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TRANSOSSEOUS OSTEOSYNTHESIS – THE ILIZAROV METHOD

TRANSOSEALNA OSTEOSINTEZA – ILIZAROVA METODA

Ivica LALIĆ

The Ilizarov method is a valuable alternative option to more conventional methods in the treatment of severe wound contamination and in cases of soft tissue or bone loss. Ilizarov named his method transosseous compression-distraction osteosynthesis and formulated its principles [1, 2]. Today it is simply called the Ilizarov method, although the method is a collective result of a large team of talented scientists, surgeons and engineers, he had gathered around him. It is a system of techniques that induce compression or distraction (or the combination of both) by moving bone fragments via transosseous wires with the adjustments of the external ring fixator for bone union, growth or spatial transformation that finally ends in osteosynthesis, consolidation and new bone remodelling. It is used for skeletal injuries, their complications, congenital disorders, degenerative diseases and tumors. The treatment of bone loss occurring as a result of acute trauma has traditionally been a complex surgical problem. In an attempt to avoid the problems associated with deficient graft materials and free tissue transfers, internal bone transport is a technique that has been a successful method for bony reconstruction in acute bone loss [1–3]. In addition, the Ilizarov apparatus may provide stability even in cases of bone comminution when internal fixation devices can do no better than tenuous fixation. Nonunion of long bones is often associated with a significant loss of function of the affected extremity, joint stiffness, muscular atrophy, diffuse osteopenia, and even an extremity amputation or systemic manifestations in the case of infection. Indications for appropriate treatment are often unclear [4]. In complex nonunion, intramedullary nailing is preferred, in delayed consolidation and hypertrophic nonunion without angular defects or hypometria, while the Ilizarov method is more often indicated in atrophic nonunion, and in hypertrophic nonunion with hypometria and angular defects [5]. The ring frame supports and stabilizes the underlying bone by means of transfixion wires and half pins. The frame stability increases with the increasing wire diameter and tension, the use of more wires per ring, placing wires on opposite sides of the ring and inserting wires in different planes. Increasing crossing angles of wires to 90° provides maximal stability and crossing angles of less than 60° may allow the bone to slide along the wires requiring the use of opposing olive wires or the addition of a half

Figure 1. G. A. Ilizarov (1921 – 1992)
Slika 1. *G. A. Ilizarov (1921–1992)*

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pin. Olive wires provide an important buttress effect in the correction of angular deformity [6].

Gavril Abramovich Ilizarov was born in Białowieża, Poland in 1921 (**Figure 1)**. His father died when Gavril was very young. There was little support for Ilizarov to attend school and consequently, his formal education was delayed until the age of 11. By the age of 16, Ilizarov had completed the equivalent of ten years of education in five consecutive years. In the setting of the Second World War, Ilizarov was forcibly evacuated from Simferopol, Crimea, where he studied at the Simferopol Medical Institute. Finally, in 1943, he finished his studies in Kzyl–Orda, Kazakhstan and at the age of 22, he was awarded a medical degree. In 1944, Ilizarov returned to a rural setting when he was assigned to practice general medicine in Dolgovka, Siberia, a remote region that had previously been used for the exile of Tsars and their families [6]. Ilizarov came across a shaft–bow harness connecting a horse to its carriage through shafts. This served as an inspiration and he attempted to incorporate this mechanism into a prototype to repair fractures. Before ever testing on a living subject, Ilizarov first created an apparatus based on the shaft–bow harness to "treat" broken broomsticks. He made several rudimentary versions of the device, trying each time to further reduce mobility of the broken broomstick. Ilizarov would eventually seek help from a local metal–worker to fashion ring–shaped wires that would be suitable for use on human limbs. The Ilizarov apparatus became a system of external fixators consisting of stainless steel rods, rings, and Kirschner wires. The method was distinct from conventional external fixators in that the apparatus encased the limb and formed an external cylinder around it **(Figure 2).** The circular construction afforded early weight bearing for patients since it provided greater support than monolateral fixators. A key biophysical element was that the superior rings of the apparatus allowed force to be transferred from the bone distal to the fracture site, through the external frame (bypassing the fracture site), directly to the bone proximal to the fracture. Although its initial application was to effectively stabilize severe fractures for healing, Ilizarov realized that the apparatus could also be used to lengthen a limb. The Ilizarov external fixator apparatus' use for this purpose relied on the principle of distraction osteogenesis; when two ends of a bone are distracted but the periosteum remains intact, new bone is laid down to fill the space. This regeneration of bone was applied to correct limb length discrepancies [7]. Ilizarov's studies proved that the ideal setting for new bone formation consisted of a low–energy osteotomy followed by one week of latency and a distraction of the bone at a rate of one mm/day in four divided increments [8].

In 1950, G. A. Ilizarov moved to Kurgan, Siberia. Practicing in this larger center allowed Ilizarov to develop his apparatus and broaden its scope. Though formally trained in general medicine, Ilizarov was promoted to Director of the Kurgan Research Institute for Experimental Orthopaedics and Traumatology because of his experience with his innovative apparatus. He chose former Russian soldiers in the Second

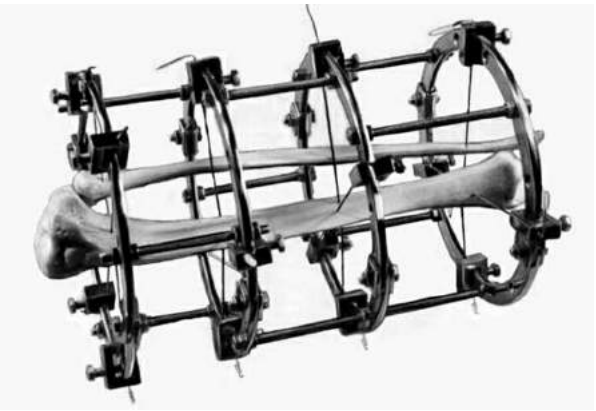

Figure 2. Model of Ilizarov apparatus
Slika 2. *Model Ilizarovog aparata*

World War as his initial patients. Ilizarov was disheartened by the time required for severe fractures to heal and wished to use his technique in an effort to repay veterans for their service. In 1964, Soviet high jumper and Olympic champion, Valery Brumel, found his career cut short after a severe automobile accident. Brumel sustained comminuted fractures resulting in the near complete loss of the use of both legs. In desperation, Brumel sought help from Ilizarov and was successfully treated in 1967. By 1981, a group of Italian orthopedic surgeons learned of his technique, mastered it and subsequently published it in didactic textbooks. In order to disseminate the device and the technique, these Italian orthopedic surgeons organized national societies throughout the world called the Association for the Study and Application of the Methods of Ilizarov. More recently, the method was introduced in North America, where it has been adopted primarily by pediatric orthopedic surgeons for limb lengthening. Some American orthopedic surgeons have expanded their practice to include the Ilizarov method for adults with severe deformities such as nonunions and bone deficiencies from trauma, infections, and tumors. Many research centers have utilized the method to study bone formation. Coincidentally, these efforts corroborated Ilizarov's own research and in part extended insights into the regeneration of both bone and soft tissues under mechanical distraction. Ilizarov first introduced this method both experimentally and clinically over his 40-year career in Siberia. The University of Toronto orthopedic surgery resident, Dr. Paley, was motivated to learn of this new technique and bring its benefits to North America. But during the tensions in the Cold War era, Soviet Union made it extremely difficult for people from the Western World to gain access to this "closed" Soviet city. In 1986, Ilizarov and other Soviet–based surgeons hosted an International Conference on Transosseous Osteosynthesis, giving Paley the perfect justification to visit Kurgan. Ilizarov felt it was important to disseminate his method to American audience but was simultaneously wary of not receiving credit from American scientists and clinicians, especially given the air of secrecy between the two nations. Paley helped to assuage these tensions

by being able to communicate with Ilizarov in Russian. Since Ilizarov was more willing to share the confidential details on the use of his apparatus in the absence of an intermediary translator, Paley enjoyed greater access to the intricate details of mastering the technique. Also, being from Canada, as opposed to the United States, made him less threatening. For Paley, learning Russian provided an opportunity to make sense of many of Ilizarov's original documents [4]. The Ilizarov procedure of transosseous osteosynthesis was alive and well by the late 1980s [8]. When Ilizarov eventually went to Rome in 1982 to lecture on his innovation, the Ilizarov apparatus had already begun to acquire global fame. Italian surgeons dubbed Ilizarov "The Michelangelo of Orthopedics" [9, 10].

In the Republic of Serbia, this method has been successfully applied at the Institute for Orthopedic Surgical Diseases "Banjica" - Belgrade for 35 years, and at the Clinic for Orthopedic Surgery and Traumatology in Novi Sad in the last 15 years. The leading surgeons of these institutions were also educated at the World Science Center in Kurgan. Their papers were presented in domestic and foreign books and journals [11–14].

Ilizarov had been bestowed the rare honour of Hero of Socialist Labour in 1981, and became a member of the Russian Academy of Sciences ten years later. He was awarded the Lenin Prize in 1979. In 1992, Ilizarov died of heart failure at the age of 71 in his hometown of Kurgan. The Ilizarov Centre for Orthopedic Surgery in Kurgan has been repurposed to focus on the surgical correction of congenital limb abnormalities **(Figure 3).** A scientific journal "Orthopaedic Genius" ("Genii Orthopedii") was created shortly after his death in honour of Ilizarov [10].

The basic principle of Ilizarov technique is the use of a percutaneous corticotomy that minimizes trauma to the periosteum and preserves the blood supply of the bone marrow and periosteum [15]. The use of a corticotomy instead of osteotomy emphasizes the importance of the blood supply to osteogenesis. Both the periosteal and medullary blood supply can be preserved by cutting only the cortex. Significant retardation of osteogenesis has been observed in animal studies with damage to the intramedullary nutrient vessels in osteotomized bones. Ilizarov's experiments with distraction osteogenesis made it clear that both rate and rhythm of distraction affect the quality of the regenerate [1, 15, 16]. Ilizarov performed his experiments in canine tibiae. He found that 0.5 mm of distraction per day often resulted in premature consolidation of the regenerate, while 2 mm of distraction per day produced a poor regenerate often with intervening fibrous tissue [15, 17].

The biomechanical goals of external fixation in bone transport are threefold: to maintain the bone ends in stable alignment, to control the movement of the bone projectile, and to allow compression of the bone in the target zone [18, 19]. Stable fixation of the bone fragments is one of the most important principles in the Ilizarov technique [15]. A stable frame permits full weight bearing, does not restrict the function of adjacent joints, and permits physiologic function of the entire limb, ensuring optimal mechanical and biologic conditions [15]. Secure fixation limits translational micro motion between the bone fragments. Weight bearing and active muscle function enhance local circulation and shorten the period required for osseous callus formation and remodelling [15]. The degree of stability of the apparatus depends on multiple factors. The number of and tension on the wires influences the stability of the construct. In addition, the angles between the wires can affect stability. Optimal stability with two transfixation wires is obtained when the wires are perpendicular to each other. When anatomic and functional constraints prevent perpendicular wire placement, supplementary wires may be required [15, 17]. Stability also depends on the number and size of the rings in the apparatus and the rigidity of the fixator construct. The amount of compression or distraction incorporated into the configuration can also contribute to the stability. The shape, cross-sectional area, and the density of the bone fragments, as well as the shape, location, and plane of the fracture or osteotomy relative to the longitudinal axis of the bone, are also important determinants of the stability of the construct.

Distraction osteogenesis has been applied in the treatment of a wide variety of problems including severe limb length discrepancy, acquired and congenital deformities, fracture nonunion, and osteomyelitis [1, 17, 20]. Regardless of the reason, the general technique remains unchanged: bone division, stable fixation of the fragments, a 7 to 14 day latency period, distraction, consolidation, assessment of regenerate bone, and removal of the frame [16, 21–24]. Care must be taken to allow for at least 2 to 3 days of consolidation for each day of distraction. Prior to removal of the frame, a careful assessment of the regenerate and docking site, if present, is required. Similarly, this technique may also be applied in acute limb shortening followed by limb lengthening [21, 25–27]. The theoretical advantage of this technique is that it allows faster healing of a traumatic defect as it does not require waiting until docking is achieved to begin callous healing. Shortening assists with the closure of soft tissue defects, though it may result in soft tissue redundancy and swelling. These techniques can be readily applied to the treatment of various types of nonunions. Atrophic nonunions may be treated with bifocal osteosynthesis, which involves resection of the nonunion site, compression at the nonunion site, and bone lengthening at an osteotomy site remote to the nonunion site [22, 28]. This technique also allows alignment if deformity is also present. Hypertrophic nonunions have traditionally been treated by revision to rigid fixation. These fractures have a vital blood supply from each bone end and a dense collagenous interface. These characteristics allow the nonunion to be treated by stimulating bone formation with primary distraction and realignment when necessary. Nonunions with bone loss can be transported with bone transport or shortening distraction [22, 29]. Infected nonunions require resection of the focus of infection, with removal of all necrotic and poorly-vascularized tissues [22]. The

remaining osseous segments must have an adequate blood supply to promote bone formation at its trailing end and healing at its leading end [19]. Bone transport can then subsequently be used to eliminate the residual defect. When compared to treatment with bone grafts, antibiotic beads, and vascularized bone grafts, patients who are treated with bone transport experience similar rates of healing, duration of treatment, final deformity, complication rates, and total number of operative procedures [22, 30, 31]. However, patients who undergo bone transport end up with improved limb length discrepancy, decreased cost of treatment, and shorter duration of disability than those treated with bone graft, antibiotic beads, and vascularized bone graft [22, 32]. The benefits of treating nonunions with bone transport include the ability to achieve regeneration of living bone with the same strength and width as that of native bone [22]. In addition, this technique can be used to treat very large defects, up to 30 cm, in both children and adults [32]. Moreover, it allows simultaneous deformity correction as well as treatment of concomitant soft tissue problems. Unfortunately, bone transport is not the answer for all nonunions. Its use requires specialized training and equipment. Frequently, bone transport requires a long treatment duration during which numerous complications may occur.

The technique of transformational osteogenesis is also described by Ilizarov, which involves the mechanical stimulation of pathologic bony interfaces through variations in compression and distraction to induce osteogenesis and regenerate normal bony continuity [15, 33, 34]. He recommended this technique for the treatment of nonunions. Transformational osteogenesis is less well documented histologically than distraction osteogenesis. Success of the technique depends on the stability and composition of the pathologic interface. Ilizarov argued that a stiff, fibrous nonunion should be treated with initial distraction [34]. Tension is applied across the nonunion site using the Ilizarov device to induce distraction neogenesis. Once new bone formation is visualized radiographically, the bone ends can then be compressed to transform the osteogenic bridge into a solid cylinder of bone [18, 34]. Transformational osteogenesis can be used similarly to treat a site of mobile pseudoarthrosis. The site must first be compressed progressively at a rate of 1 mm per day for 10 to 15 days [16]. Compressive forces induce local necrosis and subsequent neovascularization of the cartilaginous interface. When local resorption occurs, distraction of the site then renews osteoneogenesis. Following the induction of local osteogenesis, compression can then be reapplied to successfully unite the bone ends.

Early complications are pain, bleeding that can result in hematoma or compartment syndrome, deep vein thrombosis and pulmonary embolism, and nerve injury as a result of stretching. Immediate complications involve direct damage to neurovascular structures. Infection, especially of the pin sites, has been reported to be as high as 95%; however, with local pin care, with or without oral administration of antibiotics for 5 days, 97% of these resolved [35]. Soft tissue contractures, subluxation, and contracture of the joint are more serious complications. They can, however, be minimised with pre-operative planning, including protection against subluxation by spanning of the joint with the fixator and with intensive therapy and splinting during the fixation period [36]. Late complications include chronic recurrent pin-site infections, osteomyelitis, premature union if distraction is too slow or delayed or nonunion, hardware failure, reflex sympathetic dystrophy, late bowing and fracture. The rate of complications decreases substantially as the experience of the surgeon increases. In one study, major complications followed 69% of Ilizarov lengthening procedure performed in the first 6-month period of experience, but only 35% in the third 6-month period [37]. The rate of minor complications remained constant and independent of the experience of the surgeon and type of a fixator.

Ilizarov frames provide a versatile fixation system that gives stability, soft tissue preservation, adjustability and functionality. All these factors are vital for bone to realise its full osteogenic potential. A preoperative plan is essential with careful selection of patients who will be able to adhere to strict postoperative regimen of lengthening, and angular correction to avoid late complications.

Russian studies of the Ilizarov method (Popova and Khodesecich, 1984) as cited by (Ilizarov & Rozbruch, 2007) showed that the use of this method significantly reduced the time and cost of treatment, and disability payments. When used for the treatment of fractures and post traumatic nonunions, primary disability was decreased by 3–5 times, and 8-fold compared with traditional treatments in the case of open fractures. This meant that more patients were able to return to work sooner, which is advantageous for the economy of the country. Extensive knowledge of human anatomy is essential, in order to reduce the risk of nerve or vascular damage. It takes a vast amount of time to acquire the necessary knowledge and there are few surgeons adequately trained to perform this technique.

References

1. Ilizarov GA. Transosseous osteosynthesis. Theoretical and clinical aspects of the regeneration and growth of tissue. Berlin: Springer; 1992.

2. Ilizarov GA. Basic principles of transosseous compression and distraction osteosynthesis. Ortop Travmatol Protez. 1971;32(11):7–15.

3. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues: part I. The influence of stability of fixation and sort tissue preservation. Clin Orthop Relat Res. 1989;(238):249–81.

4. Lawrence B. Management of polytrauma. In: Champan MW, Madison M, editors. Operative orthopaedics. Philadelphia: JB Lippincott Company; 1993. p. 299-304.

5. Iacobellis C, Cacciato F. Aseptic nonunion and delay in consolidation in the tibia: treatment by intramedullary nailing and using the Ilizarov method. Chir Organi Mov. 2001;86(3):199-210.

6. Rozbruch SR, Ilizarov S. Limb lengthening and reconstruction surgery. New York: Informa Healthcare; 2007.

7. Littlewood R. The benefits and risks of the Ilizarov technique for limb reconstruction [Internet]. Oxford University Hospitals Limb Reconstruction. 2016 [cited 2016 Jul 08]. 1 p. Available from: http://www.ouh.nhs.uk/limbreconstruction/academia/documents/rebecca-littlewood-article.pdf .

8. Spiegelberg B, Parratt T, Dheerendra SK, Khan WS, Jennings R, Marsh DR. Ilizarov principles of deformity correction. Ann R Coll Surg Engl. 2010;92(2):101–5.

9. Ogunyemi B. Surgical innovation in the cold war era: Gavril Ilizarov and his apparatus as a device for external fixation and limb lengthening. UBCMJ. 2017;8(2):29-30.

10. Paley D. Historical vignettes on how the Ilizarov method came to the West [Internet]. [2010] [cited 2016 Jul 8]. Available from: www.lengthening.us/Ilizarov_method_and_how_it_came_to_the_west.html .

11. Lalić I, Daraboš N, Stanković M, Gojković Z, Obradović M, Marić D. Treatment of complex tibial plateau fractures using Ilizarov technique. Acta Clin Croat. 2014;53(4):437-48.

12. Lalić I, Obradović M, Lukić-Šarkanović M, Đan V. Definite management of bilateral lower leg nonunion fractures by Ilizarov apparatus in polytraumatized patient - case report. Med Pregl. 2015;68(3-4):137-42.

13. Lalić I, Harhaji V, Kecojević V, Ninković S, Dulić O, Rašović P. Analysis of Ilizarov apparatus application in acute traumatic lesions and treatment of complications of different parts of musculoskeletal system at the Department of Orthopedic Surgery and Traumatology in Novi Sad. Med Pregl. 2016;69(Suppl 1):23-33.

14. Tomić S. Pseudoartroze i defekti kostiju – metod Ilizarova. Beograd: Želnid; 2001.

15. Ilizarov GA. Clinical application of the tension-stress effect for limb lengthening. Clin Orthop Relat Res. 1990;(250):8-26.

16. Murray JH, Fitch RD. Distraction histiogenesis: principles and indications. J Am Acad Orthop Surg. 1996;4(6):317-27.

17. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues: part II. The influence of the rate and frequency of distraction. Clin Orthop Relat Res. 1989;(239):263-85.

18. Aronson J, Harrison BH, Stewart CL, Harp JH Jr. The histology of distraction osteogenesis using different external fixators. Clin Orthop Relat Res. 1989;(241):106-16.

19. Aronson J, Johnson E, Harp JH. Local bone transportation for treatment of intercalary defects by the Ilizarov technique. Biomechanical and clinical considerations. Clin Orthop Relat Res. 1989;(243):71-9.

20. De Bastiani G, Aldegheri R, Renzi-Brivio L, Trivella G. Limb lengthening by callus distraction (callotasis). J Pediatr Orthop. 1987;7(2):129-34.

21. DeCoster TA, Gehlert RJ, Mikola EA, Pirela-Cruz MA. Management of posttraumatic segmental bone defects. J Am Acad Orthop Surg. 2004;12(1):28-38.

22. Tsuchiya H, Tomita K. Distraction osteogenesis for treatment of bone loss in the lower extremity. J Orthop Sci. 2003;8(1):116-24.

23. Green SA, Jackson JM, Wall DM, Marinow H, Ishkanian J. Management of segmental defects by the Ilizarov intercalary bone transport method. Clin Orthop Relat Res. 1992;(280):136-42.

24. Ilizarov GA, Ledyaev VI. The replacement of long tubular bone defects by lengthening distraction osteotomy of one of the fragments. 1969. Clin Orthop Relat Res. 1992;(280):7-10.

25. Betz AM, Hierner R, Baumgart R, Stock W, Sebisch E, Kettler M, et al. [Primary shortening- -secondary lengthening. A new treatment concept for reconstruction of extensive soft tissue and bone injuries after 3rd degree open fracture and amputation of the lower leg]. Handchir Mikrochir Plast Chir. 1998;30(1):30-9.

26. Meffert RH, Inoue N, Tis JE, Brug E, Chao EY. Distraction osteogenesis after acute limb-shortening for segmental tibial defects. Comparison of a monofocal and a bifocal technique in rabbits. J Bone Joint Surg Am. 2000;82(6):799-808.

27. Meffert RH, Tis JE, Inoue N, McCarthy EF, Brug E, Chao EY. Primary resective shortening followed by distraction osteogenesis for limb reconstruction: a comparison with simple lengthening. J Orthop Res. 2000;18(4):629-36.

28. Paley D, Catagni MA, Argnani F, Villa A, Benedetti GB, Cattaneo R. Ilizarov treatment of tibial nonunions with bone loss. Clin Orthop Relat Res. 1989;(241):146-65.

29. Catagni MA, Guerreschi F, Holman JA, Cattaneo R. Distraction osteogenesis in the treatment of stiff hypertrophic nonunions using the Ilizarov apparatus. Clin Orthop Relat Res. 1994;(301):159-63.

30. Cattaneo R, Catagni M, Johnson EE. The treatment of infected nonunions and segmental defects of the tibia by the methods of Ilizarov. Clin Orthop Relat Res. 1992;(280):143-52.

31. Cierny G 3rd, Zorn KE. Segmental tibial defects. Comparing conventional and Ilizarov methodologies. Clin Orthop Relat Res. 1994;(301):118-23.

32. Lerner A, Fodor L, Stein H, Soudry M, Peled IJ, Ullmann Y. Extreme bone lengthening using distraction osteogenesis after trauma: a case report. J Orthop Trauma. 2005;19(6):420-4.

33. Aronson J, Harrison B, Boyd CM, Cannon DJ, Lubansky HJ, Stewart C. Mechanical induction of osteogenesis. Preliminary studies. Ann Clin Lab Sci. 1988;18(3):195-203.

34. Ilizarov G. Basic principles of tranosseous compression and distraction osteosynthesis. Ortop Travmatol Protez. 1971;32(11):7-15.

35. Aronson J. Experimental and clinical experience with distraction osteogenesis. Cleft Palate Craniofac J. 1994;31(6):473–81.

36. Coglianese DB, Herzenberg JE, Goulet JA. Physical therapy management of patients undergoing limb lengthening by distraction osteogenesis. J Orthop Sports Phys Ther. 1993;17(3):124–32.

37. Velazquez RJ, Bell DF, Armstrong PF, Babyn P, Tibshirani R. Complications of use of the Ilizarov technique in the correction of limb deformities in children. J Bone Joint Surg Am. 1993;75(8):1148–56.

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Errata

At the request of Dr. Maja Bogdan, author of the paper: THE ROLE OF PHYSICAL THERAPY IN THE TREATMENT OF POST-TRAUMATIC CONTRACTURE OF THE ELBOW IN CHILDREN, published in the journal *Medical Review*, 1- 2/2017, pages 58 - 61, we hereby provide the correct email address of the author: maja.bogdan@mf.uns.ac.rs.

Na molbu asist. dr Maje Bogdan, autorke rada „Značaj fizikalne terapije u lečenju posttraumatskih kontraktura zgloba lakta kod dece“, objavljenog u dvobroju 1-2/2017, na stranama 58-61, objavljujemo ispravke koje se odnose na elektronsku adresu koja bi trebalo da glasi maja.bogdan@mf.uns.ac.rs.

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At the request of Prof. Dr. Vuk Sekulić, author of the paper: LAPAROSCOPIC LIVING DONOR LEFT NEPHRECTOMY, published in the journal *Medical Review*, 3 - 4/2017, pages 95 - 98, we hereby provide the correct email address of the author: vuk.sekulic@mf.uns.ac.rs.

Na molbu prof. dr Vuka Sekulića, autora rada „Laparoskopska levostrana donorska nefrektomija“, objavljenog u dvobroju 3-4/2017, na stranama 95-98, objavljujemo ispravke koje se odnose na elektronsku adresu koja bi trebalo da glasi vuk.sekulic@mf.uns.ac.rs.

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At the request of the Assistant Stanislava Nikolić, Ph.D., author of the paper “THE IMPORTANCE AND USAGE OF PARAMETERS OF THE HOMEOSTASIS MODEL IN THE ASSESSMENT OF GLYCOREGULATORY MECHANISMS”, published in the journal Medical Review, 5 - 6/2017, pages 155 - 161, we hereby provide the correct list of coauthors of this paper: Stanislava Nikolić, Nikola Ćurić, Branislava Ilinčić, Romana Mijović, Branka Miličić, and Damir Benc.

Na molbu asist. dr Stanislave Nikolić, autorke rada „Značaj i upotreba parametara modela homeostaze u proceni glikoregulatornih mehanizama“, objavljenog u dvobroju 5-6/2017, na stranama 155-161, objavljujemo ispravke koje se odnose na navode i redosled autora i koautora rada, a koji bi trebalo da glasi: Stanislava Nikolić, Nikola Ćurić, Branislava Ilinčić, Romana Mijović, Branka Miličić, Damir Benc.

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OUTCOME OF INTRAVENOUS THROMBOLYSIS FOR ACUTE ISCHEMIC STROKE IN PATIENTS WITH AND WITHOUT ATRIAL FIBRILLATION

ISHOD NAKON AKUTNOG ISHEMIJSKOG MOŽDANOG UDARA LEČENOG INTRAVENSKOM TROMBOLIZOM KOD PACIJENATA SA ATRIJALNOM FIBRILACIJOM I BEZ NJE

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Summary

Introduction. Atrial fibrillation is associated with an increased risk of ischemic stroke. The benefit of intravenous thrombolysis in patients with acute ischemic stroke and atrial fibrillation is still unclear. The aim of the study was to assess and compare the effects of intravenous thrombolysis in stroke patients with and without atrial fibrillation. **Material and Methods**. We analyzed stroke patients who were treated with intravenous thrombolysis. Patients were divided into two groups according to the presence of atrial fibrillation. Demographic, clinical and radiological characteristics of patients were compared between the two groups. The treatment efficacy was evaluated in relation to the improvement of neurological status after 24 hours, and functional recovery after three months. Binary logistic regression was used to evaluate predictors of outcome. **Results**. From a total of 188 patients, 39.4% presented with atrial fibrillation. Patients with atrial fibrillation were older (69.4 vs. 62.6 years; p <0.0001), with female predominance (43.2% vs. 28.9%, p = 0.04) and had clinically more severe stroke (National Institutes of Health Stroke Scale, score on admission 15.4 vs. 12.1; p = 0.0001). Significantly more patients without atrial fibrillation (61.4% vs. 43.2%, p = 0.01) had a favorable clinical outcome at three months after stroke. Nevertheless, atrial fibrillation was not an independent predictor of poor outcome at three months after stroke (p=0.66). **Conclusion**. Acute ischemic stroke patients, with atrial fibrillation, treated with intravenous thrombolysis, had worse outcomes than patients without atrial fibrillation did. However, it is mainly due to older age and a more severe stroke in patients with atrial fibrillation.

Key words: Stroke; Brain Ischemia; Thrombolytic Therapy; Atrial Fibrillation; Treatment Outcome; Neurologic Manifestations; Fibrinolytic Agents; Tissue Plasminogen Activator; Severity of Illness Index; Risk Factors

Sažetak

Uvod. Atrijalna fibrilacija nosi povišen rizik za nastanak ishemijskog moždanog udara. Benefit intravenske trombolize kod pacijenata sa moždanim udarom nastalim zbog atrijalne fibrilacije je još uvek nedovoljno jasan. Cilj rada bio je procena efikasnosti intravenske trombolize kod pacijenata sa moždanim udarom i atrijalnom fibrilacijom u poređenju sa onima bez atrijalne fibrilacije. **Materijal i metode.** Analizirani su pacijenati koji su lečeni intravenskom trombolizom zbog akutnog ishemijskog moždanog udara. Pacijenti su podeljeni u dve grupe u odnosu na prisustvo atrijalne fibrilacije. Demografske, kliničke i radiološke karakterstike pacijenata poređene su između dve grupe. Efikasnost je procenjivana u odnosu na poboljšanje neurološkog nalaza nakon 24 sata i funkcionalni oporavak nakon tri meseca od moždanog udara. Binarnom logističkom regresijom određivani su prediktori ishoda. **Rezultati.** Od ukupno 188 pacijenata, njih 39,4% imalo je atrijalnu fibrilaciju. Ovi pacijenti su bili stariji (69,4 naspram 62,6 godina, p < 0,0001), češće ženskog pola (43,2% naspram 28,9%, p = 0,04) i imali su klinički teži moždani udar (Nacionalni vodič za skor kod akutnog ishemijskog moždanog udara na prijemu 15,4 naspram 12,1, p = 0,0001) u odnosu na pacijente bez atrijalne fibrilacije. Dobar klinički ishod nakon tri meseca imalo je značajno više pacijenata bez atrijalne fibrilacije (61,4% naspram 43,2%, p = 0,01). Ipak atrijalna fibrilacija nije bila nezavisan prediktor lošeg ishoda (p =0.66). **Zaključak.** Pacijenti sa ishemijskim moždanim udarom i atrijalnom fibrilacijom lečeni intravenskom trombolizom imaju lošiji ishod u odnosu na one bez atrijalne fibrilacije, ali je to prevashodno posledica starijeg životnog doba i težeg moždanog udara ovih pacijenata.

Ključne reči: moždani udar; moždana ishemija; trombolitička terapija; atrijalna fibrilacija; ishod lečenja; neurološke manifestacije; fibrinolitički agensi; aktivator tkivnog plazminogena; indeks težine bolesti; faktori rizika

Introduction

Atrial fibrillation (AF) is the most common heart rhythm disorder, occurring in 2% of the general population, and in 20% of persons older than 80 years [1]. Its incidence increases with age, and doubles in each decade of life after the age of 55 years. Due to the extremely high embolic potential,

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Abbreviations

AF	– atrial fibrillation
IVT	– intravenous thrombolysis
rtPA	– recombinant tissue plasminogen activator
AIS	– acute ischemic stroke
NIHSS	– national institutes of health stroke scale
ICH	– intracranial hemorrhage
CT	– computed tomography
ASPECT	– Alberta stroke program early computed tomography
OTT	– onset-to-treatment time
HT	– hemorrhagic transformation
sICH	– symptomatic intracranial hemorrhage
ECASS III	– European cooperative acute stroke study III
OCSP	– Oxfordshire community stroke project
TACI	– total anterior circulation infarction
PACI	– partial anterior circulation infarction
POCI	– posterior circulation infarction
LACI	– lacunar infarction
MR	– magnetic resonance
mRS	– modified Rankin score
ECG	– electrocardiography
OR	– odds ratio
CI	– confidence interval

AF is the most important cardiac risk for the development of cardio-embolic stroke [2–4]. The cardio-embolic stroke clinically manifests as a severe neurological deficit, and radiologically as a territorial (non-lacunar) infarction.

Until recently, intravenous thrombolysis (IVT) with recombinant tissue plasminogen activator (rtPA) was the only approved therapy for the treatment of patients with acute ischemic stroke (AIS) [5, 6], improving the outcome if applied within 4.5 hours after the onset of symptoms [7]. However, whether IVT is effective in AIS patients with AF is still insufficiently clear. Large studies have proven the benefits of IVT, but without testing the treatment efficacy in regard to the type and etiology of stroke [7]. Some authors have shown that AF is a predictor of a poor outcome in AIS patients treated with IVT [8], although there are also opposite results [9]. Even today, the attitudes on the efficacy and safety of IVT in AIS patients with AF are still quite controversial.

The aim of our study was to evaluate the efficacy and safety of IVT in AIS patients with AF compared to AIS patients without AF.

Material and Methods

This cross-sectional study analyzed the data of patients with AIS who were treated with IVT at the Clinical Center of Vojvodina in Novi Sad, Serbia. The data were prospectively collected in the period from November 2008 to April 2015. On admission, all patients were examined by a neurologist and their National Institute of Health Stroke Scale (NIHSS) score was determined. This score is used for assessment of severity of stroke and correlates with the size of cerebral infarction [10]. All patients underwent brain computed tomography (CT) in order to exclude intracranial hemorrhage (ICH). The Alberta Stroke Program Early CT (ASPECT) score, that reflects the size of the cerebral ischemia [11], presence of a hyperdense artery sign and leukoaraiosis, were assessed by a radiologist. The following data were also recorded: age, gender, risk factors (arterial hypertension, diabetes mellitus, hyperlipidemia, and smoking), glucose level on admission, previous use of antiplatelet and statin therapy, and the symptom onset-to-treatment time (OTT).

Twenty-four hours following the IVT treatment, the NIHSS score was determined again and brain CT was repeated. Neurological improvement showed a reduction in the NIHSS score of $\geq 50\%$ compared with the NIHSS score on admission, or as NIHSS score ≤ 3. Based on repeated CT findings, we determined the type of cerebral infarction, and possible presence of hemorrhagic transformation (HT). Development of symptomatic intracranial hemorrhage (sICH) in the early stages of AIS (within seven days) was assessed according to the European Cooperative Acute Stroke Study III (ECASS III) criteria [12]. The type of brain infarction was determined in accordance with the Oxfordshire Community Stroke Project (OCSP) classification [13]. According to OCSP classification, there are four types of cerebral infarction: total anterior circulation infarction (TACI), partial anterior circulation infarction (PACI), posterior circulation infarction (POCI) and lacunar infarction (LACI). All patients underwent neurovascular status evaluation with ultrasound (carotid duplex ultrasound, transcranial Doppler) and/or CT or magnetic resonance (MR) angiography examinations. Functional outcome was assessed after three months by the modified Rankin score (mRS) and favorable outcome was defined as mRS 0 – 2 and unfavorable as mRS 3 – 6.

The presence of AF was defined as an evidence of these arrhythmias on at least one electrocardiographic (ECG) recording, obtained either from medical history or during hospitalization. After admission, patients were monitored with ECG for at least 72 hours and, if necessary, had additional recordings.

Patients were divided into two groups, according to the presence or absence of AF, and they were compared with regard to demographic, clinical and radiological characteristics, outcomes and adverse events. The two-sample student t-test was used for comparisons of parametric variables and chi-square (χ^2) test or Fisher's exact test for categorical variables. A p value of less than 0.05 ($p < 0.05$) was regarded statistically significant. Binary logistic regression analysis was used to evaluate predictors of neurological improvement, favorable outcome and HT. The variables which were analyzed included age, gender, baseline NIHSS score, OTT, glucose level on admission, presence of AF and other risk factors (arterial hypertension, diabetes mellitus, hyperlipidemia, smoking). Odds ratio (OR) was calculated with 95% confidence interval (95% CI). Data were analyzed with the SPSS/PC Win package version 20.0. The study was approved by the Ethical Board of the Clinical Center of Vojvodina.

