doi:10.20944/preprints202207.0405.v1

MORPHOLOGICAL FEATURES OF CONSOLIDATION IN DAMAGED BODIES OF THORACIC AND LUMBAR VERTEBRAS AT DIFFERENT TIME POINTS AFTER INJURY

Ostrovskij V.V., Shulga A.E., Ulyanov V.Yu.,
Zaretskov V.V., Bazhanov S.P., Likhachev S.V.

Abstract. Reparative osteogenesis in the damaged vertebrae is a complex cascade of morphological and biochemical processes that result in the consolidation of the vertebral body. This research was aimed at studying the features of reparative osteogenesis in damaged thoracic and lumbar vertebral bodies at different time points after injury. We analyzed the morphological findings of the vertebral tissues harvested during surgical interventions in 43 patients with recent, subacute and long-standing injuries to thoracic and lumbar spine, and found that cell differentiation in osteogenesis is closely related to angiogenesis and the metabolic cascade. In areas with sufficient oxygenation, good partial pressure of oxygen, and active growth of microvasculature the normal cycle of development and differentiation of osteoblasts and osteocytes occurs, and hypoxia and acidosis lead to pathological osteogenesis. The reclination maneuver with dorsal tools on Day 10-12 of the injury may be ineffective due to the formation of adhesions between fragments, and reclination of the body of the damaged vertebra two weeks or more after the injury is apparently doomed to failure. Timely ventral fusion performed for objective indications is the key to successful rehabilitation of patients, on the one hand, and reduction of the surgical trauma volume in the future, on the other.

Keywords: reparative osteogenesis, vertebrae, injury, trauma, thoracic spine, lumbar spine

Vladimir V. Ostrovskij*, MD, DSc
https://orcid.org/0000-0002-8602-2715
e-mail sarniito@yandex.ru

Aleksey E. Shulga*, MD, PhD
https://orcid.org/0000-0001-8476-0231
e-mail doc.shulga@yandex.ru

Vladimir Yu. Ulyanov*, MD, DSc, Associate Professor (corresponding author)
https://orcid.org/0000-0002-9466-8348
e-mail: sarniito504@gmail.com

Vladimir V. Zaretskov*, MD, DSc
https://orcid.org/0000-0003-3643-9560
e-mail vvzaretskov@mail.ru

Sergey P. Bazhanov*, MD, DSc
https://orcid.org/0000-0001-9474-9095
e-mail baj.s@mail.ru

Sergey V. Likhachev*, MD, PhD
https://orcid.org/0000-0003-1874-2507
e-mail likha4@mail.ru

*Scientific Research Institute of Traumatology, Orthopedics and Neurosurgery, Federal State Budgetary Educational Institution of Higher Education ‘V.I. Razumovsky Saratov State Medical University’, the Russian Federation Ministry of Healthcare (NIITON SSMU)
148, N.G. Chernyshevskogo str, Saratov 410002, Russia

Relevance. Reparative osteogenesis in the damaged vertebrae is a complex cascade of morphological and biochemical processes that result in the consolidation of the vertebral body. If the damage to the vertebral bodies is severe the consolidation is usually incomplete and slow, and over a long period of time the supporting function of the vertebrae is performed by a posterior stabilization system that often suffers from fatigue fractures of the metal elements [1]. Therefore, the indications for anterior fusion as the second stage of surgical intervention have been significantly expanded [2]. However, when the delayed corporodesis is performed in adequate reclination, the consolidated vertebral body is often resected, which turns the surgery both traumatic and completely unjustified in this situation [3]. This research was **aimed at** studying the features of reparative osteogenesis in damaged thoracic and lumbar vertebral bodies at different time points after injury.

