inner surface of the cortical diaphyseal plate and was located horizontally, creating the base of the residual limb together with the plate. Like the tubular bone diaphysis, the stump was cylindrical in shape (Fig. 1). At slightly higher muscle tension, there developed a slight resorption of the cortical plate and a slight slanting of the base was formed. The bone closing plate was of osteon-beam structure. The reparative process was coming to an end. In the intramedually canal of the residual limb end, the microcirculation in the residual limb was approaching that of the bone. At the end of the stump, the vessels were defined as small branches in the adipoid bone marrow. Larger branches were not detected. Moderate reparative processes were observed in the cortical diaphyseal plate mainly along the vascular canals, but the main contours of tubular bone and the structure of compact bone in the cortical layer remained unchanged.

In the IV-X series the described morphological criteria for the optimal flow of the reparative process with the restoration of intraosseous microcirculation were violated

Fig. 1. Cylindrical stump histotope with adjacent muscles. Tinction is made by hematoxylin and eosin. X6.

Fig. 2. A histotopogram of the end of the residual limb. Periorostal bone formation with cartilage tissue in the form of exostosis. Tinction is made by hematoxylin and eosin. X6.

Fig. 3. Cult end histotopogram with cortical diaphyseal plate fracture and periosteal regenerate. Tinction is made by hematoxylin and eosin. X6.

Fig. 4. Bone residual limb end histotopogram with bone exostosis. Tinction is made by hematoxylin and eosin. X6.

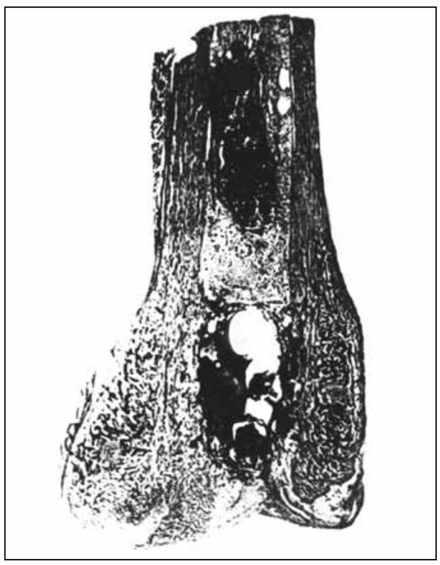

Fig. 5. Histotopogram of the end of the residual limb, which took the form of an epiphysar. Tinction is made by hematoxylin and eosin. X6.

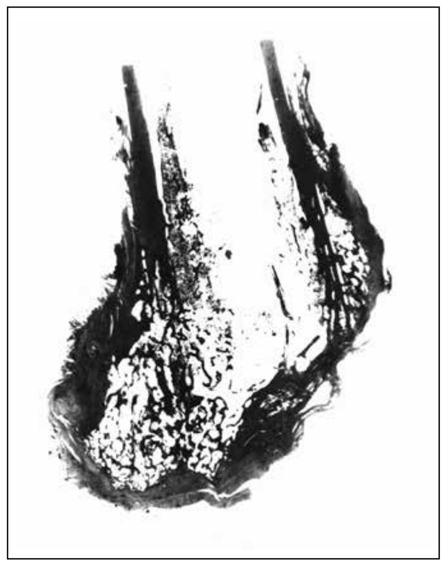

Fig. 6. Histotopogram of irregularly shaped stump with angular deviation of the end. Tinction is made by hematoxylin and eosin. X6.

in most observations. Emphasizing that in all 144 observations, the fundamental in reparative regeneration was the bone formation activity of the endothelium, it should be noted that the periosis was involved in bone formation only under certain conditions associated with increased muscle tone. Tension of muscles or their state of tone were the biomechanical factors that significantly influenced the formation of the stump.

In a number of observations with myoplasty along with muscle tone increase, as well as fascioplasty, in case of contractual contraction of unfixed muscles, the ends of stumps were sharply increased in volume due to the growth of massive periosteal regenerate (Figs. 2, 3). The lower part of the intramedually canal for 1-1.5 cm was filled with spongy regenerate in the form of a network of immature beams. The contours of the cortical diaphyseal plate at the end of the residual limb are almost undefined; it merges with the periosteum bone regenerates at a long range. In the inter-beam spaces of endostal formed bone tissue, the loose fibrous and fibrore-reticular tissue with the presence of sinusoid type vessels filled with ink and tissue cysts is determined. The oedematous adipoid bone marrow of the proximal intramedually canal has areas with loose fibrous connective tissue steeped in ink, which indicates a porous vascular wall. There revealed the sharply enlarged lumen of the feeding artery, filled with ink, which in most

Fig. 7. Histotopogram of a residual limb with conic end and disturbance of the cortical diaphyseal plate integrity. Tinction is made by hematoxylin and eosin. X6.

observations went into the soft tissues surrounding the residual linb.