Table 1. Patients' characteristics
Tabela 1. *Kliničke karakteristike pacijenata*

	Group with AF (n = 74)/*Grupa sa AF (n = 74)*	Group without AF (n = 114)/*Grupa bez AF (n = 114)*	p (significance) *p (statistička značajnost)*
Demographic/*Demografske karakteristike*			
Age, years/*Starost, godine*	69.4±9.2	62.6±10.2	<0.0001
Gender, male/*Pol, muški*	42 (56.8%)	81 (71.1%)	0.04
Risk factors/*Faktori rizika*			
Arterial hypertension/*Arterijska hipertenzija*	68 (91.9%)	100 (87.7%)	0.36
Diabetes/*Dijabetes*	10 (13.5%)	23 (20.2%)	0.24
Hyperlipoproteinemia/*Hiperlipoproteinemija*	38 (51.4%)	64 (56.1%)	0.51
Smoking/*Pušenje*	13 (17.6%)	40 (35.1%)	0.009
Current stroke/*Aktuelno stanje*			
NIHSS score on admission/*NIHSS skor na prijemu*	15.0±4.1	12.1±4.5	0.0001
Glycemia on admission/*Glikemija na prijemu, mmol/l*	7.7±2.8	8.1±3.7	0.42
Previous antiplatelet therapy *Prethodna antiagregaciona terap*ija	36 (48.7%)	32 (28.1%)	0.004
Previous statin therapy/*Prethodna terapija statinima*	8 (10.8%)	8 (7.0%)	0.36
ASPECT score/*ASPECT skor*	9.2±1.1	9.2±1.1	0.67
Leukoaraiosis/*Leukoarajoza*	12 (16.2%)	17 (14.9%)	0.08
OTT on admission, min./*OTT na prijemu, min.*	164.9±72.9	167.5±52.7	0.77
Stroke subtype/*Tip infarkta mozga*			
TACI	35 (47.3%)	22 (19.3%)	
PACI	34 (45.9%)	51 (44.5%)	<0.0001
LACI	2 (2.7%)	25 (21.9%)	
POCI	3 (4.1%)	15 (13.2%)	

Legend: AF – Atrial fibrillation; NIHSS score - The National Institutes of Health Stroke Scale; MAP-Mean Arterial Pressure; ASPECT score - Alberta Stroke Program Early CT score; OTT- Onset to-treatment time; TACI - total anterior circulation infarction; PACI - partial anterior circulation infarction; LACI – lacunar infarction; POCI - posterior circulation infarction

Legenda: AF – atrijalna fibrilacija; NIHSS skor – Nacionalni vodič za skor kod akutnog ishemijskog moždanog udara; MAP – srednji arterijski pritisak; ASPECT skor – Alberta program moždanog udara – rani rezultat kompjuterizovane tomografije; OTT – vreme početka tretmana; TACI – totalni anteriorni cirkulatorni infarkt; PACI – delimični anteriorni cirkulatorni infarkt; LACI – lakunarni infarkt; POCI – infarkt posteriorne cirkulacije

Results

The study included a total of 188 patients. Among them, 74 patients (39.4%) had AF and 114 patients (60.6%) were without AF. Demographic, clinical and radiological characteristics, as well as stroke subtypes of the two groups are shown in **Table 1**. Patients with AF were older (69.4 vs. 62.6, p<0.0001) and there were more female patients (43.2% vs. 28.9%, p=0.04). In regard to risk factors, only smoking was significantly more frequent among patients without AF (35.09% vs. 17.57%; p=0,009). Time from symptom onset to thrombolytic treatment (OTT) did not differ significantly between the two groups of patients. Regarding other clinically significant characteristics, only previous use of antiplatelet therapy was significantly more frequent in patients with AF (48.65% vs. 28.07%, p=0.004). On average, patients with AF had a more severe stroke, i.e., a higher NIHSS score on admission (15.0 vs. 12.1, p<0.0001). The average baseline ASPECT score on brain CT did not differ significantly between the two groups of patients. In patients with AF, the most frequent were total and partial anterior circulation infarctions (TACI 47.3%; PACI 45.9%), whereas lacunar infarctions were significantly more frequent in patients without AF (LACI type 21.9% vs. 2.7%).

The outcome at 90 days post treatment is shown in **Graph 1**. Approximately half of the patients without AF had a mRS score of 0 or 1 (mRS 0 – 23.1 %, mRS 1 – 25.4%), whereas majority of patients with AF had mRS 6 (29.7%).

Comparison of outcomes and complications after application of IVT is shown in **Table 2**. Neurological improvement at 24 hours was more frequent in patients without AF (42.1% vs. 31.1%), although without a statistical significance. A good outcome at three months (mRS 0 - 2) was significantly more frequent in patients without AF (61.4% vs. 43.2%; p=0.01). Furthermore, patients with AF commonly had a significant hemorrhagic transformation (29.7% vs. 10.5%; p=0.0008), as well as symptomatic intra-cerebral hemorrhage (9.5% vs. 0%;

Table 2. Outcome of intravenous thrombolysis
Tabela 2. *Ishod nakon intravenske trombolize*

	Group with AF n = 74/*Grupa sa AF (n = 74)*	Group without AF n = 114/*Grupa bez AF (n = 114)*	p (significance) *p (statistička značajnost)*
Neurological improvement at 24 h *Neurološko poboljšanje nakon 24 h*	23 (31.1%)	48 (42.1%)	0.12
Good outcome (mRS 0-2)/*Povoljan ishod (mRS 0-2)*	32 (43.2%)	70 (61.4%)	0.01
Hemorrhagic transformation/*Hemoragijska transformacija*	22 (29.7%)	12 (10.5%)	<0.001
sICH/*Simptomatska intracerebralna hemoragija*	7 (9.5%)	0 (0.0%)	<0.001
mRS 6/*Modifikovana Rankinova skala*	23 (31.1%)	9 (7.9%)	<0.001

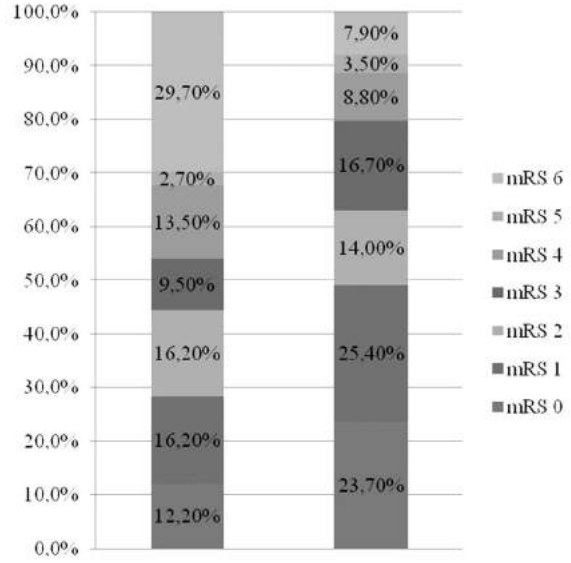
Legend: AF – atrial fibrillation, mRS – modified Rankin Scale; sICH – symptomatic intracerebral hemorrhage.
Legenda: AF – atrijalna fibrilacija, mRS – modifikovana Rankinova skala; sICH – simptomatska intracerebralna hemoragija.

p=0.001). Lethal outcome (mRS 6) within 90 days post treatment was also more frequent in patients with AF (31.08% vs. 7.89%; p<0.0001).

The binary logistic regression analysis did not show AF to be an independent predictor of poor (p=0.66) or lethal outcome (p=0.17) at three months post IVT for AIS. In our study, predictors of an unfavorable outcome (mRS 3 - 6) at three months were: older age (p<0.0001; OR 1.08, 95% CI 1.03-1.13), a higher NIHSS score on admission (p<0.0001; OR 1.31, 95% CI 1.19-1.45), a lower ASPECT score on admission (p=0.013; OR 0.64, 95% CI 0.45-0.91) and higher glucose levels on admission (p<0.0001; OR 1.28, 95% CI 1.13-1.45). Predictors of lethal outcome (mRS 6) at three months were older age (p<0.0001; OR 1.15, 95% CI 1.08-1.23), a higher NIHSS score on admission (p<0.0001; OR 1.35, 95% CI 1.18-1.54) and presence of diabetes (p=0.003; OR 5.95, 95% CI 1.86-18.97). In addition, AF was not independently associated with neurological improvements at 24 hours (p=0.38). Predictors of neurological improvement at 24 hours were a lower NIHSS score on admission (p=0.003; OR 0.89, 95% CI 0.83-0.96), a higher ASPECT score (p=0.027; OR 1.44, 95% CI 1.04-1.99) and absence of diabetes (p=0.008; OR 0.27, 95% CI 0.1-0.7). However, AF was independently associated with development of hemorrhagic transformation (p<0.0001; OR 4.44, 95% CI 1.92 - 10.27).

Discussion

Our study showed that AIS patients with AF treated with IVT had a worse outcome than patients without AF. Patients with AF were older (7 years, on average) and clinically had a more severe stroke (NIHSS score higher by around 3 points) compared to patients without AF. Higher NIHSS score in patients with AF was a result of larger cerebral infarction in patients with AF. Lacunar infarction was found only in 2.7% of patients (two patients) with AF, versus 21.9% of patients without AF. Development of HT and sICH was significantly more frequent in patients with AF. Similar results have been reported in some other studies [8, 14, 15]. In a Japanese study of 85 patients, including 51.8% of patients with AF, neurological improvement at seven days post IVT was considerably lower in patients with AF (31.8% vs. 60%; p = 0.007) [8]. In addition, a favorable outcome (mRS 0 - 2) at three months was also recorded in significantly fewer patients with AF compared to those without AF (15.9% vs. 46.3%, p = 0.002). Another study included 734 patients, of whom 21.1% had AF, a poor outcome (mRS 3-6) was reported in 52.3% of patients with AF and in 35.2% of patients without AF (p < 0.001) [14]. The mortality at 3 months following stroke was 21.9% in the group with AF and 9% in the group without AF. Correspondingly, we found a higher percentage of poor outcome and a higher mortality rate in the group of patients with AF within three months following IVT. In a recent study which examined predictors of early neurological improvement, AF was independently associated with the absence of the major neurological improvement 24 h after IVT [15]. In our study, early neurological im-



Graph 1. Outcome at 90 days
Grafikon 1. *Ishod nakon 90 dana*

provement was more common in patients without AF, but it was not significant.

In the above-mentioned studies [8, 14], patients with AF were also older and had a higher NIHSS score and more frequent HT, compared to patients without AF, which is consistent with our findings. All of these characteristics (older age, higher NIHSS score, common HT) are typical of ischemic stroke caused by AF. As numerous studies have shown that age and the NIHSS score on admission are the most important predictors of outcome, independent of risk factors and the type of ischemic stroke, it is expected that patients with AF would have a worse outcome because of these characteristics [8, 14]. Thus, AF was not an independent predictor of poor outcome, unlike older age and a higher NIHSS score. Similarly, in our study, AF was also not an independent predictor of poor outcome, as opposed to older age, higher NIHSS score and lower ASPECT score on admission, as well as higher blood glucose levels on admission. Moreover, some studies have shown that among patients with severe clinical ischemic stroke (NIHSS score $\geq$ 10) AF was associated with a better outcome [9]. A study by Shang Feng et al. analyzed the outcomes in subgroups of patients, depending on their NIHSS scores (scores above and below 10). In the group of patients with a NIHSS score over 10, a favorable outcome after three months was seen in 31% of patients with AF, and 8% of patients without AF ($p = 0.005$), and mortality rates were 8% among cases with AF and 17% among patients without AF ($p = 0.168$). This study concluded that, if NIHSS score on admission was above 10, patients with AF had a better outcome after IVT than those without AF. However, the most reliable results on the efficacy of IVT in AIS patients with AF were obtained from studies that included only patients with AF and compared those who were treated with IVT and those not treated with IVT [16]. The conclusion of those studies was that patients treated with IVT had a much better outcome compared to those who were not treated with IVT. Similar results were obtained in a multicenter study in China [17]. In this study, administration of IVT was an independent predictor of a favorable outcome (OR 5.73, 95% CI 2.4 - 13.7; $p < 0.001$) in patients with AIS and AF.

From the pathophysiological point of view, the success of IVT treatment in ischemic stroke caused by AF should be the most conclusive. In most cases, cardio-embolic stroke is caused by the red thrombus, which essentially consists of erythrocytes and fibrin [18, 19]. In cases with red thrombus, IVT is much more effective, as demonstrated by animal studies [20]. This finding is supported by clinical studies that have demonstrated successful recanalization after the IVT therapy in ischemic stroke caused by AF [21, 22]. The controversial results about the efficacy of IVT in patients with AIS and AF can be explained by additional factors that are often not considered when assessing the outcome at three months after IVT. Namely, AF is associated mainly with chronic heart failure [23, 24], which often causes reduced premorbid functionality (premorbid mRS $\geq$ 1) and a reduced ability for maximum mobility during rehabilitation treatment. Given that patients with AF are older and have a clinically more severe ischemic stroke, they often, even after successful recanalization and initial neurological improvement, have persisting neurological deficits requiring extended hospitalization [24], which itself carries the risk of further complications and after three months, patients do not reach mRS $\leq$ 2, i.e., a favorable outcome.

Limitations of our study were the small number of patients with AF. Secondly, the lack of a control group of AIS patients with AF who were not treated with IVT, whose outcome would be compared with thrombolysis patients. Also, the study included only patients with the same type of cerebral infarction (TACI, PACI or POCI), and if it included patients with different types, it would have given more reliable and perhaps different results. However, this would require more patients, and it should be a topic for future researches.

Conclusion

Patients with acute ischemic stroke and atrial fibrillation, treated with intravenous thrombolysis, had a worse outcome compared with patients without atrial fibrillation. However, patients with atrial fibrillation were older, had more severe neurological deficits, larger cerebral infarction and more commonly developed hemorrhagic transformation, which were likely the main reasons for their poorer treatment outcome.

References

1. Dewar RI, Lip GY. Identification, diagnosis and assessment of atrial fibrillation. Heart. 2007;93(1):25-8.

2. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke. 1993;24(1):35-41.

3. Kernan WN, Ovbiagele B, Black HR, Bravata DM, Chimowitz MI, Ezekowitz MD, et al. Guidelines for the prevention of stroke in patients with stroke and transient ischemic attack: a guideline for healthcare professionals from the American Heart Association/ American Stroke Association. Stroke. 2014;45(7):2160-236.

4. Durić S, Rabi Zikić T, Zikić M. Risk factors of the first stroke. Med Pregl. 2015;68(1-2):17-21.

5. Jauch EC, Saver JL, Adams HP Jr, Bruno A, Connors JJ, Demaerschalk BM, et al. Guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke. 2013;44(3):870-947.

6. Wahlgren N, Moreira T, Michel P, Steiner T, Jansen O, Cognard C, et al. Mechanical thrombectomy in acute ischemic stroke: Consensus statement by ESO-Karolinska Stroke Update 2014/2015, supported by ESO, ESMINT, ESNR and EAN. Int J Stroke. 2016;11(1):134-47.

7. Emberson J, Lees KR, Lyden P, Blackwell L, Albers G, Bluhmki E, et al. Effect of treatment delay, age, and stroke severity on the effects of intravenous thrombolysis with alteplase for acute ischaemic stroke: a meta-analysis of individual patient data from randomised trials. Lancet. 2014;384(9958):1929-35.

8. Kimura K, Iguchi Y, Shibazaki K, Iwanaga T, Yamashita S, Aoki J. IV t-PA therapy in acute stroke patients with atrial fibrillation. J Neurol Sci. 2009;276(1-2):6-8.

9. Sung SF, Chen YW, Tseng MC, Ong CT, Lin HJ. Atrial fibrillation predicts good functional outcome following intravenous tissue plasminogen activator in patients with severe stroke. Clin Neurol Neurosurg. 2013;115(7):892-5.

10. Lyden P, Brott T, Tilley B, Welch KM, Mascha EJ, Levine S, et al. Improved reliability of the NIH Stroke Scale using video training. NINDS TPA Stroke Study Group. Stroke. 1994;25 (11):2220-6.

11. Barber PA, Demchuk AM, Zhang J, Buchan AM. Validity and reliability of aquantitative computed tomography score in predicting outcome of hyperacute stroke before thrombolytic therapy. ASPECTS Study Group. Alberta Stroke Programme Early CT Score. Lancet. 2000;355(9216):1670-4.

12. Hacke W, Kaste M, Bluhmki E, Brozman M, Dávalos A, Guidetti D, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med. 2008;359(13):1317-29.

13. Bamford J, Sandercock P, Dennis M, Burn J, Warlow C. Classification and natural history of clinically identifiable subtypes of cerebral infarction. Lancet. 1991;337(8756):1521-6.

14. Padjen V, Bodenant M, Jovanovic DR, Ponchelle-Dequatre N, Novakovic N, Cordonnier C, et al. Outcome of patients with atrial fibrillation after intravenous thrombolysis for cerebral ischaemia. J Neurol. 2013;260(12):3049-54.

15. Yaghi S, Hinduja A, Bianchi N. Predictors of major improvement after intravenous thrombolysis in acute ischemic stroke. Int J Neurosci. 2016;126(1):67-9.

16. Ogata J, Yutani C, Otsubo R, Yamanishi H, Naritomi H, Yamaguchi T, et al. Heart and vessel pathology underlying brain infarction in 142 stroke patients. Ann Neurol. 2008;63(6):770-81.

17. Zhao Q, Li X, Dong W, Ye M, Cao Y, Zhang M, et al. Factors associated with thrombolysis outcome in ischemic stroke patients with atrial fibrillation. Neurosci Bull. 2016;32(2):145-52.

18. Jang IK, Gold HK, Ziskind AA, Fallon JT, Holt RE, Leinbach RC, et al. Differential sensitivity of erythrocyte-rich and platelet-rich arterial thrombi to lysis with recombinant tissue-type plasminogen activator. A possible explanation for resistance to coronary thrombolysis. Circulation. 1989;79(4):920-8.

19. Whitesell RT, Steenburg SD. Imaging findings of acute intravascular thrombus on non-enhanced computed tomography. Emerg Radiol. 2014;21(3):271-7.

20. Molina CA, Montaner J, Arenillas JF, Ribo M, Rubiera M, Alvarez-Sabín J. Differential pattern of tissue plasminogen activator-induced proximal middle cerebral artery recanalization among stroke subtypes. Stroke. 2004;35(2):486-90.

21. Puig J, Pedraza S, Demchuk A, Daunis-I-Estadella J, Termes H, Blasco G, et al. Quantification of thrombus hounsfield units on noncontrast CT predicts stroke subtype and early recanalization after intravenous recombinant tissue plasminogen activator. Am J Neuroradiol. 2012;33(1):90-6.

22. Molina CA, Alexandrov AV, Demchuk AM, Saqqur M, Uchino K, Alvarez-Sabin J. Improving the predictive accuracy of recanalization on stroke outcome in patients treated with tissue plasminogen activator. Stroke. 2004;35(1):151-6.

23. Wang TJ, Larson MG, Levy D, Vasan RS, Leip EP, Wolf PA, et al. Temporal relations of atrial fibrillation and congestive heart failure and their joint influence on mortality: the Framingham heart study. Circulation. 2003;107(23):2920-5.

24. Seet RC, Zhang Y, Rabinstein AA, Wijdicks EF. Risk factors and consequences of atrial fibrillation with rapid ventricular response in patients with ischemic stroke treated with intravenous thrombolysis. J Stroke Cerebrovasc Dis. 2013;22(2):161-5.

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COMPARISON OF THE DIAGNOSTIC VALUE OF TWO ASSAYS FOR SPECIFIC IMMUNOGLOBULIN E IN SUSPECTED BETA LACTAM ALLERGY

POREĐENJE DIJAGNOSTIČKE VREDNOSTI DVA TESTA ZA SPECIFIČNI IMUNOGLOBULIN E KOD PACIJENTA SA SUSPEKTNOM ALERGIJOM NA BETA LAKTAME

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Summary

Introduction. The aim of this study was to compare the diagnostic value of two imunoglobulin E assays for penicillin in a group of patients with a history compatible with hypersensitivity reaction during penicillin treatment who exhibited positive skin testing and/or a drug provocation testing. **Material and Methods.** In the first part of the study, penicillin G, V and/or amoxicillin specific immunoglobulin E, positive with ImmunoCAP system (Termo Fisher, Waltham, Massachusetts, USA), were selected from our biobank sera, and measured specific immunoglobulin E with Immulite system (Siemens, Munich, Germany). The second part of the study included skin testing, and if negative, drug provocation testing was done with the culprit penicillin in patients with a history compatible with hypersensitivity reaction during penicillin treatment. To check the impact of high values of total immunoglobulin E on performance of specific immunoglobulin E, we examined the specific immunoglobulin E to penicillin using both methods in sera of penicillin tolerant patients with total immunoglobulin E over 700 kIU/L. **Results.** Out of 2.521 in vitro specific immunoglobulin E penicillin tests, 58 were positive with ImmunoCAP. Of these tests, 52 were tested by the Immulite and they were all negative. Among 114 patients with a history compatible with hypersensitivity reaction during penicillin treatment, hypersensitivity was confirmed in 36 (31.6%). In 11 patients with positive immediate skin or drug provocation tests, ImmunoCAP was positive in 3 patients and Immulite in 1 patient. Sensitivity of ImmunoCAP and Immulite were 27.1% and 9.1%, respectively. Specificity of ImmunoCAP and Immulite were 96.1% and 100%, respectively. Specific immunoglobulin E to penicillins was detected with ImmunoCAP in 8/22 sera with total immunoglobulin E over 700 kIU/L. All sera were negative by the Immulite. **Conclusion.** Both the ImmunoCAP and Immulite specific immunoglobulin E assays have low sensitivity when assessing patients with a history of immediate hypersensitivity to penicillin, positive immediate skin, or drug provocation tests.

Key words: beta-Lactams; Hypersensitivity; Immunoglobulin E; Penicillins; Diagnosis; Skin Tests; Drug Hypersensitivity; False Negative Reactions; False Positive Reactions

Sažetak

Uvod. Naš cilj je bio da uporedimo dijagnostičke vrednosti dva IgE testa prema penicilinu u grupi pacijenata sa anamnezom hipersenzitivne reakcije tokom primanja penicilna, koji su imali pozitivan kožni test i/ili provokativni test. **Materijal i metode.** U prvom delu studije izabrali smo serume iz naše biobanke, gde su bila specificna IgE (sIge) pozitivna sa *ImmunoCAP* sistemom (*Termo Fisher*, *Waltham, Massachusetts, USA*) prema penicilinu G, V i/ili amoksicilinu. U drugom delu studije uradili smo kožni test i ukoliko je bio negativan, izvršili smo provokativni test sa penicilinom koji je prouzrokovao hipersenzitivnu reakciju tokom primanja. Da bi proverili uticaj visokih vrednosti ukupnog imunoglobulina E na uspešnost testiranja specifičnog imunoglobulina E, izmerili smo specifični imunoglobulin E prema penicilinu sa obe metode u serumu pacijenata koji su tolerantni na penicillin, i koji su imali ukupni imunoglobulin E iznad 700 kIU/L. **Rezultati.** Od 2521 *in vitro* specifični imunoglobulin penicilinskih testova, 58 je bilo pozitivno na *ImmunoCAP* sistem. 52 od tih je bilo podvrgnuto testiranju sa *Immulit* sistemom, svi su bili negativni. Kod 114 pacijenata sa anamnezom hipersenzitivne reakcije za vreme primanja penicilina, hipersenzitivnost je bila dokazana kod 36 (31,6%). Kod 11 pacijenata sa ranom kožnom reakcijom ili provokativnim testom, *ImmunoCAP* je dao pozitivan rezultat kod troje pacijenata, a *Immulite* kod jednog pacijenta. Senzitivnost *ImmunoCAP*-a i *Immulite* 27,1% odnosno 9,1%. Specifičnost *ImmunoCAP*-a i Immulite-a je bila 96,1 odnosno 100%. sIgE prema penicilinu bila je otkrivena *ImmunoCAP*-om kod 8/22 seruma sa ukupnim IgE preko 700 kIU/L. Svi serumi su bili negativni na Immulite aparatu. **Zaključak.** Oba, *ImmunoCAP* i *Immulite sIgE* testa su imala malu senzitivnost tokom testiranja pacijenata sa anamnezom rane hipersenzitivne reakcije na penicillin, pozitivne rane kožne ili provokativne reakcije.

Ključne reči: beta laktami; hipersensitivnost; imunoglobulin E; penicilini; dijagnoza; kožni testovi; preosetljivost na lekove; lažno negativne reakcije; lažno pozitivne reakcije

Introduction

Suspected beta lactam allergy is quite common referral to allergy departments, but the diagnosis is confirmed in less than 20% of suspected patients [1]. The cost of treatment of infections in patients

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Abbreviations

IgE – immunoglobulin E
sIgE – specific immunoglobulin E
DPT – drug provocation test
ST – skin test
SPT – skin prick test
IDT – intradermal test
tIgE – total IgE
DST – delayed skin test
PPL – benzylpenicilloyl poly-L-lysine
MDM – minor determinant mix

with real or perceived diagnosis of beta lactam allergy is significantly higher than in beta lactam tolerant individuals [2].

Determination of specific immunoglobulin E (sIgE) is a part of the diagnostic protocol in suspected penicillin allergy. The in-vitro testing penicillin allergy sensitivity is known to be low, and negative tests are not sufficient for allergy exclusion [3]. On the other hand, there is evidence in the literature, that ImmunoCAP sIgE assay, which is most often used in the diagnosis of drug allergy, often gives false positive results [4]. There is only scarce data on performance of other in-vitro test systems for sIgE to penicillin. The aim of this study was to compare the diagnostic value of two commonly used immunoglobulin E (IgE) assays in a group of well-defined patients with skin tests (ST) and/or drug provocation tests (DPTs) with confirmed or excluded penicillin allergy.

Material and Methods

The study was performed in a tertiary institution as a part of a research program, P3-0360 financed by a Slovenian Research Agency. The State Ethics Committee has approved the experiments (approval No 77/09/14). All patients gave their written informed consent. In the first part of the study, sera obtained in the period of 2010 – 2012, during routine diagnostic workup of patients referred to the clinic due to suspected penicillin allergy, were retrospectively analyzed. In the majority of patients with a history of penicillin allergy, the diagnostic procedure started with determination of sIgE against penicillin G, V and/or amoxicillin (allergens were selected from the medical history) with ImmunoCAP system (Termo Fisher, Waltham, Massachusetts, USA). In 2013, in all available ImmunoCAP positive sera, stored at - 20° C, sIgE against the same antibiotics were determined by Immulite system (Siemens, Munich, Germany). To check the stability of sIgE in frozen sera, the measurement of frozen sera was repeated with ImmunoCAP as well.

The second part of the study was conducted in 2014, and it prospectively included patients with a history compatible with IgE mediated penicillin allergy, namely a history of urticaria/angioedema, anaphylaxis or rash during penicillin, amoxicillin or amoxicillin-clavulanic acid treatment. Patients gave written informed consent with the procedures. The diagnostic procedure started with STs with culprit penicillin. Prick tests were considered positive if the wheal diameter after 20 minutes was at least half of that of histamine, and if the diameter of wheal increased by at least 3 mm and was surrounded by erythema [3]. When STs were negative, DPT with the culprit antibiotic was performed [6]. The blood samples were taken before testing, and sIgE-antibodies against penicillin antibiotics were measured after diagnostic work-ups were completed. The sIgE was considered truly positive if immediate STs, or DPT were positive.

Skin prick tests (SPTs) were performed with commercially available intravenous penicillin G (10,000 IU/ml) (Fagron, Belgium) and amoxicillin (20 mg/ml) (Lek, Slovenia). In case of negative SPT, intradermal tests (IDTs) with the same substance were performed with dilution of 1:10 and undiluted suspected antibiotic. If negative, tests were read 2 or 3 days latter, for possible late reaction. If IDTs were negative, oral DPT was performed [5]. An increasing amount of drug was administered at 1-hour intervals. Doses for phenoxymethylpenicillin (Ospen 1000, Lek, Slovenia) were 50 mg, 150 mg, 300 mg, and 500 mg (cumulative dose 1,000 mg) [5]. Doses for amoxicillin (Hiconcil 500, Krka, Slovenia) were 5 mg, 50 mg, 250 mg, and 500 mg (cumulative dose 805 mg). If immediate DPT was negative, patients continued taking 2 x 1000 mg of the antibiotic for 3 days, unless reaction occurred earlier. Patients were observed for possible delayed reactions [5].

To check the impact of high total IgE (tIgE) on performance of sIgE testing, sera of patients with tIgE over 700 kIU/L were taken from our biobank. Sera of patients with a history of penicillin allergy and sera of patients sensitized to molds were excluded.

The differences between groups of patients were calculated by Mann–Whitney U test. The difference between diagnostic performances of both tests was calculated by Chi-square test. The $p < 0.05$ was regarded as a statistically significant difference.

Results

In a 3-year period (2010 - 2012), 2.521 in-vitro determinations of sIgE against penicillin antibiotics were performed in 1.233 patients using ImmunoCAP. A total of 57 patients were positive (> 0.35 kIU/L). We retested 51 of these positive sera with Immulite (6 sera were not available), and all turned out negative (< 0.35 kIU/L). Out of 45 sera retested with ImmunoCAP, 33 remained positive and 12 turned out negative (9 class I positive (0.36 - 0.55 kIU/L) in fresh sample turned to borderline negative (0.14-0.33 kIU/L) in stored samples, and 3 strongly positive in fresh sample turned completely negative (< 0.1 kIU/L) in stored samples. The difference between tests was statistically significant ($p < 0.05$; Chi-square).

A total of 114 adult patients with a history compatible with hypersensitivity reaction during penicillin treatment were included into a prospective analysis. Of them, 60 reported hypersensitivity to amoxicillin with (39) or without (21) clavulanic acid, 51 to penicillin V and 3 to penicillin G.

According to patients' history, 35 developed reaction during the first hour of treatment (early reactors),

67 after 1 hour (range - 2 hours to 21 days, median 4 days) after beginning of treatment (late reactors), and 12 patients were not able to recall the time course of the reaction. Among 114 patients with a history compatible with hypersensitivity reaction during penicillin treatment, hypersensitivity was confirmed in 36 (31.6%) **(Table 1)**.

Table 1. Clinical data on patients with positive penicillin allergy tests
Tabela 1. *Klinički podaci pacijenata sa pozitivnim alergološkim testovima na peniciline*

Patient number *Broj pacijenta*	History *Anamneza*				Delay to testing (months) *Vreme do testiranja (meseci)*	sIgE CAP kIU/L *sIgE CAP kIU/L*			sIgE Immuno kIU/L *sIgE Immuno kIU/L*			Immulite kIU/L *Immulite kIU/L*	Total IgE kIU/L *Ukupan IgE kIU/L*	Skin tests *Kožni testovi*	OPT *OPT*	
1	Type *Tip*	Culprit drug/*Lek koji je prouzrokovao hipersenzitivnu reakciju*	Clinical presentation *Klinička prezentacija*	Treatment duration till reaction *Trajanje terapije do pojave eakcije*		pen V	pen G	amox	pen V	pen G	amox				Drug *Lek*	Reaction *Reakcija*
2	immediate *rana*	amoxi/clav, pen V/*amoxiclav V, pen V*	itch *svrab*	1 hour *1 čas*	14	<	<	<	<	<	<		NA	ID amoxi/clav late positive/*ID amoksi/klav kasno pozitvna*	ND	ND
3	immediate *rana*	amoxicillin *amoksicilin*	urticaria *urtikarija*	1 hour *1 čas*	100	ND	ND	<	ND	ND	<		NA	ID amoxi/clav late positive/ *ID amoksi/klav kasno pozitvna*	ND	ND
4	immediate *rana*	amoxi/clav *amoksi/klav*	urticaria *urtikarija*	1 hour *1 čas*	3	ND	ND	<	ND	ND	<		NA	ID amoxi/clav late positive/*ID amoksi/klav kasno pozitvna*	ND	ND
5	immediate *rana*	amoxi/clav *amoksi/klav*	angioedema *angioedem*	1 hour *1 čas*	24	<	ND	<	<	ND	<		NA	ID pen V, amox/clav positive/*ID pen V, amoksi/klav pozitivno*	ND	ND
6	immediate *rana*	amoxi/clav *amoxi/clav*	anaphylaxis *anafilaksa*	20 minutes *20 minuta*	6	<	<	<	<	<	<		NA	ID amox/clav positive/ *ID amoksi/klav pozitivno*	ND	ND
7	immediate *rana*	amoxi/clav *amoksi/klav*	anaphylaxis *anafilaksa*	20 minutes *20 minuta*	120	<	ND	<	ND	ND	<		NA	prick amox/clav positive/*prik amoks/klav pozitivno*	ND	ND
8	immediate *rana*	amoxi/clav *amoksi/klav*	anaphylaxis *anafilaksa*	10 minutes *10 minuta*	3	<	<	3,58	<	<	<		80,7	prick amoxicillin positive/*prik moksicillin pozitivno*	ND	ND
9	immediate *rana*	amoxi/clav *amoksi/klav*	anaphylaxis *anafilaksa*	5 minutes *5 minuta*	2	ND	ND	<	ND	ND	<		NA	prick amoxicillin positive/*prik amoksicillin pozitivno*	ND	ND
10	immediate rana	amoxi/clav *amoksi/klav*	urticaria *urtikarija*	1 hour *1 čas*	350	ND	ND	<	ND	ND	<		NA	prick amox/clav positive/*prik amoks/klav pozitivno*	ND	ND
11	immediate *rana*	amoxicillin *amoksicilin*	urticaria urtikarija	10 minutes *10 minuta*	4	ND	ND	<	ND	ND	<		NA	prick amoxicillin and ID pen V positive/*prik amoksicillin i ID pen V pozitivno*	ND	ND
12	immediate *rana*	amoxi/clav *amoksi/klav*	MPE *MPE*	1 hour *1 čas*	60	ND	ND	<	ND	ND	<		NA	negative *negativno*	amoxi/clav *amoksi/klav*	erythema *eritem*
13	immediate *rana*	pen V *pen V*	dyspnea *dispnea*	1 hour *1 čas*	24	<	<	<	<	<	<		NA	negative *negativno*	pen V	anaphylaxis *anafilaksa*
14	immediate *rana*	pen V *pen V*	MPE *MPE*	1 hour *1 čas*	1	7,42	0,78	1,26	0,41	0,48	0,44		>2000	negative *negativno*	pen V	urticaria *urtikarija*
15	delayed *kasna*	amoxi/clav *amoksi/klav*	MPE *MPE*	3 days *3 dana*	90	ND	ND	<	ND	ND	<		NA	ID amoxi/clav late positive/*ID amoksi/klav kasno* pozitivno	ND	ND
16	delayed *kasna*	amoxicillin *amoksicilin*	urticaria *urtikarija*	4 days *4 dana*	7	ND	ND	<	ND	ND	<		NA	ID amoxi/clav late positive/*ID amoksi/klav kasno pozitivno*	ND	ND
17	delayed *kasna*	amoxi/clav *amoksi/klav*	urticaria *urtikarija*	3 days *3 dana*	17	ND	ND	<	ND	ND	<		NA	ID amoxi/clav late positive/*ID amoksi/klav kasno pozitivno*	ND	ND

