Publishing Sector of the Society of Physicians of Vojvodina of the Medical Society of Serbia, Novi Sad, Vase Stajica 9

MEDICAL REVIEW

JOURNAL OF THE SOCIETY OF PHYSICIANS OF VOJVODINA OF THE MEDICAL SOCIETY OF SERBIA
THE FIRST ISSUE WAS PUBLISHED IN 1948

MEDICAL REVIEW is published bimonthly (six issues per year) with a circulation of 1.000 copies. The annual payment fee in 2018, for individuals from the territory of Serbia, is 3,000.00 dinars (the value-added tax included), 4,000.00 dinars for individuals from Serbia who are not members of the Society of Physicians of Vojvodina of the Medical Society of Serbia, 60 Euros for members outside the territory of Serbia, and 8,000.00 dinars (+ VAT) for institutions. The payment account is: 340-1861-70 or 115-13858-06, "Annual membership fee for Medical Review".

The manuscripts are submitted at: aseestant.ceon.rs/index.php/medpreg/. Editorial Office Address: Društvo lekara Vojvodine Srpskog lekarskog društva, 21000 Novi Sad, Vase Stajića 9, Tel. 021/521-096; 063/81 33 875, E-mail: dlv@sbb.rs; Website: www.dlv.org.rs

Printed by: Department for Joint Affairs of Provincial Authorities - Sector for Printing

Izdavačka delatnost Društva lekara Vojvodine Srpskog lekarskog društva, Novi Sad, Vase Stajića 9

MEDICINSKI PREGLED

ČASOPIS DRUŠTVA LEKARA VOJVODINE SRPSKOG LEKARSKOG DRUŠTVA
PRVI BROJ JE ŠTAMPAN 1948. GODINE.

MEDICINSKI PREGLED izlazi dvomesečno (šest dvobroja godišnje), u tiražu od 1000 primeraka. Pretplata za pojedince sa teritorije Srbije za 2018. godinu iznosi 3.000,00 dinara (sa uračunatim PDV-om), a 4.000,00 dinara za pojedince iz Srbije koji nisu članovi DLV-SLD, 60 eura za članove van Srbije, a za ustanove 8.000,00 dinara (uz dodavanje PDV-a). Uplate se vrše na račun broj 340-1861-70 ili 115-13858-06, s naznakom „Dodatna članarina za Medicinski pregled".

Prijem rukopisa vrši se u elektronskoj formi na stranici: aseestant.ceon.rs/index.php/medpreg/.
Adresa Redakcije: Društvo lekara Vojvodine Srpskog lekarskog društva,
21000 Novi Sad, Vase Stajića 9, Tel. 021/521-096; 063/81 33 875
E-mail: dlv@sbb.rs; Web: www.dlv.org.rs

Štamparija: Uprava za zajedničke poslove pokrajinskih organa - Odsek za poslove štamparije

M E D I C A L R E V I E W
JOURNAL OF THE SOCIETY OF PHYSICIANS OF VOJVODINA OF THE MEDICAL SOCIETY OF SERBIA
Novi Sad Vase Stajića 9 Serbia

Med Pregl 2018; LXXI (1-2): 1-78. Novi Sad: January-February.

CONTENTS

M E D I C I N S K I P R E G L E D
ČASOPIS DRUŠTVA LEKARA VOJVODINE SRPSKOG LEKARSKOG DRUŠTVA
Novi Sad *Vase Stajića 9* *Srbija*

Med Pregl 2018; LXXI (1-2): 1-78. Novi Sad: januar-februar.

SADRŽAJ

EDITORIAL
UVODNIK

University of Novi Sad, Faculty of Medicine Novi Sad
Department of Physiology

Editorial
Uvodnik
UDK 616.8-009.1-08
https://doi.org/10.2298/MPNS1802005N

SPASTICITY – A RESULT OF CENTRAL NERVOUS SYSTEM INJURY

SPASTIČNOST – POSLEDICA OŠTEĆENJA CENTRALNOG NERVNOG SISTEMA

Nada NAUMOVIĆ

Summary

Introduction. Spasticity is an interrrupted or constant hyperactivity of the sceletal muscles, caused by lesion of the upper motor neuron. The aim of this article was to gather scientifically and clinically important data about spasticity. **Morpho-functional basis on which spasticity originates.** The spasm is motoric disorder in which an increased muscle tone is present (especially of antigravity muscles), with slower movements and tendency of appearing moderate atrophy and contractures. **Etiology.** Spasticity may be caused by stroke; demyelinating diseases (sclerosis multiplex, amyotrophic lateral sclerosis); cerebral palsy; tumors; brain and spinal cord damage (trauma, ischemia, surgical intervention); other neurodegenerative diseases. **Symptoms**. There are a number of symptoms: increased muscle tone, joint stiffness, overactive reflexes, involuntary jerky movements, which may include spasms and clonus, pain, decreased functional abilities and delayed motor development, difficulty with care and hygiene, unusual posture, abnormal positioning of fingers, wrists, arms, or shoulders, contractures or muscle tightness. **Management of spasticity- therapeutic treatment:** physical therapy and rehabilitation, medicamentous and surgical treatment. **Conclusion.** Spasticity is a complex phenomenon with constant hyperactivity of the sceletal muscles, it is one component of upper motoneuron syndrome and many neurological diseases and disorders. Therapeutic treatment of spasticity should be highly specific, individualized, multidisciplinar and conducted carefully, controlled with the purpose of functional improvement and pain relief. It is significant to know that sometimes spasticity is useful and should not be removed. There is a need for standardized protocols for 'best practice' in management of spasticity.

Key words: Muscle Spasticity; Muscle, Skeletal; Motor Neuron Disease; Muscle Hypertonia; Risk Factors; Signs and Symptoms; Therapeutics

Sažetak

Uvod. Spasticitet je isprekidana ili konstantna hiperaktivnost skeletnih mišića, prouzrokovana lezijom gornjeg motornog neurona. Cilj ovog članka bio je da se sakupe naučno i klinički značajni podaci o spasticitetu. **Morfo-funkcionalna osnova na kojoj je baziran spasticitet.** Spazam je motorni poremećaj u kojem je povišen mišićni tonus (posebno antigravitacionih mišića), sa usporenim pokretima i tendencijom nastanka atrofije i kontraktura. **Etiologija**. Spasticitet može da nastane zbog moždanog udara, demijelinizacionih bolesti (sclerosis multiplex, amyotrophic lateral sclerosis), dečje paralize, tumora, oštećenja mozga ili kičmene moždine (trauma, ishemija, hirurška intervencija); drugih degenerativnih bolesti. **Simptomi**. Može da ima razne simptome: povišen tonus mišića, ukočenost zglobova, živahne reflekse, nevoljne pokrete, koji mogu da uključuju spazam i klonus, bol, snižene funkcionalne sposobnosti i usporen motorički razvoj, teškoće u aktivnostima svakodnevnog života; neuobičajena postura, abnormalno pozicioniranje prstiju, šake, ruke, ili ramena, kontrakture ili mišićna stegnutost. **Kreiranje terapijskog tretmana spasticiteta**: fizikalna terapija sa rehabilitacijom, medikamentni i hirurški tretman. **Zaključak.** Spasticitet je kompleksan fenomen, sa konstantnom hiperaktivnošću skeletnih mišića; komponenta je sindroma gornjeg motornog neurona i mnogih neuroloških bolesti i poremećaja. Terapijski tretman spasticiteta je veoma specifičan; mora da se sprovodi pažljivo, kontrolisano, a sa ciljem funkcionalnog poboljšanja i uklanjanja bola. Značajno je da se ima u vidu da je ponekad spasticitet koristan i ne treba ga uklanjati.

Ključne reči: mišićni spasticitet; skeletni mišić; oboljenje motornog neurona; mišićni hipertonus; znaci i simptomi; terapija

Introduction

A European Thematic Network to Develop Standardised Measures of Spasticity suggested the following definition: "Spasticity is an interrupted or constant hyperactivity of the skeletal muscles, caused by lesions of the upper motor neuron" [1]. It is characterized by tight or stiff muscles and an inability to control their contractions. In addition, reflex contraction may persist for too long and may be too strong. Spasticity is a physiological result of brain and/or spinal cord injury, which can be life treatening or lead to severe disabil-

Corresponding Author: Prof. dr Nada Naumović, Univerzitet u Novom Sadu, Medicinski fakultet, 21000 Novi Sad, Hajduk Veljkova 3, E-mail: nada.naumovic@mf.uns.ac.rs

Abbreviations

UMNS – upper motor neuron syndrome
UMN – upper motor neuron
SC – spinal cord
MS – medulla spinalis
GABA – gamma-aminobutyric acid

ity. It is a component of the upper motor neuron syndrome (UMNS). The aim of this article is to survey some data important for morpho-functional basis and management possibilities of spasticity.

Morpho-Functional Basis of Spasticity

The motor system consists of neurons and pathways whose integrated activity ensures posture and movements of the body and its individual parts. The upper motor neuron (UMN) controls the speed, strength and direction of the voluntary movements. The cell body (soma) is in the cerebral cortex and its axon extends to the spinal cord (SC) and all the injuries in this section of the pathway are called supranuclear lesions, while the injuries of soma of the lower motor neuron are nuclear lesions [2]. The injuries of axons of the lower motor neuron are called infranuclear lesions. The lower motor neurons of medulla spinalis are involved in many reflex mechanisms.

Of clinical significance for motor activity are myotatic reflex, inverse myotatic reflex and gamma loop reflex, which provide appropriate muscular speed and efficacy [2]. Myotatic reflex is a contraction that occurs in response to stretching within the muscle. Basically, these are annulospiral stretch receptors with Ia afferent fibers, synapse in the SC and an efferent axon of lower α motor neuron that causes muscle contraction. The inverse myotatic reflex starts from the Golgi tendon organs which register an increased tone, with Ib afferent fibers which cause excitation of spinal interneurons causing inhibition or inactivation of Ia fibers. Gamma motor neurons innervate intrafusal muscle fibers and provide the maintenance of sensitivity of the muscle spindle at the time when muscle is shortened during the contraction.

The muscle tone is permanent, basic muscle tension, which constantly exists but varies in intensity due to muscle activity, action of sensory stimuli of different modality and of current emotional state. It is physiologically controlled by excitatory presynaptic potentials of Ia afferent fibers and inhibitory postsynaptic potentials from muscle spindles of antagonistic muscles. Tonus may be altered by hypotonia or hypertonia. The hypertonia in UMNS can be a condition of increased muscle tone (especially of antigravity musculature) that occurs due to the damage or disease of UMN mediated by the strech reflex or intrinsic, non reflex hypertonia, due to contracture. If the muscle tone is increased due to the lesion of the pyramidal tract, it is called a spasm, and if it occurs due to the damage of extrapyramidal tract, a rigor.

A spasm is a motoric disorder in which an increased muscle tone (especially of antigravity muscles), with slower movements has a tendency of atrophy and/or contractures. It always involves groups of muscles. In spasm, there is an elastic resistance to passive stretching of the muscles and upon the termination of stretching, part of the body returns to its initial position. Sometimes a clonus may occur.

In rigidity, an increased muscle tone is much weaker than in spasm, but the muscles are partly constantly under contraction (especially the antagonists of antigravity muscles). Movements are slow, hypertonia is constant throughout the whole movement, and after the stretching the extremity remains in this newly-established position, i.e. it does not return to its initial position, as with spasm. It involves just one muscle.

The upper motor neuron syndrome represents the lesion of upper motor neuron which leads to the absence of inhibition and to disorder of the reflex arc with spasm. Spasticity, i.e. the hyperactivity of myotatic reflex can be caused by lowering the threshold of excitation of muscle spindles or an abnormal processing of sensory inputs in SC.

Two balanced descending systems are controlling stretch reflex activity: the inhibitory dorsal reticulospinal tract and facilitatory medial reticulospinal tract and vestibulospinal tract. Only the ventromedial bulbar reticular formation, originating from the dorsal reticulospinal tract is under cortical control. Brain damage causes spasticity due to the disruption of facilitatory corticobulbar fibers and causes inhibition of ventromedial reticular formation [3]. Spasticity appears to be caused by loss of reduced excitability of both postsynaptic (decreased reciprocal Ia or Ib inhibition, recurrent inhibition) and presynaptic inhibitory circuits (gamma-aminobutyric acid - GABA-ergic), which control the stretch reflex, postactivation depression decreases at spinal level (independent of the cortex) [4].

Spasticity may be caused by the plasticity of the nervous system. Plasticity of the nervous system is an attempt to restore the function. This tends to promote the formation of new neronal circuits that allow the formation of new movement patterns by axonal growth and sprouting and an increase in the number of postsynaptic membrane receptors [2, 3]. But the ultimate effect can be excessive, inadequate muscle reflex response to any peripheral stimulation. The overall result is reduced muscle tone and weaker tendon response which is a reason why there is no relaxation and muscle being in constant spasm. New branches toward vestibular, rubrospinal and reticulospinal tract are less selective than pyramidal tract, leading to overactivity. Furthermore, muscle fibrosis and other components of muscle contracture might even increase spasticity.

Spasticity is more often found in flexor muscles of the upper limbs, and in extensor muscles of the lower limbs [1, 3].

There are different degrees of spasticity (depending on velocity and length): the clasp knife phenomenon: increased resistance is present only at the beginning or at the end of the movement; stiffness with resistance throuhout the whole passive movement; stiffness with an intermittent resistance to the passive movement [2, 4].

Spasticity can be caused by pain: sensory disturbances, excessive stress on joint and muscles when they are stretched, disruption of some muscle fibers occur and release substances which influence nociceptors. Vs. pain increases spasticity.

The lesion of the lower motor neuron can be caused by trauma or metabolic disorder (alcoholism, diabetes mellitus). A flaccid paralysis appears, hypotonia, pronounced atrophy (70–80%) and only a segment muscles inervated by the damaged alpha motor neuron are affected [2].

Combined upper and lower motor neuron lesions cause so-called alternating hemiplegia, because the deficit of upper motor neuron is manifested contralaterally while of the lower motor neuron - ipsilaterally. This often happens if the lesions are in the brainstem. If the lesions of upper and lower motor neuron are at the level of SC, the symptoms are manifested ipsilaterally [2, 4].

Etiology

Spasticity may be caused by stroke; demyelinating diseases (multiple sclerosis, amyotrophic lateral sclerosis); cerebral palsy; tumors; brain and SC injury (trauma, ischemia, surgical intervention); other neurodegenerative diseases [2–4].

Symptoms

In the UMNS, immediately after the stroke or a trauma, "negative" symptoms appear: weakness, early hypotonia; loss of deep tendon reflexes; loss of dexterity; paresis and paralysis; increased fatigue; pain. Later, other "positive" signs appear: muscle hyperactivity; spasticity; hyper-reflection; atetosis; spastic dystonia; clonus (series of fast involuntary contractions); cocontraction; abnormal posture, contractures (permanent contraction of the muscle and tendon due to severe persistent stiffness and spasms) and bone and joint deformities. A positive Babinski sign tends to appear soon after the lesion and persists [1–3].

Management of Spasticity: Therapeutic Modalities

Before the treatment begins, it should be considered whether a patient needs a treatment and to what purpose? It is necessary to establish the cause, the degree and distrubution of spasticity; localization of injury; comorbidity (contractures, cognitive decline ...); clinical course of the disease; cognitive status of the patient; potential adverse effects; support of the family and community; passive mobility, presence of clonus, reflexes, tonus estimation and active mobility; electromyoneurography; quantitative analysis of walking; spasticity estimation using the Ashworth scale, spasm scale, Wartenberg pendulum test, and Tardieu scale [5, 6].

Sometimes spasticity may also be useful, since it may be: a warning mechanism; preservation of muscle mass; prevention of osteoporosis to a certain extent; maintenance of limb circulation; assistance in rehabilitation process; sometimes ensures stability in cases of muscle weakness – especially for walking; facilitates standing position, transfers and moving with the help of orthoses and aids.

The therapy is justified only if it is associated with intensive pain; with sleep disorders; significantly limited functional capacity of patients; limited capabilities of positioning and orthotising; in prevention of contractures. In patients with UMNS, mobilization of the affected limbs and prevention of prolonged shortened position of affected muscles are probably the most important things to do in order to prevent and treat muscle hypertonia. In this case, physiotherapy is of utmost importance, providing regular and individualized stretching program, along with correct positioning of limbs and application of splints and casts [4].

A multidisciplinary spasticity management includes:

– Prevention: nurture, proper positioning, regular skin inspection and bladder and intestinal program, stretching to maintain the amplitude of movement, prevention of complications such as decubitus, urinary retention, constipation, infection and pain.

– Therapy: physical procedures, work therapy, positioning/orthoses, medications, surgical interventions [3, 5, 6].

The objectives of rehabilitation in spasticity are to reduce muscle tone, maintain or improve the amplitude of movements, increase strength and coordination and improve quality of life; rehabilitation to improve daily life activities; alleviating pain and muscle spasms; utilization of orthoses/splints/trolleys ...; prevention of drug abuse; delay or avoid surgical interventions [7]. Physical therapy and rehabilitation includes kinesitherapy: controlled stretching, muscle strenthening (1h per day, 5 times per week), hydrotherapy, underwater massage, parafango, cryomassage, vibrations, thermotherapeutic modalities, functional electrical stimulation/biofeedback, hippotherapy, neuromuscular facilitation according to the Bobath concept [7–9]. Neuromodulation is also used with the aim of reducing the intensity of neuropathic pain, spasm and spastic pain by: low frequency direct current or injections of medications in the spinal subdural space [3, 10]. Nintendo Wii remote may serve as a convenient and cost-efficient tool for the assessment of spasticity [11].

Medications in the therapy of spasticity may be administered orally, transdermally, via an intrathecal pump, and chemodenervation (blockage). Drugs that are usually used are: medications acting on GABA system: benzodiazepines (diazepam and clonazepam); baclofen; dantrolene sodium; gabapentin; alpha-adrenergic and serotonergic agents (noradrenergic pathways modulate the presynaptic inhibition of afferent spinal neurons): tizanidine; clonidine; dexmedetomidine; cyproheptadine; central myorelaxants: cyclobenzaprine, carisoprodol, methocarbamol, metaxalone, chlorzoxazone, chlorphenesin, chlorpromazine; and cannabis and cannabinoid-like substances (dronabilon, nabilone). Some medications can be used as mesotherapy [12, 13].

Surgical treatment of spasticity is based on neurolysis, i. e. neurotoxins cause chemodenervation. Local anesthetics can also be used in the removal of spasticity symptoms. Parenteral administration includes: phenol, botulinum toxin, alcohol, lidocaine. The methods of administration are intrathecal drug application or dorsal rhizotomy of lumbar and sacral afferent nerve roots [3, 6, 14].

Conclusion

Spasticity is a complex phenomenon with constant hyperactivity of the skeletal muscles. It is part of the upper motor neuron syndrome and many neurological diseases and disorders. Therapeutic treatment of spasticity should be highly specific, individualized, multidisciplinary and conducted carefully, as well as controlled with the purpose of functional improvement and pain relief. It is of utmost importance to know that sometimes spasticity is useful and should not be removed. There is a need for standardized protocols for 'good clinical practice' in the management of spasticity.

References

1. Burridge JH , Wood DE , Hermens HJ , Voerman GE , Johnson GR , van Wijck F, et al. Theoretical and methodological considerations in the measurement of spasticity. Disabil Rehabil. 2005;27(1-2):69-80.

2. Young PA, Young PH, Tolbert DL. Basic clinical neuroscience. 3th ed. Philadelphia: Wolters Kluwer; 2015.

3. Trompetto C, Marinelli L, Mori L, Pelosin E, Currà A, Molfetta L, et al. Pathophysiology of spasticity: implications for neurorehabilitation. Biomed Res Int. 2014;2014:354906.

4. Mirbagheri MM, Settle K, Harvey R, Rymer WZ. Neuromuscular abnormalities associated with spasticity of upper extremity muscles in hemiparetic stroke. J Neurophysiol. 2007;98(2):629-37.

5. Tomašević Todorovic S, Kopčanski S, Mikov A, Bošković K, Petrovic, Popović Petrović S, et al. Functional status of patients after stroke. Med Pregl. 2015;68(5-6):181-6.

6. Ansari NN, Naghdi S, Arab TK, Jalaie S. The interrater and intrarater reliability of the Modified Ashworth Scale in the assessment of muscle spasticity: limb and muscle group effect. NeuroRehabilitation. 2008;23(3):231-7.

7. Barak O, Ivetić V, Filipović D, Naumović N, Lukac D, Drapsin M, et al. Event-related potentials following exercise bouts of different intensity. Med Pregl. 2007;60(11-12):531-5. Serbian.

8. Naumović N. Posebna razmatranja u odnosu na lumbalnu radikulopatiju. In: Bošković K, editor. Lumbalna radikulopatija i kvalitet života. 1st ed. Novi Sad: Medicinski fakultet Univerziteta u Novom Sadu; 2009. p. 49-103.

9. Lee YH, Lee YT, Park KH, Kim SH, Jang SM, Kim TH, et al. Effect of EMG-triggered electrical stimulation in patients with chronic hemiplegia. J Korean Acad Rehabil Med. 2003;27(3):320-8.

10. Sommerfeld DK, Eek EU, Svensson AK, Holmqvist LW, von Arbin MH. Spasticity after stroke: its occurrence and association with motor impairments and activity limitations. Stroke. 2004;35(1):134-9.

11. Ward AB. A summary of spasticity management – a treatment algorithm. Europ J Neurol. 2002;9 Suppl 1:48-52.

12. Yeh CH, Hung CY, Wang YH, Hsu WT, Chang YC, Yeh JR, et al. Novel application of a Wii remote to measure spasticity with the pendulum test: proof of concept. Gait Posture. 2016;43:70-5.

13. Naumović N, Bačikin R. Mezoterapija kao komplementarna grana fizikalnoj i rehabilitacionoj medicini: pregled dosadašnjih iskustava. Balneoclimatologia. 2016;40(2):169-74.

14. Smyth MD, Peacock WJ. The surgical treatment of spasticity. Muscle Nerve. 2000;23(2):153-63.

Rad je primljen 4. XII 2018.
Prihvaćen za štampu 4. XII 2018.
BIBLID.0025-8105:(2018):LXXI:1-2:5-8.