Material and methods. We analyzed the morphological findings of the vertebral tissues harvested during surgical interventions in 43 patients with injuries to thoracic and lumbar spine who underwent treatment in the Scientific Research Institute of Traumatology, Orthopedics and Neurosurgery, Federal State Budgetary Educational Institution of Higher Education V.I. Razumovsky Saratov State Medical University, the Russian Federation Ministry of Healthcare from 2012 to 2015. The research protocol # 7 of Feb. 01, 2022 was approved by the Ethics Committee of the Federal State Budgetary Educational Institution of Higher Education V.I. Razumovsky Saratov State Medical University of the Russian Federation Healthcare Ministry. All participants entered the research freely, with full information about it, and give their consents before entering.

The patients were divided into three groups by the time that passed since their traumas occurred: Group 1 – recent injury (up to 2 weeks), Group 2 – subacute injury (from 2 weeks to 1 month), Group 3 – long-standing injury (over 1 month). 17 patients (39.5%) had thoracic traumas and 26 (60.5%) had lumbar ones. AO/ASIF classification enabled subdividing spinal injuries within the groups into relatively stable AII and AIII (19 patients – 44.2%) and unstable BII, CI-CIII (24 patients – 55.8%). The standard examination included the study of complaints and anamnesis, as well as the evaluation of somatic, neurological and orthopedic statuses of the patient. The spinal injuries were visualized with plain radiography, as well as dynamic CT and MRI. The neurological status assessment of the patients by the ASIA/IMSOP scales revealed no signs of spinal cord injury in 29 individuals (67.4%) and spinal injuries complicated by neurological deficit of varying severity in 14 individuals

(32.6%). The severity of pain syndrome before and after surgeries was assessed using the visual analogue scale (VAS).

At different time points after their injuries all patients were operated, the supporting ventral fusion was formed though anterior approach. The corporodesis was refused in the acute period of injury due to severe somatic condition of the patients. Surgical interventions involved simultaneous fixation with either dorsal (transpedicular, laminar systems) or ventral (anterior screw systems) metal structures. If a patient had his/her spine previously stabilized though posterior approach, the damaged vertebral body was isolated and substituted with an implant. The classic surgical approaches to the thoracic and lumbar spine (thoracotomy, thoracophrenotomy, thoracophrenolumbotomy, lumbotomy) were used depending on the localization of the injury.

The harvested tissue specimens were 1 cm wide and 3-4 cm deep bone fragments cut out between adjacent discs with a straight chisel. The bone tissue was fixated in 10% neutral formalin, then decalcinated in 12% nitric acid solution. It was dehydrated in an ascending alcohol series and embedded in paraffin. The paraffin blocks were then cut into 5-7 μm slices that were de-embedding and stained with haematoxylin and eosine by general technique. The specimens were embedded in balsam, covered with glass and studied in Carl Zeiss microscope at x 100 to x 1000.

We began describing the specimens with their inner and outer cortical layers: we determined the condition of various matrix elements, the microarchitectonic parameters of the trabecular meshwork (the thickness of trabeculae, their separation and number as well as the distance between them and the density of arrangement). At the points of trabeculae branching the condition of the junctions (nodes) as well as the trabeculae ends as they feature the topological properties of the trabecular network and the quality of its conjunctivity. We also searched for superficial and/or interstitial mineralization defects, changes in bone formation parameters, and osteoid status. In the interbeam spaces, the state of the microvasculature and bone marrow was assessed. At the cellular level, the nature of osteogenesis, the condition of the bone cell lineage elements and other structures were investigated.

Results

Morphological study of the vertebral bodies in Group 1 patients: the trabeculas were thinned, had many microfractures, foci of osteomalacia and hemorrhage. In many interbeam spaces the bone marrow elements and erythrocytes were observed; in the remamining islets of the bone marrow the stromal cells with a clear structure of nucleus and cytoplasm were observed. The activity of perivascular cells essential for the reparative processes of injured vertebra was quite high: more than 40% of the cell series elements (Fig. 1). In most specimens, we observed microvasculature changes (irregular course with swelling, edema of the stroma, ɣ-metachromasia of fibrous elements, and deposition of protein complexes under the endothelium) all resulting in changes of intercellular contacts, metabolic disorders, reactive inflammation, hemorrhages into bone structures where dystrophy and necrosis prevailed.