18	delayed *kasna*	amoxi/clav *amoksi/klav*	MPE *MPE*	4 days *4 dana*	9	ND	ND	<	ND	ND	<	NA	ID amoxi/clav late positive/*ID amoksi/klav kasno pozitivno*	ND	ND
19	delayed *kasna*	amoxi/clav *amoksi/klav*	urticaria *MPE*	4 days *4 dana*	40	<	<	<	<	<	<	NA	ID amoxi/clav late positive/*ID amoksi/klav kasno pozitivno*	ND	ND
20	delayed *kasna*	pen V *pen V*	MPE *MPE*	4 days *4 dana*	10	<	<	<	<	<	<	NA	ID amoxi/clav late positive/*ID amoksi/klavkasno pozitivno*	ND	ND
21	delayed *kasna*	pen V *pen V*	MPE *MPE*	4 days *4 dana*	400	<	<	ND	<	<	ND	NA	ID penicillin late positive/*ID penicillin kasno pozitivn*o	ND	ND
22	delayed *kasna*	amoxi/clav *amoksi/klav*	MPE *MPE*	9 days *9 dana*	5	<	<	ND	<	<	ND	NA	ID penicillin late positive/*ID penicillin kasno pozitivno*	ND	ND
23	delayed *kasna*	pen V *pen V*	anaphylaxis *anafilaksa*	3 hours *3 časa*	3	19,8	<	ND	<	<	ND	71,1	prick penicilline G positive/*prik penicillin G pozitivno*	ND	ND
24	delayed *kasna*	amoxicillin *amoksicilin*	urticaria *urtikarija*	7 days *7 dana*	140	ND	ND	<	ND	ND	<	NA	negative *negativno*	amoxicillin *amoksicilin*	urticaria *urtikarija*
25	delayed *kasna*	pen V *pen V*	angioedema *angioedem*	3 days *3 dana*	300	<	<	ND	<	<	ND	NA	negative *negativno*	pen V	MPE
26	delayed *kasna*	pen V *pen V*	urticaria *urtikarija*	5 days *5 dana*	3	0,5	0,54	ND	<	<	ND	2733	negative *negativno*	pen V	MPE
27	delayed *kasna*	pen V *pen V*	MPE *MPE*	3 days *3 dana*	6	<	<	<	<	<	<	NA	negative *negativno*	pen V	MPE
28	delayed *kasna*	amoxicillin *amoksicilin*	MPE *MPE*	8 days *8 dana*	3			<			<	NA	negative *negativ*no	amoxicillin *amoksicilin*	erithema *eritem*
29	delayed *kasna*	pen V *pen V*	urticaria *urtikarija*	10 days *10 dana*	6	<	<	ND	<	<	ND	NA	negative *negativno*	pen V	MPE
30	delayed *kasna*	pen V pen V	urticaria urtikarija	12 days *12 dana*	4	<	<	ND	<	<	ND	NA	negative *negativno*	pen V	MPE
31	delayed *kasna*	amoxicillin *amoksicilin*	erythema *eritem*	2 days *2 dana*	36	ND	ND	<	ND	ND	<	NA	negative *negativno*	amoxicillin *amoksicilin*	erithema *eritem*
32	delayed *kasna*	amoxi/clav *amoksi/klav*	urticaria *urtikarija*	7 days *7 dana*	12	ND	ND	<	ND	ND	<	NA	negative *negativno*	amoxicillin *amoksicilin*	MPE
33	delayed *kasna*	pen V *pen V*	urticaria *urtikarija*	7 days *7 dana*	7	<	<	ND	<	<	<	NA	negative *negativno*	pen V	urticaria *urtikarija*
34	delayed *kasna*	pen V *pen V*	angioedema *angioedem*	8 hours *8 časova*	100	<	ND	ND	<	<	ND	NA	negative *negativno*	pen V	urticaria *urtikarija*
35	delayed *kasna*	pen V *pen V*	angioedema *angioedem*	3 days *3 dana*	10	<	ND	0,51	ND	ND	<	2375	negative *negativno*	pen V	angioedema *angioedem*
36	delayed *kasna*	amoxi/clav *amoksi/klav*	urticaria *urtikarija*	5 days *5 dana*	3	ND	ND	<	ND	ND	<	NA	negative *negativno*	amoxi/clav *amoksi/klav*	urticaria *urtikarija*
37	delayed *kasna*	amoxicillin *amoksicilin*	urticaria *urtikarija*	10 days *10 dana*	9	ND	<	7,83	ND	<	<	421	amoxicillin *amoksicillin*	amoxicillin *amoksicilin*	negative *negativno*
38	delayed *kasna*	pen V *pen V*	Dress *Dress*	10 days *10 dana*	400	1,05	0,62	ND	<	<	ND	803	pen V *pen V*	pen V	negative *negativno*
39	unknown *nepoznata*	amoxicillin *amoksicillin*	urticaria *urtikarija*	3 days *3 dana*	240	<	ND	<	<<	ND	ND	NA	ID amoxi/clav late positive/*ID amoksi/klav kasno pozitivno*	ND	ND

Legend: Delay to testing: interval between hypersensitivity reaction and diagnostics; amoxi/clav – amoxicillin plus clavulanic acid; ID – intradermal test; OPT – oral provocation test; ND – test not performed; MPE – maculopapular exantema

Legenda: Vreme do testiranja: interval između hipersenzitivne reakcije i dijagnoze; amoksi/klav – amoksicilin plus klavulanska kiselina; ID – intradermalni test; OPT – oralni provokativni test; ND – test nije bio izveden; MPE – makulo-papularni osip, sIgE – specifični imunoglobulin E

Among 35 early reactors, hypersensitivity was proven in 13 patients (37.1%). The hypersensitivity reaction occurred 54.7 ± 97.1 months before testing in patients with confirmed allergy, and 125.0 ± 170.0 months before testing in patients with negative diagnostic work-up, and the difference was not statistically significant ($p > 0.05$, t-test). As shown in **Table 1**, there were 5 with SPT, 2 with IDT, 3 with delayed skin tests (DSTs) and 3 with DPT (all immediate DPT reactions). Only in 2 of these patients (number 8 and 14) sIgE ImmunoCAP was positive (1 amoxicillin, 1 penicillin V, G and amoxicillin). One of the patients was SPT posi-

Table 2. ImmunoCAP and Immulite sIgE assays in patients with a history compatible with hypersensitivity reaction during penicillin treatment, with immediate positive skin reaction or immediate drug provocation tests
Table 2. *ImmunoCAP i Immulite testovi za specifični imunoglobulin E kod pacijaneta sa anamnezom hipersenzitvne rekacije za vreme primanja penicilina, koji su imali rane pozitvne kožne testove ili provokativno testiranje*

	Immuno CAP	Immulite
True positive (patients)/*Pozitivni (pacijenti)*	3	1
False positive (patients)/*Lažno pozitivni (pacijenti)*	4	0
False negative (patients)/*Lažno negativni (pacijenti)*	8	10
True negative (patients)/*Negativni (pacijenti)*	99	103
Sensitivity (%)/*Senzitivnost (%)*	27.2	9.1
Specificity (%)/*Specifičnost (%)*	96.1	100
Positive predictive value (%)/*Pozitivna prognostička vrednost (%)*	42.9	100
Negative predictive value (%)/*Negativna prognostička vrednost (%)*	92.5	91.2

tive to the corresponding penicillin. The other patient with a negative ST and positive DPT had positive sIgE measured by ImmunoCAP and Immulite. The other patients were Immulite negative. It is noteworthy that all 5 patients with a history of anaphylaxis were ST positive **(Table 1)**.

Among 67 delayed reactors, hypersensitivity was confirmed in 22 patients (32.8%). The hypersensitivity reaction occurred 55.0 ± 103.5 months before testing in patients with confirmed allergy and 100.2 ± 137.6 months before testing in patients with negative diagnostic work-up; the difference was not statistically significant ($p > 0.05$, t-test). As shown in **Table 1**, there was 1 patient with SPT (a patient with a history of anaphylaxis 3 hours after taking penicillin V), 8 with DST, and 13 with DPT. The ImmunoCAP sIgE was positive in 5 patients, (2 amoxicillin, 3 penicillin V). One of these patients was SPT positive, 2 were negative to DPT, and 1 was ST negative but reacted to prolonged DPT. All 5 ImmunoCAP sIgE positive patients were Immulite negative **(Table 1)**.

In 12 patients, who were not able to recall the time course of the reaction, one patient was delayed ST positive **(Table 1)**. All the others were ST and DPT negative. All patients were sIgE negative by both methods.

The diagnostic value of both sIgE assays is shown in **Table 2**. In 11 patients with positive immediate ST or DPTs, ImmunoCAP was truly positive in 3 patients and Immulite in 1 patient. Sensitivity of ImmunoCAP and Immulite were 27.2% and 9.1%, respectively. Specificity of ImmunoCAP and Immulite were 96.1% and 100%, respectively. Out of 6 positive ImmunoCAP sIgE with culprit antibiotic, 3 were truly positive, 2 were false positive and 1 was probably false positive (a patient with high tIgE), namely the patient had negative ST, but positive delayed DPT. As shown in **Table 1**, all three truly positive sIgE results (patients 8, 14, 23) were tested not more than 3 months after reaction **(Table 1)**. The sIgE was false negative in 5 patients with immediate ST positivity, and the additional 3 patients were with negative ST and immediate DPT reaction. In Immulite, 1 patient was truly positive and 10 false negative. The difference in sensitivity and specificity between tests was not statistically significant ($p > 0.05$, chi-square).

As shown in **Table 3**, sIgE against penicillin and/or amoxicillin were detected with ImmunoCAP in 0/9 penicillin tolerant subjects with tIgE between 700 and 1000 kIU/L, 3/8 with tIgE between 1000 and 2000, and 5/5 with tIgE over 2000 kIU/L. Six were positive to penicillin and 2 to amoxicillin. The concentration of sIgE was between 0.37 and 0.76 kIU/L. All sera were negative with Immulite ($p < 0.05$, chi-square).

Discussion

This prospective study was designed to compare the diagnostic efficacy of two commercially available tests for detection of sIgE in the sera of patients with suspected allergy to penicillin antibiotics (penicillin G, V and/or amoxicillin). The well known disadvantages of commercially available in vitro tests, radio allegro sorbent test (RAST) and enzyme-linked immunosorbent assay (ELISA) include their low sensitivity and ability to detect only sIgE to major but not minor penicillin (penicillin G, V and/or amoxicillin) determinants [7].

This study showed that compared to ImmunoCAP, Immulite assay has lower sensitivity, but the difference did not reach statistical significance. To our knowledge, there are no reports comparing both widely used diagnostic in-vitro assays for beta lactam allergy. There are few reports comparing assays in respiratory, food and venom allergies [8]. In a study of predominantly inhalant allergen allergy, Lee and colleagues found close association and significant correlation between both assays. Intermethod agreement based on sIgE detection was 74.1 - 100% [9]. In food allergic patients (chicken egg white, cow's milk and/or peanut) sIgE levels obtained by both methods were highly correlated regarding positivity/negativity rating, but the values obtained by Immulite were 2- to 5-fold higher than those obtained by ImmunoCAP [10, 11].

On the other side, it has been reported, that IgE sensitization does not automatically mean the presence of allergic disease [12, 13]. In ImmunoCAP penicillin sIgE assay, two technical problems were identified which may have resulted in false positive results. High tIgE sometimes resulted in low level positive result of sIgE to beta lactams in UniCAP assay [14, 15]. In as-

Table 3. ImmunoCAP and Immulite sIgE assays in penicillin tolerant patients with high levels of total IgE
Table 3. *ImmunoCAP i Immulite testovi za specifični imunoglobulin E kod pacijenta tolerantnih na penicillin sa visokim ukupnim imunoglobulinom E*

Subject number *Broj*	Immulite (kIU/l) *Immulite (kIU/l)*			ImmunoCAP (kIU/l) a *ImmunoCAP (kIU/l) a*		Total IgE kIU/L *Ukupan IgE kIU/L*
	pen G	pen V	amoxicillin	pen V	amoxicillin	
1	<	<	<	<	<	733
2	<	<	<	<	<	785
3	<	<	<	<	<	799
4	<	<	<	<	<	804
5	<	<	<	<	<	823
6	<	<	<	<	<	853
7	<	<	<	<	<	873
8	<	<	<	<	<	916
9	<	<	<	<	<	922
10	<	<	<	<	<	1040
11	<	<	<	0,76	<	1173
12	<	<	<	<	<	1221
13	<	<	<	<	<	1381
14	<	<	<	0,38	<	1424
15	<	<	<	<	<	1634
16	<	<	<	<	0,49	1813
17	<	<	<	<	<	1977
18	<	<	<	0,43	<	2001
19	<	<	<	0,55	<	2001
20	<	<	<	0,65	<	2001
21	<	<	<	0,67	<	2001
22	<	<	<	<	0,35	2001

says used before 2005, positive sIgE results were found in nearly half of sera with tIgE between 500 and 600 kIU/L, and 75% in patients with tIgE over 1000 kIU/L. In a retrospective analysis of 606 patients with a history of penicillin allergy, 49 patients (8%) were sIgE positive [16]. About one third of these had tIgE over 500 kIU/L. Four of such patients were subjected to DPT and turned out negative. In the current study, an improved ImmunoCAP assay was used [4], and false positive sIgE results were found only in sera with tIgE over 1000 kIU/L. Some other studies have also shown high rate of false positivity of sIgE against penicillin antibiotics. Silva [17] challenged patients with positive UniCAP sIgE to penicillin. Only 2 out of 7 patients were DPT positive. Macy [18] challenged SPT negative patients with a remote history of penicillin allergy regardless of the result of in-vitro tests. Six out of 150 patients were sIgE (UniCAP) positive to penicillins, and all of them were DPT negative. Johanson and colleagues found that 26% of patients with suspected IgE-mediated reaction to penicillin and positive penicillin ImmunoCAP actually might have clinically irrelevant IgE, which makes, as they stated, ImmunoCAP a poor choice for the diagnosis of penicillin allergy [4]. Unfortunately, they did not test other commercial penicillin sIgE tests for that flaw. Hjortlund and colleagues made a diagnosis of beta lactam allergy in 28.7% of tested subjects [3]. They also noticed a considerable lack of correlation between IgE and ST-positivity. They performed STs in sIgE positive patients and found that only 6 of 19 IgE-positive patients were also IDT positive. Unfortunately, DPT was not performed in sIgE positive ST negative patients. They also reported that intradermal ST might be false negative. They performed duplicate tests and showed that reproducibility of penicillin ST is only 83%. That might be one of the reasons why some patients with convincing history have negative ST and positive DPT.

There are some limitations of our study that should be considered when evaluating the results. In the retrospective part, ImmunoCAP results obtained from fresh sera were compared with Immulite results obtained from frozen sera. Thus, after testing frozen sera with ImmunoCAP, 12 out of 45 samples turned negative. We cannot explain the reason for the negativization of the ImmunoCAP results. One possibility, which is less likely, is the decrease of sIgE concentration over time in stored frozen samples. The more likely explanation is the improvement in the reagents used for sIgE determination [4]. However, in the prospective part of the study, both methods were tested simultaneously using frozen sera. A pitfall was also a selection of allergens for ST. The two main allergenic determinants currently used in the diagnosis of type I or immediate hypersensitivity to

benzylpenicillin and related antibiotics (beta-lactam), by means of SPT and IDTs are classified as major benzylpenicilloyl poly-L-lysine (PPL) formed by the bonding of the primary determinant to lysine chains and minor determinant mix (MDM), mixture of sodium benzylpenicillin, benzylpenicilloic acid, and sodium benzylpenicilloate. Both PPL and MDM are recommended standard reagents for penicillin ST. However, penicillin G is commonly suggested as an alternative source of minor determinants [20]. In this study, penicillin G (10,000 IU/ml) was used, since PPL and MDM are not available in our market. Penicillin G was also used in ST in patients with positive history following penicillin V treatment. That might be the reason for ST negativity in patients with positive DPT with penicillin

Conclusion

Both ImmunoCAP and Immulite sIgE assays showed low sensitivity when assessing patients with a history of hypersensitivity reactions during penicillin treatment, as well as positive immediate skin or immediate drug provocation tests. In addition, ImmunoCAP is nonspecific in patients with high total immunoglobulin E levels.

References

1. Trubiano J, Phillips E. Antimicrobial stewardship's new weapon? A review of antibiotic allergy and pathways to 'delabeling'. Curr Opin Infect Dis. 2013;26(6):526-37.

2. MacLaughlin EJ, Saseen JJ, Malone DC. Costs of beta-lactam allergies: selection and costs of antibiotics for patients with a reported beta-lactam allergy. Arch Fam Med. 2000;9(8): 722-6.

3. Hjortlund J, Mortz CG, Skov PS, Bindslev-Jensen C. Diagnosis of penicillin allergy revisited: the value of case history, skin testing, specific IgE and prolonged challenge. Allergy. 2013:68(8): 1057-64.

4. Johansson SG, Adedoyin J, van Hage M, Gronneberg R, Nopp A. False-positive penicillin immunoassay: an unnoticed common problem. J Allergy Clin Immunol. 2013;132(1):235-7.

5. Fransson S, Mosbech H, Kappel M, Hjortlund J, Poulsen LK, Kvisselgaard AD, et al. The importance of prolonged provocation in drug allergy - results from a Danish Allergy Clinic. J Allergy Clin Immunol Pract. In press. doi: 10.1016/j.jaip.2017.02.024.

6. Blanca M, Romano A, Torres MJ, Férnandez J, Mayorga C, Rodriguez J, et al. Update on the evaluation of hypersensitivity reactions to betalactams. Allergy. 2009;64(2):183-93.

7. Torres MJ, Blanca M, Fernandez J, Romano A, Weck A, Aberer W, et al. Diagnosis of immediate allergic reactions to beta-lactam antibiotics. Allergy. 2003;58(10):961-72.

8. Šelb J, Kogovšek R, Šilar M, Košnik M, Korošec P. Improved recombinant Api m1 - and Ves v 5-based IgE testing to dissect bee and yellow jacket allergy and their correlation with the severity of the sting reaction. Clin Exp Allergy. 2016;46(4):621-30.

9. Lee YW, Sohn JH, Lee JH, Hong CS, Park JW. Allergen-specific IgE measurement with the IMMULITE 2000 system: intermethod comparison of detection performance for allergen-specific IgE antibodies from Korean allergic patients. Clin Chim Acta. 2009;401(1-2):25-32.

10. Hamilton RG, Mudd K, White MA, Wood RA. Extension of food allergen specific IgE ranges from the ImmunoCAP to the IMMULITE systems. Ann Allergy Asthma Immunol. 2011;107(2):139-44.

11. Wang J, Godbold JH, Sampson HA. Correlation of serum allergy (IgE) tests performed by different assay systems. J Allergy Clin Immunol. 2008;121(5):1219-24.

12. Bodtger U. Prognostic value of asymptomatic skin sensitization to aeroallergens. Curr Opin Allergy Clin Immunol. 2004;4(1):5-10.

13. Bousquet J, Anto JM, Bachert C, Bousquet PJ, Colombo P, Crameri R, et al. Factors responsible for differences between asymptomatic subjects and patients presenting an IgE sensitization to allergens. A GA2LEN project. Allergy. 2006;61(6):671-80.

14. Zidarn M, Silar M, Vegnuti M, Korosec P, Kosnik M. The specificity of tests for anti-beta-lactam IgE antibodies declines progressively with increase of total serum IgE. Wien Klin Wochenschr. 2009;121(9-10):353-6.

15. Aberer W, Zidarn M, Kranke B. IgE antibodies to penicillin are indicative for but not conclusive proof of penicillin allergy. Br J Dermatol. 2006;154(6):1209-10.

16. Kopac P, Zidarn M, Kosnik M. Epidemiology of hypersensitivity reactions to penicillin in Slovenia. Acta Dermatovenerol Alp Pannonica Adriat. 2012;21(4):65-7.

17. Silva R, Cruz L, Botelho C, Castro E, Cadinha S, Castel-Branco MG, et al. Immediate hypersensitivity to penicillins with negative skin tests – the value of specific IgE. Eur Ann Allergy Clin Immunol. 2009;41(4):117-9.

18. Macy E, Goldberg B, Poon KYT. Use of commercial anti–penicillin IgE fluorometric enzyme immunoassays to diagnose penicillin allergy. Ann Allergy Asthma Immunol. 2010;105(2):136-41.

19. Wangrattanasopon P, Ruxrungtham K, Chantaphakul H, Buranapraditkun S, Klaewsongkram J. Alkali-treated penicillin G solution is a better option than penicillin G as an alternative source of minor determinants for penicillin skin test. Allergy Asthma Proc. 2012;33(3):152-9.

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WORK STATUS AND FACTORS AFFECTING WORK ENGAGEMENT OF PEOPLE WITH MULTIPLE SCLEROSIS

RADNI STATUS I FAKTORI KOJI UTIČU NA RADNO ANGAŽOVANJE OBOLELIH OD MULTIPLE SKLEROZE

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Summary

Introduction. Regular follow-up and support of people with multiple sclerosis in finding and keeping employment may enable them to stay employed longer and have better quality of work life, financial security, and a higher degree of social participation. The objective of this study was to determine the work status and factors that may affect work engagement of people with multiple sclerosis. **Material and Methods.** The study was conducted in the territory of the Autonomous Province of Vojvodina, and included 108 subjects with relapsing-remitting multiple sclerosis, aged 18 - 65 years, using the expanded disability status scale, 0 - 5.5. The data were collected using a General questionnaire and the multiple sclerosis work difficulties questionnaire. **Results.** Out of 108 subjects, only 37 were employed or had been retired for less than three years, which were the criteria for filling out the multiple sclerosis work difficulties questionnaire (in Serbian). Our results showed that subjects perceived cognitive difficulties and movement/mobility difficulties as the main factors affecting their work performance. **Conclusion.** The analysis of environmental factors and factors affecting work engagement of people with multiple sclerosis showed that other factors affected their work more than their personal preferences or the disease itself.

Key words: Multiple Sclerosis; Disabled Persons; Employment; Work Capacity Evaluation; Social Participation; Surveys and Questionnaires; Quality of Life

Sažetak

Uvod. Adekvatnim načinom praćenja i podrškom prilikom zaposlenja i tokom radnog angažovanja, osobama obolelim od multiple skleroze omogućuje se duži i kvalitetniji ostanak na poslu, finansijska sigurnost i veći stepen socijalne participacije. Cilj rada je bio da utvrdimo radni status i faktore koji mogu uticati na radno angažovanje obolelih od multiple skleroze. **Materijal i metode.** Istraživanje je sprovedeno na teritoriji Autonomne Pokrajine Vojvodine na ukupnom uzorku od 108 ispitanika sa relapsno-remitentna formom multiple skleroze, starosti 18−65 godina i EDSS skorom 0−5,5. Podaci su prikupljeni Opštim upitnikom i *The Multiple Sclerosis Work Difficulties Questionnaire* − MSWDQ koji je preveden i prilagođen za potrebe srpskog govorno-jezičkog područja. **Rezultati.** Od ukupnog uzorka (n = 108), svega 37 obolelih od multiple skleroze radi ili je u penziji manje od tri godine što je bio preduslov popunjavanja upitnika. Utvrđeno je da ispitanici kognitivne teškoće i teškoće sa pokretljivošću percipiraju kao glavne teškoće odnosno faktore koji utiču na obavljanje posla. **Zaključak.** Analizirajući faktore okruženja i faktore koji utiču na radno angažovanje obolelih od multiple skleroze, zaključili smo da drugi faktori više utiču na rad ispitanika sa multiplom sklerozom, nego njihova lična želja ili sama bolest.

Ključne reči: multipla skleroza; osobe sa invaliditetom; zapošljavanje; procena radnih sposobnosti; socijalna participacija; ankete i upitnici; kvalitet života

Introduction

Multiple sclerosis (MS) is a chronic, unpredictable neurological disease, and its onset is usually between 20 and 40 years [1], during the period of peak professional life development. It is one of the most common causes of disability among young people in Europe [2]. However, certain symptoms, acceptance of diagnosis, phases of exacerbations and numerous other circumstances, affect the individual's perception of life, present job, decision to find a new employment, and ability to function on daily basis. Moreover, prior studies indicate that both physical and cogniive functional limitations associated with MS are likely the main factors of work ability or work status (i.e. being paid vs. not paid) [3] as well as of the type of employment. Employment is an important issue for people with MS, because it directly affects the individual's level of social participation. Data show that employed people with MS have a better quality of life [4]. Furthermore, physical and cognitive functional limitations associated with the disease are probably the main determinants of work ability [3].

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Abbreviations

MS – multiple sclerosis
EDSS – expanded disability status scale
MSWDQ – Multiple Sclerosis Work Difficulties Questionnaire
SPSS – Statistical Package for the Social Sciences
AM – arithmetric mean

Serbia has a formal legal framework for employment of disabled people, including people with MS. The institutional and legal framework is in line with international recommendations and trends regarding people with disability. Currently, there is an employment quota system for disabled people. The law enables rehabilitation, facilitated employment, special forms of employment, and work engagement of disabled people [5–8].

However, due to absence of other important measures, neither getting nor keeping a job has been made easier for people with MS.

International studies indicate that more than 50% of all people with MS are unemployed [9, 10], while in Serbia, the number of unemployed people with MS is unknown.

In order to understand the work-related perceptions of people with MS better, the Multiple Sclerosis Work Difficulties Questionnaire (MSWDQ) was created [11]. It was developed using an Australian sample, a comprehensive 50-item questionnaire assessing 12 domains of workplace difficulties, each of which were highly predictive workplace outcomes and/or expectations about future employment. Difficulties experienced in the workplace, stemming directly or indirectly from MS, are therefore seen to play a key role in making a decision to withdraw from work. The perceptions of workplace difficulties of people with MS in Serbia have not been examined. Such examinations are of crucial importance, not only to understand the types of difficulties experienced by Serbian people with MS better, but also to highlight the difficulties which might exclusively be related to employment outcomes, such as work withdrawal. This information in turn is highly important for providing effective vocational and rehabilitation programs in the Serbian community within the legal context.

The aim of this study was twofold: firstly to validate and pilot the use of a translated version of the MSWDQ for MS population in Serbian language, and secondly to conduct a pilot study on the relationships between various workplace difficulties and disease-related factors and demographic variables. Such an examination should highlight factors which most likely affect work engagement in people with MS in Serbia.

Material and Methods

The study was carried out in the territory of the Autonomous Province of Vojvodina, Serbia, and included an initial sample of 108 subjects with relapsing-remitting MS. Of the 108 subjects, however, 71 participants were excluded, because they had not been employed within the last three years. Another 3 participants were excluded due to missing data on some items. The final sample comprised 34 people with MS, aged 18 - 65 years (M = 40; SD = 8.2), with the EDSS 0 - 5.5. All included subjects had been diagnosed with MS at least one year before the study. The data were collected using a General questionnaire designed for the purpose of the study.

The MSWDQ [11] was translated and adapted for use in the Serbian speaking population, and completed by participants. This translation into the Serbian language specifically involved synthesis of the translated version, evaluation of the synthesized version by experts, evaluation by a target group, and back translation as recommended by Gjersing, Caplehorn, and Clausen [12]. The MSWDQ has 12 subscales or workplace difficulty domains related to:

a. psychological/cognitive barriers: general cognitive difficulties, prospective memory difficulties, fatigue, low self-esteem, interpersonal difficulties, and non-supportive workplace;

b. physical barriers: movement/mobility difficulties, workplace inaccessibility, pain/temperature difficulties, and bladder/bowel difficulties; and

c. external barriers: financial security concerns and work/home balance difficulties.

The MSWDQ has a total of 50 items, and participants were asked to rate the extent of work difficulties during the previous 4 weeks at the current or previous job. The responses were graded on a 5-point Likert scale, from 0 (never) to 4 (almost always). Thus, higher responses showed an increased perception of disease-related difficulties. Both MSWDQ total and individual subscale scores were examined. The MSWDQ demonstrated good to excellent levels of internal consistent (Cronbach's alpha ranges for the individual subscales range from .74 to .92), and demonstrated good concurrent validity in relation to past withdrawal from work.

The Kurtzke Functional Systems and EDSS (Kurtzke, 1983) [13] is the most widely used assessment scale for measuring physical disability in MS patients [14]. The disability rating was completed by the patients' neurologist, according to a 10-point scale ranging from 0 (normal neurological function & no disability) to 10 (death due to MS). Intra-rater and inter-rater reliability coefficients for the EDSS were high, with respective coefficients of .95 and .91 [15].

Statistical data analysis was performed using the Statistical Package for the Social Sciences (SPSS) software. Descriptive statistical methods (arithmetic mean, median, range, standard deviation), and normality tests, Kolmogorov-Smirnov and Shapiro-Wilk tests were used. Statistical significance was defined as $p < 0.05$ [16]. Age differences between the groups were examined using the Mann-Whitney U test. To examine possible biases in the items, item analysis was conducted. Internal consistency, Cronbach's alpha was calculated [17]. Factor analysis with varimax rotation was conducted using principal component analysis in SPSS.

Results

General questionnaire results

Women (70/64.8%) were prevalent in the sample. In our sample (n = 108), most subjects, i.e., 73 (67.6%) had secondary education, 27 (25%) had tertiary education, while 8 (7.4%) had incomplete or complete ele-

Table 1. Reliability of the MSWDQ subscales
Tabela 1. *Pouzdanost subskala MSWDQ*

Subscales *Subskale*	Cronbach's α *Kronbahov α*	Item total correlation *Ukupna korela*cija
General cognitive difficulties/*Opšte kognitivne teškoće*	0.82	0.52 – 0.82
Movement/mobility difficulties/*Teškoće sa pokretljivošću*	0.77	0.57 – 0.77
Work/home balance difficulties/*Teškoće usklađivanja posao/kuća*	0.80	0.57 – 0.80
Non-supportive workplace/*Nedostatak podrške na poslu*	0.64	0.56 – 0.79
Bladder /bowel difficulties/*Teškoće sa crevima i bešikom*	0.81	0.53 – 0.89
Financial security concerns/*Zabrinutost za finansijsku sigurnost*	0.75	0.52 – 0.80
Low self-esteem/*Nisko samopouzdanje*	0.60	0.57 – 0.76
Prospective memory difficulties/*Prospektivne poteškoće sa memorijom*	0.73	0.51 – 0.86
Workplace inaccessibility/*Nepristupačnost radnog mesta*	0.75	0.54 – 0.89
Pain/temperature difficulties/*Teškoće sa bolovima i temperaturom*	0.70	0.60 – 0.74
Interpersonal difficulties/*Teškoće u interpersonalnim odnosima*	0.60	0.52 – 0.85
Fatigue/*Zamor*	0.70	0.53 – 0.75

mentary education. This finding is in agreement with the educational distribution in the studied region. Most subjects (n = 70; 64.8%) lived in an urban environment, as opposed to 38 subjects (35.2%) who lived in a rural environment. In regard to the marital status, 71 (68.9%) subjects were married or lived with a partner, 7 (6.6%) were separated or divorced, and 26 (24.5%) were single. Fifty-three subjects had no children, 19 had one child, 31 had two children, and 5 subjects had three or more children. Most subjects (57.8%) had their own place to live. An additional positive factor was that most subjects lived with a partner and children, 19 subjects lived with their parents, and only 10 subjects lived alone (with or without children).

Twenty-eight (26.2%) subjects were employed full-time, 4 (3.7%) were employed part-time, and 47 (43.9%) were retired. Equal number of subjects were unemployed, due to the health status and due to other reasons, 12 (11.2%) each. Only one (0.9%) subject had never looked for a job. The sample included 3 (2.8%) university students. Accordingly, in regard to the income, most subjects lived on a pension or salary, and less than 15% of all subjects were receiving unemployment benefits, such as disability-related financial support or child support. Concerning assistive technology, 63 (58.9%) subjects used some devices, most frequently eyeglasses and walking canes/sticks.

Qualitative analysis of the MSWDQ content

The time necessary to complete the questionnaire ranged from 20 to 25 minutes. All subjects reported that they understood all questions and reported that the Serbian version of MSWDQ adequately reflected the difficulties they experienced in the workplace, and was easy to complete.

Reliability of the MSWDQ

The Cronbach's Alpha Reliability Coefficient for the overall MSWDQ was excellent (0.95). The correlation coefficient between the 50 items and the total scale was high, ranging from 0.60 to 0.82. The final sample comprised 34 people with MS, and the reliability estimates were similar in various MSWDQ subscales **(Table 1)**. Inter-item correlations were considered acceptably low, and correlations of items on individual subscales did not exceed 0.90, and together with high Cronbach's α coefficient, it indicated good psychometric properties of the translated scale.

Validity of the MSWDQ

Exploratory factor analysis (principal component method) was used to assess the proposed 12-item structure of the questionnaire. Twelve factors were isolated - 10 significant and 2 insignificant (items on the financial security concerns and movement/mobility difficulties subscales). These 10 factors explain 84.94% of the variance of the questionnaire content. The addition of the two factors does not significantly increase the percentage of the explained variance (88.69%).

Exploration of MSWDQ scores

The mean scores on individual MSWDQ subscales ranged from 33.75 to 62.73. The subjects perceived most difficulties in the domain of financial security, and least difficulties in the domain of prospective memory difficulties **(Table 2)**.

Normality of score distribution on the MSWDQ and the subscales

Table 3 shows that all MSWDQ subscales, except financial security difficulties, deviate significantly from a normal distribution, so we used non-parametric statistical analysis to analyze differences.

Correlation between the MSWDQ subscales and socio-demographic variables

The Mann-Whitney U test was used to test sex differences in the 12 MSWDQ subscales. The results showed no gender-based differences associated with workplace problems on any of the 12 subscales.

The Kruskal-Wallis test did not show statistically significant differences ($p < .05$) in workplace difficul-

Table 2. The MSWDQ scores
Tabela 2. *Skorovi na upitniku MSWDQ*

	Min.	Max.	AM	SD	Skewness *Asimetrija*	Kurtosis *Homogenost distribucije*
General cognitive difficulties/*Opšte kognitivne teškoće*	25.00	83.33	43.67	14.11	1.11	1.46
Movement/mobility difficulties/*Teškoće sa pokretljivošću*	25.00	89.29	48.69	18.36	0.72	-.051
Work/home balance difficulties *Teškoće usklađivanja posao/kuća*	25.00	83.33	42.26	16.34	0.81	0.07
Non-supportive workplace/*Nedostatak podrške na poslu*	25.00	75.00	37.67	13.50	1.54	1.89
Bladder/bowel difficulties/*Teškoće sa crevima i bešikom*	25.00	112.50	45.75	23.45	1.21	0.87
Financial security concerns *Zabrinutost za finansijsku sigurnost*	25.00	118.75	62.93	26.87	0.34	-0.86
Low self-esteem/*Nisko samopouzdanje*	25.00	75.00	44.01	18.60	0.55	-1.30
Prospective memory difficulties *Prospektivne poteškoće sa memorijom*	25.00	65.00	33.75	11.67	1.28	.83
Workplace inaccessibility/*Nepristupačnost radnog mesta*	25.00	87.50	34.72	16.29	2.13	4.28
Pain/temperature difficulties *Teškoće sa bolovima i temperaturom*	25.00	91.67	40.80	19.71	1.43	1.37
Interpersonal difficulties *Teškoće u interpersonalnim odnosima*	25.00	83.33	33.88	14.99	1.87	3.03
Fatigue/*Zamor*	25.00	100.00	46.71	19.42	1.18	1.56

ties between subjects with different educational levels or marital status, and age was also not significantly associated with perceived workplace difficulties.

The time since diagnosis, in our subjects ranged from 1 to 22 years (arithmetic mean (AM) = 3.49) and time since symptom onset was 1 - 31 years (AM = 11.23). Time since diagnosis significantly negatively correlated with the following domains/subscales: non-supportive workplace (r = -0.35, p = 0.03), bowel/bladder difficulties (r = -0.43, p = 0.00), and interpersonal difficulties (r = -0.37, p = 0.02). These correlations reflect the pattern that the earlier the diagnosis was made, the more difficulties the patient had in these domains.

The time since symptom onset significantly negatively correlated with the following domains/subscales: home/work balance difficulties (r = -0.33, p = 0.04), non-supportive workplace (r = -0.36, p = 0.02), bowel/bladder difficulties (r = -0.34, p = 0.03), and interpersonal difficulties (r = -0.37, p = 0.02). The negative correlation means that the earlier the onset of symptoms, the patients had more difficulties in these domains.

There was a significant difference between subjects living in urban and rural environments, regarding the severity of difficulties in the following domains: general cognitive difficulties, non-supportive workplace, low self-esteem, and prospective memory difficulties **(Table 4)**. The mean values show that subjects from rural environments reported more difficulties in

Table 3. Tests of normality of distribution
Tabela 3. *Testovi normalnosti raspodele*

	Kolmogorov-Smirnov		
	statistic	df	p
Movement/mobility difficulties/*Teškoće sa pokretljivošću*	0.17	26	0.03
Work/home balance difficulties/*Teškoće usklađivanja posao/kuća*	0.16	26	0.07
Non-supportive workplace/*Nedostatak podrške na poslu*	0.20	26	0.00
Bladder /bowel difficulties/*Teškoće sa crevima i bešikom*	0.20	26	0.00
Financial security concerns/*Zabrinutost za finansijsku sigurnost*	0.21	26	0.00
Low self-esteem/*Nisko samopouzdanje*	0.11	26	0.20
Prospective memory difficulties/*Prospektivne poteškoće sa memorijom*	0.23	26	0.00
Workplace inaccessibility/*Nepristupačnost radnog mesta*	0.26	26	0.00
Pain/temperature difficulties/*Teškoće sa bolovima i temperaturom*	0.27	26	0.00
Interpersonal difficulties/*Teškoće u interpersonalnim odnosima*	0.22	26	0.00
Fatigue/*Zamor*	0.36	26	0.00
Movement/mobility difficulties/*Teškoće sa pokretljivošću*	0.14	26	0.14

Table 4. Association between urban/rural environment and workplace difficulties
Tabela 4. *Urbana ili ruralna sredina u odnosu na domene teškoća pri radu*

		Mean Rank *Srednji stepen*	Test statistic *Statistika testa*	p
Subscale/*Supskale*	Environment/*Sredina*			
General cognitive difficulties *Opšte kognitivne teškoće*	Urban/*Urbana*	17.04	$Z^* = 71.00$	p = 0.04
	Rural/*Ruralna*	25.11		
Non-supportive workplace *Nedostatak podrške na poslu*	Urban/*Urbana*	16.07	Z = 56.00	p = 0.01
	Rural/*Ruralna*	25.78		
Low self-esteem *Nisko samopouzdanje*	Urban/*Urbana*	16.34	Z = 51.50	p = 0.00
	Rural/*Ruralna*	27.28		
Prospective memory difficulties *Prospektivne poteškoće sa memorijom*	Urban/*Urbana*	17.02	Z = 70.50	p = 0.04
	Rural/*Ruralna*	25.17		

Z^* = Mann-Whitney test/*Man-Vitnijev test*

these four domains compared to subjects from urban environments.