ORIGINAL STUDIES
ORIGINALNI NAUČNI RADOVI

Dental Office "Dr. Repić" Novi Sad[1]
Secondary Medical School "7th April" Novi Sad[2]
Primary Health Care Center "Novi Sad", Novi Sad[3]

Original study
Originalni naučni rad
UDK 616.314.16-089
https://doi.org/10.2298/MPNS1802009R

CLINICAL AND RADIOGRAPHIC OUTCOMES OF SURGICAL MANAGEMENT OF CHRONIC PERIAPICAL LESIONS IN MULTIROOTED TEETH

KLINIČKI I RADIOGRAFSKI ISHOD HIRURŠKOG LEČENJA HRONIČNIH PERIAPIKALNIH PROMENA VIŠEKORENIH ZUBA

Igor REPIĆ[1], Gordana REPIĆ[2], Dragana ZARIĆ[3] and Anđelija PETROVIĆ[1]

Summary

Introduction. Chronic periapical lesion is a pathological process of the root apex and the surrounding alveolar bone. Granuloma, which is a term that usually describes a chronic periapical process, represents a mass of granulation tissue at the apex of a devitalized tooth. Such lesions are treated by conventional (endodontic) therapy or surgery. The aim of this study was to assess clinical and radiographic outcomes of surgical treatment of chronic periapical lesions in multirooted teeth. **Material and Methods.** This prospective study included 30 patients who underwent a standard Partsch surgical procedure. The root canals, without previous endodontic treatment, were obturated immidiately before surgery, or intraoperatively. The canals with short fillings or teeth with fixed prosthetics were retrogradely obturated. Zinc-phosphate cement condensed with gutta-percha cone was used for obturation. Evaluation of clinical outcomes was performed 6, 12 and 24 months after surgery, and radiographic outcomes after 12 and 24 months, respectively. **Results.** After surgery, a statistically significant improvements of treatment outcomes were observed between the follop-ups ($p < 0.000$). The overall success rate of chronic periapical lesions in multirooted teeth 24 months after surgery was 83.3% for clinical and 66.7% for radiographic outcomes. **Conclusion.** The results of clinical and radiographic parameters after surgical treatment outcomes in multirooted teeth were satisfying. Therefore, we may conclude that surgical treatment can be considered as a primary treatment option in the management of chronic periapical lesions.

Key words: Periapical Diseases; Chronic Disease; Periapical Granuloma; Tooth Root; Treatment Outcome; Radiography, Dental; Root Canal Obturation; Oral Surgical Procedures

Sažetak

Uvod. Hronične periapikalne lezije predstavljaju patološka stanja apeksa korena zuba i okolne kosti. Granulom je izraz koji se često koristi za hronične periapikalne lezije, a odnosi se na granulaciono tkivo koje je u kontaktu sa apeksom avitalnog zuba. Ove promene leče se konzervativno (endodontski) i hirurški. Cilj ovog istraživanja bila je analiza rezultata hirurškog lečenja hroničnih periapikalnih promena višekorenih zuba na osnovu kliničkih i radioloških parametara. **Materijal i metode.** Istraživanje je bilo po tipu prospektivne studije kojom je obuhvaćeno 30 pacijenata. Svi pacijenti operisani su standardnim hirurškim postupkom po Parču (*Partsch*). Opturacija kanala korena zuba, koji nisu prethodno endodontski tretirani, vršena je neposredno preoperativno ili intraoperativno. Kanali koji su imali kratka punjenja, odnosno zubi sa fiksnim protetskim radovima, opturisani su retrogradno. Za opturaciju je korišćen cink-fosfatni cement kondenzovan gutaperka poenom. Rezultati su klinički evaluirani nakon 6, 12 i 24 meseca nakon završene terapije, odnosno radiološki 12 i 24 meseca nakon hirurškog zahvata. **Rezultati.** Nakon hirurškog lečenja, rezultati lečenja statistički značajno su se poboljšavali između prve i druge i prve i treće kontrole ($p = 0,000$). Ukupan procenat uspeha hirurškog lečenja hroničnih periapikalnih lezija višekorenih zuba 24 meseca nakon hirurške intervencije bio je 83,3% uzimajući u obzir kliničke parametre, odnosno 66,7% uzimajući u obzir radiološke parametre. **Zaključak.** Analizom rezultata kliničkih i radioloških parametara kod pacijenata kod kojih je primenjen hirurški tretman promena na višekorenim zubima dolazi se do zaključka da hirurško lečenje ima veoma zadovoljavajuće efekte, te se smatra terapijom izbora u lečenju hroničnih periapikalnih promena.

Ključne reči: periapikalne bolesti; hronične bolesti; periapikalni granulom; koren zuba; ishod lečenja; dentalna radiografija; opturacija kanala korena zuba; oralne hirurške procedure

Introduction

Chronic periapical lesion is defined as a pathological process of the rooth apex and the surrounding alveolar bone. Granuloma, which is a term that usually describes a chronic periapical process, represents a mass of granulation tissue at the devitalized tooth. It develops as a defense mechanism and forms a margin towards the tissue destruction products within the root canal [1].

Corresponding Author: Dr Igor Repić, Stomatološka ordinacija "Repić", 21000 Novi Sad, Nikole Pašića 16, E-mail: Igorord@eunet.rs

Dental pulp degeneration may occur even before the development of complete dentin destruction and such tissue response divides pulp from the carious defect. The process of pulp degeneration inevitably leads to necrosis. Extensive chronicity of this disease affects the parodontium and at that point radiographic changes are seen as lighter shades of the periapical bone, as well as widening of the periodontal space or thickening of the lamina dura. Changes on pericapical tissues also might be seen in the stage of chronic open pulpitis, when pulp tissue is directly exposed to external effects (ie. infection). The exposed part of the pulp becomes gangrenous, while the healthy pulp reacts by creating an ulcer towards the infected part. This phenomenon has a progredient course until the whole pulp become gangrenous. Changes of the periapical tissue manifest radiographically in the stage of chronic ulcerative pulpitis. According to some authors, radiographically visible changes can be seen only when the pulp tissue is completely affected by the gangrenous process [2, 3]. However, some authors claim that clinical diagnosis does not necessarily correlate with histopathological findings [4].

In regard to the etiopathogenesis, there are four main couses of chronic periapical lesions: infective agents (bacteria, viruses, and their toxins), physical agents (mechanical trauma, heat, cold or radiation), chemical damages (organic and inorganic poisons) and immunological agents like cell-mediated and antigen–antibody reactions [5].

Most frequently, infection affects the periapex through the apical foramen (foramen apicis dentis). It is also documented that infection may spread by hematogenous and lymphogenous routes. [6]. During an intracanal treatment, it is necessary to achieve two main goals: to extract the content of radicular canal, to reshape the morphology of the existing radicular canal, and to make it fit for definitive obturation and canal restoration [2–4, 7].

Apicoectomy is reffered to as a surgical removal of the apical segment of the root. Criteria and indications for apicoectomy include:

1. Active periapical inflammation despite endodontic management
2. Perforation of non-resorbable material and gutta to periapical region
3. Severely curved apex of the root canal which does not allow instrumentation
4. Extremely long root
5. Breakage of instrument in the root canal
6. Root fracture in the apical portion
7. Continuous discharge through the root canal which does not allow adequate conservative treatment
8. If there is a need to complete the management in one visit [8].

The surgical treatment should be performed after adequate canal obturation, as part of the endodontic management of the affected tooth, or as an addition to endodontic management, respectively. If treatment was not performed before surgery, the canal has to be obturated either immidiately before the surgery or intraoperatively. It is not recommended for a root canal to be filled a day or even a few hours prior to surgery, because the restoration may cause an inflamatory process. If the canal can be dried properly, it can be filled immidiately prior to the surgery. Such filling is usually referred to as orthograde and it shortens the duration of the intervention, but in such cases it is necessary to perform a control X-ray [9].

After lifting the mucoperiosteal flap, the pathological process, which either thinned the bone or perforated it, can be found. After partial or complete removal of the periapical lesion, the root apex is being resected or curved with a rounded and fissure auger, with massive watering of the operative field, in order to prevent tissue heating. In case of solid thickness of the cortical bone layer and the absence of deterioration of the pathological process, fenestration with special trepan borers is performed, with returning the bone fragment on the original site after resection of the root tip; such procedure enables conditions for advanced coagulum organization and its later ossification. The wound has to be properly irrigated with saline solution in order to remove remains of granulation tissue, and in case of their partial removal, it has to be performed all the way to the visibly healthy bone [10].

In most cases, the healing and regeneration of the bone takes 12 – 18 months after surgery. This period of time is sufficient for complete ossification of the bone defect [11].

The aim of this study was to assess clinical and radiographic outcomes of surgical treatment of chronic periapical lesions in multirooted teeth.

Material and Methods

A prospective study was conducted at the dentist office "Dr. Repić". It included 30 patients with clinically and radiografically verified chronic periapical lesions in multi-rooted teeth. Beside the inclusion criteria, patients in whom exclusive endodonic treatment was impossible were included as well.

The basic statistical unit was an impacted tooth. The following criteria were preoperatively assessed: localization of chronic periapical lesion, previous endodonic treatment, type of previous canal restoration, canal passage, presence of fistula, X-ray characteristics and presence of clinical symptoms (spontanious pain and sensitivity to palpation or percussion, respectivelly).

During the preoperative diagnostic procedure and management planning, the passage of the root canal and presence of communication between periapical lesion and oral cavity were specifically assessed. Also, the canal was considered as fully closed if there was a fixed prosthetic work with correct canal restoration.

All of the patients were surgicaly treated using a standard Partsch procedure **(Figure 1)**. Root canal obturation of the teeth that were not previously endodontically managed was performed immidiately before the surgical procedure or intraoperatively. Zinc-phosphate cement condensed with guttapercha cone was used for obturation.

Figure 1. Suture by Partsch performed on the chronic periapical lesion of the tooth #36
Slika 1. *Pristup hroničnoj periapikalnoj leziji na zubu 36 primenom reza po Parču*

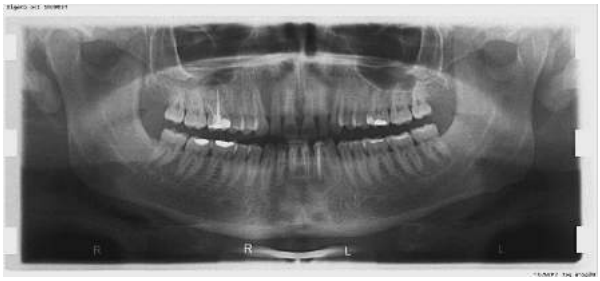


Figure 2. Bone defect after root resection of the tooth #16 and cavity replacement by Bio Oss (one month after surgery)
Slika 2. *Koštani defekt nakon resekcije korenova zuba 16 i ispunjavanja nastale šupljine veštačkom zamenom za kost (BioOss) (mesec dana nakon intervencije)*

Canals with short fillings were obturated with amalgam filling afterwards. The same procedure was performed in teeth with inadequatelly filled canals, which were also carriers of fixed prosthetic work or there was no possibility for revision of previous obturation. A classic surgical technique for apicoectomy was performed. Three types of incisions were used: according to Partsch, Novak-Peter and Pichler.

The approach to periapical lesions was done by bone trepanation with steel borers, with minimal bone extraction, at the lowest possible level which was necessary for adequate lesion approach. In a certain number of cases bone opening was done using trepan-borers and it was replaced after root resection, with the aim to enhance the healing process. If the vestibular cortex was significantly damaged, the bone cavity was filled with arteficial bone replacement (Bio Oss) after the resection and covered with guided tissue regeneration membranes **(Figure 2)**.

The mucoperiosteal flap was sutured with non-resorptive fibre (Mersilk, 3-0), and the sutures were removed seven days later. Patients were instructed about proper hygiene of the oral cavity during the immediate postoperative period.

Antibiotic prophilaxis was administrated postoperatively, as well as corticosteroid therapy, if extensive edemas were expected. Patients were also advised to take mashed foods and analgesics, if needed.

The management outcomes were evaluated six, twelve and twenty-four months after surgery. The assessment was performed based on clinical parameters, but also taking into consideration the subjective status and radiography.

All statistical analyses were conducted using SPSS version 18.0. Various descriptive measures were used (frequency distribution, mean values, standard deviation). Chi-square test and Kruskal-Wallis test were used to assess the differences with a statistical significance criterion of $p < 0.05$. The results were presented in tables.

Results

A total of 30 patients were included in the study. The male/female ratio was 36.7 : 63.3. They were aged between 20 – 62 years (M = 32.0, SD = 10.05). Almost half of the patients were aged from 20 to 29 years.

Clinical findings

The initial clinical evaluation was performed during the examination prior to the indicated apical surgery. The following criteria were examined: localization of chronic periapical lesions, obturation of molars, canal passage, tenderness to pain, presence of communication between periapical lesion and the oral cavity and clinical presentation.

In our study, most of the chronic periapical lesions were located on the lower left molars (33.3%); the same number of lesions were found on the upper right (23.3%) and lower right (23.3%) side, while six cases (20%) were found in the upper left molars.

The preoperative clinical findings suggested that in most cases the root canals were not adequately prepared for surgery, and therefore they had to be definitely restorated with zinc-phosphate cement condensed with gutta-percha cone either during the surgical procedure or immidiatelly before. In about two thirds of the cases, endodonic treatment was not performed and a lack of canal obturation was determined (66.7%); about one quarter of the patients (23.3%) had insufficiently obturated canals; only two patients had correctly obturated canals, while in one patient (3.3%) obturation was assessed as extreme.

Preoperative canal passage was also one of the parameters in the clinical evaluation prior to the apical surgery. Almost half of the canals were closed (46.7%), wether due to fixed prostethics or due to correct restoration; one third was gangrenous (33.3%) and one fifth was opened (20%).

Percussion sensitivity is a very important clinical parameter in the diagnosis of chronic periapical lesions, regardless of the presence of spontaniuos pain. Three types of pain were assessed: spontanious, by percussion and by palpation. In almost half of the patients (46.7%), the pain was provoked by percussion.

In regard to the clinical presentation, in more than half of the patents (56.7%) tumefact was found, while 43.3% were diagnosed with intraoral fistula **(Table 1)**. In our study no extraoral fistulas were found.

Table 1. Clinical findings prior to the surgery
Tabela 1. *Preoperativni klinički nalaz*

Localization of CPL *Lokalizacija HPL*	Upper left *Gore levo*	Upper right *Gore desno*	Upper left *Dole levo*	Lower right *Dole desno*
	6/20.0%	7/23.3%	10/33.3%	7/23.3%
Definitive canal obturation quality *Kvalitet definitivnog kanalnog ispuna*	Not performed *Nije izveden*	Insufficient *Nedovoljno*	Ideal *Idealno*	Extreme *Preforsirano*
	20/66.7%	7/23.3%	2/6.7%	1/3.3%
Preoperative canal passage *Preoperativna prohodnost kanala*	Closed *Zatvoren*	Gangrenous *Gangrenozni sadržaj*		Opened *Otvoren*
	14/46.7%	10/33.3%		6/20.0%
Pain *Bol*	Spontaneous *Spontani*	By percussion *Pri perkusiji*		By touch *Pri palpaciji*
	10/33.3%	14/46.7%		6/20.0%
Clinical presentation *Klinički nalaz*	Tumefaction *Tumefakt*			Intraoral fistula *Intraoralna fistula*
	17/56.7%			13/43.3%

N/%, CPL – chornic periapical lesion/*HPL*

Radiography

Radiographic assessment was based on retroalveolar imaging and orthopantomograph. Two parameters were assessed: dimension of the lesion and its diffusion. The radiographic presentation showed radiolucent lesions and all that sized up to 10 mm in diameter were considered as moderate, while those with diameter longer than 10 mm were considered as large. Also, chronic periapical lesions were considered as circumscribed if there was a clearly visible bone condenzation zone at the periphery of the lesion; they appeared as narrow shadows on the outer parts of pathological radiolucency.

Radiography was performed to determine the size of the lesions. Moderate were present in 56.7% of patients; 43.3% had large lesions. Almost half of the lesions (53.3%) were diffuse, while 46.7% were circumscribed **(Table 2)**.

Clinical evaluation 6, 12 and 24 months after surgery

In the first follow-up, scheduled six months after surgery, postoperative outcomes were assessed exclusively based on clinical parameters. In later follow-up appointments, both clinical and radiographic assessments were included.

Lack of clinical symptoms was found in most of the patients and the number of such patients increased with time elapsed after surgery. There were no patients who reported spontanious pain.

On the second follow-up (12 months after surgery), a fistula was found in one patient (3.3%). It persisted through the last follow-up in the same patient. The presence of a fistula might be discussed as the treatment failure, while the other symptoms (sensitivity to percussion or palpation) may not indicate a failure.

The number of patients with pain on percussion decreased during time, while the incidence of pain on palpation decreased between the first and second follow-up, but maintained up to the third follow-up. Statistically significal improvement was found both between the first and the second follow-up (Kruskal-Wallis $H = 21.069$, $df = 2$, $p < 0.000$) and first and third follow-up (Kruskal-Wallis $H = 19.469$, $df = 2$, $p < 0.000$) **(Table 3)**.

Radiographic evaluation 12 and 24 months after surgery

Assessment of radiographic parameters of bone healing was performed 12 and 24 months after surgery using retroalveolar and orthopantomograph images. Good ossification of the bone defect was observed in 60% of patients after 12 months, and in 66.7% of patients 24 months after surgery, respectively. Fibroseal type of bone restitution can also indicate successful bone reparement and it was observed in more than one quarter of patients (26.7%) a year after surgery, and in one fifth of respondents (20%) two years after treatment. Fibrous bone restitution persisted in 10% of pa-

Table 2. Radiographic findings prior to surgery
Tabela 2. *Preoperativni radiološki nalaz*

Size of lesion (in mm)/*Veličina lezije (u mm)*	<10 mm	>10 mm
	17/56.7%	13/43.3%
Type of lession/*Tip lezije*	Diffuse/*Difuzna*	Circumscripted/*Cirkumskriptna*
	16/53.3%	14/46.7%

N/%

Table 3. Evaluation of clinical parameters 6, 12 and 24 months after surgery
Tabela 3. *Evaluacija kliničkih pokazatelja 6, 12 i 24 meseca nakon operativnog zahvata*

Symptoms *Simptomi*	After 6 months *Nakon 6 meseci*	After 24 months* *Nakon 24 meseca**	After 48 months** *Nakon 48 meseci***
Spontaneous pain/*Spontani bol*	0/0.0%	0/0.0%	0/0.0%
Percussion pain/*Bol pri perkusiji*	6/20.0%	4/13.3%	3/10.0%
Palpation pain/*Bol pri palpaciji*	3/10.0%	2/6.7%	2/6.7%
Presence of fistula/*Prisustvo fistule*	0/0.0%	1/3.3%	1/3.3%
No symptoms/*Bez simptoma*	21/70.0%	23/76.7%	24/83.0%

N/%; *p<0.000; **p<0.000

Table 4. Evaluation of radiographic parameters 12 and 24 months after surgery
Tabela 4. *Evaluacija radiografskih pokazatelja 12 i 24 meseca nakon operativnog zahvata*

Symptoms/*Simptomi*	After 12 months/*Nakon 12 meseci*	After 24 months*/*Nakon 24 meseca**
Osseal/*Osealno*	18/60.0%	20/66.7%
Fibroosseal/*Fibroosealno*	8/26.7%	6/20.0%
Fibrous/*Fibrozno*	3/10.0%	3/10.0%
Recidives with fistula/*Recidiv sa fistulom*	1/3.3%	1/3.3%

N/%; *p<0.000

tients. Radiography confirmed clinical findings of the presence of fistula in one patient (3.3%). Statistically significant improvement was found between two radiographic follow-ups (Kruskal-Wallis H = 24.297, df = 2, p < 0.000) **(Table 4)**.

Discussion

Apical periodontitis can be defined as an inflammation and destruction of periradicular tissues caused by etiological agents of endodontic origin [12]. It is well documented that the actual prevalence of chronic periapical lesions in our region is significantly higher than in Western countries. This may be explained by poorer oral health status and proportionally higher percentage of carious teeth in our country. Moreover, it leads to the assumption that the proportion of registered periapical lesions may be higher than reported, due to insignificant symptoms. This is why in many cases, at the moment of lesion detection, massive bone destruction of the alveolar extension and development of endo-periodontal lesions has already occurred, which aggravate the possibility of adequate conventional treatment. In our study, the majority of chronic periapical lesions were closed, due to canal treatment, fixed prosthetic work or conservative restoration which were previously performed. The size of this sample might not be adequate for epidemiological conclusions (N = 30 patients), but these findings may suggest insufficiently defined concensus for determining indications for prosthetic or conservative treatment regarding assessment of pulp biological potentials [13].

The results of our study showed that the peak age prevalence was between 20–29 years; about two thirds of patients were female. The age distribution of treated patients suggests that the most probable occurrence of chronic periapical lesions may be earlier than the patients see their dentists due to the appearance of the first symptoms. However, Akinyamoju et al. analyzed medical records of 104 patients with periapical lesions and found that most cases were found among females aged from 20–29, which is in accordance with our findings [14]. The assessment of apical surgery outcome can be performed by using the combination of clinical and radiographic healing criteria [15]. In the study group, 24 months after surgery, the succes rate was 83% concerning clinical paramethers, and 66.7% concerning radiographic paramethers, respectively. The successful outcome group also included 10.0% of patients with positive percussive pain, but without any other clinical signs, 20% of patients with fibroseal tissue restitution, and 10% with fibrose tissue restitution, but without any other clinical signs. Quadir et al. rewied outcomes of periradicular surgery and came to the conclusion that success rate ranged from 30–80%. However, these studies were not consistent in sample size, type of teeth, surgical technique, type of root end filling materials and radiographic evaluation criteria. They also argued that some longitudinal studies reported a higher success rate in periradicular surgery of teeth with unsuccessful orthograde endodontic treatment [16], which is in accordance with our study. The literature review of Von Arx, presented results of success rate of apical surgeries and concluded that they widely ranged, from 44 – 90%; however, with the introduction of microsurgical methods they considerabely rased up to about 90% or more [17]. Tolarasia and Das found that periapical surgery may be the only alternative when the tooth with periapical lesion fails to respond to calcium hydroxide which was used as intracanal medication during endodontic management [18]. Singh et al. used platelet rich fibrin for stimulation and acceleration of soft tissue and bone healing with

surgical procedure combined and it proved to be very successful. Six months after surgery, complete bone regeneration was found in all patients [19].

Conclusion

The study showed that chronic periapical lesions in multiple rooted teeth can be successfully treated by combined endodontic-surgical treatment, in the environment of fully equipped private dental practice, with application of the main management principles of endodoncy and surgery. Respecting the fact that the principle of maintenance of teeth is conditio sine qua non, it is necessary to perform all possible procedures to maintain the teeth and to enable their adequate functioning in the long run, and in favour of the patients.