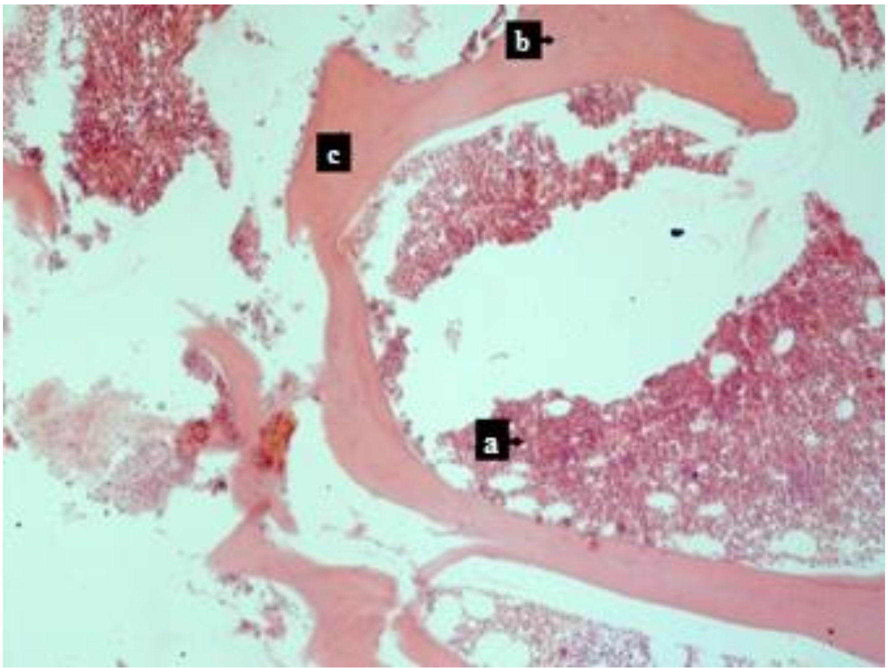

Figure 1. The detail of a 2 weeks old haematoxylin-eosine stained bone regenerate, ×100:
a) bone marrow b) osteocytes; c) primary bone plate

Morphological study of the vertebral bodies in Group 2 patients: we observed the progress of focal compensatory-adaptive processes. Tunneling developed in multiple foci of hemorrhages, granulation tissue and foci of young fibrous connective tissue began to form. The remaining signs of metabolic and circulatory hypoxia significantly stimulated proliferation of connective elements, and by the end of the month the signs of active angiogenesis and the formation of a new osteoid were clearly identified. In the spaces between trabeculas we observed the proliferation foci of the loose fibrous connective tissue elements with numerous cavernously dilated vessels as well as the bone marrow elements and occasional hemorrhage foci. The hemorrhages areas featured active arrangement with the formation of numerous crevices and tubules (tunnels) combined into the microvasculature system. More than half of the intercellular gaps reform into capillaries with an endothelial lining. In areas where the tunnel structure remained changed for a long time the interstitial blood flow was observed. The pattern of the bone tissue pathological remodeling in the injured vertebra progressed. Simultaneous remodeling occurred in several foci (islets) of small bone tissue enabling the restored bone partially fulfill its function. In the areas of extensive pathological remodeling the volume of altered bone tissue increased causing the disorder of biomechanical (supporting) function of the vertebra. Pathological osteogenesis was noted in almost all secondary deformities. The high cell activity in perivascular osteogenesis areas by the end of 3-4 weeks after injury was associated with self-maintenance of the population and its quantitative increase in the process of vessel growth (Fig. 2). This means that a combination of proliferation, differentiation and specific biosynthesis takes place in osteogenesis areas.