Such regenerates in the form of exostoses have been detected in VI, VII, VIII and especially in IX series (Fig. 4).

Exostosises were formations that significantly deform the lateral surface of the residual limb, 0.6x0.9 to 1.5x2 cm in size, with rounded contours and apex directed to the side or upwards, corresponding to the thrust of the muscles attached to the residual limb.

The histological structure of exostosises was similar: they consisted of structures of newly formed bone in the form of an irregular network of elongated under the influence of muscle traction bone beams, containing cartilage fibers. From the surface the bone structures were covered with a layer of fibrous connective tissue or fibrous cartilage, in some places with osteoblastic-type tissue. The cartilage component was found in all the exostosises studied, but it did not have the character of a fringing cartilage coating with a oriented cell arrangement, typical of most bone and cartilage exostosises removed in patients.

Uneven increased muscle draught during the contraction resulted in the formation of the end of an epiphysaric-like residual limb, similar to the femoral muscles (Fig. 5). In these cases, a sharp expansion of the cross section of the bone end was determined by resorption of the cortical diaphyseal plate and fusion of periosteum and endostal bone regenerates. Elongated longitudinal beams as related to the bone length were found along the periosteal edge of the bone regenerate. In the proximal region, the cortical diaphyseal plate is sponged. The walls of the intramedually canal in its final part are formed by sponge regenerate and the remaining of the cortical diaphyseal plate. It is infested with a narrow layer of dense fibrous tissue. In the canal there is a loose fibrous tissue with multiple tissue cysts and carcass-filled thin-walled large-diameter vessels like sinusoids, large tissue cysts. Branches of the feeding artery filled with ink penetrate from the channel into the fibrous tissue. In two observations with muscle tension of 980-1100 μN there was an angular deviation of the residual limb end from the vertical axis (Fig. 6), and in two more observations there was a curvature of the vertical axis with expansion of the intramedually canal in the proximal section due to an earlier cortical plate fracture.

At tonus increase up to 1200 μN (V series), the cone deformations of the stump with uneven thickening and resorption of the cortical diaphyseal plate were obtained (Fig. 7). Not quite mature and immature bone structures with inclusion of fibrous cartilage were detected at the bonesaw-line ends. In proximal parts, immature bone beams, often with fractures, appeared at resorption sites. In some observations, the resorption of the cortical diaphyseal plate was observed for a significant length, revealing its fragments, represented by the lamellar bone tissue. A restructuring of the bone tissue was observed. In a number of experiments, the resorbed cortical diaphyseal plate was replaced by newly formed bone tissue, in which the remaining of this plate was present as inclusions. The thickness of the formed bone wall was much thicker than that of the resorbed plate.

In the cone-shaped part of the stump in the diaphyseal plate, the elongated bone beams, which form it, were revealed. They were absent near the top of the cone. Thus, the resorption of the cortical diaphyseal plate and the formation of the conical end of a tube bone sharply different from the structure of the diaphyse was normal. There was a dense and loose fibrous connective tissue with inclusion of primitive rare bone beams and diffuse arrangement of lymph-plasma cells. The intramedually canal was filled with sharply swollen fibrous tissue with many sinusoid-type vessels, tissue cysts and the presence of lymph-plasma clusters.

The formation of the conic end of the bone stump was not the result of endometrial growth from the intramedually canal beyond the boundaries of the bonesaw-line. This was due to the significant resorption of the cortical diaphyseal plate edges and the resulting bone formation with incomplete replacement of the dispersed bone.

Negative results with the formation of a cone-shaped residual limb were also obtained in the series with loose closure of the intramedually canal by a graft embedded in it and destroying the bone marrow and the vascular network of the residual limb end. Such operation does not favor the course of forming processes and abruptly violates reparative processes.

Cortical diaphyseal plate fractures were observed in a number of preparations of different series. Above and below the level of fracture, the bone tissue of the cortical diaphyseal plate, starting from the periosteum surface, was resorbed and replaced by the newly formed immature bone tissue on the endostal surface. In the fracture zone, the edges of compact bone connected by a thin plate of dense fibrous tissue are visible. In case of fracture healing, immature bone beams were revealed. In the intramedually canal in the resorption zone in the edematous loose fibrous tissue there were diffuse macrophages, lymphatic and plasma cells. There were a large number of sinusoids filled with ink. In these observations, the closing bone plate consisted of immature bone tissue and was concave inside the channel.