References

1. Eleazer PD, Glickman GN, McClanahan SB, Webb TD, Justman BC. Glossary of endodontic terms. 8th ed. Chicago (IL): American Association of Endodontists; 2012.

2. Bergenholtz G, Horsted-Bindslev P, Reit C. Textbook of endodontology. 2nd ed. Oxford (UK): Wiley-Blackwell; 2010.

3. Chandra BS, Krishna VG, editors. Grossman's endodontic practice. 12th ed. New Delhi (India): Wolters Kluwer Health; 2010.

4. Mirković S, Tadić A, Đurđević Mirković T, Levakov A. Comparative analysis of accuracy of diagnosis of chronic periapical lesions made by clinical and histopathological examination. Med Pregl. 2012;65(7-8):277-80.

5. Kohli A, editor. Textbook of endodontics. Haryana, India: Elsevier India; 2009.

6. Newman MG, Takei H, Klokkevald PR, Carranza FA, editors. Carranza's clinical periodontology. 11 th ed. St. Louis, Missouri: Elsevier; 2012.

7. Fernandes M, de Ataide I. Nonsurgical management of periapical lesions. J Conserv Dent. 2010;13(4):240–5.

8. Fragiskos FD, editor. Oral surgery. Heidelberg, Germany: Springer-Verlag; 2007.

9. Primović S, Feher P, Marković D, Petrović Lj. Periapikalna hirurgija molara. Med Pregl. 2000;53(1-2):55-8.

10. Mueller HP. Periodontology: the essentials. Stuttgart, Germany: Thieme; 2005.

11. Đurić D. Reparatorna osteogeneza kod hroničnih periapikalnih procesa zuba gornje i donje vilice [doktorska disertacija]. Novi Sad: Univerzitet u Novom Sadu, Medicinski fakultet; 1983.

12. Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med. 2004;15(6):348-81.

13. Todorović Lj, Petrović V, Jurišić M, Kafedžiska Vračar V. Oralna hirurgija. 2. izd. Beograd: Nauka; 2007.

14. Akinyamoju AO, Gbadebo SO, Adeyemi BF. Periapical lesions of the jaws: a review of 104 cases in Ibadan. Ann Ib Postgrad Med. 2014;12(2):115-9.

15. Zuolo ML, Ferreira MO, Gutmann JL. Prognosis in periradicular surgery: a clinical prospective study. Int Endod J. 2000;33(2):91–8.

16. Quadir F, Abidi YA, Ahmed A, Syed SA. Predictable bone fill; a five year follow up of per radicular surgery: case report. Journal of The Pakistan Dental Association. 2014;23(1):36-40.

17. Von Arx T. Apical surgery: a review of current techniques and outcome. Saudi Dent J. 2011;23(1):9–15.

18. Tolasaria S, Das UK. Surgical and nonsurgical management of bilateral periapical lesions in the maxillary anterior region. J Surg Tech Case Rep. 2011;3(1):44–8.

19. Singh S, Singh A, Singh S, Singh R. Application of PRF in surgical management of periapical lesions. Natl J Maxillofac Surg. 2013;4(1):94–9.

Rad je primljen 20. XI 017.
Recenziran 28. XI 2017.
Prihvaćen za štampu 29. XI 2017.
BIBLID.0025-8105:(2018):LXXI:1-2:9-14.

General Hospital Subotica, Department of Orthopedics and Traumatology, Subotica[1]
General Hospital Bijeljina, Bijeljina, Bosnia and Herzegovina[2]
Clinical Centre of Vojvodina, Novi Sad
Clinic of Orthopedic Surgery and Traumatology[3]
University of Novi Sad, Faculty of Medicine Novi Sad[4]

Original study
Originalni naučni rad
UDK 616.718.5:616.728.3]-073
https://doi.org/10.2298/MPNS1802015R

RADIOGRAPHIC ANALYSIS OF THE TIBIAL TUNNEL POSITION AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

RADIOGRAFSKA ANALIZA POLOŽAJA TUNELA NA GOLENJAČI POSLE REKONSTRUKCIJE PREDNJEG UKRŠTENOG LIGAMENTA

Vladimir RISTIĆ[1], Nenad RISTIĆ[2], Vladimir HARHAJI[3,4], Mile BJELOBRK[3] and Vukadin MILANKOV[3,4]

Summary

Introduction. The aim of the study was to analyze the tibial tunnel position after anterior cruciate ligament reconstruction. **Material and Methods.** The study included 830 patients who underwent this operative procedure. There were four times more male than female patients. The tibial tunnel placement was analyzed on frontal and lateral radiograph images of the knee joint. **Results**. The average frontal tibial index was 55% (35 – 68%), the average frontal tibial angle was 75 degrees (58 – 90), the sagittal tibial index was 30% (15 – 52%) and the sagittal tibial angle was 68 degrees (50 – 89). **Conclusion**. A significant deviation from these values may potentially lead to failure of the anterior cruciate ligament reconstruction.

Key words: Anterior Cruciate Ligament Reconstruction; Knee Joint; Tibia; Radiography; Graft Survival; Bone-Patellar Tendon-Bone Grafts; Reconstructive Surgical Procedures; Tendons; Bone Screws

Sažetak

Uvod. Studija ima za cilj da analizira položaj tunela na golenjači nakon rekonstrukcije prednjeg ukrštenog ligamenta. **Materijal i metode.** Istraživanje je obuhvatilo 830 pacijenata kojima je izvršena rekonstrukcija ligamenta. U uzorku smo više od četiri puta imali zastupljene muškarce. Položaj tunela na golenjači analiziran je frontalnim i bočnim radiografskim snimcima kolena. **Rezultati.** Prosečna vrednost frontalnog tibijalnog indeksa iznosi 55% (35–68%), frontalnog tibijalnog ugla 75 stepeni (58–90), sagitalnog tibijalnog indeksa 30% (15–52%) i sagitalnog tibijalnog ugla 68 stepeni (50–89). **Zaključak.** Značajnija odstupanja od navedenih vrednosti potencijalno mogu dovesti do neuspeha rekonstrukcija prednjeg ukrštenog ligamenta.

Ključne reči: rekonstrukcija prednjeg ukrštenog ligamenta; zglob kolena; tibija; radiografija; preživljavanje kalema; kost-ligament čašice-kost kalemi; rekonstruktivne hirurške procedure; tetive; koštani zavrtnji

Introduction

Anterior cruciate ligament (ACL) reconstructions are successful in providing excellent results in about 80 – 90% of all performed operations [1–5]. However, the reconstructions sometimes fail, in regard to stability and symptoms that significantly reduce the patient's quality of life [1–6]. Incorrect tibial and femoral tunnel placement has been recognized as a common technical error leading to failure [1–3]. During the last decades, knowledge about normal ACL anatomy has been in focus, especially its attachements (footprints) [1, 2, 7, 8]. The aim of reconstructive surgery is to achieve proper anatomical graft tunnel placement.

A tibial tunnel placed too far anteriorly may result in pain secondary to roof impingement in extension and flexion contracture [1, 2, 7]. A tibial tunnel placed too far posteriorly will result in a vertically placed ACL graft that may lack rotational stability [2, 8].

The aim of this study is to analyze the tibial tunnel placement after ACL reconstructions. These results and comparison with the results of other studies should lead to the improvement of these surgical techniques.

Material and Methods

The Ethics Committee of the Clinical Center of Vojvodina has approved this retrospective study conducted at the Clinic of Orthopedic Surgery and Traumatology. It included 830 patients with complete ACL rupture operated in the period from January 01, 2013 to December 31, 2015. There were 677 males (81.6%) and 153 females (18.4%).

All of the ACL reconstructions were performed using a bone-patellar tendon-bone autograft. Anteroposterior and lateral radiograph images of the knee joint were made postoperatively. Anterioisterior images were made in full extension and profile images in passive extension of the knee joint. The X-ray machine was 100 cm away from the X-ray cassette, and X-rays were directed under 90 degrees above. The tibial tunnel position was determined according to X-ray images.

Corresponding Author: Dr Vladimir Ristić, Opšta bolnica Subotica, 24000 Subotica, Izvorska 3, E-mail: ristic@tippnet.rs

Abbreviations

ACL – Anterior cruciate ligament
MRI – Magnetic Resonance Imaging
BPTB – bone-patellar tendon-bone
PCL – posterior cruciate ligament
MARS – Multicenter ACL Revision Study
CL – central lateral wall
ML – medial lateral wall
AC – anterior edge of the tibial plateau
AB – depth of the tibial plateau

Measurements of the depth of the tibial plateau (AP) images were as follows **(Figure 1)**: M1: frontal tibial index (FTI) CL/ML x 100 (%) and M2: frontal tibial angle (FTA) (degrees). Profile images were used in measurements of **(Figure 2)**: M3: sagittal tibial index (STI) AC/AB x 100 (%) and M4: sagittal tibial angle (STA) (degrees).

Measurements were performed on radiographic images, using high-precision calibration. The angle measurement accuracy was 0.5 degrees, and linear measurement accuracy was 0.5 mm. The minimum, mean, maximum values and standard deviation were calculated for each monitored parameter **(Table 1)**.

The patients whose X-rays were of inadequate quality were excluded from the study, because precise measurements could not be made.

The collected data were entered into a special database created in Microsoft Excel program, and the statistical analysis was performed using the IBM SPSS software (version 23). The statistical significance level was $p < 0.05$.

Results

The analyzed radiographic images showed the following results:

M1: Frontal tibial index CL/ML x 100 (%)

The frontal tibial index ranged from 34.71 to 67.52% with an average of 54.60% (SD 3.5814). Only one patient had a ML diameter between 30 – 40%, 67 patients had 40 – 50%, 702 between 50 – 60%, and 60 of them between 60 – 70%.

M2: Frontal tibial angle

The frontal tibial angle ranged from 57.87 to 89.57 degrees, 74.90 degrees on average (SD 5.4007). The distribution of frontal tibial angle was the following: 6 images from 55 to 60 degrees, 25 from 60 to 65, 121 from 65 to 70, 130 from 80 to 85, and 19 from 85 to 90 degrees. The most frequent interval was between 75 and 80 degrees (277 images), and afterwards between 70 and 75 degrees in 252 images.

M3: Sagittal tibial index AC/AP x 100 (%)

The sagittal tibial index ranged between 15.17 and 52.44% with an average of 29.70% (SD 5.6290). The tibial tunnel was most frequently localized between 20 and 30% of AB diameter (412 patients), followed by 30 – 40% (358 patients), then 40 – 50% (32 patients), 15–20% (26) and only two patients had an AB diameter between 50 and 55%.

M4: Sagittal tibial angle

The sagittal tibial angle ranged from 50.46 to 89.10 degrees with an average of 68.03 degrees (SD 6.2026). The distribution intervals of this angle were as follows: 8 patients had 50 – 55 degrees, 71 between 55 and 60, 193 60 – 65, 249 65 – 70, 189 70 – 75, 93 75 – 80, 25 80 – 85 and only two patients had 85 – 90 degrees in the sagittal plane.

Discussion

The incidence of ACL injury has been increasing, and the most frequently injured are young, physically active persons [3–5, 9–13]. Surgical reconstruction is the method of choice in the treatment of these injuries in recreational and professional athletes who have high levels of physical activity. The primary goal of the surgery is the re-establishment of stability, allowing nor-

Table 1. Radiographic measurement analysis
Tabela 1. *Vrednosti merenja dobijene radiografskom analizom snimaka*

	N *Broj*	Minimum *Minimum*	Maximum *Maksimum*	Average *Srednja vrednost*	Standard deviation *Standardna devijacija*
CL diameter/*Izmeren CL dijametar (mm)*	830	28.1081	63.5135	48.4636	4.7042
ML diameter/*Izmeren ML dijametar (mm)*	830	50.0000	110.8333	88.8399	7.4333
Frontal tibial index CL/MLx100 (%) *Frontalni tibijalni indeks CL/MLx100 (%)*	830	34.7133	67.5276	54.5810	3.6451
AC diameter/*Izmeren AC dijametar (mm)*	830	8.5399	32.5140	17.8147	3.5956
AP diameter/*Izmeren AP dijametar (mm)*	830	34.0476	73.8888	60.0559	5.2031
Sagittal tibial index AC/APx100 (%) *Sagitalni tibijalni indeks AC/APx100 (%)*	830	15.1786	52.4419	29.7005	5.6290
Frontal tibial angle (degrees) *Frontalni tibijalni ugao (u stepenima)*	830	57.87	89.57	74.90	5.4007
Sagittal tibial angle (degrees) *Sagitalni tibijalni ugao (u stepenima)*	830	50.46	89.10	68.03	6.2026

CL – centralni lateralni zid, ML – medijalni lateralni zid, AC – prednja ivica tibijalnog platoa, AB – dubina tibijalnog platoa

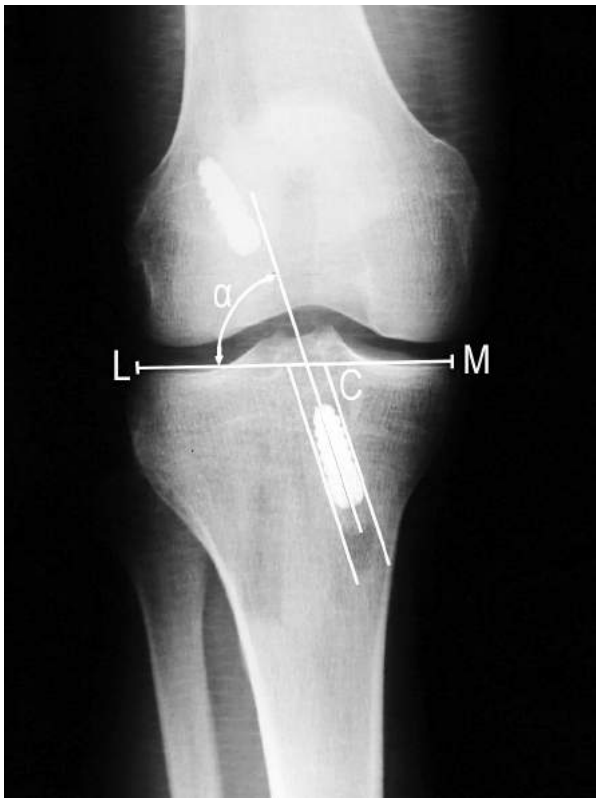


Figure 1. Anteroposterior X-ray measurements
Slika 1. *Merenja na anteroposteriornom rendgenskom snimku*

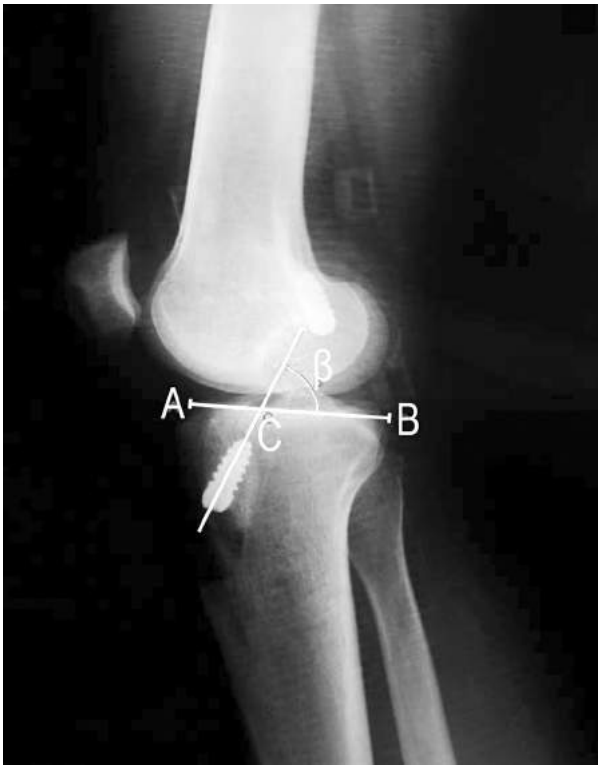


Figure 2. Lateral X-ray measurements
Slika 2. *Merenja na profilnom rendgenskom snimku*

mal knee function. All patients included in this study had arthroscopically-assisted ligament reconstruction with a modified Clancy technique [14]. Patellar tendon was used as bone-tendon-bone graft. This retrospective study included 830 patients, and there were four times more male than female patients (82%:18%). In most studies that analyzed ACL reconstructions, males were 2 – 5 times more prevalent [3–6, 9, 10], although it is known that the risk of ACL rupture is 2 – 8 times higher in females, depending on the type of sport [11–13].

The native ACL attaches in anatomical areas in front of and between the intercondylar tibial eminence to the semicircular area of the posteromedial part of the lateral femoral condyle. Its length ranges from 31 to 38 mm, and its diameter ranges from 7 to 12 mm [15]. The cross-sectional area changes in relation to the height of the section. The surface on the proximal attachment (34 mm^2, 35 mm^2) is in the middle section, while in the distal attachment it is 42 mm^2 on average [16]. Some ACL fibers on distal insertion are connected to the lateral meniscus [16].

The anterior horn of lateral meniscus and the posterior cruciate ligament (PCL) happen to be the most often used intra-articular landmarks for positioning the guiding needle in the tibia during ACL reconstruction [17–19]. Jackson and Gasser recommended using an imaginary line extended medially from the posterior border of the anterior horn of the lateral meniscus [17] and Ziegleretal et al. [18] and Morgan et al. [19] recommended using a location seven millimeters anterior to the anterior margin of the PCL with the knee flexed to 90° as an ideal place for distal attachment. Intraoperatively, we also placed a guiding wire 7 mm in front of the PCL and medially from the edge of the anterior horn of the lateral meniscus, which is consistent with most authors [20–22]. However, Heming et al., and Edwards et al. [23, 24], considered that the center of the attachment (tibial footprint) is up to 15 mm in front of the PCL fibers. Werner et al. [25] also did not agree that these landmarks were ideal, because they did not provide consistently good results.

One of the main factors which affect the final outcome of treatment and re-establishment of the passive stability of the joint is a correctly performed surgical technique. Most of the errors related to the surgical technique include generally inadequate, non-anatomical position of the graft [3, 20, 21, 26]. Its position is determined by the position of the femoral and tibial tunnels. The tibial graft position is not as important as the femoral [3, 27, 28], except in case of transtibial arthroscopically assisted reconstruction, when the tibial tunnel automatically determines the position of the femoral tunnel. Femoral tunneling by anteromedial portal has eventually overcome the transtibial technique, because the anatomical position of the graft cannot be achieved using the transtibial technique which may result in instability [3, 26–28].

The place of tibial insertion is much more accessible, manageable, and easier to determine by the surgeon. However, positioning the tibial tunnel too

far forward results in "roof impingement" or inappropriate contact of the graft with the roof of the intercondylar notch, in extended knee. This can lead to over-tightening and rupture of the graft during knee flexion [20, 21]. If the graft is placed medially to the anterior tibial eminence, there is an improper contact with PCL, which results in the impossibility of knee flexion [22]. If the graft is placed laterally to the external tibial eminence, there is a contact with the medial side of the lateral femoral condyle. Consequently, there is an anterior instability of the knee joint [29]. However, in the study of Sommer et al. [30] tibial insertion had no significant effects on the postoperative instability of the knee.

Knowledge about the anatomy of a normal ACL is a key factor to the success of reconstructive surgery. In order to determine the physiological position of the tibial attachment of ACL, Parkinson et al. [31] analyzed 76 magnetic resonance imaging (MRI) images and 26 3D computed tomography (CT) images of uninjured knees. Insertion of the ACL in the frontal plane was located at 48% ± 2% from the medial edge of the tibial plateau. In 83 subjects, Inderhaug et al. [1] found the value of the frontal tibial index of 40% (36 – 45%). Arcuri et al. [32], as we did, followed the radiographic determination of the tibial tunnel position and found that the average position of the tunnel in the frontal plane was 27.8% of the lateral edge of the tibial plateau. According to a Multicenter ACL Revision Study (MARS) conducted at 52 centers by 82 surgeons, who analyzed knee radiographs after revision ACL reconstructions [33], the distance of tibial tunnel of the medial edge of the plateau was on average 45.4% ± 3.8% of ML in diameter. In our research, the frontal tibial index (CL/ML x 100%) value was on average the same as in an non-injured knee and did not deviate from the values obtained in other studies (54.5% of the lateral, or 45.5% of the medial edge of the tibial plateau).

Frank et al. [2] used MRI images of 100 subjects and came to a conclusion that the tibial ACL insertion in the sagittal plane was on average 36 ± 6%, from 28% to 63% of the distance from the front edge of the tibial plateau of the total AP diameter of the plateau. Most authors also recommended that the tibial tunnel should be localized at 44–46% of the AP diameter [1, 32, 34]. When the position of tibial tunnel in the sagittal plane was determined relative to the anterior horn of the lateral meniscus, the average value of the sagittal tibial index was 37% ± 5.2% [25]. The majority of tunnels (66% of all) were located from 30.0–39.9% of the AP diameter; 18% of tunnels were between 40% and 44.9%; 10% over 45.0%, and 6% in the range of 25.0 - 29.9% [25]. The average values of the tibial ACL insertion in sagittal plane were 38–39% of the AP diameter [31, 33], and more than 70% of the values were in the range from 30 and 50%. The sagittal tibial index values in our study differed from the above mentioned studies, because the tibial insertion of graft in our study was set more anteriorly (29.7% of the AB diameter). Also, compared to radiographic measurements in the study conducted by Ninković et al. [5] which included 39 patients, our results were not significantly different.

Beside the localization of the tibial entry point, the tibial graft angles in the frontal and sagittal planes are also important factors. Too vertical positioning of the graft in the plane of the joint leads to its excessive tension, reduced flexion, increased anterior tibial translation, degeneration and graft rupture [20, 21]. The ideal angle is considered to be less than 75 degrees (30 to 71 degrees) [1, 20–22, 33]. When the frontal tibial angle is less than 75 degrees, it does not affect the appearance of the above-mentioned postoperative complications. Also, it is necessary to avoid a too steep angle; it is recommended to drill the tunnel at an angle of about 65 degrees, although it varies from 59 to 75 degrees [20–22]. The average value of frontal tibial angle in this study was 74.80 degrees, which is in accordance with the above recommendations.