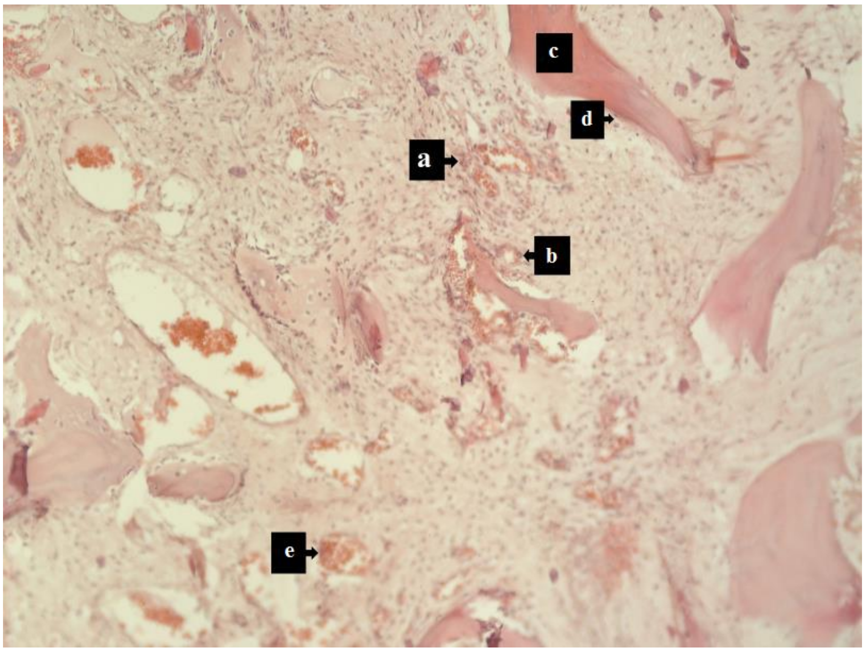

Figure 2. The detail of a 20 days old haematoxylin-eosine stained bone regenerate, ×100: a) bone marrow b) osteoblasts; c) primary bone plate; d) osteoclasts; e) blood vessel

Morphological study of the vertebral bodies in Group 3 patients: in the foci of the forming bone tissue we found all elements of histogenetic series (cell lineage) involved in regenerative processes and bone tissue remodeling. Up to 50% of these cells were differentiated bone cells that ensure the optimal structural and functional condition of the forming bone tissue using the remodeling mechanisms (resorption and plastic) participating in the mineral metabolism (Fig. 3).