Correlation between the MSWDQ subscales and the EDSS

The Spearman's correlation coefficient was used to test the association between the MSWDQ subscales and the EDSS. The results **(Table 5)** showed a significant association between the pyramidal function and movement/mobility difficulties (r = 0.41, p = 0.01). In addition, there was a significant association between the cerebellar function and interpersonal difficulties (r = 0.35, p = 0.03). Also, the bowel and bladder function was significantly associated with the following domains: movement/mobility difficulties (r = 0.36, p = 0.02), bowel/bladder difficulties (r = 0.63, p = 0.00), financial security concerns (r = 0.33, p = 0.04), workplace inaccessibility (r = 0.44, p = 0.00), pain/temperature difficulties (r = 0.35, p = 0.03), and fatigue (r = 0.40, *p* = 0.01).

Discussion

Employment continues to be of concern in persons with MS. However, few researches have been done in Serbian population to examine factors that may result in withdrawal from employment. Based on this, the aims of this study were to determine the work status and factors that may affect work engagement of people with MS.

The MSWDQ in Serbian was easy to use and our subjects completed it in less than 25 minutes. There were almost no comprehension difficulties. Internal consistency for all items was very high, which was generally in agreement with original scale developed in the Australian sample [11].

Work is an important aspect of human life, because besides livelihood it provides a feeling of self-respect, possibilities for independent life, and complete integration in the social community. Progressive diseases, such as MS, lead to difficulties in finding and keeping employment, thus affecting the

Table 5. Correlations between the MSWDQ subscales and EDSS function domains/total EDSS
Tabela 5. *Povezanost skala upitnika MSWDQ sa skorovima na funkcionalnim domenima i ukupnom Skalom za procenu neurološkog deficita*

Spearman rank correlation/*Spirmanova korelacija ranga*												
EDSS	GCD	MMD	WBD	NSW	BBD	FSC	LSE	PMD	WI	PTD	ID	FA
Visual/*Vizuelni*	-0.10	-0.14	-0.21	-0.31	-0.26	-0.26	-0.17	0.06	-0.22	-0.32	0.07	-0.23
Brainstem/*Moždano stablo*	-0.04	0.10	0.11	0.32	0.13	0.25	-0.00	0.03	0.11	0.12	-0.09	0.14
Pyramidal/*Piramidalni*	0.04	0.41*	0.21	-0.09	0.24	0.06	0.21	0.03	0.32	0.28	0.31	0.20
Cerebellar/*Cerebelarni*	0.08	0.23	0.08	-0.10	0.14	-0.10	-0.01	-0.14	0.22	0.08	0.35*	0.01
Sensory/*Senzorni*	-0.16	0.09	-0.05	0.12	0.09	0.19	0.00	-0.04	0.23	0.24	-0.04	0.16
Bowel and bladder	-0.04	0.36*	0.20	0.23	0.63**	0.33*	0.12	-0.03	0.44**	0.35*	0.26	0.40*
EDSS score/*EDSS skor za RAP creva i bešike*	-0.17	0.25	-0.07	-0.06	0.04	0.01	-0.08	-0.06	0.18	0.14	0.06	0.16

* significant at p<0.05; ** significant at p<0.01

Legend**:** GCD - General cognitive difficulties; MMD - Movement/mobility difficulties; WBD - Work/home balance difficulties; NSW - Non-supportive workplace; BBD - Bladder /bowel difficulties; FSC - Financial security concerns; LSE - Low self-esteem; PMD - Prospective memory difficulties; WI - Workplace inaccessibility; PTD - Pain/temperature difficulties; ID - Interpersonal difficulties; FA - Fatigue

Legenda: GCD – Opšte kognitivne teškoće; MMD - Teškoće s pokretljivošću; WBD – Teškoće usklađivanja posao/kuća; NSW – Nedostatak podrške na poslu; BBD – Teškoće sa crevima i bešikom; FSC – Zabrinutost za finansijsku sigurnost; LSE – Nisko samopouzdanje; PMD – Prospektivne poteškoće s memorijom; WI – Nepristupačnost radnog mesta; PTD – Teškoće sa bolovima i temperaturom; ID – Teškoće u interpersonalnim odnosima; FA - Zamor

degree of social participation. MS is associated with a high unemployment rate (50 – 60% of all patients) [10, 18]. Literature data show that most people with MS want to return to work [10, 19]. However, this is contrary to the trend in Serbia, where most people suffering from MS want to retire, in the light of the workforce in an unpredictable market.

Our study included significantly more women than men with MS. The disease, disability and the woman's role in the society, certainly affect professional life and represent a barrier in making a career when suffering from MS. A study published in 2000 [20], found that 60% of women with MS believed that lack of energy to meet all domestic and professional demands due to MS was a sufficient reason to leave employment. Personal factors also significantly influence a person's ability to cope with demands and needs of work.

The unemployment rate in people with MS increases with age, significantly more than in the general population, and according to the literature data it is attributed to increased disability [20]. The average age of our subjects was about 40 years, which is a productive age when a person should achieve professional self-actualization.

The professional status of people with MS is similar to that of the general population, since most have secondary or tertiary education and previous work experience. Even literature data from the last century confirmed that higher education was a protective factor against unemployment [21, 22]. Almost 65% of all subjects in our study came from an urban environment, which should also be a positive aspect of employment retention because it excludes commuting to work and increases chances of choosing a workplace.

Disease duration and progression have been proved to be predictive factors of the work status [18], which was partly confirmed by our study.

Due to its validity and reliability, the MSWDQ was suitable for this research. The questionnaire scores need further discussion. Although we did not find a statistical significance for the financial security concerns subscale, it is important to note that people with MS do perceive the existence of disease-related financial difficulties. In a national study of the economic impact of MS on families, Catanzaro and Weinert [23] found that 21% of families had inadequate incomes to cover medical expenses and 25% did not have sufficient means to satisfy basic living costs. In original MSWDQ, financial concerns were not predictive of work outcomes.

In the present study, the subscales that showed a high statistical significance may be grouped into subscales whose items relate to external factors – work environment (non-supportive workplace, workplace inaccessibility, interpersonal difficulties) and those whose items relate to internal factors – problems due to MS (memory difficulties, bowel/bladder difficulties, low self-esteem, pain/temperature difficulties). The results of previous similar studies also identified external factors as influential and emphasized the negative influence of poorly adjusted work structure for people with MS. This includes physical barriers, such as a lack of accommodation in the workplace or inaccessible bathrooms, inflexible work schedules and a lack of support from the employer and colleagues [24]. Our results obtained on the work environment subscales show that our subjects perceived their workplace as sufficiently supportive and accessible and had no interpersonal difficulties.

When the work status and workplace difficulties of people with MS are observed through the prism of disease and disability, it is important to consider how the EDSS, i.e. objective neurological deficit, contributes to our understanding of different domains of professional functioning of people with MS. We found a significant association between pyramidal function and mobility difficulties. Mobility difficulties may include commuting to work or workplace accessibility and spatial organization, and it is therefore not surprising that mobility is the factor that most significantly affects work hours and work ability. This was also confirmed by previous researches [25, 26]. The association of the recorded cerebellar deficits with interpersonal difficulties indicates that cognitive/mental dysfunction impairs communication at work through attention and memory problems, which was also reported by previous studies [27, 28]. These findings emphasize the importance of interpersonal relationships for job retention.

In our study, the bowel/bladder function was significantly associated with different difficulties at work, which results from the patient's fear of incontinence. This was recognized in previous studies as well [20, 29].

Although supportive measures for people with disability, including workplace accommodation and equipment, are available in Serbia, most subjects in our study did not need complex assistive devices.

Social and occupational position and factors affecting social participation of people with MS have not been studied so far in Serbia. Furthermore, no disease-specific questionnaires targeting different aspects of employment of people with MS have been used. However, considering that new definitions of disability presuppose taking into consideration the whole person, it is clear that only adequate functional assessment and rehabilitation of motor, cognitive and social abilities may enable full social participation and work engagement of people with MS.

A limitation of the present study was a small sample size and the lack of additional instruments for detection of potential cognitive and behavioral problems/impairments. For this reason, individuals who had not been working for more than three years were not eligible to fill out the questionnaire, because the data validity would be questionable, considering that a high percentage of people with MS may have unrecognized or underestimated their cognitive impairment/s. However, the goal of this pilot study was to translate the instrument and verify item performance before undertaking major data collection, and this was accomplished. The final version of the MSWDQ-Serbian is correct and idiomatic and may be widely used. It may provide faster and easier detection of workplace problems in people with MS and thus enable supportive measures for their employment retention.

Conclusion

The results suggest that people with multiple sclerosis are part of the global employment competition, and our analysis of environmental factors and factors affecting work engagement of people with multiple sclerosis showed that other factors affected their work more than their preferences or the disease. Further research in this field is required to define mechanisms that would solve practical problems affecting employment of persons with multiple sclerosis.

After adapting the multiple sclerosis work difficulties questionnaire to the Serbian language and cultural characteristics, we have obtained an instrument that does not differ much from the original English version. The final version of the multiple sclerosis work difficulties questionnaire-Serbian demonstrates satisfactory internal consistency and reliability. We have proved that the multiple sclerosis work difficulties questionnaire may be used in different countries.

References

1. Compston A, Coles A. Multiple sclerosis. Lancet. 2008;372(9648):1502-17.

2. Moore P, Harding KE, Clarkson H, Pickersgill TP, Wardle M, Robertson NP. Demographic and clinical factors associated with changes in employment in multiple sclerosis. Mult Scler. 2013;19(12):1647–54.

3. Pompeii LA, Moon SD, McCrory DC. Measures of physical and cognitive function and work status among individuals with multiple sclerosis: a review of the literature. J Occup Rehabil. 2005:15(1):69-84.

4. Miller A, Dishon S. Health-related quality of life in multiple sclerosis: the impact of disability, gender and employment status. Qual Life Res. 2006;15(2):259-71.

5. Zakon o profesionalnoj rehabilitaciji i zapošljavanju osoba sa invaliditetom. Službeni glasnik RS. 2009;(36):205-10.

6. Pravilnik o bližem načinu, troškovima i kriterijumima za procenu radne sposobnosti i mogućnosti zaposlenja ili održanja zaposlenja osoba sa invaliditetom. Službeni glasnik RS. 2010;(36).

7. Pravilnik o bližim uslovima, kriterijumima i standardima za sprovođenje mera i aktivnosti profesionalne rehabilitacije. Službeni glasnik RS. 2009;(112).

8. Pravilnik o načinu praćenja izvršavanja obaveze zapošljavanja osoba sa invaliditetom i načinu dokazivanja izvršavanja te obaveze. Službeni glasnik RS. 2010;(33).

9. Simmons RD, Tribe KL, McDonald EA. Living with multiple sclerosis: longitudinal changes in employment and the importance of symptom management. J Neurol. 2010;257(6):926-36.

10. Julian LJ, Vella L, Vollmer T, Hadjimichael O, Mohr DC. Employment in multiple sclerosis. Exiting and re-entering the work force. J Neurol. 2008;255(9):1354–60.

11. Honan CA, Brown RF, Hine DW, Vowels L, Wollin JA, Simmons RD, et al. The multiple sclerosis work difficulties questionnaire. Mult Scler. 2012;18(6):871-80.

12. Gjersing L, Caplehorn JR, Clausen T. Cross-cultural adaptation of research instruments: language, setting, time and statistical considerations. BMC Med Res Methodol. 2010;10:13.

13. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983;33(11):1444-52.

14. Sharrack B, Hughes RA. The Guy's Neurological Disability Scale: a new disability measure for multiple sclerosis. Mult Scler. 1999;5(4):223-33.

15. Ravnborg M, Gronbech-Jensen M, Jonsson A. The MS Impairment Scale: a pragmatic approach to the assessment of impairment in patients with multiple sclerosis. Mult Scler. 1997;3(1):31-42.

16. Fajgelj S. Psihometrija metod i teorija psihološkog merenja. Beograd: Centar za primenjenu psihologiju; 2009.

17. Cronbach LJ. Coefficient alpha and the internal structure of tests. Psychometrika. 1951;16(3):297–334.

18. Strober L, Chiaravalloti N, Moore N, DeLuca J. Unemployment in multiple sclerosis (MS): utility of the MS Functional Composite and cognitive testing. Mult Scler. 2014;20(1):112-5.

19. O'Connor RJ, Cano SJ, Torrenta L, Thompson AJ, Playford ED. Factors influencing work retention for people with multiple sclerosis: cross-sectional studies using qualitative and quantitative methods. J Neurol. 2005;252(9):892-6.

20. Dyck I, Jongbloed L. Women with multiple sclerosis and employment issues: a focus on social and institutional environments. Can J Occup Ther. 2000;67(5):337-46.

21. La Rocca N, Kalb R, Kendall P, Scheinberg L. The role of disease and demographic factors in the employment in people with multiple sclerosis. Arch Neurol. 1982;39(4):256.

22. Patti F, Pozzilli C, Montanari E, Pappalardo A, Piazza L, Levi A, et al. Effects of education level and employment status on HRQoL in early relapsing-remitting multiple sclerosis. Mult Scler. 2007;13(6):783-91.

23. Catanzaro M, Weinert C. Economic status of families living with multiple sclerosis. Int J Rehabil Res. 1992;15(3):209-18.

24. Johnson KL, Klasner ER, Amtmann D, Kuehn CM, Yorkston KM. Medical, psychological, social, and programmatic barriers to employment for people with multiple sclerosis. J Rehabil. 2004;70(1):38-49.

25. Johnson KL, Bamer AM, Fraser RT. Disease and demographic characteristics associated with unemployment among working-age adults with multiple sclerosis. Int J MS Care. 2009;11(3):137-43.

26. Salter AR, Cutter GR, Tyry T, Marrie RA, Vollmer T. Impact of loss of mobility on instrumental activities of daily living and socioeconomic status in patients with MS. Curr Med Res Opin. 2010;26(2):493-500.

27. Smith MM, Arnett PA. Factors related to employment status changes in individuals with multiple sclerosis. Mult Scler. 2005;11(5):602-09.

28. Sweetland J, Riazi A, Cano SJ, Playford ED. Vocational rehabilitation services for people with multiple sclerosis: what patients wants from clinicians and employers. Mult Scler. 2007;13(9):1183-9.

29. Roessler RT, Rumrill PD Jr. Multiple sclerosis and employment barriers: a systemic perspective on diagnosis and intervention. Work. 2003;21(1):17-23.

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BIODENTINE™ AS A FURCAL PERFORATION REPAIR MATERIAL – A CASE SERIES

BIODENTIN™ – MATERIJAL ZA REPARACIJU FURKALNIH PERFORACIJA – PRIKAZ SERIJE SLUČAJEVA

Sonja APOSTOLSKA[1], Marina EFTIMOSKA[1], Vasilka RENDŽOVA[1], Sašo ELENČEVSKI[2], Nadica JANEVA[2] and Aleksandra POPOVAC[3]

Summary

Introduction. So far, the most promising and most commonly used materials in endodontic treatment and retreatment were calcium silicate cements. However, due to the shortage of this material and treatment failures, a new bioactive material was introduced - Biodentine[tm]. It is a calcium silicate based technology, with excellent handling characteristics and biocompatibility. It can be used in various indications, including dentine substitution and endodontic therapy. **Case reports.** The clinical cases demonstrated excellent healing potential after the treatment with Biodentine[tm]. **Conclusion.** The bio-silicate technology is highly promising, mostly due to its chemical properties and easy clinical manipulation. The short setting time and high mechanical strength makes Biodentine[tm] a material easy to handle, highly biocompatible, with a wide range of indications.

Key words: Biocompatible Materials; Root Canal Filling Materials; Root Canal Preparation; Furcation Defects; Endodontics; Silicates; Calcium Phosphates; Treatment Outcome

Sažetak

Uvod. Do sada, najčešće korišćeni materijali u endodonciji, i ujedno materijali koji najviše i obećavaju u endodontskom tretmanu i retretmanu su bili kalcijum-silikatni cementi. Međutim, u skladu sa nedostacima ovog materijala i neuspesima tretmana, javio se novi materijal *Biodentine[tm]*. On je napravljen na kalcijum-silikatnoj tehnologiji sa odličnom biokompatibilnošću kao i karakteristikama u vezi sa aplikacijom materijala. Može da se koristi kod različitih indikacija, uključujući supstituciju dentina i endodontsku terapiju. **Prikaz slučajeva.** Klinički slučajevi pokazuju odličan potencijal lečenja nakon tretmana *Biodentinom[tm]*. **Zaključak.** Biosilikatna tehnologija veoma obećava najviše zbog hemijskih karakteristika i lake kliničke manipulacije. Kratko radno vreme i velika mehanička jačina čini *Biodentine[tm]* materijalom koji je jednostavan za korišćenje, visoke biokompatibilnosti i sa velikim opsegom indikacija.

Ključne reči: biokompatibilni materijali; materijali za punjenje zubnog kanala; preparacija zubnog kanala; defekti furkacije; endodoncija; silikati; kalcijum fosfati; ishod lečenja

Introduction

The calcium silicate cements, initially proposed as materials for retrograde obturation, have become the materials of choice for all kinds of dentine defects, including communication pathways between the root canal system and the periodontal ligament [1]. Their proven biocompatibility and ability to initiate calcium and phosphate incorporation at the interface with the periodontal tissue have a primary role in the reparation of the bone tissue [2–4]. The main disadvantage of this class of materials so far has been the slow application and complicated handling, which makes them technique-sensitive and hard to be used in everyday clinical practice [5].

Biodentine[tm] (Septodont, Saint-Maur-des-Fossés, France) is a relatively new material, which is the first all-in-one bioactive and biocompatible dentine replacement, based on the unique biosilicate technology. It can be used in a variety of indications, such as dentine substitution and in endodontic therapy. Biodentine[tm] employs a simplified clinical procedure. The healing process is an effect of the physical properties of this material, similar to the human dentine. The modified powder composition ensures the optimal physical characteristics of this material, and the pre-dosed capsule formulation highly simplifies the application procedure [6].

This paper aims to present several clinical cases treated for furcal perforation repaired with Biodentine[tm].

Case reports

Case 1. Reparation of the pulp chamber floor perforation following inappropriate endodontic treatment.

A 49-year-old male patient was referred to the Department of Restorative Dentistry and Endodontics due to a complication after a previous endodontic treatment. He complained of persistent pain on pressure in tooth 16, which started 2 weeks before. The tooth was

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Abbreviations
MTA – mineral trioxide aggregate

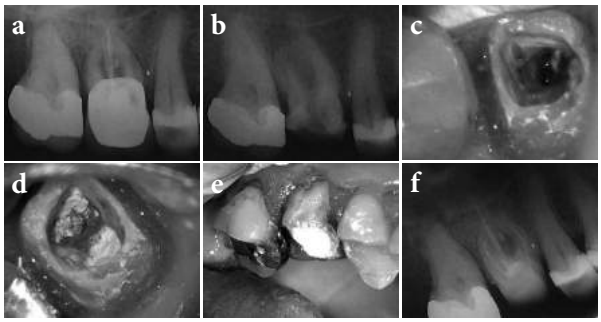

Figure 1. a. Preoperative radiographic view showing inadequate endodontic treatment and furcal perforation; **b.** Radiograph after the removal of the existing prosthetic crown and canal filling; **c.** Preoperative clinical view showing the furcation perforation; **d.** Obturation of root canals; **e.** Biodentine^{tm} used to manage the perforation and as a dentine substitute; **f.** After 6 months, complete healing of the furcal region is visible
Slika 1. **a.** *Preoperativni radiograf sa neadekvatnim endodontskim tretmanom i furkalnom perforacijom;* **b.** *Postoperativni radiograf nakon odstranjivanja protetske krune i kanalnog punjenja;* **c.** *Kavitet nakon endodontskog retretmana sa vidljivom furkalnom perforacijom;* **d**. *Obturacija kanala korena;* **e.** *Biodentin^{tm} je korišćen za prekrivanje perforacije i kao dentinska zamena;* **f.** *Nakon šest meseci, vidljivo ozdravljenje furkalne perforacije*

slightly tender on percussion and showed no mobility. The preoperative radiograph showed furcal perforation and erroneous access in the bucomesial aspect of the root canals **(Figure 1a)**. The patient had a history of root canal treatment, 3 weeks prior, which he had discontinued halfway. After the removal of the existing prosthetic crown, clinical examination revealed a large access cavity and a perforation of 1.5 mm on the floor of the chamber **(Figures 1b and 1c)**. The patient was informed about the situation and a decision was made to attempt repair the perforation followed by root canal retreatment. A signed consent was obtained from the patient and the treatment was initiated. The access cavity was refined and working lengths were determined using a No. 10 and 15 files. The canals were cleaned and shaped and sealed with calcium hydroxide for 2 weeks. After the remission of symptoms, the canals were irrigated and obturated by GuttaFlow **(Figure 1d)**. Then, the perforation and the entire cavity were restored with Biodentine^{tm}. Biodentine^{tm} was manipulated by mixing five drops of the liquid provided by the manufacturer into the capsule. The capsule was then placed on a mixing device, amalgamator for 30 seconds. The mix was carried to the site of perforation **(Figure 1e)**. Six months later, complete healing of the furcal region was visible **(Figure 1f)**.

Case 2. Endodontic retreatment, perforation repair of the pulp chamber floor following inappropriate endodontic treatment and dentine substitution.

A 32-year-old male patient presented to the Department of Conservative Dentistry and Endodontics with a complaint of pain to percussion in tooth 16. The preoperative radiograph showed inadequately sealed root canals, periapical lesions with iatrogenic lateral root perforation of tooth 16 **(Figure 2a)**. Iatrogenic lateral root perforation with incomplete obturation was diagnosed, and with the patient's consent an attempt was made to preserve the tooth by sealing the endo-perio communication. After removal of the existing restoration, the endodontic treatment was performed in two phases: after removal of the filling, the canals were instrumented using No 35 k file, calcium hydroxide was used as an intracanal medication and closure of the periodontal communication with glass ionomer cement for 2 weeks **(Figure 2b)**. After 2 weeks, the tooth was asymptomatic and the canals were dry. After the remission of symptoms, apical seal with Biodentine^{tm} and final root canal sealing was performed **(Figure 2c)**. Then, the perforation and the entire cavity were restored with Biodentine^{tm}. The follow up at two months showed no clinical signs, and the X-ray confirmed complete healing of the apical and perforation site and the patient was referred to a prosthodontist. Two years after treatment, the tooth was painless and fully functional **(Figure 2d),** as well as 7 years after treatment **(Figure 2e)**.

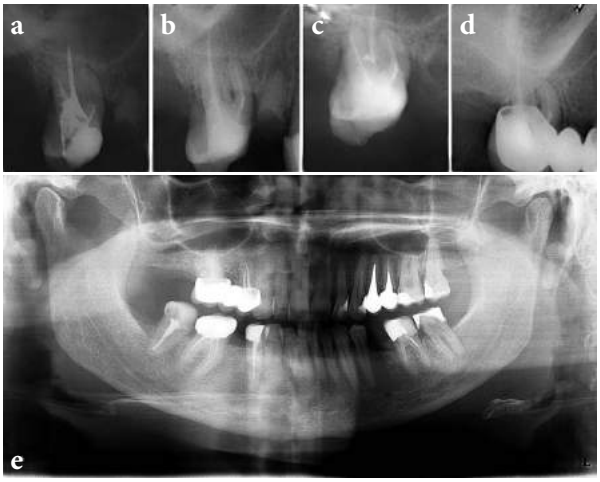

Figure 2. a. Inadequate endodontic treatment, furcal perforation; **b.** Calcium hydroxide canal filling, application of glass ionomer cement and distal closure of the periodontal communication; **c.** Definitive sealing of the root canals with guttapercha and sealer; the cavity was restored with Biodentine^{tm}; **d.** Appearance of the prosthetic appliance after 2 years; **e.** Appearance of the prosthetic appliance after 7 years
Slika 2. **a.** *Neadekvatan endodontski tretman, usled furkalne perforacije;* **b.** *Punjenje kanala kalcijum-hidroksidom, aplikacija glas-jonomer cementa i distalno zatvaranje periodontalne komunikacije;* **c.** *Definitivno zatvaranje kanala korena gutaperkom i pastom, kavitet je restauriran Biodentinom^{tm};* **d.** *Izgled zubne nadoknade nakon 2 godine;* **e.** *Izgled zubne nadoknade nakon 7 godina*

Discussion

Iatrogenic pulp floor perforation can occur if the operator becomes disorientated when trying to locate canal orifices. Perforation repair can be technically challenging, and offering referral if treatment is beyond the expertise of the operator [7]. Perforation is defined as

the pathological or iatrogenic communication between the root canal space and the periodontal tissue. Furcal perforation is usually an undesired complication that can occur during preparation of endodontic access cavities or exploring canal orifice of multirooted teeth [8]. These undesirable situations such as excess removal of tooth structure or perforation occur during attempts to locate canals or as a direct result of failing to achieve straight line access to the canals. In the process of searching for canal orifices, perforations of the crown can occur either peripherally through the sides of the crown, or through the floor of the chamber into the furcation [9]. The interval between perforation and repair is one of the critical factors for success. Immediate sealing of perforations enhances the repair process, by reducing the possibility of bacterial contamination of the defect. In the current cases, the perforation in the furcation and sufficient coronal structure was present, so we decided to repair the perforation with a biocompatible material, Biodentine^{tm}. Biodentine^{tm} is a calcium silicate-based bioactive material. It is a powder liquid system, powder composed of Tri-calcium silicate, Dicalcium silicate, Calcium carbonate and oxide, Iron oxide, Zirconium oxide. Liquid consists of Calcium chloride, Hydro soluble polymer [10, 11]. Biodentine^{tm} contains tricalcium silicate with additives such as powder and a liquid, containing Calcium chloride to speed up setting. Calcium silicate materials have excellent biocompatibility and are able to induce calcium-phosphate precipitation at the periodontal ligament interface allowing bony healing (Tay, et al., 2007, Torabinejad and Parirokh, 2010). With a reduced setting time compared to mineral trioxide aggregate (MTA), Biodentine^{tm} is perhaps more user-friendly for perforation repair [12]. It is easy to handle owing to its ease of manipulation and a short setting time - approximately 12 minutes, has high alkaline pH and is a biocompatible material that makes it a favorable material for perforation repair [13, 14]. In a study by Guneser et al., Biodentine^{tm} showed considerable performance as a perforation repair material even after being exposed to various endodontic irrigants as compared to MTA [15]. However, very few papers are available regarding the use of Biodentine^{tm} as a perforation repair material. The use of Biodentine^{tm} seems promising in the present cases. As the setting is faster, there is a lower risk of bacterial contamination than with MTA. Adding to its ability to be used as dentine substitute, Biodentine^{tm} could safely be used for each indication where dentine is damaged [16]. Therefore, it is an advantage to the clinician and the patient.

Conclusion

The use of Biodentine^{tm} for repair of furcal perforations is associated with a good short-term clinical outcome. The new bio-silicate technology, represented by Biodentine^{tm} is highly promising, mostly due to its chemical properties and easy clinical manipulation. The short setting time and high mechanical strength makes Biodentine^{tm} a material easy to handle, highly biocompatible and with wide range of indications (such as endodontic procedures and as a dentin substitute in restorations).

References

1. Jurhar-Pavlova M, Petlichkovski A, Trajkov D, Efinska- Mladenovska O, Arsov T, Strezova A, et al. Inf luence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. Vojnosanit Pregl. 2003;60(6):657-61.

2. Bogen G, Kuttler S. Mineral trioxide aggregate obturation: a review and case series. J Endod. 2009;35(6):777-90.

3. Reyes-Carmona JF, Felippe MS, Felippe WT. Biomin- eralization ability and interaction of mineral trioxide aggregate and white portland cement with dentine in a phosphate containing f luid. J Endod. 2009;35(5):731-6.

4. Tay FR, Pashley DH, Rueggeberg FA, Loushine RJ, Weller RN. Calcium phosphate phase transformation produced by the interaction of the portland cement component of white mineral trioxide aggregate with a phosphate-containing f luid. J Endod. 2007;33(11):1347-57.

5. Torabinejad M, Parirokh M. Mineral trioxide aggregate: a comprehensive literature review – Part II: leakage and bio- compatibility investigations. J Endod. 2010;36(2):190-202.

6. Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review – Part I: chemical, physical, and antibacterial properties. J Endod. 2010;36(1):16-27.

7. General Dental Council. Standards for the dental team. September 2013. [cited 2015 Jun 26]. Available from: https:/www.gdc-uk.org/Dentalprofessionals/Standards/Pages/standards.aspx.

8. Frank RJ. Endodontic mishaps: their detection, correc- tion, and prevention. In: Ingle JI, Bakland LK, editors. Endo- dontics. 5th ed. London: BC Decker Inc.; 2002. p. 769-94.

9. Roda RS. Root perforation repair: Surgical and nonsurgical management. Pract Proced Aesthet Dent 2001;13:467-72.

10. Wang X, Sun H, Chang J. Characterization of Ca3SiO5/CaCl2 composite cement for dental application. Dent Mater 2008;24:74-82.

11. Raskin A, Eschrich G, Dejou J, About I. In vitro microleakage of Biodentine as a dentin substitute compared to Fuji II LC in cervical lining restorations. J Adhes Dent 2012;14(6):535-42.

12. Wongkornchaowalit N, Lertchirakarn V. Setting time and flowability of accelerated Portland cement mixed with polycarboxylate superplasticizer. J Endodontics. 2011;37(3):387-9.

13. Priyalakshmi S, Ranjan M. Review of Biodentine - a bioactive dentin substitute. IOSR Journal of Dental and Medical Sciences. 2014;13:13-7.

14. Han L, Okiji T. Uptake of calcium and silicon released from calcium silicate–based endodontic materials into root canal dentine. International Endodontic Journal. 2011;44:1081-7.

15. Guneser MB, Akbulut MB, Eldeniz AU. Effect of Various Endodontic Irrigants on the Push-out Bond Strength of Biodentine and Conventional Root Perforation Repair Materials. J Endod. 2013:39-3. 380-4.

16. Koubi G, Colon P, Franquin JC, Hartmann A, Richard G, Faure MO, et al. Clinical evaluation of the performance and safety of a new dentine substitute, Biodentinetm, in the restoration of posterior teeth - a prospective study. Clin Oral Investig 2013;17(1):243-9.

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TRAINING FOR GENES – HOW TO DESIGN IT?

TRENING ZA GENE – KAKO GA DIZAJNIRATI?

Jelena Ž. POPADIĆ GAĆEŠA

Summary

Introduction. The aim of this short review was not to be just another systematic report, but to highlight further research hypotheses regarding the challenges in performance genomics by focusing on three papers published in 2016, which offer innovative and promising approach that would be a breakthrough in more exact application of genetic data in practical work of sports experts and training design. **Genes for sports.** More than 200 single nucleotide polymorphisms and genetic traits associated with fitness performance have been reported in numerous studies, but genes for angiotensin converting enzyme and alpha-actinin-3 are most frequently associated with enhanced physical performance. **Perspectives of epigenetics.** Genotype-phenotype interactions include a wide range of molecular mechanisms with complex effects and interconnections. **Gene adjusted training protocols.** Using genetic profiling to match individual genotype with appropriate training modality may be a powerful tool providing personalized athletic training in the future. **Conclusion.** When applying genetic profiling prior to and during training programs, special consideration should be made to avoid athlete selection; it should be only used for inclusion, not for exclusion. Also, attention must be paid to social and ethical issues. Wider approach should include training interventional studies and non-athletic population in discovering new molecular pathways of muscle adaptation to exercise through genotype-phenotype interactions.

Key words: Athletic Performance; Genotype; Gene Expression; Polymorphism, Single Nucleotide; Exercise; Programs; Peptidyl-Dipeptidase A; Actinin; Muscle, Skeletal

Sažetak

Uvod. Cilj ovog kratog revijalnog članka nije da bude još jedan sistematski izveštaj, već da naglasi moguće buduće istraživačke hipoteze o izazovima u genomici fizičke aktivnosti fokusirajući se na tri naučna rada objavljena 2016. godine, koji nude inovativni i obećavajući pristup koji bi mogao doneti proboj ka egzaktnijoj primeni genetskih podataka u praktičnom radu sportskih stručnjaka i dizajnu treninga. **Geni za sport.** Više od 200 različitih polimorfizama jednog nukleotida (*single nucleotide polymorphism*) i genetskih osobina udruženih sa sportskim postigućem objavljeni su u brojnim studijama, ali geni za angiotenzin-konvertujući enzim i alfa-aktinin-3 najčešće su povezivani sa poboljšanim posignućem. **Perspektive u epigenetici.** Interakcije između genotipa–fenotipa uključuju širok dijapazon mehanizama sa kompleksnim uticajima i međupovezanošću. **"Genu prilagođeni" trenažni protokoli.** Koristeći genetsko profilisanje radi boljeg poklapanja individualnih genotipova sa odgovarajućim trenažnim modalitetima moglo bi predstavljati moćno oruđe u postizanju detaljnije personalizovanog treninga u budućnosti. **Zaključak.** Kada se primenjuje genetsko profilisanje pre i tokom trenažnih programa, posebnu pažnju bi trebalo obratiti na to da se izbegne njegovo korištenje u selekciji; ono se može upotrebiti samo kao uključujući kriterijum, a ne kao isključujući kriterijum. Takođe, pažnja se mora posvetiti i socijalnim i etičkim pitanjima. Širi pristup bi trebalo da uključi studije sa trenažnim intervencijama i populacije nesportista u otkrivanju novih molekularnih puteva mišićne adaptacije na fizičku aktivnost kroz interakcije genotip–fenotip.

Ključne reči: sportsko postignuće; genotip; ekspresija gena; polimorfizam jednog nukleotida; vežbanje; programi; angiotenzin konvertujući enzim; aktinin; skeletni mišići

Introduction

Skeletal muscle is a tissue that possesses an intrinsic capacity to adapt to the type of physical activity it is required to perform. The adaptation is taking place during growth in the childhood, as a response to training, and its decline occurs during aging. In training, the onset and degree of adaptation depend on type, intensity and duration of the training stimulus. Highly specialized sport disciplines also demand highly specialized types of training which will favor specific metabolic muscle adaptation.

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Abbreviations

SNP	– single nucleotide polymorphism
ACE	– angiotensin converting enzyme
ACTN3	– alpha actinin 3
BRDKB2	– bradykinin receptor B2
GWAS	– genome-wide association studies
BASES	– British Association of Sport and Exercise Sciences
DNA	– deoxyribonucleic acid

The aim of training is achieving biological adaptation of the organism in line with its genetic potential, in order to accomplish specific tasks, with increased load. It is clear, therefore, that hard work is necessary to accomplish desired sports results.

Modern athletic training has two major aims: to systemize the training process and to make quantification possible. Monitoring and evaluation of training effects is possible by using functional tests, always having in mind to choose a test which has to be specific for the metabolic profile of the sport in which participant is involved in.

At the beginning of the third millennium, new perspectives have opened in modern sport in which genetics has an increased importance. By introducing new methods for gene expression profile analysis, metabolic adaptation adds a new dimension, on the molecular level, through processes of transcription and translation. Can these methods be used for designing athletic training? With the human genome decoded, is it possible to determine who has genetic potentials for top athletic achievements? The answers to these and similar questions are burdened by the fact that a variety of genetic expressions is controlled by various signaling pathways, most of them still partially known or unknown.

Since muscle cells react to mechanical, but also to chemical signals, it is probable that the training should be more scientifically based, using methods of genomics, proteomics and epigenetics in optimization the training program to gain adequate muscle mass and fitness phenotype for specific physical activity.

Still, this will probably refer only to fine tuning, rather than replacement of training that has been applied in ad hoc manner for decades. Anyhow, as 1 to 2% can make a difference between Olympic gold and failure to qualify, we can say with high certainty that this new biochemical technology will be used aiming to improve and enhance sports achievements and to develop genetic potential on a more scientifically based level.