DISCUSSION

The received data testify that the processes of bone formation and bone destruction in many respects depend on the mechanical factor which role in the mechanism of physiological, pathological rearrangement of bones is doubtless. On the model of amputation and plastic formation of residual limb and reparative process some aspects of biomechanical conditions of pathological reorganization of bone tissue are found out.

The role of biomechanical factors influence on the results of reparative regeneration of the residual limb end after amputation has been established. These factors were the degree of muscle tension in plasty, the density of closure of the open bone marrow cavity, large branches of the feeding artery coming out of the intramedually canal with its imperfect closure plate.

The results obtained in the first three series of experiments convinced us of the necessity and usefulness of a tight closure of the open bone cavity, pre-fixed to the bone and then stitched together the ends of the muscles or a bone graft, tightly placed in the entrance to the canals. The most important factor was the uniformity of muscle tension with a given tone of 916-962 μN. The result of such operations was a residual limb of cylindrical shape with preserved wall structure of cortical diaphyseal plate, normal intraosseous microcirculation, bone closing plate of osteon-beam structure at the edge of the bonesaw-line. The reparative process in such stumps was mostly completed by the month.

Absence of hermetic closure of the intramedually canal, significant or even minor disorders of muscle tension in plasty in the rest of the series already by one month caused the resorption of cortical diaphyseal plate. The degree of its severity depended on the size of the muscle tension. At the same time, intraosseous microcirculation was also impaired. With the exception of a few empirically guessed cases of creation of uniform muscle tension and closure of the intramedually canal, reparative processes were disrupted in most observations of all seven series of experiments. Intensive reactive and reparative processes did not tend to be completed even in remote periods. Immature bone structures appeared in reparative regeneration associated with the continuation of bone formation processes. These phenomena characterized the pathological reorganization of bone tissue, which always proceeded against the background of disturbed intraosseous microcirculation. Torpid flow of reparative processes in residual limb tissues accompanied by bone substance resorption and appearance of new immature bone structures during months after amputation, led to changes in its form and structure and acquisition of features not typical for tubular bone diaphysis. These are cone-shaped contractions, flask-shaped end extensions, residual limb curvature, fractures and fractures of the cortical diaphyseal plate, formation of bone-cartilage regenerate and exostosis. The disturbed process of bone-closing plate formation led to the fact that there were no canal overlapping. Large dilated branches of the feeding artery came out of it into the connective tissue. By taking up most of the lumens, they, in turn, did not allow the reparative process to be completed. The reason of bone-cartilage exostosis formation was the uneven draught of the muscles surrounding the bone, causing increased periosteum formation. This is especially noticeable in myoplastic amputation, when the muscles stitched under the bonesaw-line are mutually contracted. It is also visible during the purposeful electrostimulation of muscles in the postoperative period. Similar phenomena were observed in postoperative contractures, when the tone of one muscle group was increased.

CONCLUSIONS

1. Biomechanical factors affecting the nature of stump formation after amputation are muscle tension during plasty, tight closure of the bone cavity and intraosseous circulation.
2. Uneven muscle tension and absence of the intramedually canal closure except for microcirculation disorders leads to the increased periosteum bone formation, formation of periosteum cartilaginous exostases, club-shaped stumps, resorption and fractures of the cortical diaphyseal plate and curvatures and violations of the residual limb axis, formation of a conical stump.
3. Dense closure of the bone marrow canal and uniform muscle tension allow optimizing the reparative process and obtaining a cylindrical-shaped organotypic residual limb.

REFERENCES

1. Plucknette B.F., Krueger C.A., Rivera J.C. et al. Combat-related bridge synostosis versus traditional transtibial amputation: comparison of military-specific outcomes. Strategies Trauma Limb Reconstr. 2016;11(1):5-11.
2. Bosanquet D.C., Glasbey J.C., Stimpson A. et al. Systematic review and meta-analysis of the efficacy of perineural local anaesthetic catheters after major lower limb amputation. Eur J Vasc Endovasc Surg. 2015;50(2):241-9.
3. Chim H., Miller E., Gliniak C. et al. The role of different methods of nerve ablation of neuroma. Plast Reconstr Surg. 2013;131(5):1004-12.
4. Kahle J.T., Highsmith M.J., Kenney J. et al. The effectiveness of the bone bridge transtibial amputation technique: A systematic review of high-quality evidence. Prosthet Orthot Int. 2017;41(3):219-226.
5. Lewandowski L.R., Tintle S.M., D'Alleyrand J.C. et al. The utilization of a suture bridge construct for tibiofibular instability during transtibial amputation without distal bridge synostosis creation. J Orthop Trauma. 2013;27(10):239-42.
6. Malloy J.P., Dalling J.G., El Dafrawy M.H. et al. Tibiofibular bone-bridging osteoplasty in transtibial amputation: case report and description of technique. J Surg Orthop Adv. 2012;21(4):270-4.
7. Pet M.A., Ko J.H., Friedly J.L. et al. Does Targeted Nerve Implantation Reduce Neuroma Pain in Amputess? Clin Orthop Relat Res. 2014;472(10):2991-3001.
8. Ranz E.C., Wilken J.M., Gajewski D.A. et al. The influence of limb alignment and transfemoral amputation technique on muscle capacity during gait. Comput Methods Biomech Biomed Engin. 2017;20(11):1167-1174.