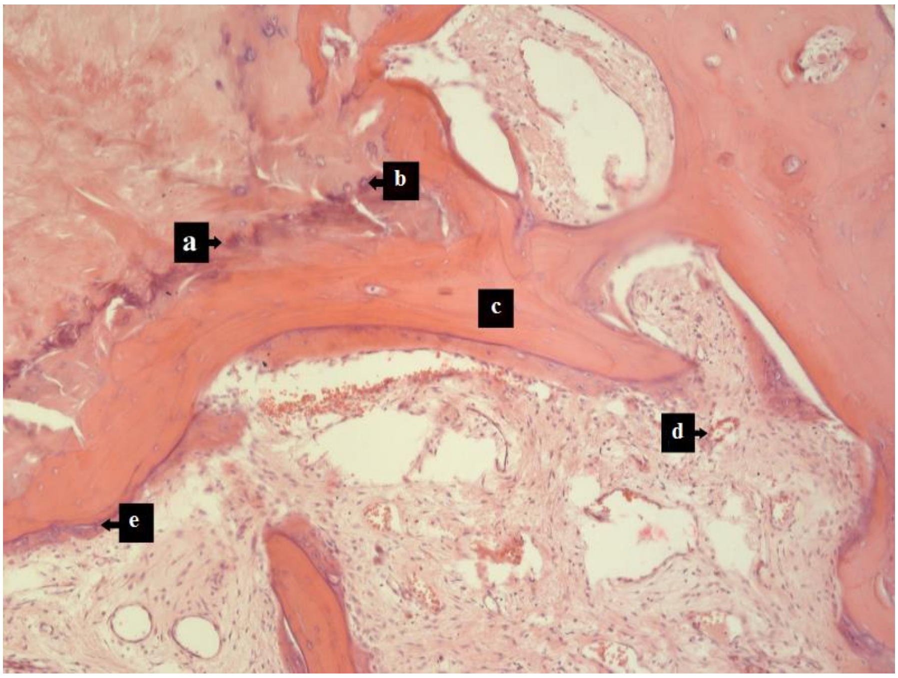

Figure 3. The detail of a 2 months old haematoxylin-eosine stained bone regenerate, ×100: a) osseogenesis area b) osteoblasts; c) primary bone plate; d) osteoclasts; e) blood vessel

Along with the described changes we detected mottled patterns forming a kind of morphological mosaic. We observed the thinning of bone trabeculae and widening of the spaces between them, a significant number of siderophages in the bone marrow, and disturbance of osteogenesis. These changes feature osteoporosis (Fig. 4).

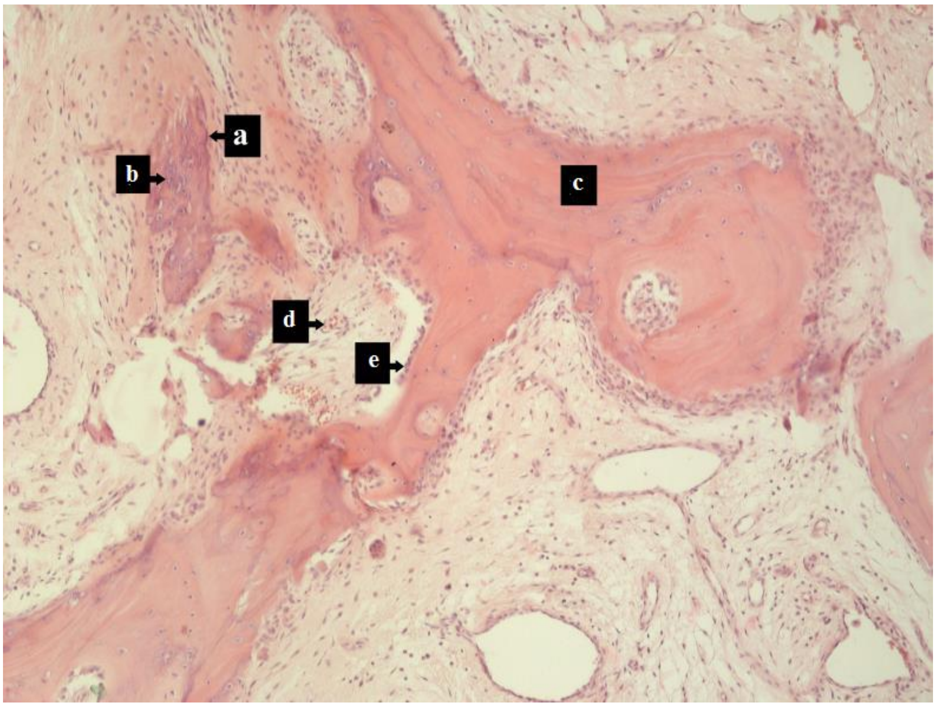

Figure 4. The detail of a 2 months old haematoxylin-eosine stained bone regenerate, ×100: a) osseogenesis area b) osteoblasts; c) primary bone plate; d) osteoclasts; e) blood vessel

The formation of provisional elements in the fracture area was accompanied by the formation of islets of cartilaginous and osteoid (new, non-calcified) bone tissue, that in some areas matured into beams and plates. Young vessels growing in there give the regenerate a resemblance to granulation tissue. There were islands of cartilaginous tissue along with osteoid and bone tissues (Fig. 5).