These landmarks on the tibial tunnel were used in a cadaveric study [19] and the resulting sagittal angle was found to be 68% (64–72%). Another study on cadavers [22] found that most of the fibers were isometric in the sagittal view if the angle was 60 degrees, and tunnel centered at 46% (42–50%) from the anterior joint line. In MARS study [33] the average value of the sagittal angle of the tibial tunnel was 83.3 degrees. Arcuri et al. [32] achieved the angle of 73.48 degrees. In a similar study on MRI images [35] the average angle was 54.5 (51–58.5) degrees, while the angle of the tunnel in the frontal plane was 72.38 degrees (69–76). In our study, the sagittal tibial angle was 68.03 degrees on average, which is in accordance with the recommendations [19–22] and does not differ significantly from the values obtained in these studies.

The main limitation of this study is that it is basically a radiographic research. With this imaging technique or inadequacy of the X-rays, it is not always possible to perform precise measurements. These difficulties can be overcome by using CT or MRI, but their price and exposure to doses of radiation restricts their use, especially in a large number of subjects. This study opens up the possibility of subsequent comparisons of clinical and radiographic results, based on which more accurate correlations and guidelines will be obtained for further researches.

Conclusion

Tibial tunnel in the frontal plane is located at the lateral edge of the tibial plateau, at 54.58% of the total plateau diameter. The average angle of the tunnel in the frontal plane is 74.80 degrees. Tibial tunnel in the sagittal plane is distant from the anterior edge of tibial plateau by 29.69% of the total anteroposterior plateau diameter. On average, the angle of the tibial tunnel in the sagittal plane is 68.03 degrees. Deviations from these values may potentially lead to the failure of anterior cruciate ligament reconstruction.

The results are in line with the results of most other studies.

References

1. Inderhaug E, Strand T, Fischer-Bredenbeck C, Solheim E. Effect of a too posterior placement of the tibial tunnel on the outcome 10–12 years after anterior cruciate ligament reconstruction using the 70-degree tibial guide. Knee Surg Sports Traumatol Arthrosc. 2014;22(5):1182–9.

2. Frank RM, Seroyer ST, Lewis PB, Bach BR Jr, Verma NN. MRI analysis of tibial position of the anterior cruciate ligament. Knee Surg Sports Traumatol Arthrosc. 2010;18(11):1607–11.

3. Milankov M, Miličić A, Savić D, Stanković M, Ninković S, Matijević R, et al. Revision anterior cruciate ligament reconstruction due to knee instability. Med Pregl. 2007;60(11-12):587-92.

4. Ristić V, Ninković S, Harhaji V, Stanković M, Savić D, Milankov M. Reconstruction of anterior crutiate ligament by using two different techniques. Med Pregl. 2010;63(11-12):845-50.

5. Ninković S, Miličić A, Savić D, Stanković M, Radić S, Milankov M. Upoređivanje kliničkih i radiografskih rezultata rekonstrukcije prednjeg ukrštenog ligamenta kolena. Med Pregl. 2006;59(9-10):421-5.

6. Ristić V, Ristić S, Maljanović M, Milankov V, Harhaji V, Đuričin A. Quality of life after bilateral anterior cruciate ligament reconstructions. Med Pregl. 2015;68(9-10):308-15.

7. Iriuchishima T, Shirakura K, Fu FH. Graft impingement in anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc. 2013;21(3):664–70.

8. Pinczewski LA, Salmon LJ, Jackson WF, von Bormann RB, Haslam PG, Tashiro S. Radiological landmarks for placement of the tunnels in single-bundle reconstruction of the anterior cruciate ligament. J Bone Joint Surg Br. 2008;90(2):172–9.

9. Ristić V, Ninković S, Harhaji V, Milankov M. Causes of anterior cruciate ligament injuries. Med Pregl. 2010;63(7-8):541-5.

10. Ristić V, Ristić S, Maljanović M, Đan V, Milankov V, Harhaji V. Risk factors for bilateral anterior cruciate ligament injuries. Med Pregl. 2015;68(5-6):192-7.

11. Silvers HJ, Mandelbaum BR. Prevention of anterior cruciate ligament injury in the female athlete. Br J Sports Med. 2007;41(Suppl 1):i52-9.

12. Cimino F, Volk BS, Setter D. Anterior cruciate ligament injury: diagnosis, management and prevention. Am Fam Physician. 2010;82(8):917-22.

13. Bahr R, Krosshaug T. Understanding injury mechanisms: a key component of preventing injuries in sport. Br J Sports Med. 2005;39(6):324-9.

14. Clancy WG Jr, Narechania RG, Rosenberg TD, Geiner JG, Wisnefske DD, Lange TA. Anterior and posterior cruciate ligament reconstruction in rhesus monkeys. J Bone Joint Surg. 1981;63(8):1270-84.

15. Duthon VB, Barea C, Abrassart S, Fasel JH, Fritschy D, Menetrey J. Anatomy of the anterior cruciate ligament. Knee Surg Sports Traumatol Arthrosc. 2006;14(3):204-13.

16. Davarinos N, O'Neill BJ, Curtin W. A brief history of anterior cruciate ligament reconstruction. Advances in Orthopedic Surgery. 2014;2014:706042.

17. Jackson DW, Gasser SI. Tibial tunnel placement in ACL reconstruction. Arthroscopy. 1994;10(2):124–31.

18. Ziegler CG, Pietrini SD, Westerhaus BD, Anderson CJ, Wijdicks CA, Johansen S, et al. Arthroscopically pertinent landmarks for tunnel positioning in single-bundle and double-bundle anterior cruciate ligament reconstructions. Am J Sports Med. 2011;39(4):743–52.

19. Morgan CD, KalmanVR, Grawl DM. Definitive landmarks for reproducible tibial tunnel placement in anterior cruciate ligament reconstruction. Arthroscopy. 1995;11(3):275–88.

20. Howell SM, Gittins ME, Gottlieb JE, Traina SM, Zoellner TM. The relationship between the angle of the tibial tunnel in the coronal plane and loss of flexion and anterior laxity after anterior cruciate ligament reconstruction. Am J Sports Med. 2001;29(5):567-74.

21. Howell SM, Taylor MA. Failure of reconstruction of the anterior cruciate ligament due to impingement by the intercondylar roof. J Bone Joint Surg Am. 1993;75(7):1044-55.

22. Simmons R, Howell SM, Hull ML. Effect of the angle of the femoral and tibial tunnels in the coronal plane and incremental excision of the posterior cruciate ligament on tension of an anterior cruciate ligament graft: an in vitro study. J Bone Joint Surg Am. 2003;85-A(6):1018-29.

23. Heming JF, Rand J, Steiner ME. Anatomical limitations of transtibial drilling in anterior cruciate ligament reconstruction. Am J Sports Med. 2007;35(10):1708-15.

24. Edwards A, Bull AM, Amis AA. The attachments of the anteromedial and posterolateral fibre bundles of the anterior cruciate ligament: Part 1: tibial attachment. Knee Surg Sports Traumatol Arthrosc. 2007;15(12):1414-21.

25. Werner BC, Burrus MT, Gwathmey FW, Miller MD. A prospective evaluation of the anterior horn of the lateral meniscus as a landmark for tibial tunnel placement in anterior cruciate ligament (ACL) reconstruction. Knee. 2016;23(3):478–81.

26. Harhaji V, Ninković S, Milojević Z, Till V, Ristić V, Harhaji S, et al. Komparativna analiza položaja kalema u butnoj kosti posle rekonstrukcije prednjeg ukrštenog ligamenta kolena tehnikom kroz prednje-unutrašnji portal i tehnikom kroz golenjaču. Acta Chir Iug. 2013;60(2):81-5.

27. Dargel J, Schmidt-Wiethoff R, Fischer S, Mader K, Koebke J, Schneider T. Femoral bone tunnel placement using the transtibial tunnel or the anteromedial portal in ACL reconstruction: a radiographic evaluation. Knee Surg Sports Traumatol Arthrosc. 2009;17(3):220-7.

28. Tudisco C, Bisicchia S. Drilling the femoral tunnel during ACL reconstruction: transtibial versus anteromedial portal techniques. Orthopedics. 2012;35(8):e1166-72.

29. Muneta T, Yamamoto H, Ishibashi T, Asahina S, Murakami S, Furuya K. The effects of tibial tunnel placement and roofplasty on reconstructed anterior cruciate ligament knees. Arthroscopy. 1995;11(1):57-62.

30. Sommer C, Friederich NF, Muller W. Improperly placed anterior cruciate ligament grafts: correlation between radiological parameters and clinical results. Knee Surg Sports Traumatol Arthrosc. 2000;8(4):207-13.

31. Parkinson B, Gogna R, Robb C, Thompson P, Spalding T. Anatomic ACL reconstruction: the normal central tibial footprint position and a standardised technique for measuring tibial tunnel location on 3D CT. Knee Surg Sports Traumatol Arthrosc. 2017;25(5):1568-75.

32. Arcuri F, Barclay F, Nacul I. Anterior cruciate ligament reconstruction: transtibial vs transportal radiographic evaluation on femoral and tibial tunnel position. Orthop J Sports Med. 2014;2(12 Suppl 4).

33. MARS group. Radiographic findings in revision anterior cruciate ligament reconstructions from the MARS Cohort. J Knee Surg. 2013;26(4):239–47.

34. Staubli HU, Rauschning W. Tibial attachment area of the anterior cruciate ligament in the extended knee position. Anatomy and cryosections in vitro complemented by magnetic resonance arthrography in vivo. Knee Surg Sports Traumatol Arthrosc. 1994;2(3):138–46.

35. Vermesan D, Inchingolo F, Patrascu JM, Trocan I, Prejbeanu R, Florescu S, et al. Anterior cruciate ligament reconstruction and determination of tunnel size and graft obliquity. Eur Rev Med Pharmacol Sci. 2015;19(3):357-64.

Rad je primljen 16 XII 2017.
Recenziran 24. XII 2017.
Prihvaćen za štampu 7. I 2018.
BIBLID.0025-8105:(2018):LXXI:1-2:15-20.

University of Novi Sad, Faculty of Medicine Novi Sad
Department of Pharmacy

Original study
Originalni naučni rad
UDK 615.322.07:582.929.4
https://doi.org/10.2298/MPNS1802021S

BIOMEDICAL POTENTIAL OF HOREHOUND EXTRACT (*MARRUBIUM VULGARE, LAMIACEAE*)

BIOMEDICINSKI POTENCIJAL EKSTRAKTA OČAJNICE (MARRUBIUM VULGARE, LAMIACEAE)

Nebojša SALAJ, Jelena BARJAKTAROVIĆ, Nebojša KLADAR, Neda GAVARIĆ and Biljana BOŽIN

Summary

Introduction. Horehound (*Marrubium vulgare, Lamiaceae*) is a widely used plant in traditional medicine used for prevention and treatment of various diseases. High content of phenolic compounds makes it a significant source of natural antioxidants. The aim of this research was to examine *in vitro* antioxidant properties and anticholinesterase activity of horehound water-alcoholic extract, followed by preliminary chemical characterization of horehound. **Material and Methods.** The *in vitro* antioxidant potentials of horehound water-alcoholic extract were assessed using several antioxidant test-systems (neutralization of 2,2-diphenyl-1-picrylhydrazyl, hydroxyl, and nitroso radical, determination of ferric reducing potential, as well as inhibition of lipid peroxidation). Preliminary chemical profiling of the extract included estimation of total phenolic and flavonoid contents, while anticholinesterase potential of the examined extract was evaluated by spectrophotometry. **Results.** The amounts of total phenolics and total flavonoids in the obtained extract were 59.87 ± 7.31 mg gallic acid equivalents/g of dry extract and 14.47 ± 0.54 mg quercetin equivalents/g of dry extract respectively. Furthermore, significant antioxidant potential was noticed in the ferric reducing potential assay (64.07 ± 2.68 mg ascorbic acid equivalents/g of dry extract), while concentrations needed for neutralization of 50% (IC_{50}) of generated 2,2-diphenyl-1-picrylhydrazyl, nitroso and hydroxyl were 13.41 μg/mL, 64.86 μg/mL and 63.99 μg/mL, respectively. The potential of the extract to inhibit lipid peroxidation process was modest (IC_{50} = 823.82 μg/mL), while in the case of anticholinesterase potential, the recorded IC_{50} value was 2821.15 μg/mL. **Conclusion.** Horehound represents a significant natural antioxidant, mostly as a result of high levels of phenolic compounds. In addition, the examined ethanol extract has a certain anticholinesterase activity.

Key words: Marrubium; Lamiaceae; Phenols; Antioxidants; Flavonoids; Spectrophotometry; Acetylcholinesterase; Cholinesterase Inhibitors; Biomedical Research

Sažetak

Uvod. Očajnica (*Marrubium vulgare Lamiaceae*) široko je primenjivana u tradicionalnoj medicini u prevenciji i terapiji različitih oboljenja, a zbog visokog sadržaja fenolnih jedinjenja predstavlja i značajan izvor prirodnih antioksidanata. Cilj istraživanja bilo je ispitivanje *in vitro* antioksidativnog potencijala i antiholinesterazne aktivnosti očajnice, uz preliminarnu hemijsku karakterizaciju ekstrakta. **Materijal i metode.** Određen je sadržaj ukupnih fenola i flavonoida u vodenoalkoholnom ekstraktu *Marrubium vulgare*, *in vitro*, antioksidativni (neutralizacija 2,2-difenil-1-pikrilhidrazil, hidroksil i nitrozo radikala, određivanje potencijala redukovanja Fe3+, inhibicija lipidne peroksidacije) i antiholinesterazni potencijal. **Rezultati**. Sadržaj ukupnih fenola iznosio je 59,87 ± 7,31 mg ekvivalenta galne kiseline/g suvog ostatka, a flavonoida 14,47 ± 0,54 mg ekvivalenata kvancetina/g suvog ostatka u ispitivanom ekstraktu. Testirana vrednost redukcionog potencijala u redukovanja Fe^{3+} testu iznosila je 64,07 ± 2,68 mg ekvivalenata askorbinske kiseline/g suvog ostatka. Koncentracija pri kojoj je 50% slobodnih radikala inhibirano (IC_{50}) za 2,2-difenil-1-pikrilhidrazil iznosila je 13,41 μg/mL, za nitrozo radikal 64,86 μg/mL, za hidroksilni radikal 63,99 μg/mL, dok je u procesu inhibicije lipidne peroksidacije IC_{50} vrednost iznosila 823,82 μg/mL. Koncentracija ispitivanog ekstrakta neophodna za inhibiciju 50% aktivnosti acetilholinesteraze iznosila je 2821,15 μg/mL. **Zaključak**. Očajnica predstavlja značajan prirodan izvor antioksidantih supstancija. Značajan antioksidativni potencijal etanolnog ekstrakta je direktno povezan sa visokim sadržajem ukupnih fenola i flavonoida. Pored toga, etanolni ekstrakt *Marrubium vulgare* ispoljava i određenu antiholinesteraznu aktivnost.

Ključne reči: Marrubium; Lamiaceae; fenoli; antioksidanti; flavonoidi; spektrofotometrija; acetilholinesteraza; inhibitori holinesteraze; biomedicinska istraživanja

Introduction

Horehound (*Marrubium vulgare, Lamiaceae*) is a widely used species in traditional medicine of many cultures. However, further researches are necessary to clarify the correlation between high levels of phenolic compounds and benefits in the treatment of certain pathological conditions. *Genus Marrubium* includes about 30 species, mostly growing in the Mediterranean region, and less in central Europe, northern Africa and temperate region of Asia. *Marrubium vulgare* (*M. vulgare*) is a typical representative of the genus [1, 2].

Due to significant pharmacological effects, horehound is also used in conventional medicine for the

Corresponding Author: Mr pharm. Nebojša Salaj, Medicinski fakultet Novi Sad, Katedra za farmaciju, 21000 Novi Sad, Hajduk Veljkova 3, E-mail: nebojsha_salaj@yahoo.com; nebojsa.salaj@mf.uns.ac.rs

Abbreviations

DPPH – 2,2-diphenyl-1-picrylhydrazyl
OH – hydroxyl
NO – nitroso
FRAP – ferric reducing potential
M. vulgare – Marrubium vulgare
AChE – acetylcholinesterase
ROS – reactive oxygen species
AD – Alzheimer's disease
FC – Folin-Ciocalteu
GAE – gallic acid equivalents
QE – quercetin equivalent
MDA – malondialdehyde
RSC – radical scavenging capacity
TBA – thiobarbituric acid
d.e. – dry extract
AsAE – ascorbic acid equivalents

preparation of bitter tonics, as an additional therapy for digestive disorders, loss of appetite and dyspepsia [3]. Secondary metabolites of *M. vulgare* exhibit the following potentials: antinociceptive [4], antihypertensive [5], antiedematogenic [6], analgesic [7], antiinflammatory [8], antimicrobial [9] (Anti-Helicobacter pylori [10]), insecticidal [11] and citoprotective [12]. In addition, hypoglycemic and hypolipidemic effects have also been confirmed [13]. Aqueous and hydroalcoholic extracts of the aerial plant parts have been reported for treatment of cough and digestive and biliary disorders [14]. Recently, the potential role in the inhibition of cyclooxygenase-1 (COX_1) and acetylcholinesterase (AChE) has been demonstrated [15].

Previous investigations of the chemical composition of *M. vulgare* leaves revealed the presence of flavonoids like apigenin and luteolin and their 7-O-glucosides, quercetin and its 3-O-glucoside and 3-O-rhamnoside. Also, recently ladanein (5,6-dihydroxy-7-4'-dimethoxyflavone) was isolated, with possible therapeutic effects in the treatment of some leukemia forms [16]. Significant chemical constituents of horehound are diterpenes marrubiin and marrubenol, with confirmed vasodilatory effects.

Reactive oxygen species (ROS) are produced by the physiological processes in all aerobic organisms. Various factors such are environmental factors, poisoning, increased physical activity, biotransformation of xenobiotics or inflammation processes, can lead to imbalance between production of ROS and antioxidative defense of the organism resulting in wide spectra of pathophysiological conditions [17, 18]. Furthermore, β-amyloid peptides, one of pathohistological markers of patients suffering from Alzheimer's disease (AD), induce inflammatory processes and formation of free radicals. Antioxidants represent free radical scavengers and they can prevent or decrease the intensity of inflammatory processes. Drugs used in the treatment of AD, by inhibition of acetylcholinesterase (AChE), increase the level of acetylcholine (ACh), reducing the symptoms of AD, but hardly exhibit any antioxidant potential and therefore do not interfere with the inflammatory processes. Currently applied synthetic drugs used in the treatment of cognitive impairment and memory loss show severe side effects, which implicates an increasing of interest in finding better AChE inhibitors from natural sources which could additionally, by several mechanisms, target different pathophysiological processes [19, 20].

Plants are known resources of phenolic compounds, terpenoids and vitamins. Recently, flavonoids have begun attracting attention of scientists because of their potential healing effects in diseases caused by free radical processes. Flavonoids, like many other polyphenols, are efficient free radical scavengers due to high reactivity and activity as hydrogen or electron donors [21].

The investigation of biological potentials of aqueous-alcoholic extracts of horehound included a hypothesis that active compounds possess significant antioxidative potentials and ability to inhibit the AChE.

The aim of the research was *in vitro* evaluation of antioxidant potentials (neutralization of 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl (OH) and nitroso (NO) radicals, determination of reducing ability of ferric reducing potential (FRAP), and inhibition of lipid peroxidation) followed by preliminary chemical characterization of the horehound extract through quantification of the content of total phenolics and flavonoids. Also, the *in vitro* anticholinesterase potential of *M. vulgare* extract was evaluated.

Material and Methods

The chemicals used in this research: ethanol (p. a.) – Zorka Pharma (Serbia), DPPH radical, S-acetylthiocholine iodide, sulphanilamide (SA), N-(1-naphthyl)-ethylenediamine dihydrochloride (NEDA) – Alpha Aesar (Germany); Folin-Ciocalteu (FC) reagent – Merck (Germany); sodium bicarbonate, iron(II)-sulphate, acetic acid and methanol *(pro aanalysis)* – POCH (Poland); gallic acid, aluminium chloride, 2-deoxy-D-ribose, 2-thiobarbituric acid – Sigma Aldrich (USA); hydrogen peroxide – Lach-Ner (Czech Republic); quercetin – Extrasynthese (France); 5,5'-dithiobis-(2-nitrobenzoic acid) – J. T. Baker (USA); commercial solution of acetylcholinesterase – Roche (Switzerland); sodium nitroprusside (SNP) – Centrohem (Serbia) and distilled water.

The aerial parts of the horehound (*M. vulgare*, *Lamiaceae*) were collected at the full blossom stage in southeastern part of Republic of Srpska (locality: Korićka jama; Global Positioning System coordinates: 43.055518, 18.503914), in June 2015. The sample was identified at the Department of Biology and Ecology, Faculty of Natural Sciences, University of Novi Sad. The voucher specimen of *M. vulgare* was confirmed and deposited in the BUNS Herbarium (Herbarium of the Department of Biology and Ecology, Faculty of Natural Sciences and Mathematics, University of Novi Sad; Voucher No. 2-1510). The plant material was stored at room temperature, at the Laboratory of Pharmacognosy, Department of Pharmacy, Faculty of Medicine, University of Novi Sad until starting the experiments.

The extract was prepared by maceration with 70% ethanol during 24 hours, according to the instructions

for the preparation of commercially available extracts of horehound available on the market [22] and recommendations proscribed by the European Pharmacopoeia (6th edition) [23]. The ethanol extract was filtered, and then evaporated to dryness to determine the content of dry extract (extraction yield 20.88%). For further experiments, 20% of aqueous solution of the extract was prepared and preserved at -20°C until the experiments were performed.

The total phenolics content (TPC) was determined by previously mentioned FC spectrophotometric method [24]. Phenolics in reaction with FC reagent (mixture of phospho-molybdic and phospho-wolframic acid) form a blue-colored compound, with a maximum of absorbance at 760 nm. The content of total phenolics was expressed based on previously designed calibration curve of standard solution of gallic acid as mg of gallic acid equivalents (GAE) per g of dry extract (d.e.) (mg GAE/g d.e.).

The content of total flavonoids was quantified by a previously mentioned spectrophotometric method [24]. The result was expressed as mg of quercetin equivalents (QE) per g of dry extract (mg QE/g d.e.).

Determination of DPPH•, OH• and NO• neutralization

The ability of investigated extract to neutralize DPPH, OH and NO radicals was examined using the previously described spectrophotometric methods [25, 26]. Different concentrations of investigated extract were added in solution of purple colored stable DPPH• and change of color was monitored spectrophotometrically at 515 nm. Neutralization of OH radicals, which were generated in a Fenton reaction, was also monitored spectrophotometrically, based on the degradation of 2-deoxy-D-ribose to malondialdehyde (MDA), whereby MDA forms a compound with thiobarbituric acid. The ability of the extract to inhibit generated NO radicals was examined by the use of Griess reagent.