Genetic era has certainly brought novelties into sports medicine. How will the development of young top athletes be designed in the future? Can gene therapy be used in sports and misused as well? We cannot get answers to these and other questions concerning modern sports soon, but they will certainly raise new questions and challenges in this area. One thing is certain – all the possibilities to enhance and raise limits of human performance are not yet exhausted.

The exercise genomics is also very promising in the population of non-athletes in the research of muscle adaptation processes in various interactions and interconnections between genotype and environmental stimuli, knowing that genes are not sufficient for achievement, but we do not know exactly how to train them to reach maximum performance.

More than 200 single nucleotide polymorphisms (SNPs) associated with performance and fitness-related traits have been reported in the literature. These genetic traits have been identified as effective in muscle morphology, function, adaptation and physical performance in humans, and reported in numerous studies [1–6]. The literature in this area is rich, with extensive reviews published on the subject, and regular annual review reports about current status of research in exercise genomics. However, advances in this area are not as fast as expected.

Therefore, the aim of this review was not to be just another systematic report, but to highlight new and promising approaches in performance genomics by focusing on three interesting papers published in 2016 [7–9]. They offer a refreshing and innovative approach that would be a breakthrough in exact application of genetic data in practical work of sports experts.

Genes for sports

Skeletal muscles can adapt to different levels of physical activity by increasing muscle size and strength. The capacity of adaptation depends on multiple factors, like genetic predisposition, hormonal influences, neural components, metabolic factors, muscle fiber composition and the training protocol [10–19].

The genetic factors are estimated to account for 20 – 80% of various aspects of athletic performance [20]. A number of genes have been discovered that likely influence specific performance both in athletes and non-athletes. In the decades since the gene for angiotensin converting enzyme (ACE) was first proposed to be a "human gene for physical performance" [21, 22], there have been numerous studies examining the effects of ACE and other genes on athletic status through its association with strength/power, aerobic capacity, muscle fiber composition [6, 23–26]. So far, only ACE, alpha-actibnin 3 (ACTN3) and bradykinin receptor B2 (BRDKB2) (due to connections with ACE) [27, 28], are genes most frequently connected with enhanced performance.

For ACE gene, D allele is associated with increased ACE activity, which converts angiotensin I to angiotensin II (potent vasoconstrictor) and degrades bradykinin (vasodilator), while allele I is associated with increased endurance performance and increased kinin activity. Effect of bradykinin on muscular blood flow and metabolism was also studied [29]. The combination of ACEI/BDKRB2-9 is associated with increased muscle efficiency and endurance through elevation of kinin activity [28].

The ACTN3 gene encodes actin-binding protein alpha-actinin-3 which has a structural role of the Z-line only in type II fibers. X allele leads to complete deficiency of actinin, and XX genotype is rarely seen in athletes. ACTN3 showed no association with muscle metabolic characteristics, and in knockout mice study, although muscles had similar fiber proportions as the wild type mice, reduced muscle mass with decreased fast-twitch fiber diameter, increased aerobic enzymes concentration, contraction time, and decreased recovery period were reported [30].

Beside the above described genes, there are more than 200 other genes reported to be linked with some variations in human performance, mainly in the aspects of strength/power and endurance. However, some genes are connected to cardiovascular, pulmonary, endocrine, or central nervous system regulation related to exercise [31–33]. **Table 1** presents several polymorphisms for genes most frequently connected with the physical performance.

Although numerous studies examined effects of different genes on athletic status and physical performance, only 25% of these genetic markers were positively associated with performance in at least two studies for polymorphism responsible for the substantial heritability of performance-related phenotypes [34, 35]. This indirectly implies relative magnitude of the genetic contribution to sporting performance.

Advanced molecular technologies with simultaneous genotyping of numerous markers shifted study designs from family studies, like HERITAGE study [36] to population-based association studies, which can be candidate-gene association studies or genome-wide association studies (GWAS).

Genome-wide association studies approach was introduced to identify new SNPs that confer susceptibility to sprint/endurance performance in world-class athletes. The study is ongoing, and 17 SNPs were identified by Jamaican group that may be connected to sprint performance in elite athletes [35]. Some new traits were recently discovered and published by using GWAS approach [37]. But, the most recent study of Rankinen et al. from GWAS failed to show that world-class athletes possess higher concentrations of endurance performance alleles than sedentary controls [7]. As Rankinen et al. reported, GWAS studies were undertaken on two cohorts of elite endurance athletes and controls (GENATHLETE and Japanese endurance runners), from which a panel of 45 promising markers was identified, then tested for replication in seven additional cohorts and controls, with a total of 1.520 endurance athletes and 2.760 controls. Although world-class athletes were more likely expected to have an endurance genetic profile, only one statistically relevant marker was found, and the study did not identify a panel of genomic variants common to these elite endurance athlete groups. Further analyses of some suggestive alleles (as the study was underpowered to identify alleles with small effect sizes) in controlled exercise training studies could expand our knowledge in this area [7], as a novel direction in exercise genomics research.

So far, extensive literature analysis marked several important advantages, as well as disadvantages in the research approach in exercise genomics. Advantages include development of new technology, low cost analyses, higher availability of genotyping, designing GWAS, inclusion of top athlete cohorts, with fine mapping/targeted resequencing and multi-centric study models. On the other hand, disadvantages include the following: most known genes may have only accidental association with phenotypes of interest, many genes are involved in sporting performance, large cohorts are unachievable for SNPs, small effect size, acute training effects, and in many cases only association with genotype, which does not have to mean connection with gene variant. Also, many other factors directly or indirectly influence the final athletic performance, like social and psychological ones.

Rees et al. recently elaborated and discussed in detail current knowledge on the development of best sporting talents, reviewing extensive literature on what is the most important contributor to the development of super-elite performance in sport [8]. Taking into consideration personality, environment, training and other factors, authors reported only moderate quality of the evidence that any of these parameters individually are important contributors to the development of super-elite performance. The authors emphasized that genetics, together with anthropometric and physiological factors, presents a significant contributor in talent development strategy, with a recommendation to sports practitioners to use these personality profiling for talent development, but not for talent selection purposes. Also, the trajectory to super-athletes status appears distinctly non-linear, involving repeated selection and de-selection, rather than linear progression within athletes support programs, which suggests that early athletes support programs are not the sole route to the development of the talent, that they should be open for access at all age ranges, and to monitor de-selected athletes for potential return, with special attention paid to progress fail in many talented athletes at transition from junior elite to elite level. Thus, early specialization should be avoided if not necessary, with promoting opportunities for young athletes to experience non-organized play and sampling in variety of sports. Other factors include socio-economic status, the reminiscence effect (improvement of performance that occurs while the subject is resting), emotional factors, and other complex interactions of an athlete and environment during growth, which all make discovering and nourishing a sports talent so difficult, as well as impossible to be completely scientifically based and predicted [8].

As Lucia et al. pointed in their review [38], being an athletic champion takes much more than having "champion genes", with making sport achievement of excellence even from the scientific and theoreti-

Table 1. The best known genes associated with physical performance
Tabela 1. *Najpoznatiji geni povezani sa fizičkim postignućem*

Gene *Gen*	Product *Produkt*	Functions *Funkcije*	SNP/*JNP - Jednonukleotidni polimorfizam*	Effects *Efekti*
ACE	Angiotensin I converting enzyme *Angiotenzin I konvertujući enzim*	Synthesis of angiotensin II (vasoconstriction) and degradation of vasodilator kinins/*Sinteza angiotenzina II (vazokonstrikcija) i razgradnja vazodilatatora kinina*	Alu I/D (rs4646994) l: 17q23.3	I: endurance D: power *I: izdržljivost* *D: snaga*
ACTN3	α-actinin-3 *α-aktinin-3*	Muscle contractility in fast-twitch muscle fibers/*Mišićna kontraktilnost u brzokontra- hujućim mišićnim vlaknima*	R577X (rs1815739 C/T) l: 11q13.1	T: endurance C: power *T: izdržljivost* *C: snaga*
BDKBR2	Bradykinin receptor B2 *Bradikinin receptor B2*	Endothelium-dependent vasodilation/*Endotelijalno-zavisna vazodilatacija*	+9/-9 (exon1) rs1799722 C/T l: 14q32.1-q32.2	-9: endurance T: endurance *T: izdržljivost*
CNTF	Ciliary neurotrophic factor/*Cilijarni neurotrofni faktor*	Differentiation and survival of motor neurons/*Diferencijacija i preživljavanje motornih neurona*	rs1800169	Power *Snaga*
IGF1	Insulin-like growth factor-1/*Insulinu sličan faktor rasta-1*	Muscle growth and development *Mišićni rast i razvoj*	rs6220, rs7136446	Power *Snaga*
IL6	Interleukin-6 *Interleukin 6*	Cytokine engaged in regulation of differentiation, proliferation and survival of target cells/*Citokini angažovani u regulaciji diferencijacije, proliferacije i preživljavanja ciljnih ćelija*	-174 C/G (rs1800795 C/G) l: 7p21	G: power *Snaga*
PPARA	Peroxisome proliferator-activated receptor α *Peroksizom roliferatorni-aktivirajući receptor α*	Regulates liver, heart and skeletal muscle lipid metabolism, glucose homeostasis, mitochondrial biogenesis, cardiac hypertrophy/*Reguliše metabolizam masti u jetri, srcu i skeletnom mišiću, homeostazu glukoze, biogenezu mitohondrija, hipertrofiju srčanog mišića*	G/C (rs4253778 G/C) l: 22q13.31	G: Endurance C - Power *G: Izdržljivost* *C: Snaga*
PPARGC1A	Peroxisome proliferator-activated receptor γ coactivator 1α *Peroksizom roliferatorni-aktivirajući receptor γ koaktivator 1α*	Regulates fatty acids oxidation, glucose utilization, mitochondrial biogenesis, thermogenesis, angiogenesis, formation of muscle fibers/*Reguliše oksidaciju masnih kiselina, utilizaciju glukoze, biogenezu mitohondrija, termogenezu, angiogenezu, formiranje mišićnih vlakana*	Gly482 (G) (rs8192678 G/A)	G: Endurance *G: Izdržljivost*

cal point very unpredictable, genetic profiling should not be used for selection, but for development of an individual potential. All the information should be implemented in interventional studies with non-athletic population as well, which could also represent an important new dimension of exercise genomics.

Perspectives of epigenetics

Epigenetics has a growing influence in the field of exercise genomics. A predisposition to achieve excellence in sports does not only depend on genes, but also on the level of their expression, and therefore on numerous mechanisms influencing transcriptional and post-transcriptional regulation processes [39].

The regulation of gene expression is a fundamental process that establishes and impacts the phenotype of each tissue. Each cell type possesses its own gene expression mechanisms, which are conducted by a specific epigenetic pattern. Epigenetics focuses on accessibility of deoxyribonucleic acid (DNA) to the transcriptional processes, association of the epigenetic modifications with gene expression patterns, and their potential effects on the phenotype [38–40]. Epigenetic modifications include DNA methylation and histone modifications, with epigenetic inheritance through genomic imprinting [39]. Therefore, genotype-phenotype interactions include a wide range of mechanisms with complex interactions and interconnections, which additionally complicate predetermination of individual athletic physical potential.

Acute exercise effect on molecular processes broadens the horizon in epigenetic researches. Acute gene activation is associated with a dynamic and transient change in DNA methylation in skeletal muscle and DNA hypomethylation is an early event in contraction-

induced gene activation, which provides new evidence that the epigenetic marks across the genome undergo dynamic variations as a response to acute as well as chronic stimuli [40]. This and similar findings should be kept in mind when analyzing epigenetic inflow on performance genes and their expression.

New perspectives of genetic testing for prevention of sports injuries and treatment will bring new aspects of its application in the field of sports sciences but also rehabilitation medicine [41]. Also, ethical issues regarding genetic testing of athletes are very important and widely discussed in the literature [42, 43]. The British Association of Sport and Exercise Sciences (BASES) Molecular Exercise Physiology Interest Group has produced a position stand to advise on current issues in genetic research and testing in sport and exercise sciences (BASES position stand on Genetic Research and Testing in Sport and Exercise Science). However, discussion on this subject is beyond the scope of this review.

Gene adjusted training protocols

Angiotensin coverting enzyme and ACTN3 genes (and BDKRB2 due to the association with ACE) are the most frequently studied and mostly linked to enhanced performance, with physiological mechanisms of their influences studied in detail. Introduction of GWAS showed promising approach in discovering new SNPs for "fitness genes". However, it is not easy to link genotypes with athletic performance phenotypes. The most recent report from Rankinen et al. (including GENATHLETE project) failed to show that world-class athletes possess higher concentrations of endurance performance alleles compared to sedentary controls [7]. Therefore, trying to find the perfect athletic genotype will not be as easy and practical as expected, since it is a complex multifactorial interaction with variable successful outcomes in numerous super athletes worldwide.

Although numerous studies reported a variety of gene/athletic performance interactions, there are inconsistencies for most of the SNPs in different studies, as many genes are involved in sports performance with their variable expression and functional interactions which are mainly unknown and very complex. One of the most important disadvantages so far is the lack of interventional training studies which could explore training effects and their association with genotype.

Since the HERITAGE study reported individual response to training stimulus and its linkage to genetic profile [36], some other researchers correlated different polymorphisms with training effects on functional capacity [34, 44–46]. Individual variability in training response is in all these studies discovered after completing the training protocols and no modifications were done during training. It would be essential to take into consideration these individual variations when preparing the training. A personalized approach with individually dosed exercise combination is the final aim in training design, which could significantly contribute in achieving maximal sports performance based on athlete's genetic potential and its phenotypic expression and interaction. Recently, Jones et al. have for the first time, as to our knowledge, reported on a personalized approach to training based on athletes' genetic algorithm [9]. Using genetic profiling to better match individual genotype with appropriate training modality may be a powerful tool to aid personalized and precise training in the future. Numerous cohorts of participants could be included in various training interventional studies with "genetically designed" protocols and serve as solid ground for future development and better understanding of underlying physiological mechanisms. Aging studies could also further benefit from this approach. Therefore, significant scientific efforts should be shifted towards designing training protocols for non-athletes based on known SNPs. Previously prescribed training, based on genetic profiling, would allow investigators to follow up numerous functional, morphological and metabolic properties, including mitochondrial dynamics, their response to training intervention and possible association with genetic traits, illuminating physiological mechanisms and pathways in this complex interconnection. Developing gene adjusted training protocols for non-athletic populations may decrease the number of non-responders and significantly fasten positive adaptation changes, with enhanced therapeutical effect. The follow-up of the training response may broaden epigenetic approach through possible gene expression modifications as a result of training intervention.

Is training in the future going to be done in an ad hoc manner, or precisely designed, based on genetic predisposition in athletic and non-athletic populations? If applying this fine tuning prior or during the training programs, special effort should be made to avoid using genetic profiling for selection; it should only be used for inclusion, not for exclusion.

Conclusion

Physical performance represents a complex multifactorial phenotype, combination of genetics and environmental factors, through epigenetic patterns.

Numerous studies of genetic associations with performance phenotypes have been published over the past three decades. The actual number of single nucleotide polymorphisms and genetic traits that can potentially explain elite athletic performance or response to training will eventually be much higher in the future.

Further replications of these single nucleotide polymorphisms in independent cohorts are required to be taken forward for fine mapping/targeted resequencing and interventional training studies to reveal the underlying physiological mechanisms.

Also, attention must be paid to social and ethical issues regarding genetic testing in sports medicine.

As discussed in this review, genetic profiling should not be used for prediction or selection of future top

athletes, but for development of physical fitness and achievements in accordance to individual potentials. New efforts should be focused on application of exercise genomics knowledge in non-athletic populations, by designing genetically-based training algorithms. Widening the research in this direction could bring new information about muscle plasticity and more scientifically based application of different stimuli that enhance or modify skeletal muscle adaptation to exercise through various molecular mechanisms. Introducing skeletal muscle as a secretory organ, with still unknown extent of its influence in systemic response to exercise, will substantially increase its role and importance in future research.

References

1. Ahmetov II, Popov DV, Astratenkova IV, Druzhevskaya AM, Missina SS, Vinogradova OL, et al. The use of molecular genetic methods for prognosis of aerobic and anaerobic performance in athletes. Fiziol Cheloveka. 2008;34(3):86-91.

2. Ahmetov II, Fedotovskaya ON. Sports genomics: current state of knowledge and future directions. Cel Mol Exerc Physiol. 2012;1(1).

3. Kostek M, Hubal MJ, Pescatello LS. Genetic role in muscle strength. ACSMs Health Fit J. 2007;11(2):18-23.

4. Loos RJ, Hagberg JM, Perusse L, Roth SM, Sarzynski MA, Wolfarth B, et al. Advances in exercise, fitness and performance genomics in 2014. Med Sci Sports Exerc. 2015;47(6):1105-12.

5. Rankinen T, Bray MS, Hagberg JM, Perusse L, Roth SM, Wolfarth B, et al. The human gene map for performance and health-related fitness phenotypes: the 2005 update. Med Sci Sports Exerc. 2006;38(11):1863-88.

6. Rankinen T, Roth SM, Bray MS, Loos R, Perusse L, Wolfarth B, et al. Advances in exercise, fitness, and performance genomics. Med Sci Sports Exerc. 2010;42(5):835-46.

7. Rankinen T, Fuku N, Wolfarth B, Wang G, Sarzynski MA, Alexeev DG, et al. No evidence of a common DNA variant profile specific to world class endurance athletes. Plos One 2016;11(1):e0147330.

8. Rees T, Hardy L, Gullich A, Abernethy B, Cote J, Woodman T, et al. The great British medalists project: a review of current knowledge on the development of the world's best sporting talent. Sports Med. 2016;46(8):1041-58.

9. Jones N, Kiely J, Suraci B, Collins DJ, de Lorenzo D, Pickering C, et al. A genetic-based algorithm for personalized resistance training. Biol Sport. 2016;33(2):117-26.

10. Abe T, DeHoyos DV, Pollock ML, Garzarella L. Time course for strength and muscle thickness changes following upper and lower body resistance training in men and women. Eur J Appl Physiol. 2000;81(3):174-80.

11. Andersen LL, Andersen JL, Magnusson SP, Aagaard P. Neuromuscular adaptations to detraining following resistance training in previously untrained subjects. Eur J Appl Physiol. 2005;93(5-6):511-8.

12. Kraemer WJ, Patton JF, Gordon SE, Harman EA, Deschenes MR, Reynolds K, et al. Compatibility of high-intensity strength and endurance training on hormonal and skeletal muscle adaptations. J Appl Physiol. 1995;78(3):976-89.

13. Kraemer WJ, Fleck SJ, Evans WJ. Strength and power training: physiological mechanisms of adaptation. Exerc Sport Sci Rev. 1996;24:363-97.

14. Kraemer WJ, Ratamess NA. Hormonal responses and adaptations to resistance exercise and training. Sports Med. 2005;35(4):339-61.

15. Mazzetti SA, Kraemer WJ, Volek JS, Duncan ND, Ratamess NA, Gomez AL, et al. The influence of direct supervision of resistance training on strength performance. Med Sci Sports Exerc. 2000;32(6):1175-84.

16. Popadic Gacesa JZ, Kozic DB, Dragnic NR, Jakovljevic DG, Brodie DA, Grujic NG. Changes of functional status and volume of triceps brachii measured by magnetic resonance imaging after maximal resistance training. J Magn Reson Imaging. 2009;29(3):671-6.

17. Popadic Gacesa JZ, Jakovljevic DG, Kozic DB, Dragnic NR, Brodie DA, Grujic NG. Morpho-functional response of the elbow extensor muscles to twelve-week self-perceived maximal resistance training. Clin Physiol Funct Imaging. 2010;30(6):413-9.

18. Popadic Gacesa JZ, Kozic DB, Grujic N. Triceps brachii strength and regional body composition changes after detraining quantified by MRI. J Magn Reson Imaging. 2011;33(5):1114-20.

19. Popadic Gacesa JZ, Klasnja A, Grujic N. Changes in strength, endurance, and fatigue during a resistance-training program for the triceps brachii muscle. J Athl Train. 2013;48(6):804-9.

20. MacArthur DG, North KN. Genes and human elite athletic performance. Hum Genet. 2005;116(5):331-9.

21. Gayagay G, Yu B, Hambly B, Boston T, Hahn A, Celermajer DS, et al. Elite endurance athletes and the ACE I allele – the role of genes in athletic performance. Hum Genet. 1998;103(1):48-50.

22. Montgomery HE, Marshall R, Hemingway H, Myerson S, Clarkson P, Dollery C, et al. Human gene for physical performance. Nature. 1998;393(6682):221-2.

23. Ahmetov II, Rogozkin VA. Genes, athlete status and training – an overview. Med Sport Sci. 2009;54:43-71.

24. Bray MS, Hagberg JM, Perusse L, Rankinen T, Roth SM, Wolfarth B, et al. The human gene map for performance and health-related fitness phenotypes: the 2006–2007 update. Med Sci Sports Exerc. 2009;41(1):35-73.

25. Puthucheary Z, Skipworth JR, Rawal J, Loosemore M, Van Someren K, Montgomery HE. The ACE gene and human performance 12 years on. Sports Med. 2011;41(6):433-48.

26. Ma F, Yang Y, Li X, Zhou F, Gao C, Li M, et al. The association of sport performance with ACE and ACTN3 genetic polymorphisms: a systematic review and meta-analysis. Plos One. 2013;8(1):e54685.

27. Murphey LJ, Gainer JV, Vaughan DE, Brown NJ. Angiotensin-converting enzyme insertion/deletion polymorphism modulates the human in vivo metabolism of bradykinin. Circulation. 2000;102(8):829-32.

28. Williams AG, Dhamrait SS, Wootton PT, Day SH, Hawe E, Payne JR, et al. Bradykinin receptor gene variant and human physical performance. J Appl Physiol. 2004;96(3):938-42.

29. Wicklmayr M, Dietze G, Brunnbauer H, Rett K, Mehnert H. Dose-dependent effect of bradykinin on muscular blood flow and glucose uptake in man. Hoppe-Seylers Z Physiol Chem. 1983;364(7):831-3.

30. MacArthur DG, Seto JT, Chan S, Quinlan KG, Raftery JM, Turner N, et al. An Actn3 knockout mouse provides mechanistic insights into the association between α-actinin-3 deficiency and human athletic performance. Hum Mol Genet. 2008;17(8):1076-86.

31. North KN, Yang N, Wattanasirichaigoon D, Mills M, Easteal S, Beggs AH. A common nonsense mutation results in alpha-actinin-3 deficiency in the general population. Nat Genet. 1999;21(4):353-4.

32. Yang N, MacArthur DG, Gulbin JP, Hahn AG, Beggs AH, Easteal S, et al. ACTN3 genotype is associated with human elite athletic performance. Am J Hum Genet. 2003;73(3):627-31.

33. Eynon N, Hanson ED, Lucia A, Houweling PJ, Garton F, North KN, et al. Genes for elite power and sprint performance: ACTN3 leads the way. Sports Med. 2013;43(9):803-17.

34. Perusse L, Rankinen T, Hagberg JM, Loos RJF, Roth SM, Sarzynski MA, et al. Advances in exercise, fitness, and performance genomics in 2012. Med Sci Sports Exerc. 2013; 45(5):824-31.

35. Wang G, Padmanabhan S, Wolfarth B, Fuku N, Lucia A, Ahmetov II, et al. Chapter four. Genomics of elite sporting performance: what little we know and necessary advances. Adv Genet. 2013;84:123-49.

36. Bouchard C, Sarzynski MA, Rice TK, Kraus WE, Church TS, Sung YJ, et al. Genomic predictors of the maximal O2 uptake response to standardized exercise training programs. J Appl Physiol. 2011;110(5):1160-70.

37. Ahmetov II, Kulemin N, Popov D, Naumov V, Akimov E, Bravy Y, et al. Genome-wide association study identifies three novel genetic markers associated with elite endurance performance. Biol Sport. 2015;32(1):3-9.

38. Lucia A, Moran M, Zihong H, Ruiz JR. Elite athletes: are the genes the champions? Int J Sports Physiol Perform. 2010;5(1):98-102.

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39. Ehlert T, Simon P, Moser DA. Epigenetics in sports. Sports Med. 2013;43(2):93-110.

40. Barres R, Yan J, Egan B, Treebak JT, Rasmussen M, Fritz T, et al. Acute exercise remodels promoter methylation in human skeletal muscle. Cell Metab. 2012;15(3):405-11.

41. Goodlin GT, Roos TR, Roos AK, Kim SK. The dawning age of genetic testing for sports injuries. Clin J Sport Med. 2015;25(1):1-5.

42. McNamee MJ, Muller A, van Hilvoorde I, Holm S. Genetic testing and sports medicine ethics. Sports Med. 2009;39(5):339-44.

43. Lippi G, Solero GP, Guidi G. Athletes genotyping: ethical and legal issues. Int J Sports Med. 2004;25(2):159. author reply 160-1.

44. Kostek MC, Delmonico MJ, Reichel JB, Roth SM, Douglass L, Ferrell RE, et al. Muscle strength response to strength training is influenced by insulin-like growth factor 1 genotype in older adults. J Appl Physiol. 2005;98(6):2147-54.

45. Popadic Gacesa JZ, Momcilovic M, Veselinovic I, Brodie DA, Grujic NG. Bradykinin type 2 receptor -9/-9 genotype is associated with triceps brachii muscle hypertrophy following strength training in young healthy men. BMC Musculoskelet Disord. 2012;13:217.

46. Popadic Gacesa JZ, Secher NH, Momcilovic M, Grujic NG. Association between intramuscular fat in the arm following arm training and INSIG2. Scand J Med Sci Sports. 2014; 24(6):907-12.

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FUNCTIONAL ABILITIES OF CHILDREN WITH CEREBRAL PALSY

FUNKCIONALNI STATUS DECE SA CEREBRALNOM PARALIZOM

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Summary

Introduction. Cerebral palsy is one of the leading causes of neurological impairment in childhood. The dominant clinical sign is the impairment of gross motor functions; however, associated conditions may limit the child with cerebral palsy in daily activities as well. The aim of this study was to determine the functional status of children with cerebral palsy, its relationship with the types of cerebral palsy, and concomitant conditions. **Material and Methods.** The sample included 206 children with cerebral palsy. The data were obtained from medical records with clinical characteristics of cerebral palsy, and associated conditions. The clinical types of cerebral palsy were determined using the Surveillance of Cerebral Palsy in Europe registry and topographical categories. Gross motor function abilities were evaluated using the Gross Motor Function Classification System and manual abilities by the Manual Ability System Classification. **Results.** According to the Gross Motor Function Classification System, about two thirds of children with cerebral palsy (64.0%) had levels I, II or III of gross motor function impairment. A statistically significant difference was noted with respect to the distribution of various clinical types of cerebral palsy in relation to functional classification based on Gross Motor Function Classification System ($p < 0.001$), as well as in the functional classification in terms of intellectual impairment ($p < 0.001$); children with severe forms of intellectual impairment were classified at a higher level of functional limitation. Epilepsy was more prevalent in children with higher level of functional limitation ($p = 0.009$). **Conclusion.** Two thirds of children with cerebral palsy could walk independently or with walking aids. Children with quadriplegia and dyskinetic type of cerebral palsy had the most limited functional abilities. Associated conditions were more prevalent in children with higher functional limitations.

Key words: Athletic Performance; Genotype; Gene Expression; Polymorphism, Single Nucleotide; Exercise; Programs; Peptidyl-Dipeptidase A; Actinin; Muscle, Skeletal

Sažetak

Uvod. Cerebralna paraliza se nalazi među najčešćim uzrocima nastanka neuroloških oštećenja u dečjem uzrastu. U kliničkoj slici dominira oštećenje funkcionalnih motoričkih sposobnosti. Kod dece sa cerebralnom paralizom često su prisutni udruženi neurorazvojni ili senzorni problemi. Prisustvo ovih poremećaja može ograničavati pojedinca u svakodnevnim aktivnostima. Cilj rada bio je utvrđivanje funkcionalnog statusa dece sa cerebralnom paralizom i istraživanje odnosa između funkcionalnih sposobnosti, oblika cerebralne paralize i pridruženih oboljenja. **Materijal i metode.** Uzorak se sastojao od ukupno 206 dece. Iz medicinske dokumentacije su dobijeni podaci o kliničkim karakteristikama cerebralne paralize i pridruženim oboljenjima. Klinički tip cerebralne paralize određen je prema preporuci *Surveillance of Cerebral Palsy in Europe* i topografski. Grube motoričke funkcije procenjene su na osnovu istoimenog klasifikacionog sistema *Gross Motor Function Classification System*, a manuelne sposobnosti na osnovu *Manual Ability Classification System*. **Rezultati.** Oko dve trećine dece sa cerebralnom paralizom (64%) imalo je I, II ili III stepen oštećenja grube motoričke funkcije prema primenjenom klasifikacionom sistemu. Registrovana je statistički značajna razlika u distribuciji kliničkih tipova cerebralne paralize i mentalnog deficita u odnosu na funkcionalnu klasifikaciju ($p < 0,001$). Teži stepen mentalnog deficita, kvadriplegična i diskinetična forma cerebralne paralize bile su povezane sa težim nivoom funkcionalnog oštećenja. Epilepsija je bila prisutnija u grupi dece sa većim stepenom oštećenja grube motoričke funkcije ($p = 0,009$). **Zaključak.** Hod je bio moguć samostalno ili sa pomagalom kod dve trećine dece. Funkcionalna sposobnost najviše je bila limitirana kod kvadriplegičnog i diskinetičkog oblika cerebralne paralize. Teži nivo funkcionalnog oštećenja bio je povezan sa većom zastupljenošću i težim stepenom pridruženih oboljenja.

Ključne reči: sportsko postignuće; genotip; ekspresija gena; polimorfizam jednog nukleotida; vežbanje; programi; angiotenzin konvertujući enzim; aktinin; skeletni mišići

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Abbreviations
CP – cerebral palsy
GMFCS – Gross Motor Function Classification System
MACS – Manual Ability Classification System
SCPE – Surveillance of Cerebral Palsy in Europe

Introduction

Cerebral palsy (CP) is one of the leading causes of neurological impairment in childhood [1, 2]. Worldwide studies on the prevalence of CP indicate that it is much higher in developing countries, and its incidence varies from 1.5 to 3.0 per 1.000 live births [2]. The clinical picture of CP is dominated by the functional impairment of motor skills pertaining to the degree of motor function limitation in all body regions, including speech and oral motor performance. The capacity for independent movement, walking in particular, is often used as a rough measure for assessing the severity of motor deficit [3]. According to Palisano et al., classification of gross motor function is based on the Gross Motor Function Classification System (GMFCS) as follows: level I and II indicate ability to walk unaided, level III pertains to walking with hand-held support or assistance, while individuals classified at level IV and V are unable to walk [3–5]. The definition of the degree of motor function is based on functional limitations, with particular emphasis on the sitting function (upper body control) and walking, use of assistive technologies, including walking aids (walker, crutches, walking stick) and wheelchair, as well as reduced motion quality [6]. Thus, GMFCS provides guidelines for healthcare professionals, allowing them monitoring and treatment of health issues associated with CP [7].

Upper extremity functional abilities of children diagnosed with CP may be assessed using the Manual Ability Classification System (MACS) which is a systematic classification method which evaluates how children with CP use their hands to handle objects in daily activities. The MACS focuses on self-initiated manual dexterity, with specific focus on the object use in "personal space" (those in close proximity to one's body, rather than objects out of reach) [8]. Empirical evidence shows that child's motivation and cognitive ability impact his ability to handle objects manually, and therefore affect the MACS level [9].

Children diagnosed with CP often have associated conditions, such as epilepsy, impaired vision or hearing, compromised attention and communication, as well as behavioral and cognitive disorders [10]. It is necessary to identify the presence or absence of these disorders, as their presence is known to inhibit everyday activities [11, 12]. It is believed that the cognitive status, severity of motor impairment, and subtype of CP are positively correlated. Children with spastic diplegia and quadriplegia are less successful at tasks that require motor activity, compared to verbal tasks [13].

Given that the signs and symptoms of CP are primarily based on the functional impairments of motor skills, i. e. the greatest limitation of patient's abilities in everyday activities, our aim was to determine the functional status of children with CP and to investigate its relationship with the type of CP and concomitant diseases.

Material and Methods

This qualitative, clinical-epidemiological, classical study was conducted at the Clinic of Children's Habilitation and Rehabilitation, of the Institute of Child and Youth Healthcare of Vojvodina in Novi Sad. The study was approved by the Ethics Committee of the Institute and the Faculty of Medicine, University of Novi Sad. The analysis included all patients diagnosed with CP from 1990 – 2009, resulting in a sample of 206 children. Part of this comprehensive study is presented in this paper. Initially, medical histories of all patients were reviewed in order to ascertain their gestational age at birth, as well as their CP clinical characteristics and associated conditions. Clinical CP types were determined according to the Surveillance of Cerebral Palsy in Europe (SCPE) and topographically [14]. Based on clinical presentations, five-level GMFCS was performed, whereby level I indicated the highest functional ability, and level V the most severely limited motor function. For each of the five levels, the description of gross motor function included four age groups: under the age of 2, between the age of 2 and 4, between the age of 4 and 6, and between the age of 6 and 12 years [6]. Based on the clinical picture and the occupational therapists' reports, the quality of the upper extremity function was assessed using the MACS and it was classified into five levels, where level I indicated easy and successful use of objects, while level V implied that the patient was unable to manually manipulate objects [8]. Data on associated conditions (mental defects, epilepsy, visual impairments) were gathered from the patients' medical records provided by neurologists, neuro-pediatricians, psychologists, speech therapists and ophthalmologists.

The collected data were saved in a database specifically designed for the purpose of this study. The subsequent data analysis consisted of descriptive and inferential statistics. Attributive characteristics were presented through frequencies and percentages. Comparison of the observed and expected frequencies was performed via χ^2 test, whereas analysis of correlation between two characteristics was done using the Spearman correlation coefficient test. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) Statistics 17.0 computer software.

Results

According to the GMFCS, nearly two thirds of children with CP (64.0%) exhibited a motor function impairment at levels I, II or III, whereas levels IV and V were found in 17.5% and 18.6% children, respectively **(Table 1)**.

In the examined sample, a statistically significant difference was found in the distribution of clinical types of CP, according to the GMFCS functional ability (χ^2

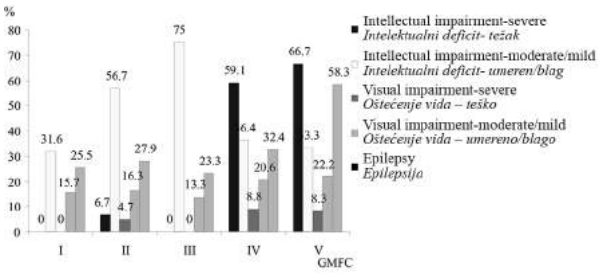
Table 1. Functional classification according to GMFCS in children with CP
Tabela 1. *Funkcionalna klasifikacija grubih motoričkih funkcija (GMF) kod dece sa cerebralnom paralizom*

GMFCS	N	%	Valid %1
I	51	24.8	26.3
II	43	20.9	22.2
III	30	14.6	15.5
IV	34	16.5	17.5
V	36	17.5	18.6
Total	194	94.2	100.0
No data	12	5.8	
Total/*Ukupno*	206	100.0	

= 148.149, p<0.001), as shown in **Table 2**. In children diagnosed with spastic unilateral CP, GMFCS levels I and II were the most prevalent (92.5%), whereas quadriplegic patients were mostly classified at levels IV and V (95.6%). The same findings were found in children with dyskinetic (64.7%) and ataxic (66.7%) type of CP. On the other hand, in 54.7% of the examined children, motor function was classified at GMFCS levels I or II, while 34.4% of the sample had level III, as shown in **Table 2**.

According to MACS classification, the majority (88.9%) of children with spastic unilateral CP were classified at level II (41.7%) or level III (47.2%). Nearly all diplegic children (92.1%) exhibited manual ability at level I (48.9%) or level II (42.2%), while 77.1% of quadriplegic patients demonstrated MACS level IV (38.7%) or level V (48.4%). Manual dexterity in children with dyskinetic CP varied from levels II − V, whereby the majority were classified at level III (33.3%) or IV (33.3%), as shown in **Table 3**.

Analyses revealed statistically significant differences (χ^2 = 93.984, p <0.001) between the mental deficit and the GMFCS functional classification. As displayed in **Graph 1**, severe cognitive impairment was absent in children at level I, while it was found in 59.1% and about 33% of children classified at GMFCS levels IV and V, respectively. Similarly, epilepsy was statistically significantly more associated with the GMFCS level (χ^2 =13.531, p = 0.009). More specifically, the percentage of CP patients with concomitant epilepsy increased with the GMFCS level, from 25.5% at GMFCS level I to 58.3% at level V. On the other hand, no significant differences were found between visual impairment and the GMFCS classification.