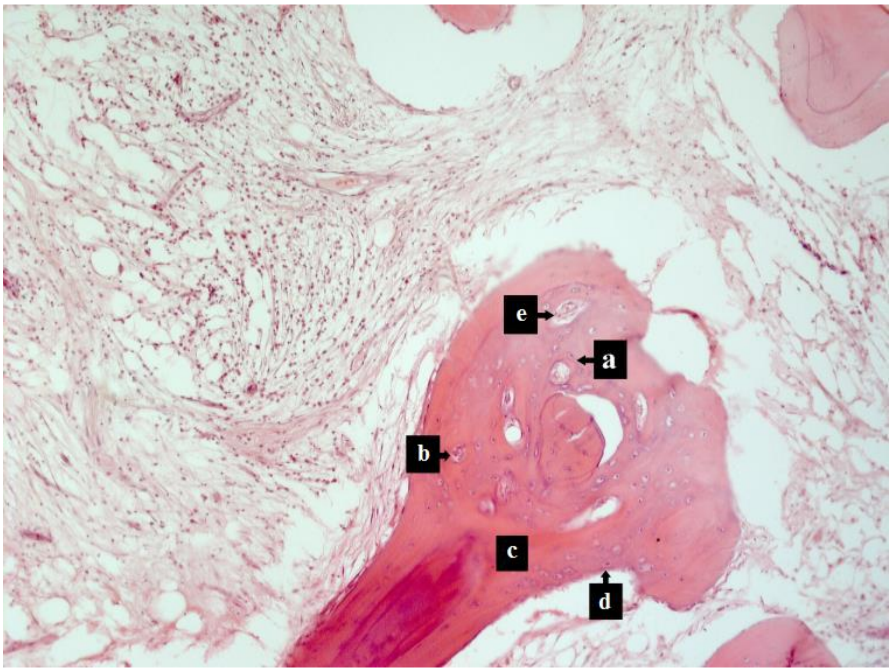

Figure 5. The detail of a 4 months old haematoxylin-eosine stained bone regenerate, ×100: a) osseogenesis area b) osteoblasts; c) primary bone plate; d) osteoclasts; e) blood vessel

The maximum amount of cartilaginous tissue was found in the areas with capillary growth retardation and in cases of significant axial loads on the damaged vertebra (incorrect or insufficient immobilization). The rearrangement of cartilage tissue into bone mainly resembled endochondral ossification. The new bone plates were devoid of Haversian canals (Fig. 6).

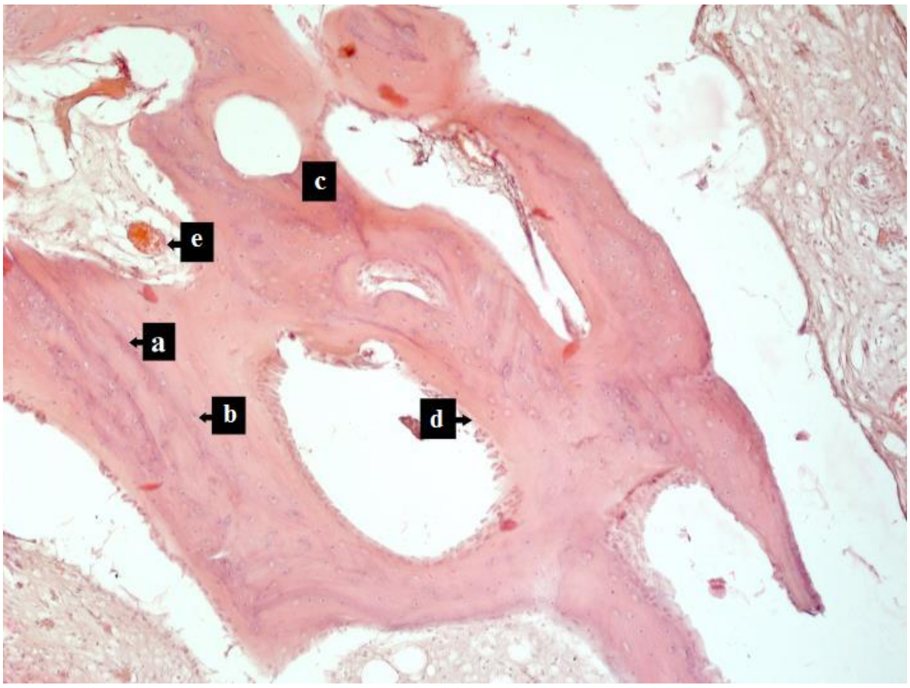

Figure 6. The detail of a 5 months old haematoxylin-eosine stained bone regenerate, ×100: a) osseogenesis area b) osteoblasts; c) primary bone plate; d) osteoclasts; e) blood vessel