All the measurements were carried out in three replications, while the free radical scavenging capacity (RSC) of different extract concentrations was calculated by the following equation (1):

– (1) RSC (%) = (1 - A/A_0) 100%; A was the absorbance of working solutions, and A_0 the absorbance of blank solutions. Based on RSC value, IC_{50} values (the extract concentration providing 50% inhibition of DPPH•, NO• and OH•) were determined by applying regression analysis.

Determination of lipid peroxidation inhibition

The extent of lipid peroxidation was determined by thiobarbituric acid (TBA) assay [25] measuring the absorbance of compound produced in the reaction between TBA and MDA, as the final product of lipid peroxidation. Liposome "PRO-LIPO S" emulsion was used as a model-system of biological membranes. All the measurements were carried out in three replications, while the percentage of lipid peroxidation inhibition was calculated by the following equation (2):

– (2) I (%) = 100 - 100 (A/A_0); A_0 was the absorbance of the control mixture, and A was the absorbance of the test mixture.

Determination of ferric-reducing antioxidant power assay

Determination of reducing potential as an indicator of antioxidative potential of the investigated extract was based on the spectrophotometric method of Benzie et al. Antioxidants at low pH values reduce iron(III)-2,4,6-tripyridyl-*s*-triazine complex to the iron(II)-2,4,6-tripyridyl-*s*-triazine complex [27]. All of the experiments were performed in triplicate. Ascorbic acid was used as a standard substance in this method, and results were expressed as mg of ascorbic acid equivalents (AsAE) per g of d.e. (mg AsAE/g d.e.).

Inhibition of acetylcholinesterase activity

The anticholinesterase activity of the extract was evaluated spectrophotometrically by modified Ellman's method with S-acetylthiocholine iodide as a substrate [28]. All the measurements were carried out in three replications, while the percentage of AChE inhibition was calculated by the following equation (3):

– (3) I (%) = 100 - (A_s/A_c) 100; A_s was the absorbance of reaction mixture containing extract, and A_c was the absorbance of control mixture.

All data were processed using the Microsoft Excel, v. 2010 software package.

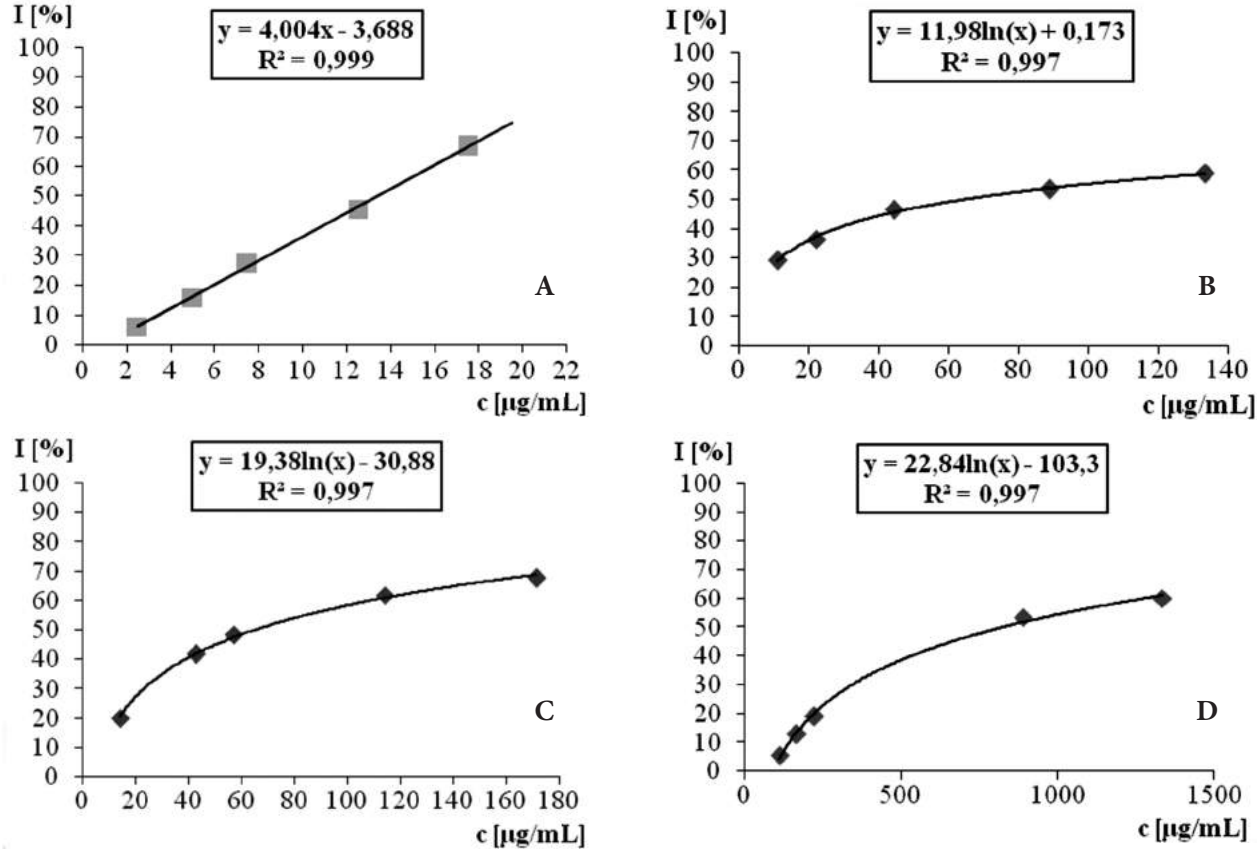
Results

Determination of total phenolics and flavonoids content

The amount of total phenolic content in the extract of *M. vulgare* was 59.87 ± 7.31 mg GAE/g d.e., while the total flavonoid content was 14.47 ± 0.54 mg QE/g d.e. **(Table 1)**, which indicates high levels of these classes of secondary metabolites occurring in the examined species.

Table 1. Content of total phenols and flavonoids in the extract of *M. vulgare*
Tabela 1. *Sadržaj ukupnih fenola i flavonoida prisutnih u ekstraktu Marrubium vulgare*

	Phenols/*Fenoli*	Flavonoids/*Flavonoidi*
A_1	0,6301	0,1398
A_2	0,6595	0,1340
A_3	0,7679	0,1285
A_{sr}	0,6858	0,1341
Content/*Sadržaj*	59,87 ± 7,31 mg GAE/g s.e.	14,47 ± 0,54 mg QE/g s.e

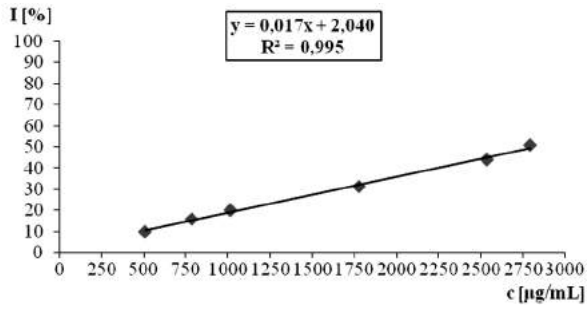


Graph 1 a, b, c and d. Dependence of the degree of inhibition: **a)** DPPH radicals, **b)** OH radicals, **c)** NO radicals, **d)** lipid peroxidation of the examined concentration of *M. vulgare* extract

Grafikon 1 a, b, c i d. *Grafikon zavisnosti stepena inhibicije:* ***a)*** *2,2-difenil-1-pikrilhidrazil radikala,* ***b)*** *hidroksi radikala,* ***c)*** *azot-monoksid radikala,* ***d)*** *lipidne peroksidacije od koncentracije ispitivanog ekstrakta M. vulgare.*

Determination of antioxidative potential

Based on the obtained values for neutralization of DPPH, OH and NO radical, as well as inhibition of the lipid peroxidation process, which negatively correlated with the applied concentrations of the extracts **(Graph 1 a, b, c and d)**, IC_{50} values were calculated. The strongest antioxidant potential was recorded in DPPH-test system (IC_{50}=13.41 µg/mL), while higher IC_{50} values were noticed in OH- and NO-test systems (IC_{50}=63.99 µg/mL and IC_{50}=64.86 µg/mL, respectively). Furthermore, the potential of the examined extract to inhibit lipid peroxidation process was relatively modest (IC_{50}=823.82 µg/mL), which was surprising, considering the strong reduction potential demonstrated in FRAP assay (64.07 ± 2.68 mg AsAE/g d.e.).



Graph 2. The inhibition of AChE activity of of the examined extract concentration

Grafikon 2. *Grafikon zavisnosti inhibicije aktivnosti acetilholinesteraze od koncentracija ispitivanog ekstrakta*

Inhibition of acetylcholinesterase activity

The potential of water–alcoholic extract of *M. vulgare* to inhibit AChE is important in pathophysiology of AD. The concentration of the extract required for the inhibition of 50% AChE activity (IC_{50} value) was 2821,15 µg/mL **(Graph 2)**.

Discussion

The results of previous studies regarding chemical characterization of *M. vulgare* vary significantly depending on the geographical origin of the plant material and the analytical parameters of the conducted analyses (type of extraction, solvent selection, duration of extraction) [29, 30].

Previous studies showed that the methanolic extract of horehound leaves contained slightly less amounts of total phenolics and flavonoids (40.57 ± 1.91 mg GAE/g d.e. and 10.25 ± 0.08 mg QE/g d.e., respectively) than the obtained results of our study [8]. On the

other hand, great variations are present within the other *Marubium* species considering the content of total phenolics. Very low amounts of the mentioned secondary metabolites are present in *M. deserti* [30], but high amounts of phenolics and flavonoids were present in extracts of *M. peregrinum* [31]. The study of *M. parviflorum* showed similar quantities of total phenolics and flavonoids in the methanolic extracts (49.8 ± 2.69 mg GAE/g d.e. and 9.77 ± 5.23 mg QE/g d.e., respectively) as in our study, but the amounts of secondary metabolites in hexane extract were 6.42 ± 2.66 mg GAE/g d.e. and 5.36 ± 1.08 mg QE/g d.e., respectively [32]. Generally, the extraction of phenolic compounds increases with the polarity of the used solvent, but variations in the content of secondary metabolites between the samples of the same species originating from different geographical locations must not be neglected because of the influence of abiotic (climatic, edaphic and orographic), as well as biotic (genetic influence on biosynthesis of active principles) factors [31].

A study dealing with horehound harvested in Algeria, where this plant is widely spread, showed a good correlation of antioxidant potential and the content of phenolic compounds [30]. The ethyl acetate extract of horehound showed similar potential of DPPH$^{\bullet}$ neutralization as in our study (IC_{50} = 11.67 ± 1.51 μg/mL), and stronger potential of $OH^{\bullet}$ neutralization (IC_{50} = 8.2 ± 0.09 μg/mL) [33]. Furthermore, the results obtained by FRAP assay in our study were similar to the previous results obtained for horehound methanol and acetone extract [30]. Current literature review showed no data for *in vitro* examination of lipid peroxidation inhibition. However, a study carried out in Canada, stated that horehound leaves extract significantly inhibited copper-induced low density lipoprotein peroxidation and enhanced reverse cholesterol transport. These antioxidant properties increase the anti-atherogenic potential of high density lipoprotein and thus offer an additional natural antioxidant source for prevention of cardiovascular diseases [34].

Inhibition of acetylcholinesterase, a key enzyme in the degradation of acetylcholine, is a significant strategy in the treatment of neurodegenerative disorders, such as AD, dementia, ataxia, myasthenia gravis and Parkinson's disease. In support of this, galanthamine - an alkaloid isolated from green snowdrop (*Galanthus woronowii, Amaryllidaceae*), has been approved recently, in the treatment of mild to moderate forms of AD [35]. Several studies demonstrated the potential of *M. vulgare* extract to inhibit the activity of various enzymes, such as acetylcholinesterase and cyclooxygenase-1 [15]. It was noticed that *M. vulgare* largely inhibits cyclooxygenase-1 as compared with the simultaneously investigated flowers of *Globularia alypum* and leaves of *Eryngium maritinum*. Data suggest that horehound possesses a large percentage of phenolic compounds (primarily flavonoids and coumarins), iridoids and monoterpenes, which could be responsible for the anti-inflammatory effects [8, 15]. The study of several Mediterranean plants, including *M. vulgare*, revealed significant anticholinesterase potential of horehound, while the obtained IC_{50} value (3062,78 μg/mL) was comparable to our results [15].

Conclusion

The obtained results indicated significant biomedical potentials of *Murrabium vulgare* extract and sug gested the possibility of its exploitation in pharmacy and phytotherapy in the future. It can be concluded that this plant is a potentially significant natural source of antioxidants, especially when polar solvents are being used for extraction. Also, the ethanolic extract of horehound exhibited an anticholinesterase potential. However, future investigations should be directed towards detailed chemical profiling followed by fractionation of extracts guided by different biological potentials, as well as towards future *in vivo* studies.

References

1. Diklic N. Thymus L. In: Josifović M, editor. Flora SR Srbije. VI tom. Beograd: SANU; 1974. p. 475-509.

2. Takeda Y, Yanagihara K, Masuda T, Otsuka H, Honda G, Takaishi Y, et al. Labdane diterpenoids from *Marrubium globosum ssp. globosum*. Chem Pharm Bull (Tokio). 2000;48(8):1234–5.

3. Blumenthal M, Goldberg A, Brinckmann J, editors. Herbal medicine. Expanded Commission E Monographs. Austin: American Botanical Council; 2000.

4. De Jesus RA, Cechinel-Filho V, Oliveira AE, Schlemper V. Analysis of the antinociceptive properties of marrubiin isolated from *Marrubium vulgare*. Phytomedicine. 2000;7(2):111–5.

5. El Bardai S, Lyoussi B, Wibo M, Morel N. Comparative study of the antihypertensive activity of *Marrubium vulgare* and of the dihydropyridine calcium antagonist amlodipine in spontaneously hypertensive rat. Clin Exp Hypertens. 2004;26(6):465–74.

6. Stulzer H, Tagliari MP, Zampirolo JA, Cechinel-Filho V, Schlemper V. Antioedematogenic effect of marrubiin obtained from *Marrubium vulgare*. J Ethnopharmacol. 2006;108(3):379–84.

7. Meyre-Silva C, Yunes RA, Schlemper V, Campos-Buzzi F, Cechinel-Filho V. Analgesic potential of marrubiin derivatives, a bioactive diterpene present in Marrubium vulgare (Lamiaceae). Farmaco. 2005;60(4):321–6.

8. Sahpaz S, Garbacki N, Tits M, Bailleul F. Isolation and pharmacological activity of phenylpropanoid esters from Marrubium vulgare. J Ethnopharmacol. 2002:79(3):389–92.

9. Zarai Z, Ben Chobba I, Ben Mansour R, Bekir A, Mejdoub H, Gharsallah N. The in-vitro evaluation of antibacterial, antifungal and cytotoxic properties of *Marrubium vulgare* L. essential oil grown in Tunisia. Lipids Health Dis. 2011;10:161.

10. Ramadan MA, Safwat NA. Antihelicobacter activity of a flavonoid compound isolated from *Desmostachya bipinnata.* Australian Journal of Basic and Applied Sciences. 2009;3 (3):2270–7.

11. Pavela R. Insecticidal activity of certain medicinal plants. Fitoterapia. 2004;75(7-8):745–9.

12. Martin-Nizard F, Sahpaz S, Furman C, Fruchart JC, Duriez P, Bailleul F. Natural phenylpropanoids protect endothelial cells against oxidized LDL-induced cytotoxicity. Planta Med. 2003;69(3):207-11.

13. Kovalewski Z, Matlwaska L. Flavonoids in *Marrubium vulgare* herbage. Herba Polonica. 1978;24:183–6.

14. Masoodi MH, Ahmed B, Zargar IM, Khan SA, Khan S, Singh P. Antibacterial activity of whole plant extract of *Marrubium vulgare*. African Journal of Biotechnology. 2008;7 (2):86–7.

15. Amessis-Ouchemoukh N, Madani K, Falé PL, Serralheiro ML, Araújo EM. Antioxidant capacity and phenolic contents of some Mediterranean medicinal plants and their potential role in the inhibition of cyclooxygenase-1 and acetylcholinesterase activities. Ind Crops Prod. 2014;53:6–15.

16. Alkhatib R, Joha S, Cheok M, Roumy V, Idziorek T, Preudhomme C, et al. Activity of ladanein on leukemia cell lines and its occurrence in *Marrubium vulgare*. Planta Med. 2010;76(1):86–7.

17. Đukić MM. Oksidativni stres - slobodni radikali, prooksidansi i antioksidansi. Beograd: Mono i Manjana; 2008.

18. Chapple IL. Reactive oxygen species and antioxidants in inflammatory diseases. J Clin Periodontol. 1997;24(5):287–96.

19. Brenner GM. Pharmacology. Philadelphia: W.B. Saunders; 2000.

20. Ur-Rahman A, Choudhary MI. Bioactive natural products as a potential source of new pharmacophores. A theory of memory. Pure and Applied Chemistry. 2001;73(3):555-60.

21. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Oxford University Press; 2003.

22. Committee on Herbal Medicinal Products. Community herbal monograph on *Marrubium vulgare* L., herba. London: European Medicines Agency; 2013.

23. European Pharmacopoeia Commission, European Directorate for the Quality of Medicines. European pharmacopoeia. Volume 2. 6th ed. Strasbourg: Council of Europe; 2007.

24. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol. 1999;299:152–78.

25. Božin B, Kladar N, Grujić N, Anačkov G, Samojlik I, Gavarić N, et al. Impact of origin and biological source on chemical composition, anticholinesterase and antioxidant properties of some St. John's wort species (*Hypericum spp., Hypericaceae*) from the Central Balkans. Molecules. 2013;18(10): 11733-50.

26. Orčić DZ, Mimica-Dukić NM, Francišković MM, Petrović SS, Jovin EĐ. Antioxidant activity relationship of phenolic compounds in *Hypericum perforatum* L. Chem Cent J. 2011;5:34.

27. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239(1):70-6.

28. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7(2):88–95.

29. Amessis-Ouchemoukh N, Abu-Reidah IM, Quirantes-Piné R, Madani K, Segura-Carretero A. Phytochemical profiling, *in vitro* evaluation of total phenolic contents and antioxidant properties of *Marrubium vulgare* (horehound) leaves of plants growing in Algeria. Ind Crops Prod. 2014;61:120–9.

30. Djeridane A, Yousfi M, Brunel JM, Stocker P. Isolation and characterization of a new steroid derivative as a powerful antioxidant from Cleome arabica in screening the in vitro antioxidant capacity of 18 Algerian medicinal plants. Food Chem Toxicol. 2010;48(10):2599–606.

31. Stanković MS. Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. Kragujevac Journal Sciences. 2011;33:63–72.

32. Yumrutas O, Saygideger SD. Determination of in vitro antioxidant activities of different extracts of *Marrubium parviflorum* Fish et Mey. and Lamium amplexicaule L. from South east of Turkey. Journal of Medicinal Plants Research. 2010; 4(20):2164–72.

33. Matkowski A, Tasarz P, Szypuła E. Antioxidant activity of herb extracts from five medicinal plants from Lamiaceae, subfamily Lamioideae. Journal of Medicinal Plants Research. 2008;2(11):321-30.

34. Berrougui H, Isabelle M, Cherki M, Khalil A. *Marrubium vulgare* extract inhibits human-LDL oxidation and enhances HDL-mediated cholesterol efflux in THP-1 macrophage. Life Sci. 2006;80(2):105–12.

35. Scott LJ, Goa KL. Galantamine: a review of its use in Alzheimer's disease. Drugs. 2000;60(5):1095–122.

Rad je primljen 1. VIII 2016.
Recenziran 19. II 2017.
Prihvaćen za štampu 2. I 2018.
BIBLID.0025-8105:(2018):LXXI:1-2:21-26.

REVIEW ARTICLES
PREGLEDNI ČLANCI

University of Novi Sad, Faculty of Medicine Novi Sad
Department of Internal Medicine, Novi Sad[1]
Clinical Center of Vojvodina, Novi Sad
Clinic of Gastroenterology and Hepatology[2]

Review article
Pregledni članak
UDK 616.33/.34-002.44:616.36-004
https://doi.org/10.2298/MPNS1802027S

VARIOUS ASPECTS OF PEPTIC ULCER IN PATIENTS WITH LIVER CIRRHOSIS

RAZLIČITI ASPEKTI PEPTIČKOG ULKUSA KOD BOLESNIKA SA CIROZOM JETRE

Željka SAVIĆ[1, 2], Dragomir DAMJANOV[1, 2], Vladimir VRAČARIĆ[1, 2], Dijana KOSIJER[1, 2], Dimitrije DAMJANOV[1, 2] and Tihomir ORLIĆ[2]

Summary
Introduction. The occurrence of peptic ulcer in patients with liver cirrhosis is intriguing due to its frequency and complexity. The aim of the present study was to investigate the incidence of peptic ulcer in patients with liver cirrhosis. **Results.** It was found that in these patients the usual aggressive factors of the gastric environment do not play a major role in ulcerogenesis; however, researches noticed the importance of reduced mucosal defense which, in portal hypertension, has the features of hypertensive portal gastropathy. The presence of *Helicobacter pylori* infection in these patients is lower, compared to other patients with peptic ulcer. The prevalence of *Helicobacter pylori* infection decreases with the severity of liver cirrhosis. Non-steroidal anti-inflammatory drugs play an important role in peptic ulcer bleeding in cirrhotic patients, but the data are limited and contradictory. Peptic ulcer bleeding is the most frequent etiology of nonvariceal bleeding and it is associated with a great number of complications. **Conclusion.** *Helicobacter pylori* infection cannot be considered the key risk factor for the development of peptic ulcer in patients with liver cirrhosis. The role of non-steroidal anti-inflammatory drugs is accepted, although the data are controversial. The treatment of peptic ulcer in cirrhotic patients is identical to the treatment of peptic ulcer in patients without liver cirrhosis, except in cases of bleeding ulcers. There are specific therapeutic protocols for peptic ulcer bleeding in patients with liver cirrhosis.
Key words: Peptic Ulcer; Liver Cirrhosis; Hypertension, Portal; *Helicobacter pylori*; Anti-Inflammatory Agents, Non-Steroidal; Gastric Mucosa; Peptic Ulcer Hemorrhage; Risk Factors

Sažetak
Uvod. Pojava peptičkog ulkusa kod bolesnika sa cirozom jetre je intrigantna zbog svoje učestalosti i kompleksnosti. Cilj rada bio je da se sagleda evolucija u istraživanju peptičkog ulkusa kod bolesnika sa cirozom jetre. **Rezultati**. Kod ovih bolesnika klasični agresivni faktori želudačne sredine nemaju velikog značaja u patogenezi peptičkog ulkusa, ali je istraživanjima utvrđen značaj smanjenja odbrambenih faktora želudačne mukoze koja u portnoj hipertenziji ima sliku hipertenzivne portne gastropatije. Zastupljenost *Helicobacter pylori* infekcije kod ovih bolesnika je manja nego kod drugih bolesnika sa peptičkim ulkusom. Prevalencija *Helicobacter pylori* infekcije opada sa težinom bolesti jetre. Nesteroidni antiinflamatorni lekovi imaju ulogu u krvarenju iz peptičkog ulkusa kod bolesnika sa cirozom jetre, ali su podaci limitirani i kontradiktorni. Krvarenje iz peptičkog ulkusa je najčešća etiologija nevariksnog krvarenja i povezano je sa velikim brojem komplikacija. **Zaključak**. *Helicobacter pylori* infekcija se ne može smatrati jednim od ključnih faktora u nastanku peptičkog ulkusa kod bolesnika sa cirozom jetre. Uloga nesteroidnih antiinflamatornih lekova u nastanku peptičkog ulkusa kod ovih bolesnika je prihvaćena, iako kontroverzna. Lečenje peptičkog ulkusa kod bolesnika sa cirozom jetre je identično lečenju peptičkog ulkusa kod bolesnika bez ciroze jetre, osim u slučaju ulkusnog krvarenja. U krvarenju iz peptičkog ulkusa kod bolesnika sa cirozom jetre postoje decidirani terapijski postulati.
Ključne reči: peptički ulkus; ciroza jetre; portna hipertenzija; *Helicobacter pylori*; nesteroidni antiinflamatorni lekovi; gastrična mukoza; krvarenje peptičkog ulkusa; faktori rizika

Introduction

Liver cirrhosis (LC) is a huge health issue. It is a disease which deteriorates the quality of life and has a high mortality rate, mainly caused by complications which are well documented and clear from the etiopathogenic, clinical, and therapeutic aspects [1].