Graph 1. Distribution of associated conditions by GMFCS levels
Grafikon 1. *Distribucija udruženih stanja sa stepenom grube motoričke funkcije*

Discussion

In the present study, the gross motor skill distribution represented by the GMFCS classification levels (64% of the sample at levels I − III, and the remaining 36% at IV or V) was in agreement with the report of Himmelmann, whereby 69% of the assessed children exhibited levels I to III, and the remaining 31% of the studied sample were classified at levels IV or V [14]. Similar findings were yielded by a study conducted in Australia [16]. On the other hand, in Sweden

Table 2. Distribution of GMFCS levels by CP types
Tabela 2. *Distribucija stepena grubih motoričkih funkacija (GMF) i tipa cerebralne paralize*

		GMFCS levels/*Stepen GMF*			
Clinical type of CP *Klinički tip cerebralne paralize*		I-II	III	IV-V	Total/*Ukupno*
Spastic unilateral/*unilateralni spastični*	N (%)	49 (92.5)	3 (5.7)	1 (1.9)	53 (100)
SB* diplegia/*SB* diplegija*	N (%)	35 (54.7)	22 (34.4)	7 (10.9)	64 (100)
SB* quadriplegia/*SB* kvadriplegija*	N (%)		2 (4.4)	43 (95.6)	45 (100)
Dyskinetic/*Diskinetični*	N (%)	5 (29.4)	1 (5.9)	11 (64.7)	17 (100)
Ataxic/*Ataksični*	N (%)	3 (33.3)		6 (66.7)	9 (100)
Total/*Ukupno*	N (%)	92 (48.9)	28 (14.9)	68 (36.2)	188 (100)

*SB – spastic bilateral/*spastična bilateralna*

Table 3. Distribution of MACS levels by CP types
Tabela 3. *Distribucija stepena manuelnih sposobnosti i tipova cerebralne paralize*

		MACS/*Sistem klasifikacije manuelnih sposobnosti*					
		1	2	3	4	5	Total/*Ukupno*
Spastic unilateral/*Spastični unilateralni*	N (%)	3 (8.3)	15 (41.7)	17 (47.2)	1 (2.8)	0 (.0)	36 (100)
SB* diplegia/*SB* diplegija*	N (%)	22 (48.9)	19 (42.2)	3 (6.7)	1 (2.2)	0 (.0)	45 (100)
SB* quadriplegia/*SB*kvadriplegija*	N (%)	.0 (.00)	1 (3.2)	3 (9.7)	12 (38.7)	15 (48.4)	31 (100)
Dyskinetic/*Diskinetični*	N (%)	.0 (.0)	1 (11.1)	3 (33.3)	3 (33.3)	2 (22.2)	9 (100)
Ataxic/*Ataksični*	N (%)	.0 (.0)	2 (66.7)	0 (.0)	1 (33.3)	0 (.0)	3 (100)
Total/*Ukupno*	N (%)	25 (20.2)	38 (30.6)	26 (21)	18 (14.5)	17 (13.7)	124 (100)

*SB – spastic biateral/*spastični bilateralni*

and Island, the researchers classified 5 − 10% fewer children at the highest GMFCS levels [17, 18]. This discrepancy may be due to the greater number of quadriplegic children in our sample. The differences in the prevalence of gross motor impairments at different GMFCS levels can also be explained by the difficulties in precisely assessing children under the age of two, compared to more mature patients. Gorter and colleagues examined the predictive value of GMFCS administered to children under the age of 2, and reporting that in 42% of cases the initially established level was revised as the children aged, typically to the higher level indicative of greater functional impairment [19]. Yet, Palisano et al. claimed that GMFCS classification was stable over time [3, 6]. Bax and colleagues believed that the disparities in these findings stem from the use of an overly wide age span, suggesting that it should be defined more precisely when using this classification system [20].

Our findings point to statistically significant differences in the distribution of CP types in relation to the GMFCS classification. The majority of children exhibiting a hemiplegic form of CP were classified at levels I or II, with only three children at level III and one at level IV. These results are in line with those reported by Beckung et al., who classified all hemiplegic patients at levels I − IV, with greater prevalence of cases at lower levels [21]. Himmelmann and colleagues, however, found that children with hemiplegic CP were classified at all GMFCS levels [22]. Nonetheless, most authors concur that gross motor function of hemiplegic children with CP is at level I [21, 23−25]. Our results pertaining to children with diplegia indicate that they also tend to exhibit gross motor function difficulties at the first four GMFCS levels. More specifically, half of this group was classified at levels I or II, while none of the children were at level V, in accordance with the findings reported by Howard et al. [16]. However, other authors noted a small number (about 5% of the examined sample) of diplegic children at level V [21, 22]. In our study, the majority of children with quadriplegia were classified at levels IV or V, with only two at level III. In several extant studies, the most severe CP type was associated with the greatest functional impairments according to the GMFCS classification, whereby level V predominated [16, 22, 25]. Some authors assume that spastic quadriplegia is associated with level V only [20]. Our findings indicate presence of dyskinetic CP et all GMFCS levels, with levels IV or V found in 2/3 of the examined cases, which is in agreement with the results yielded by a Swedish study [22]. Beckung and colleagues, on the other hand, found that the dyskinetic CP was the most prevalent at level III [21]. Ataxic CP is very rare and according to the available evidence it is distributed across the first three GMFCS levels. In our sample, children with ataxic CP were classified at levels I, II and IV, with the greatest number found at level IV, in line with the results reported by other authors [16, 21]. Significant variations in the functional abilities of ataxic children could be attributed to the predominance of symptoms indicative of hypotonic or ataxic CP, due to which some researchers separated children predominantly displaying hypotonia from the group exhibiting ataxia [16, 26].

The MACS allows manual dexterity to be classified in the evaluation of the upper extremity function in everyday life. According to the prevailing consensus, manual functional abilities are much more restricted in children with unilateral spastic CP, in relation to their gross motor functions, whereas diplegic CP tends to primarily impair gross motor functions [27]. Our findings indicated that more than 75% of children with unilateral spastic CP exhibited manual dexterity at levels II or III. Children diagnosed with hemiplegic CP have a tendency to neglect the affected upper extremity, which compromises their ability to partake in bimanual activities, resulting in classification at MACS level II [8]. Our results showed that children with diplegia typically exhibit manual functions at levels I or II, while those with quadriplegia are classified at level IV or V, and the dyskinetic CP mostly corresponds to levels III, IV or V. According to Carnahan et al., quadriplegic and dyskinetic CP forms usually correspond to MACS levels IV or V. In children with these types of CP, upper extremity function can be improved through occupational therapy, provided that they do not suffer any cognitive impairment [27]. It is widely established that the upper extremity function depends on both cognitive status and patient's self-directed motor control [28].

According to our findings, higher GMFCS levels, indicative of greater functional impairment, are associated with greater prevalence and severity of concomi-

tant conditions, such as mental deficit, visual impairment and epilepsy. Consequently, GMFCS classification level can be viewed as a reliable indicator of the overall extent of disability in children with CP. In their work, Himmelmann and colleagues correlated the higher GMFCS levels with the greater number of associated impairments, while in children born at term, GMFCS classification reflected the severity of adverse perinatal or neonatal events, such as intracranial bleeding or cerebral infarction and hypoxic-ischemic encephalopathy [22].

Conclusion

In the examined sample, two thirds of the children with cerebral palsy were capable of walking, either independently or with assistance (corresponding to Gross Motor Function Classification System - levels I – III), while the remaining third of the examined children were immobile (and were thus classified at Gross Motor Function Classification System – levels IV or V). In terms of functional classification, about 50% and 90% of children with bilateral and unilateral spastic cerebral palsy, respectively, were classified at levels I or II, pointing to the highest functional ability. Functional ability was most compromised in quadriplegic and dyskinetic cases of cerebral palsy, and those children were classified at levels IV or V. According to the Gross Motor Function Classification System classification, more severe functional impairments were related to higher prevalence and greater extent of associated conditions, such as mental deficit, visual impairment and epilepsy.

References

1. Stelmach T, Pisarev H, Talvik T. Ante- and perinatal factors for cerebral palsy: case-control study in Estonia. J Child Neurol. 2005;20(8):654-60.

2. Himmelmann K, Hagberg G, Wiklund LM, Eek MN, Uvebrant P. Dyskinetic cerebral palsy: a population based study of children born between 1991 and 1998. Dev Med Child Neurol. 2007;49(4):246-51.

3. Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B. Development and reliability of a system to classify gross motor function in children with cerebral palsy. Dev Med Child Neurol. 1997;39(4):214-23.

4. Dolk H, Parkes J, Hill N. Trends in the prevalence of cerebral palsy in Northern Ireland, 1981-1997. Dev Med Child Neurol. 2006;48(6):406-12.

5. Palisano R, Rosenbaum P, Walter S, Russel D, Wood E, Galuppi B. Gross Motor Function Classification System. Dev Med Child Neurol. 1997;48(6):424-8.

6. Palisano R, Cameron D, Rosenbaum PL, Walter SD, Russell D. Stability of the gross motor function classification system. Dev Med Child Neurol. 2006;48(6):424-8.

7. Harvey A, Rosenbaum P, Graham HK, Palisano RJ. Current and future uses of the Gross Motor Function Classification System. Dev Med Child Neurol. 2009;51(4):328-9.

8. Eliasson AC. Manual Ability Classification System for children with cerebral palsy. Dev Med Child Neurol. 2008;50(Suppl s114):37.

9. Steenbergen B. Using the MACS to facilitate communication about manual abilities of children with cerebral palsy. Dev Med Child Neurol. 2006;48(12):948.

10. Knežević Pogančev M. Cerebralna paraliza i epilepsija. Med Pregl. 2010;64(7-8):527-30.

11. Bax M, Goldstein M, Rosenbaum P, Leviton A, Paneth N, Dan B, et al. Proposed definition and classification of cerebral palsy, April 2005. Dev Med Child Neurol. 2005;47(8):571-6.

12. Pakula AT, Van Naarden Braun K, Yeargin-Allsopp M. Cerebral palsy: classification and epidemiology. Phys Med Rehabil Clin N Am. 2009;20(3):425-52.

13. Sigurdardottir S, Eiriksdottir A, Gunnarsdottir E, Meintema M, Arnadottir U, Vik T. Cognitive profile in young Icelandic children with cerebral palsy. Dev Med Child Neurol. 2008;50(5): 357-62.

14. Bax M, Goldstein M, Rosenbaum P, Leviton A, Paneth N, Dan B, et al. Proposed definition and classification of cerebral palsy, April 2005. Dev Med Child Neurol. 2005;47(8):571-6.

15. Himmelmann K. Cerebral palsy in Western Sweden - epidemiology and function [dissertation]. Goteborg: Goteborg University, The Sahlgrenska Academy; 2006.

16. Howard J, Soo B, Graham HK, Boyd RN, Reid S, Lanigan A, et al. Cerebral palsy in Victoria: motor types, topography and gross motor function. J Paediatr Child Health. 2005;41(9-10):479-83.

17. Sigurdardottir S, Thorkelsson T, Halldorsdottir M, Thorarensen O, Vik T. Trends in prevalence and characteristics of cerebral palsy among Icelandic children born 1990 to 2003. Dev Med Child Neurol. 2009;51(5):356-63.

18. Nordmark E, Hagglund G, Lagergen J. Cerebral palsy in southern Sweden I. Prevalence and clinical features. Acta Paediatr. 2001;90(11):1271-6.

19. Gorter JW, Ketelaar M, Rosenbaum P, Helders PJ, Palisano R. Use of the GMFCS in infants with CP: the need for reclassification at age 2 years or older. Dev Med Child Neurol. 2009;51(1):46-52.

20. Bax M. Diagnostic assessment of children with cerebral palsy. Lancet Neurol. 2004;3(7):395.

21. Beckung E, Carlsson G, Carlsdotter S, Uvebrant P. The natural history of gross motor development in children with cerebral palsy aged 1 to 15 years. Dev Med Child Neurol. 2007;49 (10):751-6.

22. Himmelmann K, Beckung E, Hagberg G, Uvebrant P. Gross and fine motor function and accompanying impairments in cerebral palsy. Dev Med Child Neurol. 2006;48(6):417-23.

23. Voorman JM, Dallmeijer AJ, Knol DL, Lankhorst GJ, Becher JG. Prospective longitudinal study of gross motor function in children with cerebral palsy. Arch Phys Med Rehabil. 2007; 88(7):871-6.

24. Demeši Drljan Č, Mikov A, Filipović K, Tomašević Todorović S, Knežević A, Krasnik R. Cerebral palsy in preterm infants. Vojnosanit Pregl. 2016;74(4):343-8,

25. Caram LH, Funayama CA, Spina CI, Giuliani LdeR, de-Pina Neto JM. Investigation of neurodevelopment delay etiology: resources and challenges. Arq Neuropsiquiatr. 2006;64(2B):466-72.

26. Pfeifer LI, Silva DB, Funayama CA, Santos JL. Classification of cerebral palsy: association between gender, age, motor type, topography and Gross Motor Function. Arq Neuropsiquiatr. 2009;67(4):1057-61.

27. Carnahan KD, Arner M, Hagglund G. Association between gross motor function (GMFCS) and manual ability

(MACS) in children with cerebral palsy. A population based study of 359 children. BMC Musculoscelet Disord. 2007;8:50.

28. Tieman BL, Palisano RJ, Gracely EJ, Rosenbaum PL. Gross motor capability and performance of mobility in children with cerebral palsy: a comparison across home, school and outdoors/community settings. Phys Ther. 2004;84(5):419-29.

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CASE REPORTS
PRIKAZI SLUČAJEVA

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Case report
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CEREBRAL ARTERIOVENOUS MALFORMATION RADIOSURGERY AFTER INTRACRANIAL HEMORRHAGE – A CASE REPORT AND LITERATURE REVIEW

RADIOHIRURŠKI TRETMAN CEREBRALNE ARTERIO-VENSKE MALFORMACIJE NAKON INTRAKRANIJALNE HEMORAGIJE – PRIKAZ SLUČAJA I PREGLED LITERATURE

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Summary

Introduction. Treatment of cerebral arteriovenous malformations is very challenging and controversial in spite of current recommendations. Surgery is recommended in patients with hemorrhagic stroke, but in patients with good neurological status, when symptoms improve rapidly, the risk of surgical morbidity may be much higher than the risk of rebleeding. **Case report.** We report a case of a patient with an intracranial hemorrhage due to a ruptured arteriovenous malformation located in the right temporal region of the brain. Because of angiographic and anatomical features of the arteriovenous malformation (deep location and deep venous drainage, but also small arteriovenous malformation nidus size), radiosurgery was the preferred treatment modality. The patient was treated conservatively in the acute stage, and the arteriovenous malformation was subsequently completely eradicated with gamma knife radiosurgery. During the 3-year imaging follow-up, no sings of rebleeding were found. Also, angiography demonstrated that the arteriovenous malformation was completely excluded from the cerebral circulation. The patient was in a good condition and presented without neurological deficits or seizures during the follow-up period. **Conclusion.** All treatment modalities carry a risk of neurological compromise, but gamma knife radiosurgery may be a good option, even in cases with hemorrhagic presentation. It needs to be mentioned that complete obliteration takes approximately 1 to 3 years after the treatment, and in some cases it cannot be obtained.

Key words: Radiosurgery; Arteriovenous Malformations; Stroke; Cerebral Hemorrhage; Treatment Outcome; Risk Factors; Embolization, Therapeutic; Diagnosis

Sažetak

Uvod. Tretman cerebralnih arterio-venskih malformacija vrlo je zahtevan i uprkos savremenim preporukama za lečenje, i dalje postoje brojne kontroverze. Za pacijente sa znacima intrakranijalne hemoragije preporučuje se hirurško lečenje, ali postoje slučajevi kada je pacijent u dobrom opštem stanju i neurološki intaktan, sa blagom simptomatologijom koja brzo regredira, te rizik od hirurškog morbiditeta može biti veći od prirodnog rizika od ponovnog krvarenja. **Prikaz slučaja.** Prikazali smo pacijentkinju koja je imala intrakranijalnu hemoragiju zbog rupture arterio-venskih malformacija u slepoočnoj regiji mozga sa desne strane. Na osnovu angiografskih i anatomskih karakteristika arterio-venskih malformacija − u smislu dubokog položaja malformacija, prisustva duboke venske drenaže, ali i relativno male veličine nidusa, radiohirurški tretman je određen kao najoptimalniji. Bolesnica je inicijalno tretirana konzervativno, a potom je radiohirurški tretman doveo do potpune obliteracije. Tokom tri godine smo sproveli adekvatno neuroradiološko praćenje pacijentkinje i nije bilo znakova ponovne hemoragije. Takođe angiografski nalaz ukazuje na kompletno isključenje malformacije iz cerebralne cirkulacije. Pacijentkinja je u dobrom stanju i nema neurološkog deficita, niti je bilo epi-simptomatologije tokom perioda praćenja. **Zaključak.** Svi modaliteti lečenja nose rizik od mogućeg neurološkog pogoršanja, ali radiohirurški tretman može biti dobra opcija u dobro selektovanim slučajevima i za pacijente sa znacima intrakranijalne hemoragije. Važno je naglasiti da kompletna obliteracija zahteva period od jedne do tri godine nakon tretmana i da u nekim slučajevima nije uvek moguća.

Ključne reči: radiohirurgija; arteriovenske malformacije; cerebralno krvarenje; ishod lečenja; faktori rizika; terapijska embolizacija; dijagnoza

Introduction

Brain arteriovenous malformations (AVMs) are abnormal, congenital connections between arteries and veins leading to arteriovenous shunting without intervening capillary network of vessels [1]. The vascular conglomerate, the so called nidus of AVM, has no capillary bed and the feeding arteries drain directly to the draining veins. Nidus is the weak spot, due to abrupt transition from arterial to the venous

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Abbreviations

AVM – arteriovenous malformation
MRI – magnetic resonance imaging
CT – computerized tomography
SAH – subarachnoid hemorrhage

system, from high to low pressure vessels, and may result in hemorrhage, perifocal edema and/or subsequent irritation of the surrounding brain tissue [2, 3]. Therefore, brain AVMs usually manifest with headache, seizures, focal neurological deficits, and in case of rupture, AVMs manifest with intracranial hemorrhage [4–6]. Seizures and neurological deficits are due to mass effect or steal phenomenon [4].

Brain AVMs are responsible for 3% of strokes and 9% of subarachnoid hemorrhages [7, 8], affecting about 0.1% of population. The risk of hemorrhage is 2 – 4% per year, and the average annual mortality from untreated AVMs is 1.0% [9, 10]. The annual rupture rate is at least 0.9%, but in patients with risk factors such as deep AVM location and/or deep venous drainage it is much higher [9]. Also, the risk of subsequent hemorrhage is increased when the brain AVM presents with hemorrhage, and it may be from 4.5% to as high as 34% [10]. The main diagnostic tool is angiography, but magnetic resonance imaging (MRI) is also mandatory, because precise location of AVM and its relationship with the surrounding brain tissue is a key factor in further treatment planning [11]. Depending on the size, location and angioarchitecture of AVM, surgery and radiosurgery are the primary treatment options. Endovascular embolization can be considered as an individual treatment, or as an additional option before surgical resection [12]. Using the original 5-tier Spetzler–Martin (SM) classification and the 3-tier modification, we can estimate the surgical risk, risk of complications, and the outcome [13, 14].

In this case report, we present a patient with a deep seated AVM in the right temporal region of the brain with a hemorrhage; it was treated conservatively in the acute stage, and subsequently completely eradicated with gamma knife radiosurgery.

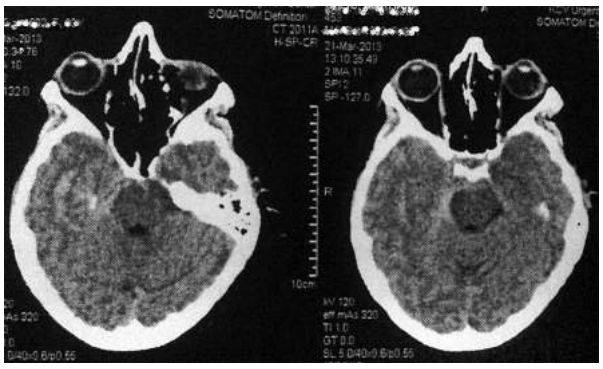

Figure 1. CT of the head revealed a right temporal subarachnoid hemorrhage
Slika 1. *Kompjuterizovana tomografija glave koja ukazuje na prisutvo subarahnoidalne hemoragije u temporalnoj regiji sa desne strane*

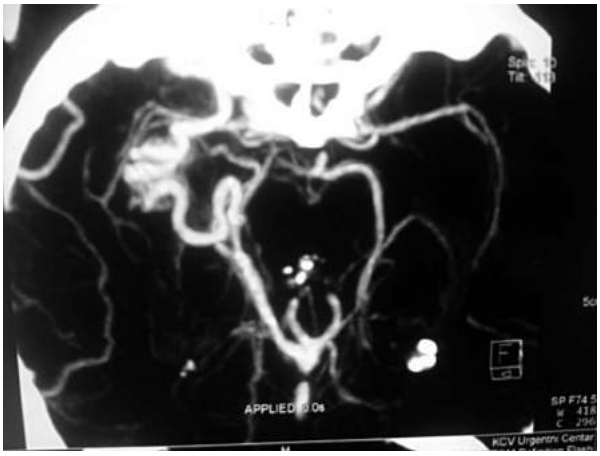

Figure 2. CT angiography demonstrated deep seated AVM in the temporal region of brain
Slika 2. *Kompjuterizovana tomografska angiografija ukazuje na duboko položenu arterio-vensku malformaciju u desnoj temporalnoj regiji mozga*

Case Report

A 31-year-old right handed female, with no major comorbidities, presented with sudden onset of headache accompanied by nausea and vomiting. On admission, the results of physical and neurological examinations were normal. The laboratory blood test results were within normal limits. The computerized tomography (CT) scans of the head revealed a right temporal subarachnoid hemorrhage (SAH) and CT angiography demonstrated a deep seated AVM in the temporal lobe of the brain **(Figures 1 and 2)**. She was initially managed at the neurocritical care unit with supportive treatment and blood pressure control. Subsequent MRI and digital subtraction angiography showed a right temporal AVM with the nidus dimension approximately 2 cm and branches supplied mainly from the right middle cerebral artery, some smaller branches from the right posterior communicating artery, and drainage through the basal vein of Rosenthal into the sinus rectus (modified Spetzler-Martin AVM grade 2) **(Figures 3 and 4)**. The available treatment options were discussed with the patient. The symptoms subsided after several days of conservatory treatment, and the patient was discharged after 10 days. One month after the hemorrhage, she underwent gamma knife radiosurgery with a prescription dose of 24 Gy with a 40% isodose line and treatment volume of 1.2 mL. During the 3-year follow-up, several MRI exams were performed, showing progressive reduction in flow voids and noticeably reduced enhancement **(Figure 5)**. A cerebral angiogram was performed 3 years after the treatment showing no residual AVM **(Figure 6)**. The patient is in a good condition, without neurological deficits or seizures during the follow-up period.

Discussion

The treatment of brain AVM must be carefully planned, especially in patients with previous hemorrhage. Surgical treatment is recommended for Spetzler-

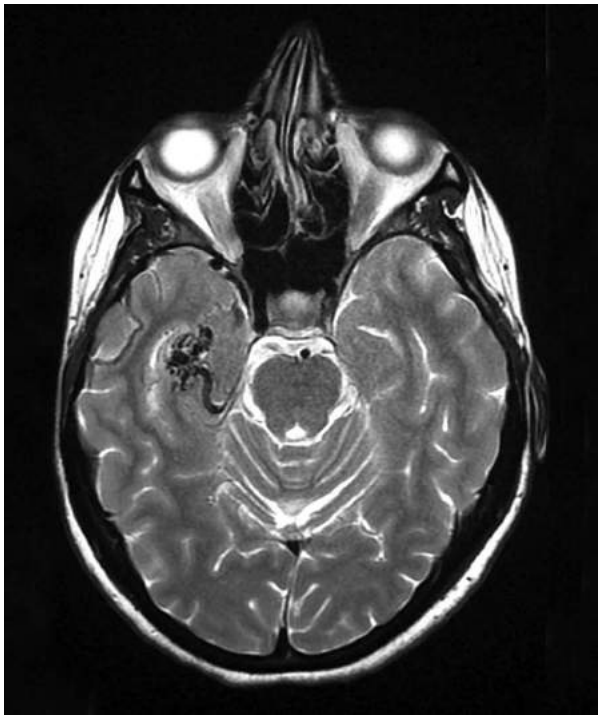

Figure 3. MRI of the head showing a right temporal AVM with the nidus size approximately 2 cm
Slika 3. *Magnetna rezonancija glave ukazuje na arteriovensku malformaciju u desnoj temporalnoj regiji sa dimenzijom nidusa od oko 2 cm*

Martin AVMs grade A, multimodal treatment must be considered for grade B, and observation with some reservations is suggested for grade C [14]. It is hard to estimate the risk of neurological injury, and risks of potential rehemorrhage in a patient who is in excellent condition. Microsurgical interventions seem to have a low risk of complications in small malformations in non-eloquent areas, and result in immediate cure [15]. Stereotactic radiosurgery (SRS) can be effective in small brain AVMs and it is a minimally invasive procedure, but complete obliteration of circulation requires approximately 1 to 3 years after treatment [16]. The two most important factors for complete obliteration of the AVM with radiosurgery are the diameter of the lesion, and maximum without deficit dose of radiation. Hemorrhage in the latency period and radiation edema or necrosis may occur as late complications [16–18].

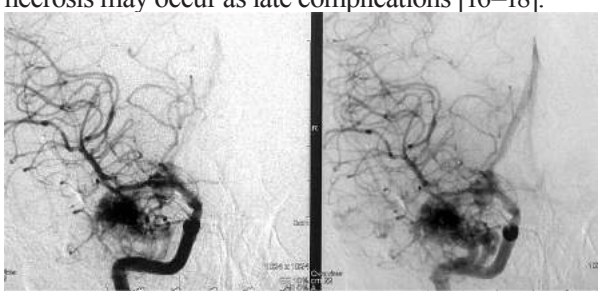

Figure 4. Angiography showing AVM angioarchitecture
Slika 4. *Angiografija ukazuje na angioarhitektoniku arterio-vensku malformaciju*

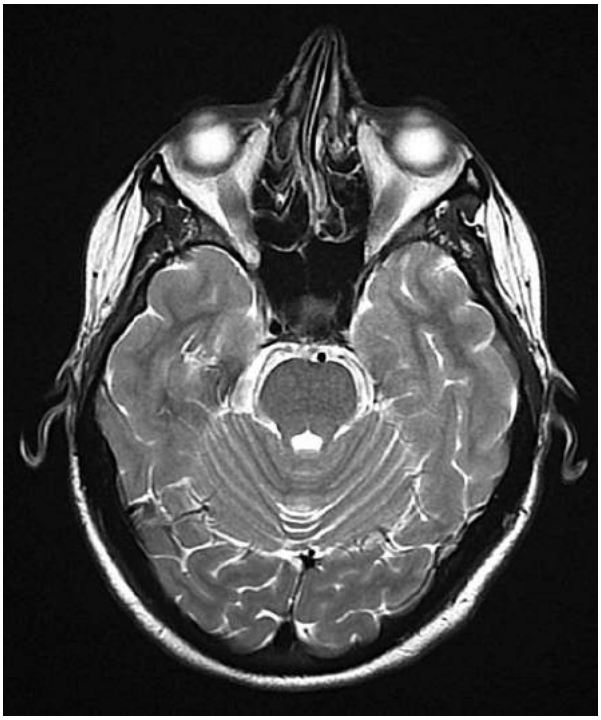

Figure 5. Two years after the treatment head MRI showing a significant flow void reduction
Slika 5. *Dve godine nakon tretmana, magnetna rezonancija glave ukazuje na značajnu redukciju protoka*

In our case, it was a small, but deep seated AVM, with deep venous drainage, so the treatment options included surgical resection or radiosurgery. Together with

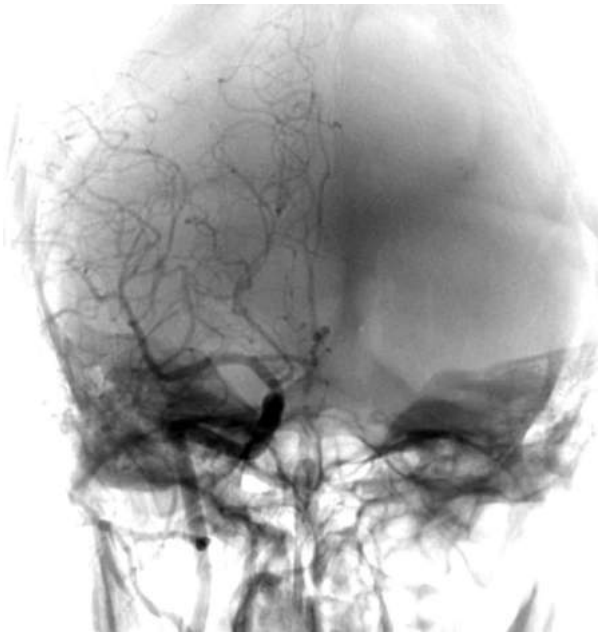

Figure 6. Three years after surgery, control angiography demonstrates no pathological circulation
Slika 6. *Kontrolna angiografija nakon tri godine ne ukazuje na prisustvo patološke cirkulacije*

the patient, we decided to perform radiosurgery, due to her good neurological condition, small amount of subarachnoid hemorrhage (Fischer scale grade 2) and quick resolution of symptoms. The previously mentioned disadvantage of radiosurgery is the risk of hemorrhage, until the AVM is completely obliterated, while surgical resection is not associated with such a possibility. Therefore, follow-up neuroradiological imaging was conducted even one year after the radiosurgery. Reduction of flow voids and reduced contrast enhancement on head MRI showed a successful treatment. Also, there is strong evidence in a large series of patients, showing reduced risk of hemorrhage during the follow up period [8].

Some case series reported that AVMs that are 3 cm in size or smaller, show high response rate to radiation [19–21]. A large series from the Karolinska Institute, including 1.319 patients showed an 80% overall obliteration rate [22]. Other authors reported results showing complications of radiosurgery mostly depending on two factors: lesion location and the total volume of treatment [23].

Conclusion

A great number of factors must be considered when choosing the optimal treatment for individual patients, and all of them carry risk of neurological compromise. However, gamma knife radiosurgery may be a good option, even in cases with hemorrhagic presentation. It needs to be mentioned that complete obliteration takes approximately 1 to 3 years after treatment, and in some cases it cannot be obtained.

References

1. Doppman JL. The nidus concept of spinal cord arteriovenous malformations. A surgical recommendation based upon angiographic observations. Br J Radiol. 1971;44(526):758-63.

2. Miyachi S, Negoro M, Handa T, Sugita K. Contribution of meningeal arteries to cerebral arteriovenous malformations. Neuroradiology. 1993;35(3):205-09.

3. Valavanis A. The role of angiography in the evaluationof cerebral vascular malformations. Neuroimaging Clin N Am. 1996;6(3):679-704.

4. Brown RD Jr, Wiebers DO, Forbes G, O'Fallon WM, Piepgras DG, Marsh WR, et al. The natural history of unruptured intracranial arteriovenous malformations. J Neurosurg. 1988;68(3):352-7.

5. Mast H, Young WL, Koennecke HC, Sciacca RR, Osipov A, Pile-Spellman J, et al. Risk of spontaneous haemorrhage after diagnosis of cerebral arteriovenous malformation. Lancet. 1997;350(9084):1065-8.

6. Golubović J, Vuleković P, Đilvesi Đ, Karan M, Jelača B, Cigić T. Semiology of pathological conditions in patients indicated for stereotactic biopsy. Med Pregl. 2016;69(11-12):345-50.

7. Schäuble B, Cascino GD, Pollock BE, Gorman DA, Weigand S, Cohen-Gadol AA, et al. Seizure outcomes after stereotactic radiosurgery for cerebral arteriovenous malformations. Neurology. 2004;63(4):683-7.

8. Maruyama K, Kawahara N, Shin M, Tago M, Kishimoto J, Kurita H, et al. The risk of hemorrhage after radiosurgery for cerebral arteriovenous malformations. New Engl J Med. 2005;352(2):146-53.

9. Stapf C, Mast H, Sciacca RR, Choi JH, Khaw AV, Connolly ES, et al. Predictors of hemorrhage in patients with untreated brain arteriovenous malformation. Neurology. 2006;66(9):1350-5.

10. Da Costa L, Wallace MC, Ter Brugge KG, O'Kelly C, Willinsky RA, Tymianski M. The natural history and predictive features of hemorrhage from brain arteriovenous malformations. Stroke. 2009;40(1):100-5.

11. Al-Shahi R, Warlow C. A systematic review of the frequency and prognosis of arteriovenous malformations of the brain in adults. Brain. 2001;124(Pt 10):1900-26.

12. Friedlander RM. Clinical practice. Arteriovenous malformations of the brain. New Engl J Med. 2007;356(26):2704-12.

13. Spetzler RF, Martin NA. A proposed grading system for arteriovenous malformations. J Neurosurg. 1986;65(4):476-83.

14. Spetzler RF, Ponce FA. A 3-tier classification of cerebral arteriovenous malformations. J Neurosurg. 2011;114(3):842-9.

15. Starke RM, Komotar RJ, Hwang BY, Fischer LE, Garrett MC, Otten ML, et al. Treatment guidelines for cerebral arteriovenous malformation microsurgery. Br J Neurosurg. 2009;23(4):376-86.

16. Starke RM, Komotar RJ, Hwang BY, Fischer LE, Otten ML, Merkow MB, et al. A comprehensive review of radiosurgery for cerebral arteriovenous malformations: outcomes, predictive factors, and grading scales. Stereotactic Funct Neurosurg. 2008;86(3):191-9.

17. Sun DQ, Carson KA, Raza SM, Batra S, Kleinberg LR, Lim M, et al. The radiosurgical treatment of arteriovenous malformations: obliteration, morbidities, and performance status. Int J Radiat Oncol Biol Phys. 2011;80(2):354-61.

18. Se YB, Kim DG, Park SH et al. Radiation-induced osteosarcoma after Gamma Knife surgery for vestibular schwannoma: a case report and literature review. Acta Neurochir. 2017;159(2):385-91.

19. Pollock BE, Meyer FB, Friedman WA. Radiosurgery for arteriovenous malformations. J Neurosurg. 2004;101(3):390-2.

20. Liščák R, Vladyka V, Šimonová G, Urgošík D, Novotný J Jr, Janoušková L, et al. Arteriovenous malformations after Leksell gamma knife radiosurgery: rate of obliteration and complications. Neurosurgery. 2007;60(6):1005-14.

21. Flickinger JC, Pollock BE, Kondziolka D, Lunsford LD. A dose-response analysis of arteriovenous malformation obliteration after radiosurgery. Int J Radiat Oncol Biol Phys. 1996;36(4):873-9.

22. Karlsson B, Lindquist C, Steiner L. Prediction of obliteration after gamma knife surgery for cerebral arteriovenous malformations. Neurosurgery. 1997;40(3):425-30.

23. Skjøth-Rasmussen J, Roed H, Ohlhues L, Jespersen B, Juhler M. Complications following linear accelerator based stereotactic radiation for cerebral arteriovenous malformations. Int J Radiat Oncol Biol Phys. 2010;77(2):542-7.

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Case report
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SEVERE INFECTION OF THE ANTERIOR ABDOMINAL WALL IN A PATIENT WITH DIABETES MELLITUS – A CASE REPORT

TEŠKA INFEKCIJA PREDNJEG ZIDA ABDOMENA KOD PACIJENTA SA DIJABETESOM MELITUS – PRIKAZ SLUČAJA

Džemail S. DETANAC[1], Dženana A. DETANAC[1], Mersudin MULIĆ[2], Merima A. ĆERANIĆ[1] and Anida I. ADEMOVIĆ[3]

Summary

Introduction. Necrotizing soft tissue infection is a severe, life threatening infection, with high mortality rate, especially in patients with comorbidities. **Case report**. We are presenting a 53-year-old female patient with diabetes mellitus and a severe infection of the anterior abdominal wall resulting from a vulval infection. The treatment consisted of an extensive excision of the abdominal wall necrosis and surgical eradication of the deep infection source, hyperbaric oxygen therapy, and antibiotic conservative therapy. **Conclusion**. Prompt diagnosis, aggressive medical treatment and radical surgical debridement, as soon as possible, are the key to successful treatment.