Discussion

The body reacts to damage from the moment of injury (hormones, biologically active substances, cytokines, interleukins, *shock molecules*). In the area of injury (fracture) opsonin proteins in the form of albumins, fibrinogen, immunoglobulin G, and complement proteins concentrate [4]. The fracture area turns into a *hot spot* with the biologically active material that signals of what has happened to all body systems. The rapid response system gets engaged: the number and activity of fibroblasts increases, they begin to synthesize collagen. These cells are *recruited* from the surrounding connective tissue (CT) affected by perifocal elements of the preserved bone tissue in hematomas. This signaling cascade initiates resorption and further formation of bone tissue that may result in its regeneration and remodeling.

The analysis of the morphological findings suggests that within the first 2 weeks after the injury the reactive processes occur in the body of the damaged vertebra. They feature circulatory disorders (hemorrhages, thrombosis, blood separation, blood flow sequestration), inflammatory responses, vascular changes (wrong course with swelling, edema of the stroma, ɣ-metachromasia of fibrous elements and deposition of protein complexes under the endothelium). These result in dystrophy and necrosis dominance in bone structures which are most clearly represented in foci with many primary microfractures.

The first signs of union, or rather fibrous adhesions (gluing) appear on Day 10-12 of the trauma. This is evidenced by active fibroblasts and fibrocytes in the alteration area. 3 weeks later the connective tissue gets better organized, and we observed foci of CT element proliferation, fields of angiogenesis, formation of chondrogenic islets and osteoid. These findings indicate that any surgical interventions aimed at reclining the damaged vertebral body within subacute period (2 to 3 weeks after injury) are doomed to failure. The presence of a non-straightened body in dorsal fixation suggests the need for its replacement with an implant in the early stages to prevent secondary deformities.

Reparative osteogenesis of damaged vertebral bodies in different (remote) points of time after injury is of interest in terms of determining the timing of the vertebra full consolidation. The average compete union time (when the vertebral body becomes a support) is 5 months. Consolidation of multi-comminuted (burst) fractures is slower (6 months) due to multifocal foci of bone tissue destruction in vertebral bodies and the same nature of reparative osteogenesis. The islands of bone recovery and other CT elements differed significantly in cellular composition, angiogenesis rate and the differentiation of the main structures respectively.

Conclusion

1. Cell differentiation in osteogenesis is closely related to angiogenesis and the metabolic cascade. In areas with sufficient oxygenation, good partial pressure of oxygen, and active growth of microvasculature the normal cycle of development and differentiation of osteoblasts and osteocytes occurs, and hypoxia and acidosis lead to pathological osteogenesis.

2. The reclination maneuver with dorsal tools on Day 10-12 of the injury may be ineffective due to the formation of adhesions between fragments, and reclination of the body of the damaged vertebra two weeks or more after the injury is apparently doomed to failure.

3. Timely ventral fusion performed for objective indications is the key to successful rehabilitation of patients, on the one hand, and reduction of the surgical trauma volume in the future, on the other.

Conflict of interest: this research was part of the project Development of the predicting and preventing system for adverse outcomes of surgical treatment of thoracic and lumbar spine injuries based on a comprehensive personalized analysis of the vertebral repair rate, Nat. Reg. No 122022700112-7. It received no specific grants from any funding agency in the public, commercial, or not-for-profit sectors. All authors give their consents to distribute the full text of this paper to Ulyanov V.Yu., MD, DSc, Associate Professor.

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