Global liver cirrhosis deaths increased from around 676.000 deaths in 1980 (1.54% of global deaths) to 1.029.0420 deaths in 2010 (1.95% of the global total). In the Republic of Serbia, there were 822 (537 – 1212), 854 (607 – 1.185), 1.041 (833 – 1.378) and 951 (721 – 1.203) deaths (95% uncertainty intervals) in 1980, 1990, 2000, 2010, respectively [2].

Corresponding Author: Dr Dimitrije Damjanov, Klinički centar Vojvodine, Klinika za gastroenterologiju i hepatologiju, 21000 Novi Sad, Hajduk Veljkova 1-7, E-mail: ddamjan.dd@gmail.com; dimitrije.damjanov@mf.uns.ac.rs

Abbreviations

PU – peptic ulcer
GU – gastric ulcer
DU – duodenal ulcer
PUB – peptic ulcer bleeding
LC – liver cirrhosis
HVPG – hepatic venous pressure gradient
BAO – basal acid output
MAO – maximal acid output
PHG – portal hypertensive gastropathy
PH – portal hypertension
HCl – hydrochloric acid
H. pylori – Helicobacter pylori
NSAID – non-steroidal anti-inflammatory drug
PPI – proton pump inhibitors
GI – gastrointestinal

Peptic ulcer (PU) is a continuous active disease with a significant social aspect. The cumulative life-prevalence of PU ranges from 8% to 14% [3].

Due to its incidence and complexity, the occurrence of PU in patients with LC has been intriguing clinicians and researchers for decades. The etiopathogenic mechanisms of PU in LC patients are not completely clear yet, as opposed to the incidence of PU in general population.

Earlier researches have established a connection between the severity of liver disease and PU incidence, and it was considered that the liver disease was the "primum movens" for PU, which was called "hepatogenic ulcer". The clinical findings of increased incidence of PU in LC, despite reduction in gastric acid output, may be explained by relative disturbance of the balance between aggressive and protective mechanism, the latter being diminished [4].

The point prevalence of PU in patients with liver cirrhosis is 11.7%. The annual incidence of PU in these patients is 4.3%, with 2.8% accounting for duodenal ulcer (DU), and 1.4% for gastric ulcer (GU), which is 20 – 47 times higher incidence rate compared to the non-cirrhotic population [5, 6]. Other authors have found a similar point prevalence of PU in patients with liver cirrhosis, 10.5% [7], or even higher (24.3–38.5%) [8, 9].

A significantly higher prevalence of GU was found in patients with LC who have hepatic venous pressure gradient (HVPG) >12 mmHg, amounting to 20.8% compared to 4% in healthy controls [10].

The prevalence of GU in patients with LC is higher compared to the general population. Aggressive factors taking part in the pathogenesis of GU are lower, however, the defensive factors of gastric mucosa are also reduced due to portal hypertension (PH) [11].

In healthy (non-cirrhotic) population, the prevalence of GU accounts for 2% or less. Tomoda et al. found a prevalence of GU of 20% in patients with LC [12]. Other researches found the presence of GU in 15% of patients with decompensated LC and ascites, 3.3% in compensated patients, and 1.7% in healthy controls [13].

Aggressive Factors of Gastric Mucosa in Patients with PU and LC

In patients with LC, the secretion of gastric acid (Hydrochloric acid - HCl) is reduced or possibly normal [14].

Tabaqchali and Dawson did not find a disorder of HCl secretion in patients with LC, except after portacaval anastomosis where it was found that basal secretion of HCl was increased, but without an accompanying increase to the maximum histamine stimulation [15].

Scobie and Summerskill found a significant decrease of basal and maximal (histamine stimulated) HCl secretion in patients with LC and concluded that this was not connected to the etiology or severity of the liver disease, nor with the presence or degree of the collateral portal circulation [16].

Lam examined the basal acid output (BAO) and the maximal acid output (MAO) after pentagastrin stimulation, and fasting and postprandial gastrin levels in patients with LC. The mean values of BAO and MAO were significantly lower compared to healthy controls. The fasting gastrin level was significantly higher, and the postprandial gastrin response was significantly increased and prolonged [17].

Gaur et al. examined the basal and pentagastrin-stimulated gastric secretion in patients with LC, in patients with non-cirrhotic portal fibrosis and in control groups. They found that the maximum volume and secretion of the gastric acid was significantly lower in the first two groups compared to the control groups. The authors did not establish a connection between the gastric hyposecretion and the degree of hepatocellular dysfunction, but they believed that the aforementioned could be secondary to portal hypertension and collateral circulation [18].

Savarino et al. examined 24-hour gastric pH-metry in patients with LC and found that these patients had significant hypoacidity during the entire circadian cycle compared to the control group of healthy subjects [19].

Patients with LC have normal basal pepsin levels, but a reduced response to stimulation. In patients with atrophic gastritis, pepsinogen levels are significantly lower in patients with LC compared to patients without liver diseases [20].

Classic aggressive factors of gastric environment, such as HCl and pepsin, do not have a significant impact on the pathogenesis of PU in LC.

Defensive Factors of Gastric Mucosa in PU Occurrence in Patients with LC

The increased susceptibility of gastric mucosal damage, associated with portal hypertensive gastropathy (PHG), usually caused by alcohol, aspirin and bile salts, was first described during the 1980s. Gastric mucosa in PH has PHG features with unique functional and morphological disruptions which make it susceptible to harmful agents. Such gastric mucosa has microvascular changes, even though the total gastric blood flow is unchanged. The essence lies in the redis-

tribution of gastric blood flow with disrupted inflow of blood, and therefore oxygen in the gastric mucosa itself [21, 22].

Patients with LC and PH also have increased plasma endothelin-1 level, which is a powerful vasoconstrictor, as well as increased tumor necrosis factor-a, which has cytotoxic properties. The gastric mucus secretion, the content of gastric mucin and their precursor hexosamine, is reduced. Production of bicarbonates is also reduced. The mucosa of the stomach with PHG has a reduced proliferation of mucosal epithelial cells. The prostaglandin E2 content is significantly lower, especially in mucosal congestion. The increase in nitric oxide may contribute to the increased sensitivity to mucosal noxae in PHG [11].

Ulcer Healing

Proliferation of epithelial cells in the ulcer margin and angiogenesis at the bottom of the ulcer are essential for ulcer healing. The gastric ulcer healing in PHG is slow, primarily due to the inhibition of epithelial proliferation in the ulcer margin [23].

In the study of Siringo et al., after 8 weeks of therapy, GU healing occurred in 67% of patients with LC and 84% of patients without LC. Ulcer recurrence was higher in patients with LC (50%) than in patients without LC (30%) [5].

***Helicobacter Pylori* Infection – the Role in Ulcerogenesis and PU Bleeding in Patients with LC**

Helicobacter pylori (H. pylori) is a human pathogen that is transferred from one human to another causing chronic active gastritis in all colonized subjects. This may lead to PU, atrophic gastritis, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma. Eradication of H. pylori results in healing of gastritis and may prevent long-term complications of the infection [24].

In populations of Northern Europe and North America, about one-third of adults are still infected, whereas in south and east Europe, South America, and Asia, the prevalence of H. pylori is often higher than 50%. Low socioeconomic conditions in childhood are confirmed to be the most important risk factors for H. pylori infection [25].

In duodenal ulcer (DU), H. pylori infection is present in around 90 – 100% of cases, and in gastric ulcer (GU) in around 60 – 100% of cases. Eradication of H. pylori drastically reduces the annual recurrence of DU [26].

A lower incidence of H. pylori infection is recorded in PU bleeding (PUB). Authors from Novi Sad have found the presence of H. pylori in GU bleedings to be 58.33%, and 69.8% in DU bleedings [27]. They have also found that H. pylori *per se* is not a risk factor for PUB, but that it does have a synergistic effect with taking non-steroidal anti-inflammatory drugs (NSAIDs), OR =3.63, $p < 0.01$. It was concluded that taking alcohol significantly increases the probability of PUB (OR = 3.25, $p < 0.01$) [28]. The use of alcohol in certain regions of Serbia is a significant problem [29].

H. pylori infection is significantly less present in patients with LC, even compared to the general population of medium-developed countries. Gastric mucosa in PHG is not a suitable element for H. pylori colonization. Colonization of gastric mucosa with H. pylori is 26% in patients with LC and PH, and 38% in patients without PH [30].

Alempijević et al. found that the infection with H. pylori is present in 36% of patients with LC, regardless of the presence of PU, and that in this case H. pylori infection does not impact the development of the ulcer disease [31].

Kim et al. found the presence of H. pylori infection in patients with LC and PU in 35.6% of cases, and in 34.9% of cases in patients with LC without PU. The presence of H. pylori infection decreases, and the frequency of PU increases proportionally to the severity of the LC [8].

Other authors have found PU incidence in a group of patients with LC, who were infected with H. pylori, to be eight times higher compared to the non-infected patients [32].

Certain researches show that H. pylori infection was present in 40 – 89% of patients with LC, which was probably conditioned by the methods used to establish the infection. Studies that used serological tests found greater incidence of H. pylori infection [13, 33, 34].

H. pylori infection rate in cirrhotic patients with PU is 35.5 – 51.92%. Although H. pylori is not the predominant etiologic factor of PU in LC, it should be treated. Early eradication of H. pylori infection is connected to the reduced risk of recurrent PU in patients with LC [35].

This is in contrast with the research done by Lo et al. who concluded that eradication of H. pylori infection in patients with LC and DU was not efficient in preventing ulcer recurrence [36]. In general population the approach to diagnostics and treatment of H. pylori infection significantly reduced the incidence of DU [37].

To sum up, the presence of H. pylori infection in patients with PU in LC is significantly lower compared to the presence of this infection in patients with PU without LC. The prevalence of H. pylori infection is not different in patients with LC with PU and those without PU. The prevalence of H. pylori infection decreases with the severity of liver disease, but there is no simultaneous change in the incidence of PU. Therefore, this infection cannot be considered one of the key factors of PU incidence in patients with LC.

Non-steroidal Anti-Inflammatory Drugs: the Role in Ulcerogenesis and Peptic Ulcer Bleeding in Patients with Liver Cirrhosis

Aspirin and other NSAIDs are a significant cause of PU in general population. Their use can be found in up to 60% of patients with PU [38].

In a study done by Bang et al., the prevalence of PU in LC was 18%. The prevalence of taking ulcero-

genic drugs (NSAID, aspirin, clopidogrel, ticlopidine, and steroids) was 4.9% in patients with LC. It was proven that these ulcerogenic drugs are associated with the occurrence of PU in patients with LC (OR 4.34, p = 0.04). Other variables (age, sex, alcohol, smoking, HVPG) in a univariate analysis showed no significant difference between cirrhotic patients with and without PU [39].

It has been proven that these drugs play a role in PUB in LC, which would indicate a similarity to the etiology of ulcer bleedings in general population [40].

In general population, NSAIDs have a significant role in damaging the gastrointestinal tract mucosa. It is believed that they play a role in PUB in patients with LC, however the data are still limited and sometimes contradictory [41].

Rudler et al. measured differences in the etiology of PUB between patients with LC and population of non-cirrhotic patients. The results viewed in such a manner were as follows: H. pylori = 10.3% versus 48.8%, NSAIDs = 17.2% versus 54.0%, idiopathic ulcer bleeding = 79.3% versus 23.8%. Considering that 85% of patients had alcoholic etiology of LC, the authors stated that the possible cause of PUB in these patients could be PH with alcohol intake [42].

Luo et al. found that in patients with LC significant risk factors for PUB were age, male sex, diabetes, chronic renal insufficiency, use of NSAIDs and previous variceal bleeding [40].

Patients with LC have bleeding of non-variceal origin in 1/4 to 1/3 of the cases. PUB accounts for a significant percentage of non-variceal bleeding in patients with LC. Results presented by a group of authors from Novi Sad showed that patients with alcoholic LC and acute upper gastrointestinal (GI) bleeding had bleeding from esophagogastric varices in 71% of the cases, whereas in 29% of the cases the bleeding was of non-variceal origin. In 29% of patients with non-variceal bleeding, PUB was present in 16.3% of patients [43].

Svoboda et al. found non-variceal bleeding in 37% of the total number of patients with LC and acute upper GI bleeding. PUB was present in 18.2% of these patients. The authors found H. pylori infection in 36% of patients with PUB, and the use of NSAIDs in 8% of patients. The mortality in patients with variceal bleeding was 18.6%, and 7.8% in other patients who had non-variceal bleeding [44].

Another study found mortality in variceal bleeding measured during five days/six weeks to be 9.2%, 20.8% respectively, and with non-variceal bleeding 5.3%, 14.9% respectively [45].

In patients with LC and upper GI bleeding, Ardevol et al. concluded that after first line therapy, the continuation of bleeding was more frequent with variceal (18%) than with ulcer bleeding (10%). The risk of 45-day mortality was the same for both groups of patients. Most patients died due to liver insufficiency and comorbidity, and only 2% of patients with PUB and 3% of patients with variceal bleeding died due to uncontrollable bleeding [46].

Kuo et al. defined high Rockall score (age, shock, comorbidity, endoscopic diagnosis, bleeding stigmata), the number of blood transfusions, and no antibiotic prophylaxis as predictors of ulcer bleeding recurrence. The total mortality of PUB in LC was 17%; higher in decompensated (28.6%) than in compensated disease (7.7%) [47].

The therapy for PUB in patients with LC requires the application of proton pump inhibitors (PPI), volume resuscitation by crystalline solution, and transfusions of packed erythrocytes, fresh frozen plasma, vitamin K and sometimes transfusions of thrombocyte concentrate. Application of antibiotics is mandatory in order to prevent bacterial infections [41].

Peptic Ulcer Therapy in Patients with Liver Cirrhosis

Treatment of non-complicated PU in patients with LC is not different from treatment of PU in patients without LC. The therapy of PUB in patients with LC has its own specificities, regarding the basic liver disease.

It is necessary to point out the extensive application of PPI within chronic therapy of patients with LC without PU. These drugs are used by 25% to 40% of patients with cirrhosis without clearly documented indications. Without arguments for the application of these drugs, patients with LC are at risk for developing bacterial infections and sepsis with multiple organ dysfunction, deterioration of liver function and bad prognosis [48, 49].

Conclusion

In patients with liver cirrhosis there is a significant presence of peptic ulcer. Aggressive factors taking part in the pathogenesis of peptic ulcer are reduced, as well as defensive factors of gastric mucosa. *Helicobacter pylori* infection cannot be considered one of the key factors in the development of peptic ulcer in these patients. The importance of using non-steroidal anti-inflammatory drugs is undeniable, though controversial. Peptic ulcer appears more frequently in advanced liver cirrhosis. Peptic ulcer bleeding is the most frequent cause of non-variceal bleeding in these patients.

Treatment of non-complicated peptic ulcer in patients with liver cirrhosis does not differ from the treatment of peptic ulcer in patients without liver cirrhosis. The peptic ulcer bleeding in patients with liver cirrhosis has some specificities and therapeutic postulates. In the therapy of patients with liver cirrhosis without peptic ulcer, it is necessary to take a restrictive approach in applying proton pump inhibitors due to the risk for developing serious bacterial infections.

References

1. Savić Ž, Vračarić V, Hadnađev L, Petrović Z, Damjanov D. Iskustvo u lečenju nekih komplikacija portne hipertenzije kod alkoholne ciroze jetre. Vojnosanit Pregl. 2011;68(11):917-22.

2. Mokdad AA, Lopez AD, Shahraz S, Lozano R, Mokdad AH, Stanaway J, et al. Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. BMC Med. 2014;12:145.

3. Del Valle J, Chey WD, Scheiman JM. Acid peptic disorders. In: Yamada T, Alpers DH, editors. Textbook of gastroenterology. 4th ed. Philadelphia: Lippincott Williams and Wilkins; 2003. p. 1322-76.

4. Thiel H. "Hepatogenic ulcer" theories and facts. Z Gastroenterol. 1978;16(2):73–84.

5. Siringo S, Burroughs AK, Bolondi L, Muia A, Di Febo G, Miglioli M, et al. Peptic ulcer and its course in cirrhosis: an endoscopic and clinical prospective study. J Hepatol. 1995;22 (6):633-41.

6. Siringo S, Vaira D, Menegatti M, Piscaglia F, Sofia S, Gaetani M, et al. High prevalence of Helicobacter pylori in liver cirrhosis. Dig Dis Sci. 1997;42(10):2024-30.

7. Calvet X, Navarro M, Gil M, Lafont A, Sanfeliu I, Brullet E, et al. Epidemiology of peptic ulcer disease in cirrhotic patients: role of Helicobacter pylori infection. Am J Gastroenterol. 1998;93(12):2501-7.

8. Kim DJ, Kim HY, Kim SJ, Hahn TH, Jang MK, Baik GH, et al. Helicobacter pylori infection and peptic ulcer disease in patients with liver cirrhosis. Korean J Intern Med. 2008;23(1):16-21.

9. Kamalaporn P, Sobhonslidsuk A, Jatchavala J, Atisook K, Rattanasiri S, Pramoolsinsap C. Factors predisposing to peptic ulcer disease in asymptomatic cirrhotic patients. Aliment Pharmacol Ther. 2005;21(12):1459-65.

10. Chen LS, Lin HC, Hwang SJ, Lee FY, Hou MC, Lee SD. Prevalence of gastric ulcer in cirrhotic patients and its relation to portal hypertension. J Gastroenterol Hepatol. 1996;11(1):59-64.

11. Kitano S, Dolgor B. Does portal hypertension contribute to the pathogenesis of gastric ulcer associated with liver cirrhosis? J Gastroenterol. 2000;35(2):79-86.

12. Tomoda J, Mizuno M, Sugihara T, Itano T, Tsuji T. Gastric mucosal lesion in liver disease: impaired gastric mucosal defence mechanism in rats with induced liver injury and in patients with liver cirrhosis. J Gastroenterol Hepatol. 1989;4(Suppl 1):136–9.

13. Wu CS, Lin CY, Liaw YF. Helicobacter pylori in cirrhotic patients with peptic ulcer disease: a prospective, case controlled study. Gastrointest Endosc. 1995;42(5):424–7.

14. Fraser AG, Pounder RE, Burroughs AK. Gastric secretion and peptic ulceration in cirrhosis. J Hepatol. 1993;19(1):171–82.

15. Tabaqchali S, Dawson AM. Peptic ulcer and gastric secretion in patients with liver disease. Gut. 1964;5:417-21.

16. Scobie BA, Summerskill WH. Reduced gastric acid output in cirrhosis: quantitation and relationships. Gut. 1964;5:422-8.

17. Lam SK. Hypergastrinaemia in cirrhosis of liver. Gut. 1976;17(9):700-8.

18. Gaur SK, Vij JC, Sarin SK, Anand BS. Gastric secretion in cirrhosis and non-cirrhotic portal fibrosis. Digestion. 1988;39(3):151-5.

19. Savarino V, Mela GS, Zentilin P, Mansi C, Mele MR, Vigneri S, et al. Evaluation of 24-hour gastric acidity in patients with hepatic cirrhosis. J Hepatol. 1996;25(2):152-7.

20. Mitsunaga A, Yokoyama S, Hashimoto H, Hirose H, Adachi H, Kurokawa K, et al. Clinical study of the aggressive factors of the gastric mucosa in liver cirrhosis. J Gastroenterol Hepatol. 1989;4 Suppl 1:266–7.

21. Sarfeh IJ, Soliman H, Waxman K, Coccia M, Rypins EB, Bui HX, et al. Impaired oxygenation of gastric mucosa in portal hypertension. The basis for increased susceptibility to injury. Dig Dis Sci. 1989;34(2):225-8.

22. Auroux J, Lamarque D, Roudot-Thoraval F, Deforges L, Chaumette MT, Richardet JP, et al. Gastroduodenal ulcer and erosions are related to portal hypertensive gastropathy and recent alcohol intake in cirrhotic patients. Dig Dis Sci. 2003;48(6):1118-23.

23. Baatar D, Kitano S, Yoshida T, Bandoh T, Ninomiya K, Tsuboi S. Delayed healing of acetic acid-induced gastric ulcer in portal hypertensive rats. Eur Surg Res. 1999;31(4):340-6.

24. Sugano K, Tack J, Kuipers EJ, Graham DY, El-Omar EM, Miura S, et al. Kyoto global consensus report on Helicobacter pylori gastritis. Gut. 2015;64(9):1353-67.