Key words: Abdominal Wall; Diabetes Mellitus; Soft Tissue Infections; Skin Diseases, Infectious; Fasciitis, Necrotizing; Anti-Bacterial Agents; Debridment; Hyperbaric Oxygenation

Sažetak

Uvod. Nekrotizirajuća infekcija mekih tkiva je veoma ozbiljna i po život opasna infekcija, sa veoma visokom stopom mortaliteta, posebno u grupi pacijenata sa komorbiditetima. **Prikaz slučaja.** Predstavljamo 53-godišnju pacijentkinju, sa dijabetesom melitus, koja je imala tešku infekciju prednjeg abdominalnog zida, koja je bila posledica infekcije kože vulvarnog regiona. Lečenje je podrazumevalo eksciziju velikog, nekrotičnog dela abdominalnog zida i hiruršku eradikciju infektivnog žarišta, hiperbaričnu terapiju sa antibiotskom i propratnom konzervativnom terapijom. **Zaključak.** Momentalno i agresivno lečenje i radikalna hirurška intervencija su ključ uspeha u lečenju pacijenata sa nekrotizirajućom infekcijom tkiva.

Ključne reči: trbušni zid; dijabetes melitus; infekcije mekih tkiva; kožne infekcije; nekrotizirajući fasciitis; antibiotici; debridman; hiperbarična oksigenacija

Introduction

Skin and soft tissue infections encompass a wide spectrum of inflammatory diseases of the skin, subcutaneous tissue, fascia and muscles [1]. The necrotizing soft tissue infection (NSTI) is a life threatening bacterial infection, characterized by rapid progression through deep tissue planes, resulting in necrosis, involving the scrotum, perineum, the abdominal wall, or the extremities [2]. The mortality rate is high (median mortality 32.2%). The prognosis becomes poorer in patients with comorbidities. The most frequent comorbidity in patients with necrotizing fasciitis is diabetes mellitus [2, 3].

Case Report

A 53-year-old patient, suffering from diabetes, receiving oral hypoglycemic therapy, was admitted to the Surgery Department of the General Hospital Novi Pazar, due to the anterior abdominal wall infection and stomach pain, in October 2015. The symptoms started two days earlier. On admission, the patient was in a bad general condition, confused, febrile, hypotensive, tachycardic, tachypneic, overweight and barely mobile. In the inguinal and vulvar regions, on the left side, there were signs of skin infection, with a purulent foul-smelling discharge. A serohemorrhagic bullous lesion, approximately 10 x 10 cm in size was visible on the front left abdominal wall in the periumbilical region, with local erythema and small epithelial lesions. In the lateral part of the anterior abdominal wall a stiffness zone was palpable, with a smaller area of crepitation. The rest of the skin was without clinically significant changes. The patient suffered from hypertension, which was considered comorbidity. Ten years earlier, the patient underwent left nephrectomy, due to renal calculus of the left kidney.

Common laboratory tests were performed at the department, as well as radiography and ultrasonography. On admission, the laboratory test results were: C-reactiv protein (CRP) 308,7 mg/L; white blood cells (WBC) $36{,}3\times10^9$/l; red blood cells (RBC) 5,02 x 10^{12}/l; hemoglobin (HGB) 152 g/l; glycemia 5,2 mmol/l; alkaline phosphatase 140 U/L, urea 6 mmol/L. The treat-

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Abbreviations

NSTI – necrotizing soft tissue infection
SIRS – systemic inflammatory response syndrome
HBOT – hyperbaric oxygen therapy
LRINEC – Laboratory Risk Indicator for Necrotizing Fasciitis
HBG – hemoglobin
CRP – C-reactiv protein
WBC – white blood cells
RBC – red blood cells

ment started with intravenous application of analgesics, triple antibiotic and supportive therapy, with intensive monitoring of vital parameters. After the general state and hemodynamic parameters were stabilized, 4 hours upon admission, a wide surgical excision of the necrotizing tissue of the anterior abdominal wall up to the zone without visible cutaneous changes was performed under general anesthesia. The surgery was performed and followed by rinsing with hydrogen and other antiseptic solutions, finished by placing a hydrogen peroxide dressing on the wound, and specific and delicate care of the infection-free skin. During the surgery, signs of anterior abdominal wall infection were noticed.

The supportive therapy and conservative antibiotic treatment continued (penicillin G, cephalosporins and metronidazole) empirically, until the specific antibiogram test results came. On the second day of hospitalization, a reoperation was performed with a repeated debridement and necrectomy, due to extensive infection **(Figure 1)**. From the wound smear, Pseudomonas spp. was isolated and the treatment was continued with carbapenem and metronidazole, according to the antibiogram, with regular monitoring of laboratory parameters **(Table 1)**. During the period of treatment, consultations were done with an internist, cardiologist, nephrologist, endocrinologist, and pneumophthisiologist, due to the comorbidities.

The patient was transferred to a tertiary medical center on the fifth day of hospitalization with stable vital parameters and early signs of wound stabilization. The conservative treatment continued, with a daily wound treatment, as well as hyperbaric oxygen therapy (HBOT). Twenty days later, the patient was referred to our department, for further treatment. After 7 days, the patient was released from the hospital, with advice on bandaging the wound and occasional check-ups with the surgeon. Five months after the release, the anterior abdominal wall healed, and presented without signs of infection **(Figure 2)**.

Discussion

Necrotizing soft tissue infection is a severe, multifactorial, life-threatening condition with diverse microbiological etiology characterized by rapid spread of infection which may cause extensive soft tissue damage [4]. Globally, the prevalence of NSTI has been reported to be 0.40 cases per 100.000 populations; it commonly affects men, with a male-to-female ratio of 3:1. The disease affects all age groups, but it occurs commonly

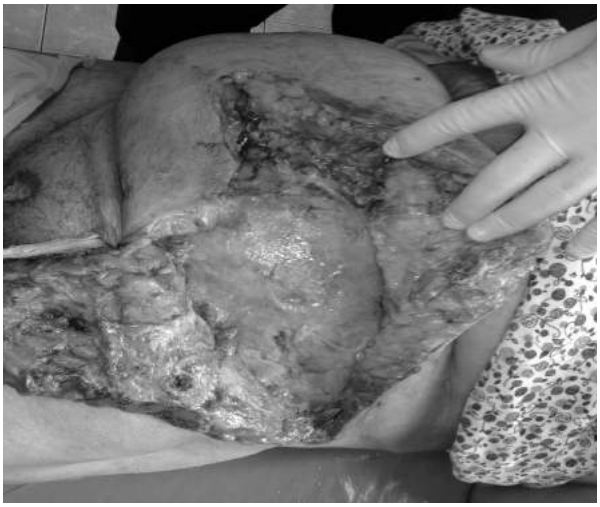

Figure 1. The infection of anterior abdominal wall after necrectomy
Slika 1. *Infekcija prednjeg trbušnog zida nakon nekrektomije*

among those over 50 years of age [3, 5]. The age is a very important predictor of lethal outcome of necrotizing infection and older patients have the poorest survival rate [6]. The most common comorbidity is diabetes mellitus (up to 60%). Other risk factors are comorbidities such as cirrhosis, obesity, alcohol abuse, corticosteroid therapy, smoking, immunodeficiency, hypertension, chronic renal failure [3, 7, 8]. There are differences between studies; Wong et al. reported 70.8% of patients with diabetes, but van Stigt et al. reported only 24.1% of patients with diabetes. Van Stigt et al. found cardiovascular disease to be the most frequent comorbidity in almost 40% of patients [7, 9].

Stone et al. and Yanar et al. reported 40–63% of deaths from NSTI [3, 10, 11]. The age of patients was considered to be an essential predicting factor in the potential lethal outcome. However, Kalaivani et al. believe that older age, combined with more comorbidities

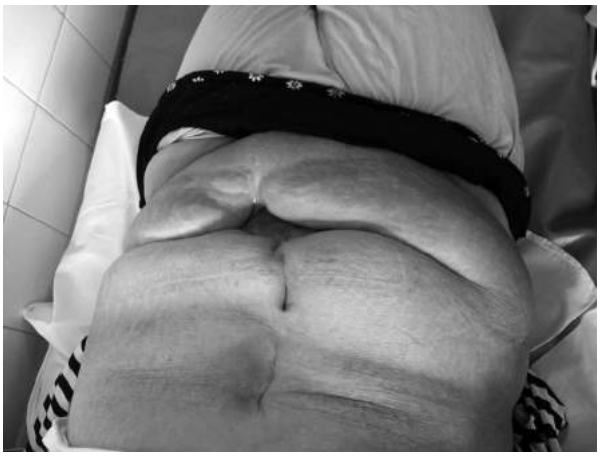

Figure 2. Anterior abdominal wall after successful treatment
Slika 2. *Prednji trbušni zid nakon uspešnog lečenja*

Table 1. Laboratory test results during the first hospitalization
Tabela 1. *Laboratorijske analize tokom prve hospitalizacije*

	CRP (mg/l)	WBC (x10^9/l)	RBC x10^{12}/l)	HGB (g/l)	Glycemia (mmol/l)	Creatinine (Umol/l)
09.10.2015.	308.7	36.3	5.02	152	5.2	76
10.10.2015.	310.1	40.1	4.71	144	8.8	73
11.10.2015.	300.2	34.0	4.43	136	9.1	80
12.10.2015.	276.5	31.3	4.28	125	20.1	72
13.10.2015.	208.3	22.7	4.42	122	7.5	

Legenda: CRP – C-reaktivni protein, WBC – bela krvna zrnca, RBC – crvena krvna zrnca, HGB – hemoglobin

leads to an increased risk of lethal outcome with a more frequent occurrence of severe forms of diseases [12].

We are presenting a 53-year-old female patient with several risk factors: 10-year history of diabetes mellitus, hypertension, obesity and only one kidney (left nephrectomy 10 years ago). This case is in the group of higher risk patients, which is in correlation with other studies.

A great amount of microorganisms (microbes), aerobes as well as anaerobes, with a synergistic effect cause infections. Elliot et al. reported that NSTIs are commonly polymicrobial, while a single pathogen was responsible for causing an infection in 28 out of 182 patients [13]. There was no possibility of isolating anaerobic microorganisms out of the tissue sample in our facility. Therefore, it may be the reason why merely Pseudomonas spp. was isolated from the necrotizing samples.

The etiology of NSTIs is usually associated with a pathological process from cutaneous sources, anorectal or genitourinary region [14]. The disease usually involves the perineum (50%), scrotum (30%), and the anterior abdominal wall (20%) [14, 15]. Chalia et al. [5] reported that scrotum was the most frequent anatomical site involved in more than 75% of patients in their study. In women, the most common entry site for infections and the basis for wide spreading are Bartholin abscesses and vulval skin infections [3].

The infection begins in the above mentioned areas and progresses as an inflammatory reaction that involves the superficial and deep-tissue planes. The extension of the infection and necrosis is facilitated by the synergy between different bacteria and toxins, and the enzymes they produce. The fascial and hypodermic necrotic spread is greater than the overlying skin changes [3]. The microorganisms produce various endotoxins and exotoxins. These toxins are also released into the systemic circulation, resulting in systemic inflammatory response syndrome (SIRS) and septic shock [14]. The detection of microorganisms is essential for implementation of a causal therapy for infectious diseases. The knowledge of the pathogen and its antibiotic susceptibility enables a targeted antimicrobial therapy to be conducted and guides the subsequent patient management [16].

Patients with this kind of infection usually have symptoms such as local pain, local warmth, erythema, tachycardia and fever, followed by hypotension and tachypnea. The infected site displays tenderness, skin necrosis, and hemorrhagic bullae [3, 17].

In our patient, the entry site for infection was the vulval infection, which spread in the left inguinal region, from where it expanded to the other parts of the anterior abdominal wall. Clinical signs and local findings on admission are in agreement with the mentioned studies.

Early diagnosis, aggressive resuscitation of the patient, administration of broad spectrum antibiotics, and aggressive radical surgical debridement, as soon as possible, are the key to successful treatment [5]. The shorter the interval between the infection onset and initiation of the treatment, the greater are the odds for better prognosis.

Although the diagnosis is based on clinical signs and physical examination, laboratory tests and radiological methods may help to delineate the extent of the disease.

The most important laboratory scoring system for early diagnosis is the Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC) score. A LRINEC score ≥ 6 indicates high rates of mortality [6, 9, 18]. In our patient the LRINEC score was six on admission, placing her in the high-risk group.

Emergency surgical debridement is the primary management modality for NSTI. Surgical intervention is life-saving and must be performed as early as possible. It can be repeated in the next 24 h or later, depending on the clinical course of the necrotizing infection [3]. In the study of Benjelloun et al., all patients underwent radical surgical debridement, ranging from 1 to 10 procedures, with an average of 2.5 [19]. Glass et al. reported that the median number of debridements in their study was 2, and the median number of total surgical procedures was 5 (range: 1 – 17) [2]. Aggressive surgical debridement (< 24 h) is associated with a lower mortality [7, 20].

The utility of HBOT in the treatment of NSTIs, as an essential part of treatment, has not been proved. The study of Shaw J. J. et al. showed that using HBOT was associated with increased survival in the severe cases, whereas in all other patients HBOT was associated with increased cost, without an immediate survival benefit [21].

Before surgery, our patient underwent fluid resuscitation and treatment with parenteral broad-spectrum triple antimicrobial agents, continuous monitoring and other conservative therapy. Bearing

in mind that the patient was admitted two days after the symptoms onset, the first surgical intervention was made within 4 hours after admission, after correction of her general condition. The reintervention, with additional debridement, was done in the next 24 hours, due to the expansion of the infection. The following treatment included referral of the patient into the tertiary healthcare center, where the wound treatment was continued with daily use of HBOT.

The patient reacted well to the prescribed therapy.

Conclusion

Necrotizing soft tissue infection is a very severe and unpredictable disease. It requires fast diagnosis and aggressive treatment, which primarily means wide surgical excision of the infected tissue and use of wide spectrum antibiotic treatment. A multidisciplinary approach to the treatment is crucial, in order to increase the odds of full recovery.

References

1. Gurlich R, Adamkova V, Ulrych J, Brodska H, Janík V, Lindner J, et al. Skin and soft tissue infections (SSTIs). Rozhl Chir. 2016;95(4):141-6.

2. Glass GE, Sheil F, Ruston JC, Butler PE. Necrotising soft tissue infection in a UK metropolitan population. Ann R Coll Surg Engl. 2015;97(1):46-51.

3. Misiakos EP, Bagias G, Patapis P, Sotiropoulos D, Kanavidis P, Machairas A. Current concepts in the management of necrotizing fasciitis. Front Surg. 2014;1:36.

4. Hansen MB, Simonsen U, Garred P, Hyldegaard O. Biomarkers of necrotizing soft tissue infections: aspects of the innate immune response and effects of hyperbaric oxygenation—the protocol of the prospective cohort BIONEC study. BMJ Open. 2015;5(5):e006995.

5. Chalya PL, Igenge JZ, Mabula JB, Simbila S. Fournier's gangrene at a tertiary health facility in northwestern Tanzania: a single centre experiences with 84 patients. BMC Res Notes. 2015;8:481.

6. Kojič M, Mikič D, Nožić D, Rakonjac B. Streptococcal necrotizing fasciitis with toxic shock syndrome and rapid fatal outcome. Srp Arh Celok Lek. 2015;143(7-8):476-9.

7. van Stigt SF, de Vries J, Bijker JB, Mollen RM, Hekma EJ, Lemson SM, et al. Review of 58 patients with necrotizing fasciitis in the Netherlands. World J Emerg Surg. 2016;11:21.

8. Angoules AG, Kontakis G, Drakoulakis E, Vrentzos G, Granick MS, Giannoudis PV. Necrotising fasciitis of upper and lower limb: a systematic review. Injury. 2007;38 Suppl 5:S19-26.

9. Wong CH, Khin LW, Heng KS, Tan KC, Low CO. The LRINEC (Laboratory Risk Indicator for Necrotizing Fasciitis) score: a tool for distinguishing necrotizing fasciitis from other soft tissue infections. Crit Care Med. 2004;32(7):1535-41.

10. Stone HH, Martin JD Jr. Synergistic necrotizing cellulitis. Ann Surg. 1972;175(5):702-11.

11. Yanar H, Taviloglu K, Ertekin C, Guloglu R, Zorba U, CabiogluN, et al. Fournier's gangrene: risk factors and strategies for management. World J Surg. 2006;30(9):1750-4.

12. Kalaivani V, Hiremath BV, Indumathi VA. Necrotising soft tissue infection-risk factors for mortality. J Clin Diagn Res. 2013;7(8):1662-5.

13. Elliott D, Kufera JA, Myers RA. The microbiology of necrotizing soft tissue infections. Am J Surg. 2000;179(5):361-6.

14. Singam P, Wei KT, Ruffey A, Lee J, Chou TG. Fournier's gangrene: a case of neglected symptoms with devastating physical loss. Malays J Med Sci. 2012;19(3):81-4.

15. Ullah S, Khan M, Asad Ullah Jan M. Fournier's gangrene: a dreadful disease. Surgeon. 2009;7(3):138-42.

16. Schmitz RP, Brunkhorst FM. Sepsis biomarkers and pathogen detection methods: state of the art. Sanamed. 2014;9(1):49-61.

17. Frazee BW, Fee C, Lynn J, Wang R, Bostrom A, Hargis C, et al. Community-acquired necrotizing soft tissue infections: a review of 122 cases presenting to a single emergency department over 12 years. J Emerg Med. 2008;34(2):139-46.

18. Tilkorn D, Citak M, Fehmer T, Ring A, Hauser J, Al Benna S, et al. Characteristics and differences in necrotizing fasciitis and gas forming myonecrosis: a series of 36 patients. Scand J Surg. 2012;101(1):51-5.

19. Benjelloun el B, Souiki T, Yakla N, Ousadden A, Mazaz K, Louchi A, et al. Fournier's gangrene: our experience with 50 patients and analysis of factors affecting mortality. World J Emerg Surg. 2013;8(1):13.

20. Cheung JP, Fung B, Tang WM, Ip WY. A review of necrotising fasciitis in the extremities. Hong Kong Med J. 2009;15(1):44-52.

21. Shaw JJ, Psoinos C, Emhoff TA, Shah SA, Santry HP. Not just full of hot air: hyperbaric oxygen therapy increases survival in cases of necrotizing soft tissue infections. Surg Infect (Larchmt). 2014;15(3):328-35.

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TRAUMATIC BRAIN INJURY AND ADJUSTMENT DISORDERS

TRAUMATSKE POVREDE MOZGA I POREMEĆAJI PRILAGOĐAVANJA

Jovan MILATOVIĆ

Summary

Introduction. Traumatic brain injury and reactive psychiatric disorders are universal health problems, both individually and in comorbidity. Traffic accidents are the most common cause of traumatic head injury, followed by falls, violence, and sports injuries. Due to the fact that they are associated with rapid, stressful events, they clearly trigger or generate reactive psychiatric disorders. What makes them special in this area is their organic substrate. Almost all patients with severe head injuries, more than half with moderate, and one tenth with mild head injuries suffer neuropsychiatric sequelae. **Discussion and Conclusion.** Among the published papers on this topic, prospective epidemiological analytical studies are dominant. Most articles deal with injured soldiers, injured children or adolescents. Recent papers emphasize the need for a timely, multidisciplinary care for the affected people and the primary community. It is very important to initiate early rehabilitation and psychotherapy. Due to non-specific and limited pharmacotherapeutic options, especially evident in organ damage and pediatric population, special attention is given to occupational, psychological rehabilitation, and cognitive-behavioral psychotherapy, as well as psycho-pharmacological drugs in case of clear clinical indications. As potentially the most important for further research, are the results on the genetic predisposition of individuals for clinical outcomes of associated conditions, structural and functional visualization of brain regions associated with specific psychological symptoms, and psycho-protective role of morphine and amnesia. Involvement of the wider community in a range of activities that contribute to poor outcomes is of utmost importance.

Key words: Brain Injuries, Traumatic; Craniocerebral Trauma; Adjustment Disorders; Stress, Psychological; Stress Disorders, Post-Traumatic; Memory Disorders; Risk Factors

Sažetak

Uvod. Traumatske povrede mozga i reaktivna psihijatrijska stanja predstavljaju ubikvitarne zdravstvene probleme, pojedinačno i u komorbiditetu. Većinu incidenata koji dovode do povreda glave čine saobraćajne nesreće, slede padovi, nasilje i sportske povrede. Pošto predstavljaju naglo nastale stresne događaje, jasno je uočljiva njihova uloga kao okidača ili generatora reaktivnih psihijatrijskih poremećaja. Ono što ih u ovom domenu čini posebnim jeste njihov organski supstrat. Gotovo svi pacijenti sa teškom povredom glave, više od polovine sa umerenom i desetina sa blagom povredom glave zadobije neuropsihijatrijske sekvele. **Diskusija i zaključak.** Među objavljenim radovima na ovu temu dominiraju epidemiološke prospektivne analitičke studije. Najbrojniji su radovi u kojima su posmatrani povređeni vojnici, kao i studije u kojima je zahvaćena dečja i adolescentna populacija. Aktuelni radovi potenciraju potrebu za pravovremenim multidisciplinarnim pristupom, usmerenim na pogođenog pojedinca i primarnu društvenu zajednicu. Veoma je značajno započeti sa ranom rehabilitacijom i psihoterapijom. Zbog nespecifičnih i limitiranih farmakoterapijskih mogućnosti, pogotovo kod izraženijih organskih oštećenja i u pedijatrijskoj populaciji, trenutno stanje ovog područja daje primat okupacionoj, psihorehabilitacionoj i kognitivno-bihejvioralnoj terapiji, sa adjuvantnom upotrebom psihofarmaka u slučaju jasnih kliničkih indikacija. Kao potencijalno najznačajniji za dalja istraživanja, izdvajaju se rezultati koji govore o genetskoj predisponiranosti pojedinca u odnosu na kliničke ishode udruženih stanja, strukturalnoj i funkcionalnoj vizuelizaciji moždanih regiona povezanih sa određenim psihološkim simptomima, kao i o psihoprotektivnoj ulozi morfina i amnezije. Posebno značajno je angažovanje šire društvene zajednice radi smanjenja rizičnih ponašanja i borbe protiv aktivnosti koje doprinose lošim ishodima.

Ključne reči: traumatske povrede mozga; kraniocerebralna trauma; poremećaji prilagođavanja; psihološki stres; posttraumatski stresni poremećaj; poremećaji memorije; faktori rizika

Introduction

Traumatic brain injury (TBI) is a non-degenerative, non-congenital brain tissue damage, caused by external mechanical force, associated with reduced or altered state of consciousness, with a high possibility of temporary or permanent occurrence of cognitive, physical, and psychosocial disorders [1]. The

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Abbreviations

TBI – traumatic brain injury
PTSD – post-traumatic stress disorder
MRI – magnetic resonance imaging
H MRS – proton magnetic resonance spectroscopy
NAAG – N-acetyl-aspartyl-glutamate
GCS – Glasgow coma scale

TBIs can roughly be divided into open (penetrating) and closed (blunt) injuries, whereas in regard to the severity of clinical manifestations into: mild, moderate, and severe injuries. Blunt traumas are more common than penetrating traumas [2].

Head injuries are frequent clinical conditions. It is estimated that about two million incidents are associated with head injuries annually. The most commonly affected are people aged 15 to 25 years, with triple predominance of males. Traffic accidents are responsible for more than half of all incidents that lead to blunt head injury. Falls, violence and sports injuries are responsible for most of the remaining cases. Almost all patients with severe injuries, more than half with moderate, and about one tenth with mild head injuries have neuropsychiatric sequelae resulting from head injury [2].

Memory disorders occurring after head injuries usually appear after retrograde amnesia, up to the moment of injury, sometimes including amnesia for the traumatic event itself. Injury severity may somewhat correlate with the duration and severity of amnesic syndrome, and the best predictive indicator of recovery is the memory recovery during the first week after regaining consciousness [3]. After a period of post traumatic amnesia, the recovery usually takes 6 to 12 months, and its symptoms are likely to remain permanent [2].

The most common cognitive problems after a head injury include slow processing of information, increased distractibility and consequent attention disorders, difficulty solving problems and a reduced ability to endure faith in oneself, problems with memory and learning new information, and diverse speech disorders [2]. Dementia can also be caused by head injury. Specifically, the so-called "punch-drunk" syndrome, or "dementia pugilistica", which occurs in boxers receiving repeated blows to the head for years, and is characterized by emotional instability, dysarthria and impulsivity [4].

Behavioral sequelae of traumatic head injuries primarily include depressive syndromes, increased impulsivity and irritability, and personality changes. The symptoms can be intensified by the use of alcohol, which may lead to traumatic incidents [2]. A recently published study of patients with TBI in the Swedish population, particularly distinguishes alcoholism, drug addiction and depression among psychiatric disorders that occur before and after the injury. Particularly important are the findings that show that comorbidity of psychiatric disorders and TBIs are significantly associated with an increased suicide rate and mortality in general, affirming that the premorbid psychiatric diagnosis seems to have a greater impact than the one made after the injury [5]. As a direct result of abrupt and sudden severe stress events or chronic stress, the following reactive psychiatric disorders may arise: acute stress reaction, post-traumatic stress disorder (PTSD), and adjustment disorders: short and prolonged depressive reaction, anxious-depressive and behavioral disorders [6]. Since traumatic head injury is certainly an abrupt and sudden stressful event, it is a trigger, or the generator of reactive mental disorders which is clearly visible. What is certainly special in this domain is their organic substrate.

The aim of this paper is to review the current literature on TBIs and adjustment disorders. Articles published in the last five years were analyzed using the PubMed database.

Discussion

Among the papers dealing with the problems of adjustment after TBI, prospective epidemiological analytical studies are most predominant. In regard to the number of the examined population, most articles studied soldiers who participated in the war zones of Afghanistan and Iraq, as well as children and adolescents.

The above mentioned Swedish study accurately provides data on psychiatric comorbidity in patients with TBIs leading to a significant increase in the mortality rate - 7.6 times higher than in patients without psychiatric disorders, if the six month period or more after the injury is considered [5]. Even worse, the mortality rate among psychiatric patients is more than 10 times higher in those diagnosed with substance abuse. Regarding the suicide rate, comorbidity of depression or substance abuse with TBIs leads to a statistically significant increase [5]. There is a strong association reported between premature deaths and both psychiatric disorder and substance abuse, with 61% of premature deaths in TBI patients having a lifetime psychiatric or substance abuse diagnosis. Among those with moderate to severe TBI and psychiatric or substance abuse comorbidity, 1 in 12 died prematurely. Another two important implications of this study show that the suicide rates and death are greater six months or more after the injury in patients with focal head injuries, but the assumption of a higher suicide rate among people with lower incomes and psychosocial deprivation has not been supported [5].

These findings are connected with the results of other studies dealing with social, demographic and psychiatric aspects of head injuries. Thus, a study conducted in Taiwan showed that people with mental disorders had almost twice the risk of such injuries, with predominance of male gender, older age, living in urban areas, and lower incomes as risk factors. The intensity of psychiatric treatment and pharmacotherapy is positively correlated with the risk of injury and mortality, which rises proportionally with the frequency of outpatient treatment, hospitaliza-

tion, and emergency psychiatric intervention [7]. The examination of the work-related psychiatric disorders in Korea showed that the main compensated occupational problems are as follows: 1) personality and behavior disorders caused by disease, injury or brain dysfunction, 2) other mental diseases due to cerebral injury/dysfunction and somatic diseases, 3) reaction to severe stress and adjustment disorders, and 4) depressive episodes [8]. The assumption that the incidence of stressful life events is a strong predictor of poor outcome after head trauma, is supported by the results of the study published in 2011, which reported that several potentially life-changing-stressful events experienced before the accident are present in at least 25% of the tested participants [9]. One group of studies examined the impact of psychological symptoms early after injury in the further course of the recovery and broader life-social consequences. Thus, a pilot study which included 142 patients with mild to moderate traumatic head injury found that the intensity of acute pain, post-traumatic adjustment, depression and acute trauma symptoms, as well as the use of alcohol, were significant predictors of the severity of pain, depression, post traumatic stress symptoms, as well as physical mobility 6 months after the injury [10]. Early multidisciplinary treatment, which involves pain therapists, physical medicine specialists, psychotherapists, occupational therapists, and clinical psychologists in the first and third months after injury, leads to a significant relief of pain symptoms 6 months later. This model of a therapeutic approach appears protective against the development of PTSD symptoms. Namely, in contrast to the 24% of patients undergoing conventional health care after the injury presenting with PTSD symptoms, none of the patients assigned to a multidisciplinary model had the characteristic symptoms following exposure to trauma 6 months after the injury [10]. Another research indicates that the poor outcome associated with memory, executive functions, attention and information processing speed, are connected with self-reported depression and anxiety after TBI, and adaptive coping strategies have greater influence on the level of depression in individuals with a lower information processing speed [11]. One-year follow-up of patients after traumatic axonal injury has shown that fatigued patients had subjective experience of significantly higher cognitive dysfunction and a lower quality of life than shown on the application scales [12]. The presence of alexithymia, in persons with TBIs, turned out to be a significant factor affecting the quality of relationships and satisfaction in relationships, especially from the point of view of the partners of injured patients, showing much lower estimates of the overall relationship quality, customization, consensus and cohesion [13].

In some studies, in addition to psychological tests, patients were subjected to radiological "neuroimaging" procedures, in order to achieve the visualization of the possible organic and functional changes that correlate with psychiatric symptoms. A five-year trial showed that scores of the peritraumatic dissociation levels are positively correlated with the activation of the right occipital lobe - lingual, fusiform and parahippocampal gyri, regions responsible for vivid autobiographical memory of highly emotional events. The results suggest that peritraumatic dissociation directly leads to the formation and intrusive memories. The peritraumatic dissociation represents a valuable predictor of PTSD development from the fifth week until the third month after the injury [14]. By comparing the findings of behavioral measurements with magnetic resonance imaging (MRI), there are three significant results: 1) impulsivity is associated with an elevated coefficient of diffusivity present bilaterally into the orbitofrontal gyrus, the insula and the caudate nucleus; 2) an abnormal adjustment risk correlates with an increased coefficient of diffusion shown in the right thalamus, the dorsal striatum and the left caudate nuclei; 3) damage of performing rational choices corresponds to displayed high diffusion coefficient in the bilateral dorsolateral prefrontal cortical areas of the frontal and top gyri, right ventrolateral prefrontal cortex, the striatum dorsal and ventral hippocampus and the left hippocampus. These findings support the theory that disruption of key monoaminergic neurotransmitter systems, such as dopamine, may play a key role in a widespread cognitive dysfunction seen after traumatic axonal injury [15]. Abnormalities of the proton magnetic resonance spectroscopy (H MRS) in the limbic system, with a reduced ratio of N-acetyl-aspartyl-glutamate (NAAG) - creatinine in the left hippocampus, together with the neuronal loss and/or dysfunction, correlated significantly with psychosocial adjustment. The change appears independent from the age of the patient and cortical atrophy. Also, the reduction of this ratio in the left frontal part of the cingulate cortex is present in patients with TBI and clinical diagnosis of mood disorders, independently of the age and severity of injury. This technique (H-MRS), may give valuable information about traumatic injuries of the brain which are not shown best in structural MRI [16]. The studies that compared psychometric scales with the findings obtained by neuro-radiological diagnostic procedures suggest that in vivo detection of brain damage could have important implications for the organization of the patient's medical care, research choice, and help understanding complex neurocognitive pathways [15].

Apart from the psychiatric, other comorbidities stand out as very important in the context of TBI. A thirteen-year monitoring of United States patients discharged with TBI has concluded that the worst outcomes of injured are found in patients with epilepsy and convulsive disorders, due to potentially more often repeated and therefore severe injuries [17]. The finding that seizure control is of utmost importance in the prevention of TBI and comorbid

conditions gets important if we take into account the results of the study indicating that the neurological and psychiatric conditions (substance abuse, psychosis, bipolar affective disorder, schizophrenia and depression) represent significant predictors of new onset epilepsy in older adults of both genders [18].

Since the treatment of cognitive and behavioral disorders in patients with head injuries are similar to the approaches in other patients with these symptoms, a difference in the fact that such patients may be particularly susceptible to side effects of psychotropic medications [2], most of the papers dealing with the treatment of these conditions are focused on the psychotherapeutic and rehabilitation approaches. Changes in the structural and functional connections after cognitive rehabilitation suggest that the pace of adjustment in the activities on the tasks and the rest in regions related to the site of injury, most likely is the mechanism responsible for the recovery of function. Behavioral interventions that target these processes emphasize the need for metacognitive and emotional regulation, as well as a very welcome role of subjective experience and beliefs as central in the process of rehabilitation [19]. Post-acute rehabilitation interventions improve integration into society, with special emphasis on the role of occupational therapy [20]. The need for a convenient, cost-effective and accessible education and training after injury is very important, but plenty of large barriers are present (problems finding a job, coexisting diagnosis, and limited self-representation). Opportunities for inter-agency cross-training and education, particularly in terms of symptoms of psychological adjustment risk assessment, can reduce the resulting disconnection, improve guardians' security and ease the crisis. Developing multi-professional teams to maximize the availability of services, either face to face or virtual, is essentially important. These perspectives highlight opportunities to improve access to services and strengthen the relationship between the guardian and agencies [21]. A new semiological approach to meet the best possible integration of people with severe TBI is needed, not only limited to medical care, including social-psychological care that is tailored to the needs of each person and family that lives in its environment, which is currently minimal even in developed countries [22].

As already mentioned, the most numerous among the analyzed papers are those dealing with soldiers who participated in war zones in this century and pediatric patients.

Children and adolescents

The most common causes of head injuries in children and adolescents, causing developmental disorders, including seizures, are traffic accidents. However, there are head injuries caused by home accidents: falls off the table, open windows and stairs, as well as traumas due to child abuse [23].

Epidemiological studies of pediatric population in the United States have shown TBI as the leading cause of morbidity, mortality and deficits in cognitive, behavioral and social functioning, as well as in quality of life related to health [24]. A Taiwanese study, which included over eight thousand pediatric patients, showed a significantly increased risk of TBI in socioeconomically disadvantaged children, especially among children with mental disorders, epilepsy, or both. It emphasized that children from low income families should be monitored with special care to reduce the risk of head injury and the subsequent morbidity [25].

Some studies examined the impact of age at the time of injury on the outcome of head injury. From the aspect of intellectual development, younger children are more resistant to the effects of TBI compared to the older, because of greater neuroplasticity. The view that young children have a greater capacity of cerebral reorganization may find support in early, focal vascular lesions, but not in severe diffuse white matter traumas. Permanent intellectual impairment probably arises from injury involving acceleration-deceleration of a high level, due to the resulting diffuse or multifocal severe injury of the brain tissue [26]. A research including children divided into four age categories (newborn, preschool, middle childhood, late childhood) shows the worst outcome of TBI in patients injured during the middle childhood or early school-period [27].

A cohort prospective comparative study, which compared children with TBIs and orthopedic injuries, shows that children with TBI presented with greater acquired anxiety problems compared with children with orthopedic injuries. With the passage of time, children who suffered brain injuries at an earlier age had higher levels of anxiety, attention and hyperkinetic disorders than children who were older at the time of injury [28]. Pediatric patients with mild TBI presented with more post concussion symptoms than those with orthopedic injury [29]. A 6-month follow-up of children aged 3 to 6 years suggested that children with head injury showed more sleep problems than children with orthopedic injury. Sleep problems, on the other hand, considerably increased the risk of poor psychosocial performance over time, but did not show worse neuropsychological test results [30]. An interesting study observed three groups of patients, aged 24 to 47 months, with acquired brain lesions (trauma injuries, vascular tumors and/or infectious damage). About half of the total sample showed psychological and behavioral problems, which varied depending on the etiology. Children with traumatic injuries achieved average scores on most behavioral scales for children aged 2 to 3 years, as opposed to the more distinct internalizing problems of children who have survived a brain tumor and higher scores on externalizing scales in children with vascular or infectious damage. The relevance and impact of brain lesions must necessarily be taken into account in the organization and development of psychological treatment, rehabilitation and social re-entry [31].

In addition to expected results, significantly lower values of intelligence quotient were found in chil-

dren with mild, moderate and severe TBIs sustained under the age of 3 years, compared to the control group, approximately after 40 months of injury; it is important to point out that the child's environment affects the cognitive-behavioral functioning after the injury [32]. Good maternal care and psychological support contribute to normal deoxyribonucleic acid methylation and brain development. In this sense clinically useful psychological instruments are dialogue, symbolic play, drawing and storytelling [33].