9. Bezsmertnyi Y.O., Shevchuk V.I., Grushko O.V. et al. Information model for the evaluation of the efficiency of osteoplasty performing in case of amputations on below knee. Proc. SPIE 10808, Photonics Applications in Astronomy, Communications, Industry, and High-Energy Physics Experiments 2018: 108083H. doi: 10.1117/12.2501558.
10. Preißler S., Htielemann D., Dietrich C. et al. Preliminary Evidence for Training-Induced Changes of Morphology and Phantom Limb Pain. Front Hum Neurosci. 2017; 11:319.
11. Taylor B.C., Poka A. Osteomyoplastic Transtibial Amputation: The Ertl Technique. J Am Acad Orthop Surg. 2016;24(4):259-65.
12. Tintle S.M., Keeling J.J., Forsberg J.A. et al. Operative complications of combat-related transtibial amputations: a comparison of the modified burgess and modified Ertl tibiofibular synostosis techniques. J Bone Joint Surg Am. 2011;93(11):1016-21.
13. Tosun B., Selek O., Gok U. et al. Medial gastrocnemius muscle flap for the reconstruction of unhealed amputation stumps. J Wound Care. 2017;26(8):504-507.
14. Vasil'ev A.Ju., Egorova E.A., Vykljuk M.V. Kliniko-luchevaja diagnostika izmenenij kul'ti bedra i goleni posle amputacij vsledstvie minno-vzryvnoj travmy [Clinical and radiation diagnosis of changes in the stump of the thigh and lower leg after amputations due to mine explosion injury]. Medicinskaja vizualizacija. 2011;1:107-116. (in Russian).
15. Vasil'ev A.Ju., Egorova E.A., Smyslenova M.V. Luchevaja diagnostika izmenenij kul'tej nizhnih konechnostej pri protezirovanii [Radiation diagnosis of changes in the stump of the lower extremities during prosthetics]. Klinicheskaja medicina. 2013;5(1):51-7. (in Russian).
16. Pavlov V.S., Bezsmernyi Y.O., Zlepko S.M. et al. The photonic device for integrated evaluation of collateral circulation of lower extremities in patients with local hypertensive-ischemic pain syndrome. 2017. https://doi.org/10.1117/12.2272324.
17. Weng W., Zhao B., Lin D. et al. Significance of alpha smooth muscle actin expression in traumatic painful neuromas: a pilot study in rats. Sci Rep. 2016;6:23828.
18. Xin Zhang, Yongming Xu, Jin Zhou et al. Ultrasound-guided alcohol neurolysis and radiofrequency ablation of painful stump neuroma: effective treatments for post-amputation pain. J Pain Res. 2017;10:295-302.
19. Yan Yin, Lan Zhang, Hong Xiao et al. The preamputation pain and the postoperative deafferentation are the risk factors of phantom limb pain: a clinical survey in a sample of Chinese population. BMC Anesthesiol. 2017;17:69.

The study was carried out as part of the research work "Discover the patterns of postamputation pain syndrome formation", state registration number 0120U101372.

ORCID and contributionship:
Viktor I. Shevchuk: 0000-0003-1105-4795 [A,B,D,F]
Yurii O. Bezsmertnyi: 0000-0002-1388-7910 [B,E]
Halyna V. Bezsmertna: 0000-0003-1505-4872 [B,D,E]
Tetyana V. Dovgalyuk: 0000-0003-1614-9021 [B,D,E]
Yankai Jiang: 0000-0001-8100-3438 [B,D]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Yurii O. Bezsmertnyi
Research Institute of Rehabilitation of
National Pirogov Memorial Medical University
56 Pirogov St., 21018 Vinnytsia, Ukraine
tel: +380972815160
e-mail: bezsmertnyiyurii@gmail.com

Received: 2020-04-21
Accepted: 2021-06-01

A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article