25. Eusebi LH, Zagari RM, Bazzoli F. Epidemiology of Helicobacter pylori infection. Helicobacter. 2014;19(Suppl 1):1-5.

26. Kuipers EJ, Thijs JC, Festen HP. The prevalence of Helicobacter pylori in peptic ulcer disease. Aliment Pharmacol Ther. 1995;9 Suppl 2:59-69.

27. Savić Ž. Faktori rizika za nastanak krvarenja iz peptičkog ulkusa gastroduodenuma [Magistarska teza]. Novi Sad: Medicinski fakultet Novi Sad; 2004.

28. Savić Ž, Hadnadjev Lj, Damjanov D, Knežević A, Petrović Z, Pešić T, et al. Faktori rizika za krvarenje iz peptičkog ulkusa (solisti ili orkestar). Medicina danas. 2011;10(4-6):113-8.

29. Savić Ž, Dickov A, Hadnađev Lj, Damjanov D, Petrović Z, Knežević A, et al. Epidemiološki i klinički profil alkoholizma i konsekutivne ciroze jetre na teritoriji Novog Sada. Medicina danas. 2011;10(7-9):266-70.

30. McCormick PA, Sankey EA, Cardin F, Dhillon AP, McIntyre N, Burroughs AK. Congestive gastropathy and Helicobacter pylori: an endoscopic and morphometric study. Gut. 1991;32(4):351-4.

31. Alempijević T, Krstić M, Antonić V, Krstić S. Učestalost infekcije s Helicobacter pylori kod osoba obolelih od ciroze jetre. Srp Arh Celok Lek. 2007;135(9-10):536-40.

32. Dore MP, Mura D, Deledda S, Maragkoudakis E, Pironti A, Realdi G. Active peptic ulcer disease in patients with hepatitis C virus-related cirrhosis: the role of Helicobacter pylori infection and portal hypertensive gastropathy. Can J Gastroenterol. 2004;18(8):521-4.

33. Calvet X, Navarro M, Gil M, Mas P, Rivero E, Sanfeliu I, et al. Seroprevalence and epidemiology of Helicobacter pylori infection in patients with cirrhosis. J Hepatol. 1997;26(6):1249-54.

34. Chen JJ, Changchien CS, Tai DI, Chiou SS, Lee CM, Kuo CH. Role of Helicobacter pylori in cirrhotic patients with peptic ulcer. A serological study. Dig Dis Sci. 1994;39(7):1565-8.

35. Chang SS, Hu HY. Helicobacter pylori: effect of coexisting diseases and update on treatment regimens. World J Gastrointest Pharmacol Ther. 2015;6(4):127-36.

36. Lo GH, Yu HC, Chan YC, Chen WC, Hsu PI, Lin CK, et al. The effects of eradication of Helicobacter pylori on the recurrence of duodenal ulcers in patients with cirrhosis. Gastrointest Endosc. 2005;62(3):350-6.

37. Jovanović I, Đurić P, Alempijević T, Sokić-Milutinović A, Krstić M, Milosavljević T. Ulkusna bolest dvanaestopalačnog creva tokom dvadeset godina nakon otkrića Helicobacter pylori - iskustvo iz jednog centra. Med Pregl. 2010;63(3-4):258-61.

38. Quan C, Talley NJ. Management of peptic ulcer disease not related to Helicobacter pylori or NSAIDs. Am J Gastroenterol. 2002;97(12):2950-61.

39. Bang CS, Baik GH, Kim JH, Kim JB, Suk KT, Yoon JH, et al. Peptic ulcer disease in liver cirrhosis and chronic hepatitis: impact of portal hypertension. Scand J Gastroenterol. 2014;49(9):1051-7.

40. Luo JC, Leu HB, Hou MC, Huang CC, Lin HC, Lee FY, et al. Cirrhotic patients at increased risk of peptic ulcer bleeding: a nationwide population-based cohort study. Aliment Pharmacol Ther. 2012;36(6):542–50.

41. Nojkov B, Cappell M. Distinctive aspects of peptic ulcer disease, Dieulafoy's lesion, and Mallory-Weiss syndrome in patients with advanced alcoholic liver disease or cirrhosis. World J Gastroenterol. 2016;22(1):446-66.

42. Rudler M, Rousseau G, Benosman H, Massard J, Deforges L, Lebray P, et al. Peptic ulcer bleeding in patients with or without cirrhosis. different diseases but the same prognosis? Aliment Pharmacol Ther. 2012;36(2):166-72.

43. Savić Ž, Hadnadjev Lj, Damjanov D, Petrović Z, Mrdja Z, Knežević A, et al. Upper gastrointestinal bleeding in patients with alcohol liver disease. In: V Congress of Internal Medicine for the South-Eastern Europe: abstract book; 2009 March 6-9; Beograd, Serbia. Internist. 2009;2(Suppl 1):27.

44. Svoboda P, Konecny M, Martinek A, Hrabovsky V, Prochazka V, Ehrmann J. Acute upper gastrointestinal bleeding in liver cirrhosis patients. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2012;156(3):266-70.

45. D'Amico G, De Franchis R, Cooperative Study Group. Upper digestive bleeding in cirrhosis. Post-therapeutic outcome and prognostic indicators. Hepatology. 2003;38(3):599-612.

46. Ardevol A, Ibanez-Sanz G, Profitos J, Aracil C, Castellvi JM, Alvarado E, et al. Survival of patients with cirrhosis and acute peptic ulcer bleeding compared with variceal bleeding using current first-line therapies. Hepatology. In press. doi: 10.1002/hep.29370.

47. Kuo MT, Yang SC, Lu LS, Hsu CN, Kuo YH, Kuo CH, et al. Predicting risk factors for rebleeding, infections, mortality following peptic ulcer bleeding in patients with cirrhosis and the impact of antibiotics prophylaxis at different clinical stages of the disease. BMC Gastroenterol. 2015;15:61.

48. Picardi A, Vespasiani-Gentilucci U. Proton pump inhibitor prescription abuse and sepsis in cirrhosis. World J Gastrointest Pharmacol Ther. 2016;7(1):1-4.

49. Bajaj JS, Ratliff SM, Heuman DM, Lapane KL. Proton pump inhibitors are associated with a high rate of serious infections in veterans with decompensated cirrhosis. Aliment Pharmacol Ther. 2012;36(9):866-74.

Rad je primljen 22. XI 2017.
Recenziran 23. XI 2017.
Prihvaćen za štampu 2. I 2018.
BIBLID.0025-8105:(2018):LXXI:1-2:27-32.

PROFESSIONAL ARTICLES
STRUČNI ČLANCI

University of Novi Sad, Faculty of Medicine Novi Sad
Department of General Education Subjects[1]
Department of Pharmacy[2]

Professional article
Stručni članak
UDK 616-051:331.101.32(497.113)
UDK 614.23:316.334.55/.56(497.113)
https://doi.org/10.2298/MPNS1802033G

MOTIVATION AND JOB SATISFACTION OF HEALTHCARE PROFESSIONALS IN URBAN AND RURAL AREAS IN THE AUTONOMOUS PROVINCE OF VOJVODINA, SERBIA

MOTIVACIJA I ZADOVOLJSTVO POSLOM ZDRAVSTVENIH RADNIKA U URBANIM I RURALNIM SREDINAMA NA PODRUČJU AUTONOMNE POKRAJINE VOJVODINE, SRBIJA

Maja GRUJIČIĆ[1], Jelena JOVIČIĆ BATA[2] and Budimka NOVAKOVIĆ[2]

Summary
Introduction. Motivation and job satisfaction of healthcare professionals represent the basis for providing quality health care. The aim of the study was to establish whether there is a difference in motivation and job satisfaction among healthcare professionals in urban and rural areas in Vojvodina, Serbia. **Material and Methods.** The study included 574 healthcare professionals in urban area, and 145 in rural setting, from three health centers. Data collection was performed by a self-administered questionnaire. **Results.** Urban healthcare professionals, compared to rural healthcare workers, were significantly more motivated by the factor of work motivation – achieving the goals of the health center. In comparison with rural healthcare professionals, urban healthcare workers are significantly more satisfied with personal qualities of their immediate supervisors, job security guaranteed by their institution, immediate support at work they received from managers, and professional supervision of their work. **Conclusion.** Compared to rural healthcare professionals, urban healthcare workers are more work motivated and job satisfied.
Key words: Health Personnel; Motivation; Job Satisfaction; Rural Health Services; Urban Health Services; Surveys and Questionnaires; Physicians; Nurses

Sažetak
Uvod. Motivacija i zadovoljstvo zdravstvenih radnika poslom predstavljaju osnovu pružanja kvalitetne zdravstvene zaštite. Cilj rada je bio da se ispita da li postoji razlika u motivaciji i zadovoljstvu poslom između zdravstvenih radnika zaposlenih u urbanim i ruralnim sredinama u Vojvodini, Srbija. **Materijal i metode.** Istraživanjem su obuhvaćena 574 zdravstvena radnika u urbanoj i 145 u ruralnoj sredini, zaposlena u tri doma zdravlja. Podaci su prikupljeni upitnikom koji su ispitanici samostalno popunjavali. **Rezultati.** Zdravstveni radnici u urbanoj sredini, u odnosu na zdravstvene radnike u ruralnoj sredini, bili su značajno više motivisani faktorom radne motivacije – postizanje ciljeva zdravstvene ustanove. U poređenju sa zdravstvenim radnicima u ruralnoj sredini, zdravstveni radnici u urbanoj sredini su bili značajno više zadovoljni ličnim kvalitetima neposrednog rukovodioca, sigurnošću zaposlenja koju im garantuje ustanova u kojoj su zaposleni, neposrednom podrškom na poslu koju im pruža rukovodilac i stručnim nadzorom nad njihovim radom. **Zaključak.** Zdravstveni radnici zaposleni u urbanoj sredini su više motivisani za rad i zadovoljniji poslom u odnosu na zdravstvene radnike zaposlene u ruralnoj sredini.
Ključne reči: zdravstveno osoblje; motivacija; zadovoljstvo poslom; seoske zdravstvene službe; gradske zdravstvene službe; ankete i upitnici; lekari; medicinske sestre

Introduction

The quality of health care represents one of the most important characteristics of a health care system [1]. As a product of work of health care system and healthcare professionals, the quality of health care services is connected with motivation and satisfaction of the employees in health care [2].

Motivation, as the most comprehensive notion, is the process of initiation of activity of individual directed to achiveing particular goals [3]. As a reliable indicator of motivation, it is considered to be job satisfaction expressed via attitudes toward job [3].

Job satisfaction is formally defined as cognitive, affective and evaluative reactions of an individual, actually employees to their job [4]. Job satisfaction of the empoyees in a healthcare institution represents

Corresponding Author: Doc. dr Maja Grujičić, Univerzitet u Novom Sadu, Medicinski fakultet Novi Sad, Katedra opšteobrazovnih predmeta, 21000 Novi Sad, Hajduk Veljkova 3, E-mail: maja.grujicic@mf.uns.ac.rs

Table 1. Work motivation factors: comparison of urban and rural healthcare professionals
Tabela 1. *Faktori radne motivacije: poređenje zdravstvenih radnika zaposlenih u urbanoj i ruralnoj sredini*

Work motivation factors *Faktori radne motivacije*	I am motivated by *Motiviše me*	Urban area *Urbana sredina*		Rural area *Ruralna sredina*		p
		n	%	n	%	
Achieving the goals of the health center *Postizanje ciljeva zdravstvene ustanove*	No/*Ne*	42	7.3	22	15.2	0.007
	I am not sure/*Nisam siguran/a*	91	15.9	26	17.9	
	Yes/*Da*	441	76.8	97	66.9	
Professional recognition *Priznanje za dobro obavljen posao*	No/*Ne*	77	13.4	23	15.9	0.353
	I am not sure/*Nisam siguran/a*	75	13.1	24	16.5	
	Yes/*Da*	422	73.5	98	67.6	
Good work relationships *Dobri međuljudski odnosi*	No/*Ne*	48	8.4	13	9.0	0.934
	I am not sure/*Nisam siguran/a*	81	14.1	19	13.1	
	Yes/*Da*	445	77.5	113	77.9	
Opportunities for promotion and improvement/*Mogućnost unapređenja i napredovanja*	No/*Ne*	84	14.6	26	17.9	0.217
	I am not sure/*Nisam siguran/a*	109	19.0	34	23.4	
	Yes/*Da*	381	66.4	85	58.6	
Personal qualities of immediate supervisors/*Lični kvaliteti neposrednih rukovodilaca*	No/*Ne*	62	10.8	9	6.2	0.194
	I am not sure/*Nisam siguran/a*	81	14.1	25	17.2	
	Yes/*Da*	431	75.1	111	76.6	
Monthly income *Novčani iznos mesečne zarade*	No/*Ne*	154	26.8	48	33.1	0.291
	I am not sure/*Nisam siguran/a*	87	15.2	18	12.4	
	Yes/*Da*	333	58.0	79	54.5	
Working conditions *Uslovi na radu*	No/*Ne*	84	14.6	18	12.4	0.776
	I am not sure/*Nisam siguran/a*	108	18.8	29	20.0	
	Yes/*Da*	382	66.6	98	67.6	
Positive working atmosphere *Kooperativna radna atmosfera*	No/*Ne*	51	8.9	10	6.9	0.711
	I am not sure/*Nisam siguran/a*	95	16.5	23	15.9	
	Yes/*Da*	428	74.6	112	77.2	
Training opportunities *Mogućnost usavršavanja*	No/*Ne*	81	14.1	24	16.5	0.101
	I am not sure/*Nisam siguran/a*	90	15.7	32	22.1	
	Yes/*Da*	403	70.2	89	61.4	
Secure job/*Siguran posao*	No/*Ne*	35	6.1	7	4.8	0.432
	I am not sure/*Nisam siguran/a*	66	11.5	22	15.2	
	Yes/*Da*	473	82.4	116	80.0	
Support by supervisors *Podrška rukovodilaca*	No/*Ne*	53	9.2	10	6.9	0.269
	I am not sure/*Nisam siguran/a*	69	12.0	24	16.6	
	Yes/*Da*	452	78.7	111	76.5	
Indepedance at work *Autonomija prilikom obavljanja posla*	No/*Ne*	44	7.7	11	7.6	0.657
	I am not sure/*Nisam siguran/a*	78	13.6	24	16.5	
	Yes/*Da*	452	78.7	110	75.9	
Current equipment *Posedovanje savremene opreme za rad*	No/*Ne*	68	11.8	20	13.8	0.776
	I am not sure/*Nisam siguran/a*	87	15.2	23	15.9	
	Yes/*Da*	419	73.0	102	70.3	
Bonuses for exceptional work *Nagrada za posebno dobro urađen posao*	No/*Ne*	106	18.5	27	18.6	0.782
	I am not sure/*Nisam siguran/a*	71	12.4	21	14.5	
	Yes/*Da*	397	69.2	97	66.9	
Professional supervision *Stručan nadzor nad radom*	No/*Ne*	83	14.5	23	15.9	0.442
	I am not sure/*Nisam siguran/a*	115	20.0	35	24.1	
	Yes/*Da*	376	65.5	87	60.0	

one of the parameters of quality of health care which influences the productivity of the employees, quality of the provided health service, satisfaction of the users, as well as the costs allocated for health care [1, 5–7]. Numerous studies of job satisfaction of healthcare professionals were conducted [8–15]. The factors defined as important for the feeling of job satisfaction and/or dissatisfaction of the healthcare professionals are the following: financial incentives, secure employment, social interaction, superiors, equipment, possibility for education, etc. [8–15]. Place of work can also represent a significant predictor of job satisfaction of healthcare professionals [16].

Managers of healthcare institutions should direct their attention to motivate their employees in the right way, so that the employees invest their efforts and skills in such a way to do their job the best and achieve the aims of the institution [17, 18]. Managers should also modify and improve managerial performances in order to increase the motivation of the employees, their level of job satisfaction and, finally, achieve better quality and outcome of work of a healthcare institution [17, 18].

So far, there have not been studies dealing with motivation and job satisfaction of healthcare professionals in urban and rural areas in the Autonomous Province of Vojvodina, Republic of Serbia.

The aim of this study was to establish whether there is a difference in motivation and job satisfaction in a sample of healthcare professionals in urban and rural areas in the Autonomous Province of Vojvodina, Republic of Serbia.

Material and Methods

A cross-sectional study was conducted among healthcare professionals in one Health Center of each of the three districts of Vojvodina: "Dr. Milorad – Mika Pavlović" in Inđija (Srem), "Dr. Boško Vrebalov" in Zrenjanin (Banat) and Health Center in Apatin (Bačka), in the period from March to June 2011. All of the Health Centers include objects on the territory of the very city and surrounding village settlements belonging to their municipalities. A convenience sampling of health facilities was used.

In the three health centers there was a total of 879 (100%) of healthcare professionals. Data collection was performed by a self-administered questionnaire which was anonymous. The questionnaire was designed by the author. The distribution and collection of the questionnaire was in charge of the author. Aiming at providing anonymity and confidentiality, the author personally offered questionnaire in the envelope to all healthcare professionals present at work on the day of the survey, 809 (92.0%). The self-administered questionnaire was on a voluntary basis. The questionnaire was refused to be filled by 52 (5.9%) healthcare professionals. A total of 757 (86.1%) health professionals completed the questionnaires. Only the data from completely filled in questionnaires were included in the further analysis. There were 719 (81.8%) completely filled in questionnaires, out of which 574 (79.8%) were completed by the healthcare professionals from urban setting (176 physicians and 398 nurses), and 145 (20.2%) healthcare professionals from rural area (54 physicians and 91 nurses). After completion, the respondents returned a blank envelope containing a questionnaire to the researcher.

The questionnaire consisted of three parts (about demographic characteristics of the respondents, work motivation factors and job satisfaction).

In order to evaluate motivation of healthcare professionals we used fifteen questions about the importance of certain factors of work motivation. Respondents answered each of the questions by choosing one of the five offered answers - five-point Likert scale ranging from 1 (It does not motivate me at all), 2 (It motivates me a little), 3 (I am not sure), 4 (It motivates me a lot), to 5 (It motivates me the most). For each of the respondents, the total number of points, i.e. average score, was calculated according to the given answers. The respondents whose total number of points was over 45, i.e. average score over 3.00, were characterized as motivated, while the others were characterized as unmotivated. In that way, a new variable (dichotomous variable), called "Motivation category" (0 = unmotivated, 1 = motivated) was created.

For the evaluation of job satisfaction we used fifteen questions about the attitudes of healthcare professionals towards satisfaction/fulfillment of certain factors of work motivation. Five possible answers were offered - five-point Likert scale ranging from 1 (I strongly disagree), 2 (I partially disagree), 3 (I neither agree nor disagree), 4 (I partially agree), to 5 (I strongly agree).

Statistical analysis was performed using Student *t-test*, χ^2 *test,* Spearman's rank correlation coefficient, Receiver Operating Charasteristic Curve (ROC) analysis and binary logistic regression analysis (p value of < 0.05 was considered statistically significant).

Statistical analysis was done using the SPSS version 17.

Receiver Operating Charasteristic Curve analysis (motivation in relation to age and years of service) was used to calculate the optimal values for categorisation/grouping the respondents on the basis of age (40 years) and years of service (15 years). The binary logistic regression analysis (method Enter)was used to analyze the impact of the respondents' demographic characteristics (gender, age), profession, years of service, and place of employment (urban area or rural area) on the motivation of the respondents. The independent variables, in the binary logistic regression analysis, were coded as follows: gender (0 = male, 1 = female), age (0 = older than 40 years, 1 = younger than 40 years), profession (0 = nurse, 1 = physician), years of service (0 = over 15 years of service, 1 = up to 15 years of service), and place of employment (0 = rural area, 1 = urban area). As dependent variable (dichotomous variable), in models of binary logistic regression, there was used the value "Motivation category".

Table 2. Job satisfaction: comparison of urban and rural healthcare professionals
Tabela 2. *Zadovoljstvo poslom: poređenje zdravstvenih radnika zaposlenih u urbanoj i ruralnoj sredini*

Attitudes related to job satisfaction *Stavovi u vezi sa zadovoljstvom poslom*	I agree *Slažem se*	Urban area *Urbana sredina*		Rural area *Ruralna sredina*		p
		n	%	n	%	
The manager supports me to achieve my professional goals/*Rukovodilac mi pomaže da dostignem svoje radne ciljeve*	No/*Ne*	89	15.5	23	15.9	0.397
	I am not sure/*Nisam siguran/a*	107	18.6	34	23.4	
	Yes/*Da*	378	65.9	88	60.7	
The manager gives me praises when it is necessary, appropriate/*Rukovodilac me, kada je to potrebno/prikladno, pohvali*	No/*Ne*	127	22.1	29	20.0	0.481
	I am not sure/*Nisam siguran/a*	113	19.7	35	24.1	
	Yes/*Da*	334	58.2	81	55.9	
Work relationships are good in my institution/*U ustanovi u kojoj radim vladaju dobri međuljudski odnosi*	No/*Ne*	86	15.0	30	20.7	0.247
	I am not sure/*Nisam siguran/a*	185	32.2	43	29.6	
	Yes/*Da*	303	52.8	72	49.7	
The manager supports me to get a better position or a promotion/*Rukovodilac mi pomaže da radim na svojoj promociji*	No/*Ne*	137	23.9	36	24.8	0.316
	I am not sure/*Nisam siguran/a*	143	24.9	44	30.3	
	Yes/*Da*	294	51.2	65	44.8	
My direct supervisor has good personal qualities/*Neposredni rukovodilac poseduje dobre lične kvalitete*	No/*Ne*	70	12.2	14	9.6	0.004
	I am not sure/*Nisam siguran/a*	96	16.7	42	29.0	
	Yes/*Da*	408	71.1	89	61.4	
I am satisfied with my income *Zadovoljan/a sam visinom novčanog iznosa mesečne zarade*	No/*Ne*	329	57.3	87	60.0	0.838
	I am not sure/*Nisam siguran/a*	116	20.2	27	18.6	
	Yes/*Da*	129	22.5	31	21.4	
My institution ensures good working conditions/*Ustanova u kojoj radim obezbeđuje dobre radne uslove*	No/*Ne*	136	23.7	34	23.4	0.670
	I am not sure/*Nisam siguran/a*	127	22.1	37	25.5	
	Yes/*Da*	311	54.2	74	51.0	
There is a positive working atmosphere in my institution/*U ustanovi u kojoj radim vlada kooperativna radna atmosfera*	No/*Ne*	81	14.1	21	14.5	0.920
	I am not sure/*Nisam siguran/a*	154	26.8	41	28.3	
	Yes/*Da*	339	59.1	83	57.2	
My institution offeres me opportunities for continous training/*Ustanova u kojoj radim mi pruža mogućnost stalnog usavršavanja*	No/*Ne*	85	14.8	32	22.1	0.106
	I am not sure/*Nisam siguran/a*	109	19.0	25	17.2	
	Yes/*Da*	380	66.2	88	60.7	
My institution guarantees secure employment/*Ustanova u kojoj radim garantuje zaposlenima sigurnost zaposlenja*	No/*Ne*	74	12.9	17	11.7	0.028
	I am not sure/*Nisam siguran/a*	122	21.2	46	31.7	
	Yes/*Da*	378	65.9	82	56.6	
The manager provides me immediate support at work/*Rukovodilac mi obezbeđuje neposrednu podršku na poslu*	No/*Ne*	88	15.3	14	9.6	0.045
	I am not sure/*Nisam siguran/a*	116	20.2	41	28.3	
	Yes/*Da*	370	64.5	90	62.1	
The suvervisor allows me independence at routine tasks/*Rukovodilac mi omogućava da upotrebim samostalnost kod rutinskih zadataka*	No/*Ne*	56	9.8	14	9.7	0.904
	I am not sure/*Nisam siguran/a*	94	16.4	26	17.9	
	Yes/*Da*	424	73.9	105	72.4	
My institution provides current equipment/*Ustanova u kojoj radim mi obezbeđuje savremenu opremu za obavljanje posla*	No/*Ne*	124	21.6	37	25.5	0.152
	I am not sure/*Nisam siguran/a*	141	24.6	43	29.7	
	Yes/*Da*	309	53.8	65	44.8	
My institution pays bonuses for exceptional work/*Ustanova u kojoj radim obezbeđuje zaposlenima nagradu za posebno dobro urađen posao*	No/*Ne*	251	43.7	68	46.9	0.055
	I am not sure/*Nisam siguran/a*	149	26.0	47	32.4	
	Yes/*Da*	174	30.3	30	20.7	
The supervisor is qualified to supervise my work/*Rukovodilac je stručan za obavljanje nadzora nad mojim radom*	No/*Ne*	70	12.2	11	7.6	0.002
	I am not sure/Nisam siguran/a	77	13.4	36	24.8	
	Yes/*Da*	427	74.4	98	67.6	

The Management Boards of health centers gave permission for the study to be conducted. The Ethics Committee of the author's institutions gave ethical approval for the study.