Pediatric patients with TBI are at risk of deterioration in the social sphere. The six-month follow-up of social functioning after brain trauma and contributing injuries, cognitive and environmental impact testing has shown serious violations and consequently scarce communication skills leading to greater social problems - adjustment and participation in social life disorders, with significant influence on family functioning. Processing speed, younger age and male gender contribute to social outcomes [34]. This study builds upon the results of similar ones that examined long-term psychosocial outcomes and quality of life. Parents of adolescents aged 15 to 18 years, who have suffered TBIs under the age of five years, reported that their children's quality of life was lower in comparison to their peers in the control group who did not experience a TBI [35]. An Icelandic cohort study of children and adolescents, who were sent questionnaires to scale clinical outcomes and issues related to socioeconomic status, sixteen years after the injury, showed that impact strength, the number and severity of injuries are predictors of poor results. Socioeconomic status of parents and demographic factors had limited effects [36]. One study registered more prominent vulnerability of children with mild TBIs in functional families, with better material and existential status, which is especially noticeable in the subsequent somatic symptoms [29].

Armed forces

Most of the published papers report on the development of adjustment disorders after TBI among American, British and Canadian soldiers who participated in the wars in Iraq and Afghanistan, as a major psychiatric comorbidity, namely PTSD. Although PTSD is usually a consequent psychiatric condition after head injury in military personnel of both genders, it was noted that females have fewer prospects for its development, but on the other hand, are twice as likely to develop depression, and increased risk for developing other anxiety disorders and PTSD with depression [37]. While less severe brain traumas reported by soldiers typically have neuropsychological consequences of limited duration, post traumatic stress and depression are associated with a more permanent cognitive loss [38].

Returnees from the battlefields with TBI associated with psychiatric comorbidity (PTSD, depression, alcohol abuse) represent major challenges: persistence in relationships, schools, physical health, sleep and ride [39]. Psychosocial adaptation is compromised by declining ability to cope with anger - the type reduction threshold of irritability, impaired memory and emotional instability, which is why these patients face problems when searching for job, have trouble with the full reintegration into society [40], and there are also significant denominated financial difficulties [41]. Their behavioral and personality changes, resulting from the co-morbidity of brain trauma and psychiatric disorders, often give rise to actions that are subject to criminal prosecution (criminally violent behavior, traffic violations), and a specific systematic approach is recommended for this population during forensic formulation cases, with particular note of the relationship between exposure combat debit/combat zone and associated clinical conditions [42]. A study which used driving simulation to analyze and compare the performance of veterans and people from the general population, registered that soldiers with mild TBI, and post-traumatic stress make more critical errors than the control group (due to speed and much slower reactions to sudden stimuli), which considerably increases the risk of traffic accidents [43].

Post concussion symptoms after mild TBI highly correlate with mental health problems that have arisen with the Canadian soldiers - participants in the war in Afghanistan [44]. Another research indicated that these symptoms were more pronounced among the soldiers with loss of consciousness after the injury, which in longer follow-up led to major psychosocial constraints [45]. Although studies show that these soldiers frequently present with moderate or severe headaches, they also affect their functional deterioration, and according to the results they are more associated with mental disorders than with mild TBIs [46].

Soldiers with PTSD and TBI often present with intermittent and restless sleep, according to a study where they used sensors in the rooms based on Doppler, sensors of light, sound and binder clips in the room environment and autographs observation [47]. Another study showed that frequent sleep disruptions in this population may interfere with the recovery and rehabilitation. Insomnia is a risk factor for PTSD, depression and suicide. Preliminary data suggest that the combination of cognitive-behavioral therapy for insomnia and "therapy practicing imagination" (imagery rehearsal therapy) demonstrated positive results in reduction of sleep disorders [48].

The greatest attention should probably be given to the results of a study which followed up 258 patients undergoing amputation after the injury in the war zone. None of the patients (0%) with a Glasgow Coma Scale (GCS) score of 12 and below developed PTSD during the observed period, compared with 20% of patients with scores of 12 or above, who developed PTSD. As for the patients with TBI, those who were treated with intravenous morphine during the first few hours after the injury had a much low-

er prevalence of PTSD (6.3%) and mood disorders (15.6%) compared with patients who received only fentanyl (PTSD prevalence of 41.2%, the prevalence of mood disorders, 47.1%). The GCS scores and therapy using morphine or fentanyl were not significantly associated with adjustment disorders, anxiety and substance abuse disorders [49].

Finally, worthy of note is that the research suggests that both the genotype of the serotonin transporter-linked polymorphic region (5-HTTLPR) status and TBI, independently, in almost identical but opposite ways affect the resilience and the perceived limitations of social participation. Among those who have suffered brain injury resistance, perceived constraints are higher among carriers of SO SO genotype, with respect to the holders of LO genotype. While the existence of TBI seems to increase the sensitivity, the veterans with occupational injuries, carriers of LO allele genotype fared the worst, with a lower resilience and a much lower community reintegration, compared to the carriers of LO allele genotype without TBI or veterans with SO SO genotype, regardless of the cerebral trauma status [50].

Conclusion

Traumatic brain injury and reactive psychiatric conditions are highly frequent and widespread health problems, both individually and in comorbidity. Bearing in mind the polymorphism of psychological, somatic and neurological symptoms connected with their associated occurrence, the main conclusion of the current scientific paper reviews imposes the need for timely, multidisciplinary approach, directed primarily to the affected individuals, as well as to the primary community. In the earliest stage, besides stabilizing somatic and neurological conditions, it is very important to start with the early rehabilitation and psychotherapy involving, if necessary, different specialists, psychologists, social workers and therapists. With regard to the non-specific and somewhat limited psychopharamacological possibilities, especially in patients with a pronounced organic damage and pediatric population, the current state of the art therapy gives priority to psychotherapy (occupational, psychological and rehabilitation, cognitive-behavioral), with the adjuvant use of psychopharmaceuticals in the case of clear clinical indications. Potentially most important for further analysis are the results on the genetic predispositions with regard to the clinical outcomes associated conditions (genotype of the serotonin transporter-linked polymorphic region), the structural and functional visualization of brain regions, which are specifically associated with certain psychological symptoms, as well as protecting role of morphine and amnesia in the development of post-traumatic stress disorder. Prevention is still the best way to reduce morbidity, comorbidity and mortality associated with traumatic brain injuries. It is of particular significance to engage wider community to cut risky behavior and fight against activities that are associated with poor outcome. In this regard, I would personally comment on the papers dealing with brain trauma and associated reactive psychiatric conditions in recent wars. It appears that a selective choice of patients (no study was performed on the problems of the local population and civilians exposed to military aggression) leads to a pseudo-humanistic approach. If the papers fail to condemn aggressive military campaigns, their devastating effects on the health of people and the community, we are far away from ethics and good medical and research practice.

References

1. Foulkes MA, Eisenberg HM, Jane JA, Marmarou A, Marshall LF. The Traumatic Coma Data Bank: design, methods, and baseline characteristics. J Neurosurg. 1991;75(1):S8-13.

2. Sadock BJ, Sadock VA. Kaplan & Sadock`s synopsis of psychiatry: behavioral sciences/clinical psychiatry. 10th ed. Baltimore: Lippincott Williams & Wilkins; 2007. p. 350-73.

3. Sadock BJ, Sadock VA. Kaplan & Sadock`s synopsis of psychiatry: behavioral sciences/clinical psychiatry. 10th ed. Baltimore: Lippincott Williams & Wilkins; 2007. p. 344-50.

4. Sadock BJ, Sadock VA. Kaplan & Sadock`s synopsis of psychiatry: behavioral sciences/clinical psychiatry. 10th ed. Baltimore: Lippincott Williams & Wilkins; 2007. p. 329-44.

5. Fazel S, Wolf A, Pillas D, Lichtenstein P, Långström N. Suicide, fatal injuries, and other causes of premature mortality in patients with traumatic brain injury: a 41-year Swedish Population Study. JAMA Psychiatry. 2014;71(3):326-33.

6. Nedić A. Reakcija na težak stres i poremećaji prilagođavanja. In: Nedić A, Živanović O, urednici. Psihijatrija – udžbenik za studente medicine. Novi Sad: Medicinski fakultet; 2011. p. 289-98.

7. Liao CC, Chiu WT, Yeh CC, Chang HC, Chen TL. Risk and outcomes for traumatic brain injury in patients with mental disorders. J Neurol Neurosurg Psychiatry. 2012;83(12):1186-92.

8. Choi KS, Kang SK. Occupational psychiatric disorders in Korea. J Korean Med Sci. 2010;25(Suppl):S87-93.

9. van Veldhoven LM, Sander AM, Struchen MA, Sherer M, Clark AN, Hudnall GE, et al. Predictive ability of preinjury stressful life events and post-traumatic stress symptoms for outcomes following mild traumatic brain injury: analysis in a prospective emergency room sample. J Neurol Neurosurg Psychiatry. 2011;82(7):782-7.

10. Browne AL, Appleton S, Fong K, Wood F, Coll F, de Munck S, et al. A pilot randomized controlled trial of an early multidisciplinary model to prevent disability following traumatic injury. Disabil Rehabil. 2013;35(14):1149-63.

11. Spitz G, Schönberger M, Ponsford J. The relations among cognitive impairment, coping style, and emotional adjustment following traumatic brain injury. J Head Trauma Rehabil. 2013;28(2):116-25.

12. Esbjörnsson E, Skoglund T, Sunnerhagen KS. Fatigue, psychosocial adaptation and quality of life one year after traumatic brain injury and suspected traumatic axonal injury; evaluations of patients and relatives: a pilot study. J Rehabil Med. 2013;45(8):771-7.

13. Williams C, Wood RL. The impact of alexithymia on relationship quality and satisfaction following traumatic brain injury. J Head Trauma Rehabil. 2013;28(5):E21-30.

14. Daniels JK, Coupland NJ, Hegadoren KM, Rowe BH, Densmore M, Neufeld RW, et al. Neural and behavioral correlates of peritraumatic dissociation in an acutely traumatized sample. J Clin Psychiatry. 2012;73(4):420-6.

15. Newcombe VF, Outtrim JG, Chatfield DA, Manktelow A, Hutchinson PJ, Coles JP, et al. Parcellating the neuroanatomical basis of impaired decision-making in traumatic brain injury. Brain. 2011;134(Pt 3):759-68.

16. Capizzano AA, Jorge RE, Robinson RG. Limbic metabolic abnormalities in remote traumatic brain injury and correlation with psychiatric morbidity and social functioning. J Neuropsychiatry Clin Neurosci. 2010;22(4):370-7.

17. Wilson DA, Selassie AW. Risk of severe and repetitive traumatic brain injury in persons with epilepsy: a population-based case-control study. Epilepsy Behav. 2014;32:42-8.

18. Martin RC, Faught E, Richman J, Funkhouser E, Kim Y, Clements K, et al. Psychiatric and neurologic risk factors for incident cases of new-onset epilepsy in older adults: data from U.S. Medicare beneficiaries. Epilepsia. 2014 ;55(7):1120-7.

19. Cicerone KD. Facts, theories, values: shaping the course of neurorehabilitation. The 60th John Stanley Coulter memorial lecture. Arch Phys Med Rehabil. 2012;93(2):188-91.

20. Kim H, Colantonio A. Effectiveness of rehabilitation in enhancing community integration after acute traumatic brain injury: a systematic review. Am J Occup Ther. 2010;64(5):709-19.

21. Meixner C, O'Donoghue CR, Witt M. Accessing crisis intervention services after brain injury: a mixed methods study. Rehabil Psychol. 2013;58(4):377-85.

22. Truelle JL, Fayol P, Montreuil M, Chevignard M. Community integration after severe traumatic brain injury in adults. Curr Opin Neurol. 2010;23(6):688-94.

23. Sadock BJ, Sadock VA. Kaplan & Sadock`s synopsis of psychiatry: behavioral sciences/clinical psychiatry. 10th ed. Baltimore: Lippincott Williams & Wilkins; 2007. p. 1138-58.

24. Schneier AJ, Shields BJ, Hostetler SG, Xiang H, Smith GA. Incidence of pediatric traumatic brain injury and associated hospital resource utilization in the United States. Pediatrics. 2006;118(2):483-92.

25. Liao CC, Chang HC, Yeh CC, Chou YC, Chiu WT, Chen TL. Socioeconomic deprivation and associated risk factors of traumatic brain injury in children. J Trauma Acute Care Surg. 2012;73(5):1327-31.

26. Levin HS. Long-term intellectual outcome of traumatic brain injury in children: limits to neuroplasticity of the young brain? Pediatrics. 2012;129(2):e494-5.

27. Crowe L, Catroppa C, Babl FE, Rosenfeld JV, Anderson V. Timing of traumatic brain injury in childhood and intellectual outcome. J Pediatr Psychol. 2012;37(7):745-54.

28. Karver CL, Wade SL, Cassedy A, Taylor HG, Stancin T, Yeates KO, et al. Age at injury and long-term behavior problems after traumatic brain injury in young children. Rehabil Psychol. 2012;57(3):256-65.

29. Yeates KO, Taylor HG, Rusin J, Bangert B, Dietrich A, Nuss K, et al. Premorbid child and family functioning as predictors of post-concussive symptoms in children with mild traumatic brain injuries. Int J Dev Neurosci. 2012;30(3):231-7.

30. Shay N, Yeates KO, Walz NC, Stancin T, Taylor HG, Beebe DW, et al. Sleep problems and their relationship to cognitive and behavioral outcomes in young children with traumatic brain injury. J Neurotrauma. 2014;31(14):1305-12.

31. Pastore V, Colombo K, Villa F, Galbiati S, Adduci A, Poggi G, et al. Psychological and adjustment problems due to acquired brain lesions in pre-school-aged patients. Brain Inj. 2013;27(6):677-84.

32. Crowe LM, Catroppa C, Babl FE, Anderson V. Intellectual, behavioral, and social outcomes of accidental traumatic brain injury in early childhood. Pediatrics. 2012;129(2):e262-8.

33. Bouras G, Lazaratou E. [Emergence of early childhood trauma in adult psychiatric symptomatology]. Psychiatriki. 2012;23 Suppl 1:39-48.

34. Anderson V, Beauchamp MH, Yeates KO, Crossley L, Hearps SJ, Catroppa C. Social competence at 6 months following childhood traumatic brain injury. J Int Neuropsychol Soc. 2013;19(5):539-50.

35. Green L, Godfrey C, Soo C, Anderson V, Catroppa C. A preliminary investigation into psychosocial outcome and quality-of-life in adolescents following childhood traumatic brain injury. Brain Inj. 2013;27(7-8):872-7.

36. Halldorsson JG, Arnkelsson GB, Tomasson K, Flekkoy KM, Magnadottir HB, Arnarson EO. Long-term outcome of medically confirmed and self-reported early traumatic brain injury in two nationwide samples. Brain Inj. 2013;27(10):1106-18.

37. Iverson KM, Hendricks AM, Kimerling R, Krengel M, Meterko M, Stolzmann KL, et al. Psychiatric diagnoses and neurobehavioral symptom severity among OEF/OIF VA patients with deployment-related traumatic brain injury: a gender comparison. Women's Health Issues. 2011;21(4 Suppl):S210-7.

38. Vasterling JJ, Brailey K, Proctor SP, Kane R, Heeren T, Franz M. Neuropsychological outcomes of mild traumatic brain injury, post-traumatic stress disorder and depression in Iraq-deployed US Army soldiers. Br J Psychiatry. 2012;201(3):186-92.

39. Plach HL, Sells CH. Occupational performance needs of young veterans. Am J Occup Ther. 2013;67(1):73-81.

40. Siddharthan K. The effect of post traumatic stress disorders on rehabilitation among combat-wounded veterans. Stud Health Technol Inform. 2012;182:114-24.

41. Elbogen EB, Johnson CS, Wagner HR, Newton VM, Beckham JC. Financial well-being and post-deployment adjustment among Iraq and Afghanistan war veterans. Mil Med. 2012;177(6):669-75.

42. Sreenivasan S, Garrick T, McGuire J, Smee DE, Dow D, Woehl D. Critical concerns in Iraq/Afghanistan war veteran-forensic interface: combat-related postdeployment criminal violence. J Am Acad Psychiatry Law. 2013;41(2):263-73.

43. Classen S, Levy C, Meyer DL, Bewernitz M, Lanford DN, Mann WC. Simulated driving performance of combat veterans with mild tramatic brain injury and posttraumatic stress disorder: a pilot study. Am J Occup Ther. 2011;65(4):419-27.

44. Garber BG, Rusu C, Zamorski MA. Deployment-related mild traumatic brain injury, mental health problems, and post-concussive symptoms in Canadian Armed Forces personnel. BMC Psychiatry. 2014;14:325.

45. Verfaellie M, Lafleche G, Spiro A 3rd, Tun C, Bousquet K. Chronic postconcussion symptoms and functional outcomes in OEF/OIF veterans withself-report of blast exposure. J Int Neuropsychol Soc. 2013;19(1):1-10.

46. Rona RJ, Jones M, Goodwin L, Hull L, Wessely S. Risk factors for headache in the UK military: cross-sectional and longitudinal analyses. Headache. 2013;53(5):787-98.

47. Yardibi T, Cleary D, Wood J, Stachura M, Wood E, Dicks A. Sleep and activity monitoring for Returning Soldier Adjustment Assessment. Conf Proc IEEE Eng Med Biol Soc. 2012;2012:2144-8.

48. Bramoweth AD, Germain A. Deployment-related insomnia in military personnel and veterans. Curr Psychiatry Rep. 2013;15(10):401.

49. Melcer T, Walker J, Sechriest VF 2nd, Lebedda M, Quinn K, Galarneau M. Glasgow Coma Scores, early opioids, and posttraumatic stress disorder among combat amputees. J Trauma Stress. 2014;27(2):152-9.

50. Graham DP, Helmer DA, Harding MJ, Kosten TR, Petersen NJ, Nielsen DA. Serotonin transporter genotype and mild traumatic brain injury independently influence resilience and perception of limitations in veterans. J Psychiatr Res. 2013;47(6):835-42.

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Primaju se samo radovi koji su napisani na engleskom jeziku, uz sažetak rada i naslov rada koji treba da budu napisani na engleskom i srpskom jeziku.

Radove koji su pristigli u časopis *Medicinski pregled* pregleda jedan ili više članova Uređivačkog odbora Časopisa. Oni radovi koji su napisani prema pravilima Časopisa šalju se na anonimnu recenziju kod najmanje dva recenzenta, stručnjaka iz odgovarajuće oblasti biomedicine. Načinjene recenzije radova pregleda glavni urednik ili članovi Uređivačkog odbora i one nisu garancija da će rad biti prihvaćen za štampu. Materijal koji je pristigao u časopis ostaje poverljiv dok se rad nalazi na recenziji, a identitet autora i recenzenata su zaštićeni, osim u slučaju ako oni odluče drugačije.

U časopisu *Medicinski pregled* objavljuju se: uvodnici, originalni članci, prethodna ili kratka saopštenja, pregledni članci, stručni članci, prikazi slučajeva, članci iz istorije medicine i drugi članci.

1. Uvodnici – do 5 strana. Sadrže mišljenja ili diskusiju o posebno značajnoj temi za Časopis, kao i o podacima koji su štampani u ovom ili nekom drugom časopisu. Obično ih piše jedan autor po pozivu.

2. Originalni članci – do 12 strana. Predstavljaju rezultate istraživanja autora rada i njihovo tumačenje. Istraživanje treba da bude obrađeno i izloženo na način da se može ponoviti, a analiza rezultata i zaključci jasni da bi se mogli proveriti.

3. Pregledni članci – do 10 strana. Predstavljaju sistematsko, sveobuhvatno i kritičko izlaganje problema na osnovu analiziranih i diskutovanih podataka iz literature, a koji oslikavaju postojeću situaciju u određenom području istraživanja. Literatura koja se koristi u radu mora da sadrži najmanje 5 radova autora članka iz uže naučne oblasti koja je opisana u radu.

4. Prethodna ili kratka saopštenja – do 4 strane. Sadrže izuzetno važne naučne rezultate koje bi trebalo objaviti u što kraćem vremenu. Ne moraju da sadrže detaljan opis metodologije rada i rezultata, ali moraju da imaju sva poglavlja kao originalni članci u sažetoj formi.

5. Stručni članci – do 10 strana. Odnose se na proveru ili prikaz prethodnog istraživanja i predstavljaju koristan izvor za širenje znanja i prilagođavanja originalnog istraživanja potrebama postojeće nauke i prakse.

6. Prikazi slučajeva – do 6 strana. Opisuju retke slučajeve iz prakse. Slični su stručnim člancima. U ovim radovima prikazuju se neuobičajeni oblici i tokovi oboljenja, neočekivane reakcije na primenjenu terapiju, primene novih dijagnostičkih procedura ili retke i nove bolesti.

7. Članci iz istorije medicine – do 10 strana. Ovi članci opisuju događaje iz prošlosti sa ciljem da omoguće očuvanje medicinske i zdravstvene kulture. Imaju karakter stručnih članaka.

8. Ostali članci – U časopisu Medicinski pregled objavljuju se feljtoni, prikazi knjiga, izvodi iz strane literature, izveštaji sa kongresa i stručnih sastanaka, saopštenja o radu pojedinih zdravstvenih organizacija, podružnica i sekcija, saopštenja Uredništva, pisma Uredništvu, novosti u medicini, pitanja i odgovori, stručne i staleške vesti i članci napisani u znak sećanja (*In memoriam).*

Priprema rukopisa

Kompletan rukopis, uključujući tekst rada, sve priloge i propratno pismo, treba poslati na elektronsku adresu koja je prethodno navedena.

Propratno pismo:

– mora da sadrži izjavu svih autora da se radi o originalnom radu koji prethodno nije objavljen niti prihvaćen za štampu u drugim časopisima;

– autori svojim potpisom preuzimaju odgovornost da rad ispunjava sve postavljene uslove i da ne postoji sukob interesa i

– autor mora navesti kategoriju članka (originalni rad, pregleni rad, prethodno saopštenje, stručni rad, prikaz slučaja, rad iz istorije medicine, itd.).

Rukopis

Opšta uputstva

Tekst rada treba da bude napisan u programu *Microsoft Word* za *Windows*, na A4 formatu stranice (sve četiri margine 2,5 cm), proreda 1,5 (isto važi i za tabele), fontom *Times New Roman*, veličinom slova 12 *pt*. Neophodno je koristiti međunarodni sistem mernih jedinica (*SI*), uz izuzetak temperature (° *C*) i krvnog pritiska (*mmHg*).

Rukopis treba da sadrži sledeće elemente:

1. Naslovna strana

Naslovna strana treba da sadrži: kratak i sažet naslov rada, bez skraćenica, skraćeni naslov rada (do 40 karaktera), imena i prezimena autora (ne više od 6) i afilijacije svih autora. Na dnu strane treba da piše ime, prezime i titula autora zaduženog za korespondenciju, njena/njegova adresa, elektronska adresa, broj telefona i faksa.

2. Sažetak

Sažetak ne može da sadrži više od 250 reči niti skraćenice. Treba da bude strukturisan, kratak i sažet, sa jasnim pregledom problema istraživanja, ciljevima, metodama, značajnim rezultatima i zaključcima.

Sažetak originalnih i stručnih članaka treba da sadrži uvod (sa ciljevima istraživanja), materijale i metode, rezultate i zaključak.

Sažetak prikaza slučaja treba da sadrži uvod, prikaz slučaja i zaključak.

Sažetak preglednih članaka treba da sadrži Uvod, podnaslove koji odgovaraju istima u tekstu i Zaključak.

Navesti do 10 ključnih reči ispod sažetka. One su pomoć prilikom indeksiranja, ali autorove ključne reči mogu biti izmenjene u skladu sa odgovarajućim deskriptorima, odnosno terminima iz *Medical Subject Headings*, *MeSH*.

Sažetak treba da bude napisan na srpskom i engleskom jeziku. Sažetak na srpskom jeziku trebalo bi da predstavlja prevod sažetka na engleskom, što podrazumeva da sadrži jednake delove.

3. Tekst članka

Originalni rad treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima istraživanja), Materijal i metode, Rezultati, Diskusija, Zaključak, spisak skraćenica (ukoliko su

korišćene u tekstu). Nije neophodno da se u posebnom poglavlju rada napiše zahvalnica onima koji su pomogli da se istraživanje uradi, kao i da se rad napiše.

Prikaz slučaja treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima), Prikaz slučaja, Diskusija i Zaključak.

Uvod

U poglavlju Uvod potrebno je jasno definisati predmet istraživanja (prirodu i značaj istraživanja), navesti značajne navode literature i jasno definisati ciljeve istraživanja i hipoteze.

Materijal i metode

Materijal i metode rada treba da sadrže podatke o vrsti studije (prospektivna/retrospektivna, uslove za uključivanje i ograničenja studije, trajanje istraživanja, demografske podatke, period praćenja). Detaljno treba opisati statističke metode da bi čitaoci rada mogli da provere iznesene rezultate.

Rezultati

Rezultati predstavljaju detaljan prikaz podataka koji su dobijeni istraživanjem. Sve tabele, grafikoni, sheme i slike moraju biti citirani u tekstu rada i označeni brojevima po redosledu njihovog navođenja.

Diskusija

Diskusija treba da bude koncizna, jasna i da predstavlja tumačenje i poređenje rezultata studije sa relevantnim studijama koje su objavljene u domaćoj i međunarodnoj literaturi. U poglavlju Diskusija potrebno je naglasiti da li su postavljene hipoteze potvrđene ili nisu, kao i istaknuti značaj i nedostatke istraživanja.

Zaključak

Zaključci moraju proisteći isključivo iz rezultata istraživanja rada; treba izbegavati uopštene i nepotrebne zaključke. Zaključci koji su navedeni u tekstu rada moraju biti u saglasnosti sa zaključcima iz Sažetka.

4. Literatura

Potrebno je da se literatura numeriše arapskim brojevima redosledom kojim je u tekstu navedena u parentezama; izbegavati nepotrebno velik broj navoda literature. Časopise bi trebalo navoditi u skraćenom obliku koji se koristi u *Index Medicus* (*http://www.nlm.nih.gov/tsd/serials/lji.html*). Pri citiranju literature koristiti Vankuverski sistem. Potrebno je da se navedu svi autori rada, osim ukoliko je broj autora veći od šest. U tom slučaju napisati imena prvih šest autora praćeno sa *et al*.

Primeri pravilnog navođenja literature nalaze se u nastavku.

Radovi u časopisima

∗ Standardni rad

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ Organizacija kao autor

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ Bez autora

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ Volumen sa suplementom

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ Sveska sa suplementom

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ Sažetak u časopisu

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Knjige i druge monografije

∗ Jedan ili više autora

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ Urednik (urednici) kao autor (autori)

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ Poglavlje u knjizi

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ Zbornik radova sa kongresa

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

∗ Disertacija

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Elektronski materijal

∗ Članak iz časopisa u elektronskom formatu

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ Monografija u elektronskom formatu

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ Kompjuterska datoteka

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Prilozi (tabele, grafikoni, sheme i slike)

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

Tabele, grafikoni, sheme i slike se postavljaju kao posebni dokumenti.

– Tabele i grafikone bi trebalo pripremiti u formatu koji je kompatibilan programu u kojem je napisan tekst rada. Slike bi trebalo poslati u jednom od sledećih oblika: *JPG*, *GIF*, *TIFF*, *EPS*.

– Svaki prilog mora biti obeležen arapskim brojem prema redosledu po kojem se navodi u tekstu rada.

– Naslovi, tekst u tabelama, grafikonima, shemama i legende slika bi trebalo da budu napisani na srpskom i engleskom jeziku.

– Nestandardne priloge označiti u fusnoti uz korišćenje sledećih simbola: *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– U legendi slika trebalo bi napisati korišćeno uveličanje okulara i objektiva mikroskopa. Svaka fotografija treba da ima vidljivu skalu.

– Ako su tabele, grafikoni, sheme ili slike već objavljene, navesti originalni izvor i priložiti pisano odobrenje autora za njihovo korišćenje.

– Svi prilozi će biti štampani kao crno-bele slike. Ukoliko autori žele da se prilozi štampaju u boji, obavezno treba da plate dodatne troškove.

6. Dodatne obaveze

AUTORI I SVI KOAUTORI RADA OBAVEZNO TREBA DA PLATE GODIŠNJU PRETPLATU ZA ČASOPIS *MEDICINSKI PREGLED*. U PROTIVNOM, RAD NEĆE BITI ŠTAMPAN U ČASOPISU.

INFORMATION FOR AUTHORS

Medical Review publishes papers (previously neither published in nor submitted to any other journals) from various fields of biomedicine intended for broad circles of doctors.

Since January 1th, 2013 the Medical Review has been using the service e-Ur: Electronic Journal Editing. All users of the Registration system, i.e. authors, reviewers, and editors have to be registered users with only one e-mail address. Registration should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/user/register.

Manuscript submission should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/

A SUPPLEMENTARY FILE, WITH THE STATEMENT THAT THE PAPER HAS NOT BEEN SUBMITTED OR ACCEPTED FOR PUBLICATION ELSEWHERE AND A CONSENT SIGNED BY ALL AUTHORS, HAVE TO BE ENCLOSED WITH THE MANUSCRIPT.

Authors may not send the same manuscript to more than one journal concurrently. If this occurs, the Editor may return the paper without reviewing it, reject the paper, contact the Editor of the other journal(s) in question and/or contact the author's employers.

Papers should be written in English language, with an abstract and title page in English, as well as in Serbian language.

All papers submitted to ***Medical Review*** are seen by one or more members of the Editorial Board. Suitable articles are sent to at least two experts to be reviewed, thier reports are returned to the assigned member of the Editorial Board and the Editor. Revision of an article gives no guarantee of acceptance and in some cases revised articles are rejected if the improvements are not sufficient or new issues have arisen. Material submitted to *the Journal* remains confidential while being reviewed and peer-reviewers' identities are protected unless they elect to lose anonymity.

Medical Review publishes the following types of articles: editorials, original studies, preliminary reports, review articles, professional articles, case reports, articles from history of medicine and other types of publications.

1. Editorials – up to 5 pages – convey opinions or discussions on a subject relevant for the Journal. Editorials are commonly written by one author by invitation.

2. Original studies – up to 12 pages – present the authors' own investigations and their interpretations. They should contain data which could be the basis to check the obtained results and reproduce the investigative procedure.

3. Review articles – up to 10 pages – provide a condensed, comprehensive and critical review of a problem on the basis of the published material being analyzed and discussed, reflecting the current situation in one area of research. Papers of this type will be accepted for publication provided that the authors confirm their expertise in the relevant area by citing at least 5 self-citations.

4. Preliminary reports – up to 4 pages – contain scientific results of significant importance requiring urgent publishing; however, it need not provide detailed description for repeating the obtained results. It presents new scientific data without a detailed explanation of methods and results. It contains all parts of an original study in an abridged form.

5. Professional articles – up to 10 pages – examine or reproduce previous investigation and represent a valuable source of knowledge and adaption of original investigations for the needs of current science and practice.

6. Case reports – up to 6 pages – deal with rare casuistry from practice important for doctors in direct charge of patients and are similar to professional articles. They emphasize unusual characteristics and course of a disease, unexpected reactions to a therapy, application of new diagnostic procedures and describe a rare or new disease.

7. History of medicine – up to 10 pages – deals with history with the aim of providing continuity of medical and health care culture. They have the character of professional articles.

8. Other types of publications – The journal also publishes feuilletons, book reviews, extracts from foreign literature, reports from congresses and professional meetings, communications on activities of certain medical institutions, branches and sections, announcements of the Editorial Board, letters to the Editorial Board, novelties in medicine, questions and answers, professional and vocational news and In memoriam.

Preparation of the manuscript

The complete manuscript, including the text, all supplementary material and covering letter, is to be sent to the web address above.

The covering letter:

– It must contain the proof given by the author that the paper represents an original work that it has neither been previously published in other journals nor is under consideration to be published in other journals.

– It must confirm that all the authors meet criteria set for the authorship of the paper, that they agree completely with the text and that there is no conflict of interest.

– It must state the type of the paper submitted (an original study, a review article, a preliminary report, a professional article, a case report, history of medicine).

The manuscript:

General instructions.

Use Microsoft Word for Windows to type the text. The text must be typed in font *Times New Roman*, page format A4, space 1.5 (for tables as well), margins set to 2.5 cm and font size 12pt. All measurements should be reported in the metric system of the International System of Units – SI. Temperature should be expressed in Celsius degrees (°C) and pressure in mmHg.

The manuscript should contain the following elements:

1. The title page.

The title page should contain a concise and clear title of the paper, without abbreviations, then a short title (up to 40 characters), full names and surnames of the authors (not more than 6) indexed by numbers corresponding to those given in the heading along with the full name and place of the institutions they work for. Contact information including the academic degree(s), full address, e-mail and number of phone or fax of the corresponding author (the author responsible for correspondence) are to be given at the bottom of this page.

2. Summary.

The summary should contain up to 250 words, without abbreviations, with the precise review of problems, objectives, methods, important results and conclusions. It should be structured into the paragraphs as follows:

– Original and professional papers should have the introduction (with the objective of the paper), materials and methods, results and conclusion

– Case reports should have the introduction, case report and conclusion

– Review papers should have the introduction, subtitles corresponding to those in the paper and conclusion.

The authors should provide up to 10 keywords below the summary. These keywords will assist indexers in cross-indexing the article and will be published with the summary, but the authors' keywords could be changed in accordance with the list of Medical Subject Headings, MeSH of the American National Medical Library.

The summary should be written in both languages, English as well as Serbian. The summary in Serbian language should be the translation of the summary in English; therefore, it has to contain the same paragraphs.

3. The text of the paper.

The text of original studies must contain the following: introduction (with the clearly defined objective of the study), materials and methods, results, discussion, conclusion, list of abbreviations (if used in the text) and not necessarily, the acknowledgment mentioning those who have helped in the investigation and preparation of the paper.

The text of a case report should contain the following: introduction (with clearly defined objective of the study), case report, discussion and conclusion.

Introduction contains clearly defined problem dealt with in the study (its nature and importance), with the relevant references and clearly defined objective of the investigation and hypothesis.

Materials and methods should contain data on design of the study (prospective/retrospective, eligibility and exclusion criteria, duration, demographic data, follow-up period). Statistical methods applied should be clear and described in details.

Results give a detailed review of data obtained during the study. All tables, graphs, schemes and figures must be cited in the text and numbered consecutively in the order of their first citation in the text.

Discussion should be concise and clear, interpreting the basic findings of the study in comparison with the results of relevant studies published in international and national literature. It should be stated whether the hypothesis has been confirmed or denied. Merits and demerits of the study should be mentioned.

Conclusion must deny or confirm the attitude towards the 0based solely on the author's own results, corroborating them. Avoid generalized and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

4. References are to be given in the text under Arabic numerals in parentheses consecutively in the order of their first citation. Avoid a large number of citations in the text. The title of journals should be abbreviated according to the style used in Index Medicus (http://www.nlm.nih.gov/tsd/serials/lji.html). Apply Vancouver Group's Criteria, which define the order of data and punctuation marks separating them. Examples of correct forms of references are given below. List all authors, but if the number exceeds six, give the names of six authors followed by 'et al'.

Articles in journals

∗ *A standard article*

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ *An organization as the author*

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ *No author given*

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ *A volume with supplement*

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ *An issue with supplement*

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ *A summary in a journal*

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Books and other monographs

∗ *One or more authors*

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ *Editor(s) as author(s)*

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ *A chapter in a book*

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ *A conference paper*

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

∗ *A dissertation and theses*

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Electronic material

∗ *A journal article in electronic format*

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ *Monographs in electronic format*

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ *A computer file*

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Attachments (tables, graphs, schemes and photographs).

THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

– Tables, graphs, schemes and photographs are to be submitted as separate documents, on separate pages.

– Tables and graphs are to be prepared in the format compatible with Microsoft Word for Windows programme. Photographs are to be prepared in JPG, GIF, TIFF, EPS or similar format.

– Each attachment must be numbered by Arabic numerals consecutively in the order of their appearance in the text

– The title, text in tables, graphs, schemes and legends must be given in both Serbian and English languages.

– Explain all non-standard abbreviations in footnotes using the following symbols *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– State the type of color used and microscope magnification in the legends of photomicrographs. Photomicrographs should have internal scale markers.

– If a table, graph, scheme or figure has been previously published, acknowledge the original source and submit written permission from the copyright holder to reproduce it.

– All attachments will be printed in black and white. If the authors wish to have the attachments in color, they will have to pay additional cost.

6. Additional requirements

SHOULD THE AUTHOR AND ALL CO-AUTHORS FAIL TO PAY THE SUBSCRIPTION FOR MEDICAL REVIEW, THEIR PAPER WILL NOT BE PUBLISHED.