Results

There were significantly more male respondents among urban healthcare professionals than among the healthcare professionals in rural area (19.3% vs. 7.6%). Between the examined groups no statistically significant difference was obtained in relation to age.

Urban healthcare professionals, compared to rural healthcare workers, were significantly more motivated by the factor of work motivation – achieving the goals of the health center (health promotion, prevention of diseases, early diagnosis and therapy of the diseased) **(Table 1)**. For all the other factors no statistically significant difference was obtained between the examined groups of healthcare professionals.

In comparison with rural healthcare professionals, urban healthcare workers were significantly more satisfied with personal qualities of their immediate supervisors, job security guaranteed by their institution, immediate support at work they received from

Table 3. Correlation between the importance of work motivation factors among urban healthcare professionals (n=574) and the level of their fulfillment/satisfaction provided by their healthcare institutions
Tabela 3. *Korelacija između značaja faktora radne motivacije zdravstvenih radnika u urbanoj sredini (n=574) i stepena njihovog ispunjenja/zadovoljstva od zdravstvene ustanove u kojoj su zaposleni*

Work motivation factors (urban healthcare professionals) *Faktori radne motivacije (zdravstveni radnici zaposleni u urbanoj sredini)*	Mean score related to the level of importance of factors *Prosečna ocena za nivo značaja faktora* ($\bar{X} \pm$ SD)	Mean score related to the level of fulfillment of factors *Prosečna ocena za stepen ispunjenja faktora* ($\bar{X} \pm$ SD)	*t*-test (p)*	Spearman's ρ Spirman ρ (p)†
Achieving the goals of the health center *Postizanje ciljeva zdravstvene ustanove*	4.0 ± 1.0	3.7 ± 1.2	4.970 (< 0.001)	0.299 (< 0.001)
Professional recognition *Priznanje za dobro obavljen posao*	3.9 ± 1.2	3.5 ± 1.4	6.174 (< 0.001)	0.318 (< 0.001)
Good work relationships *Dobri međuljudski odnosi*	4.1 ± 1.1	3.5 ± 1.1	11.839 (< 0.001)	0.436 (< 0.001)
Opportunities for promotion and improvement *Mogućnost unapređenja i napredovanja*	3.8 ± 1.2	3.3 ± 1.3	7.184 (< 0.001)	0.367 (< 0.001)
Personal qualities of immediate supervisors/*Lični kvaliteti neposrednih rukovodilaca*	4.0 ± 1.1	3.9 ± 1.2	1.391 (0.165)	0.459 (< 0.001)
Monthly income *Novčani iznos mesečne zarade*	3.5 ± 1.5	2.3 ± 1.3	16.475 (< 0.001)	0.242 (< 0.001)
Working conditions *Uslovi na radu*	3.8 ± 1.1	3.4 ± 1.2	6.669 (< 0.001)	0.350 (< 0.001)
Positive working atmosphere *Kooperativna radna atmosfera*	4.0 ± 1.0	3.6 ± 1.1	8.125 (< 0.001)	0.466 (< 0.001)
Training opportunities *Mogućnost usavršavanja*	3.8 ± 1.2	3.8 ± 1.2	0.468 (0.640)	0.376 (< 0.001)
Secure job *Siguran posao*	4.3 ± 1.0	3.8 ± 1.1	10.595 (< 0.001)	0.415 (< 0.001)
Support by supevisors *Podrška rukovodilaca*	4.1 ± 1.0	3.7 ± 1.2	7.300 (< 0.001)	0.474 (< 0.001)
Independence at work *Autonomija prilikom obavljanja posla*	4.1 ± 1.0	4.0 ± 1.1	1.087 (0.227)	0.507 (< 0.001)
Current equipment *Posedovanje savremene opreme za rad*	3.9 ± 1.2	3.4 ±1.3	9.502 (< 0.001)	0.420 (< 0.001)
Bonuses for exceptional work *Nagrada za posebno dobro urađen posao*	3.8 ± 1.3	2.7 ± 1.4	16.239 (< 0.001)	0.317 (< 0.001)
Professional supervision *Stručan nadzor nad radom*	3.7 ± 1.2	4.0 ± 1.1	4.876 (< 0.001)	0.394 (< 0.001)

* Level of significance p - Student *t*-test for associated samples/*Nivo značajnosti p - Studentov t-test za vezane uzorke*
† Level of significance p - Spearman's rank correlation coefficient/*Nivo značajnosti p - Spirmanov koeficijent korelacije ranga*

managers, and professional supervision of their work **(Table 2).** For all the other factors no statistically significant difference was obtained between the examined groups of healthcare professionals.

Urban healthcare professionals were significantly more work motivated than job satisfied by the fulfillment of factors of work motivation: achieving the goals of the health center, professional recognition, good work relationships, opportunities for promotion and improvement, monthly income, working conditions, positive working atmosphere, secure job, support by supervisors, current equipment, and bonuses for exceptional work **(Table 3).** Urban healthcare workers were significantly less work motivated by professional supervision of their work, compared to the degree of its satisfaction/fulfillment provided by their healthcare institutions. For all the other factors no statistically significant difference was obtained between the examined groups of healthcare professionals. According to Spearman's rank correlation coefficient, work motivation level of urban healthcare workers was higher if the level of their job satisfaction was higher. Rural healthcare professionals were significantly more work motivated than job satisfied by the fulfillment of factors of work motivation: achieving the goals of the health center, professional recognition, good work relationships, opportunities for promotion and improvement, personal qualities of immediate supervisors, monthly income, working conditions, positive working atmosphere, secure job, support by supervisors, current equi-

Table 4. Correlation between the importance of work motivation factors among rural healthcare professionals (n=145) and the level of their fulfillment/satisfaction provided by their healthcare institutions
Tabela 4. *Korelacija između značaja faktora radne motivacije zdravstvenih radnika u ruralnoj sredini (n=145) i stepena njihovog ispunjenja/zadovoljstva od zdravstvene ustanove u kojoj su zaposleni*

Work motivation factors (rural healthcare professionals) *Faktori radne motivacije (zdravstveni radnici zaposleni u ruralnoj sredini)*	Mean score related to the level of importance of factors *Prosečna ocena za nivo značaja faktora* ($\bar{X} \pm$ SD)	Mean score related to the level of fulfillment of factors *Prosečna ocena za stepen ispunjenja faktora* ($\bar{X} \pm$ SD)	*t*-test (p)*	Spearman's ρ Spirman ρ (p)†
Achieving the goals of the health center *Postizanje ciljeva zdravstvene ustanove*	3.8 ± 1.1	3.6 ± 1.1	2.229 (0.027)	0.243 (0.003)
Professional recognition *Priznanje za dobro obavljen posao*	3.7 ± 1.2	3.4 ± 1.2	2.695 (0.008)	0.303 (< 0.001)
Good work relationships *Dobri međuljudski odnosi*	4.0 ± 1.1	3.3 ± 1.2	7.209 (< 0.001)	0.378 (< 0.001)
Opportunities for promotion and improvement/*Mogućnost unapređenja i napredovanja*	3.5 ± 1.3	3.2 ± 1.2	2.353 (0.020)	0.324 (< 0.001)
Personal qualities of immediate supervisors/ *Lični kvaliteti neposrednih rukovodilaca*	4.0 ± 1.0	3.8 ± 1.1	2.155 (0.033)	0.306 (< 0.001)
Monthly income *Novčani iznos mesečne zarade*	3.3 ± 1.6	2.2 ± 1.3	8.145 (< 0.001)	0.337 (< 0.001)
Working conditions *Uslovi na radu*	3.8 ± 1.1	3.3 ± 1.2	4.274 (< 0.001)	0.383 (< 0.001)
Positive working atmosphere *Kooperativna radna atmosfera*	4.0 ± 1.0	3.4 ± 1.1	5.926 (< 0.001)	0.431 (< 0.001)
Training opportunities *Mogućnost usavršavanja*	3.6 ± 1.2	3.5 ± 1.3	1.089 (0.278)	0.332 (< 0.001)
Secure job *Siguran posao*	4.2 ± 1.0	3.6 ± 1.1	5.668 (< 0.001)	0.345 (< 0.001)
Support by supevisors *Podrška rukovodilaca*	4.0 ± 1.0	3.7 ± 1.1	2.773 (0.006)	0.242 (0.003)
Independence at work *Autonomija prilikom obavljanja posla*	4.0 ± 1.0	3.9 ± 1.1	0.328 (0.743)	0.227 (0.006)
Current equipment *Posedovanje savremene opreme za rad*	3.8 ± 1.2	3.2 ± 1.2	5.561 (< 0.001)	0.421 (< 0.001)
Bonuses for exceptional work *Nagrada za posebno dobro urađen posao*	3.7 ± 1.3	2.4 ± 1.3	9.988 (< 0.001)	0.362 (< 0.001)
Professional supervision *Stručan nadzor nad radom*	3.6 ± 1.1	3.9 ± 1.1	3.074 (0.003)	0.222 (0.007)

* Level of significance p - Student *t*-test for associated samples/*Nivo značajnosti p - Studentov t-test za vezane uzorke*
† Level of significance p - Spearman's rank correlation coefficient/*Nivo značajnosti p - Spirmanov koeficijent korelacije ranga*

Table 5. Independent variables and their impact on motivation of healthcare professionals in urban area
Tabela 5. *Nezavisne promenljive i njihov uticaj na motivisanost zdravstvenih radnika zaposlenih u urbanoj sredini*

Variables/*Promenljive*	B	S.E.	p	OR	95% CI for OR
Gender (female)/*Pol (ženski pol)*	0.166	0.327	0.612	1.180	0.622 – 2.241
Age (younger than 40 years)/*Starost (mlađi od 40 godina)*	0.423	0.464	0.362	1.527	0.614 – 3.795
Profession (physicians)/*Zanimanje (lekar)*	0.180	0.283	0.525	1.197	0.688 – 2.083
Years of service (up to 15 years)/*Dužina radnog staža (do 15 godina)*	0.860	0.464	0.064	2.364	0.951 – 5.873
Constant/*Konstanta*	1.245	0.333	0.000	3.477	

Table 6. Independent variables and their impact on motivation of healthcare professionals in rural area
Tabela 6. *Nezavisne promenljive i njihov uticaj na motivisanost zdravstvenih radnika zaposlenih u ruralnoj sredini*

Variables/*Promenljive*	B	S.E.	p	OR	95% CI for OR
Gender (female)/*Pol (ženski pol)*	1.063	0.712	0.135	2.895	0.717 – 11.682
Age (younger than 40 years)/*Starost (mlađi od 40 godina)*	2.247	0.971	0.021	9.460	1.41 – 63.491
Profession (physicians)/*Zanimanje (lekar)*	0.962	0.521	0.065	2.617	0.943 – 7.263
Years of service (up to 15 years)/*Dužina radnog staža (do 15 godina)*	0.543	0.712	0.446	0.581	0.414 – 2.347
Constant/*Konstanta*	0.023	0.711	0.974	1.023	

pment, and bonuses for exceptional work **(Table 4).** Rural healthcare workers were significantly less work motivated by professional supervision of their work, compared to the degree of its satisfaction/fulfillment provided by their healthcare institutions. For all the other factors no statistically significant difference was obtained between the examined groups of healthcare professionals. According to Spearman's rank correlation coefficient, work motivation level of rural healthcare workers was higher if the level of their job satisfaction was higher.

The binary logistic regression analysis showed no statistically significant impact of independent variables: gender, age, profession and years of service on the motivation of urban healthcare professionals **(Table 5).** It showed that only age had statistically significant effect on the motivation of rural healthcare workers **(Table 6).** Among healthcare professionals from rural area younger than 40 years, the percentage of the motivated ones was 9.460 times higher (95% CI: 1.41–63.491; p=0.021) in relation to healthcare workers older than 40 years. Gender, profession and years of service were not statistically significant predictors of work motivation among rural healthcare professionals.

Discussion

Our study shows that urban healthcare professionals, compared to rural healthcare workers, were significantly more motivated by the factor of work motivation – achieving the goals of the health center. The result of the study performed by Rakić [19] in healthcare institution in Banja Luka showed that variations in income influence the employees work motivation, but not so expressed that the financial reward would become the main determinant of the level of their work motivation. Similar to our results, the result of the study of work motivation conducted in healthcare institutions in central Serbia shows that healthcare professionals in urban area in relation to healthcare workers in rural area are significantly more motivated by achieving the goals of their institution [20].

In our study, healthcare workers in urban area in regard to healthcare workers in rural area were significantly more satisfied with personal qualities of their immediate supervisors, job security, immediate support at work they received from managers, and professional supervision. Place of employment can be a significant predictor of job satisfaction [16]. The study of Ulmer and Harris [16] conducted in Australia shows that work in urban area does not necessarily represent a predictor of high level of physicians' job satisfaction. The same study shows that the most frequent predictors of job dissatisfaction among physicians are working full-time, bad mental health and work in urban area [16]. According to the study of Prosen and Piskar [8] conducted in Slovenia, job satisfaction of nurses is mostly influenced by social interaction. The research of Mrduljaš Đujić et al. [9], conducted in Split, shows that general practitioners are least satisfied with the superiors, i.e. their payers (Croatian Institute for Health Insurance). The result of the study performed by the Institute of Public Health of Serbia shows that the highest level of job satisfaction is present in healthcare institutions in Kosovo and Metohija, then on the territory of the central Serbia and Vojvodina, and the lowest one in healthcare facilities in Belgrade [6]. In the study conducted in state hospitals in Belgrade, Kuburović et al. [10] show that the main determinants of employees' dissatisfaction are their salaries, equipment, possibilities of education and professional advancement.

The results of our study which shows that healthcare professionals were significantly more work motivated than satisfied with their monthly income and current equipment are expected and comprehensible, having in mind the fact that our country is facing a difficult economic situation [21–23]. In 2007, Minister of Health passed the "The Regulation on detailed conditions for the implementation of continuing education

for healthcare workers and associate healthcare workers" [24]. On the basis of the Regulation healthcare professionals have the right and duty to continually improve themselves [24], so it is very important as well as expected, that in our study no significant difference was obtained between the level of importance and job satisfaction regarding the possibility of improvement. The study of Ašćerić [25] conducted in the Tuzla Health Center shows that salary, secure job, professional advancement and autonomy at work are evaluated as being the most important motivational factors the least satisfied by health institution. Level of job satisfaction in itself does not show whether a particular job characteristic is at the same time important to the employees [26]. Low satisfaction rating and high level of importance of particular job characteristic point to a significant potential for improvement by modification of particular job charasteristic [26].

Our study has several limitations. Since it was a cross-sectional study, comparisons were made at one point in time, and no conclusion about cause-effect relationships could be made [27]. Also, in the institutions there is a high sensitivity of the employees, especially the managerial staff, to studies dealing with motivation and attitudes of the employees about specific aspects of work, management and the very institution, therefore the most common problem in such studies is the impossibility of researchers to determine the reliability of the respondents' responses [3]. The employees are suspicious and afraid of potential unwanted consequences and the possibility to place themselves in an undesired position in the institutions, thus their answers are often insincere [3]. For data collection we used a self-administered questionnaire, but although we emphasized the anonymity of the questionnaire and that the results would be used only for the purpose of the research, we are sure that we did not obtain completely sincere answers. The reasons lie in the fact that even the global attitude to motivation and job satisfaction in the institution whose employees were examined represents to the employees some kind of danger regarding their relationship with managers [3]. Finally, the fact that the study was conducted in the healthcare centers selected by the method of convenience sampling, and not randomly represented a limitation of this study, so the obtained results cannot be generalized to all healthcare professionals in Vojvodina.

Conclusion

Compared to rural healthcare professionals, urban healthcare workers are more motivated and job satisfied. Aiming at improving the quality of work in healthcare instititution and increasing patients' satisfaction, the management of healthcare institutions should recognize what motivates healthcare professionals and which aspects of work make them job satisfied. It is necessary that the management teams continually conduct studies of motivation and job satisfaction. The examination of motivation and job satisfaction is necessary on a representative sample of healthcare professionals, in rural as well in urban areas of the Republic of Serbia. In accordance with the results of the investigations, managers of healthcare institutions should define and undertake adequate activities, and direct them to improvement of healthcare workers' motivation and job satisfaction.

References

1. World Health Organization Regional Office for Europe. Health 21 - The health for all policy for the WHO European Region – 21 target for the 21st century. Copenhagen: WHO Regional Office for Europe; 1998.

2. Peltier J, Dahl A. The relationship between employee satisfaction and hospital patient experiences. Forum for people performance management and measurement [Internet]. 2009 [cited 2017 Oct 9]. Available from: http://www.info-now.com/typo3conf/ext/p2wlib/pi1/press2web/html/userimg/FORUM/Hospital%20Study%20-relationship%20Btwn%20Emp.%20Satisfaction%20and%20Pt.%20Experiences.pdf.

3. Mihailović D. Motivation for work: the methods and techniques of measurement. Belgrade: Yugoslav Institute for labor productivity; 1985. Serbian.

4. Greenberg J, Baron RA. Behavior in organizations: understanding and managing the human side of work. Englewood Cliffs, NJ: Prentice-Hall; 1995.

5. The Regulation on indicators of quality of health care. Službeni glasnik RS. 2010;(49). Serbian.

6. Horozović V. Analysis of satisfaction surveys of employees in state health institutions of the Republic of Serbia 2016. Belgrade: Institute of Public Health of Serbia "Dr Milan Jovanović Batut"; 2017. Serbian.

7. Jakovljević M, Vuković M, Chen CC, Antunović M, Dragojević Simić V, Veličković Radovanović R, et al. Do health reforms impact cost consciousness of health care professionals? Results from a nation-wide survey in the Balkans. Balkan Med J. 2016;33(1):8-17.

8. Prosen M, Piskar F. Job satisfaction of Slovenian hospital nursing workforce. J Nurs Manag. 2015;23(2):242-51.

9. Mrduljaš Đujić N, Kuzmanić M, Kardum G, Rumboldt M. Job satisfaction among medical doctors in one of the countries in transition: experience from Croatia. Coll Antropol. 2010;34(3):813-8.

10. Kuburović NB, Dedić V, Đuričić S, Kuburović V. Determinants of job satisfaction of healthcare professionals in public hospitals in Belgrade, Serbia - Cross-sectional analysis. Srp Arh Celok Lek. 2016;144(3-4):165-73.

11. Barać I, Plužarić J, Kanisek S, Dubac Nemet L. Job satisfaction of nurses in relation to workplace. Sestrinski glasnik. 2015;20(1):27-32. Croatian.

12. Shi L, Song K, Rane S, Sun X, Li H, Meng Q. Factors associated with job satisfaction by Chinese primary care providers. Prim Health Care Res Dev. 2014;15(1):46-57.

13. Đorđević D, Petrović D, Vuković D, Mihailović D, Dimić A. Motivation and job satisfaction of health workers in a specialized health institutions in Serbia. Vojnosanit Pregl. 2015;72(8):714-21.

14. Radević S, Mihailović N, Kocić S, Radovanović S, Milosavljević M, Živanović S, et al. Satisfaction of medical staff in primary health care of Sumadija district. Medicinski časopis. 2015;49(1):7-12.

15. Li T, Lei T, Sun F, Xie Z. Determinants of village doctors' job satisfaction under China's health sector reform: a cross-sectional mixed methods study. Int J Equity Health. 2017;16(1):64.

16. Ulmer B, Harris M. Australian GPs are satisfied with their job: even more so in rural areas. Fam Pract. 2002;19(3):300-3.

17. Miljković S. Motivation of employees and behaviour modification in health care organisations. Acta Medica Medianae. 2007;46(2):53-62. Serbian.

18. Dieleman M, Toonen J, Touré H, Martineau T. The match between motivation and performance management of health sector workers in Mali. Hum Resour Health. 2006;4:2.

19. Rakić S. Work motivation of employees of the Clinical Center of Banja Luka and contributions to its improvement [dissertation]. Banja Luka: Pan-European University "Apeiron"; 2010. Serbian.

20. Grujičić M, Jovičić Bata J, Rađen S, Novaković B, Šipetić Grujičić S. Work motivation and job satisfaction of health workers in urban and rural areas. Vojnosanit Pregl. 2016;73(8):735-43.

21. Teofilo Marković R, Deljanin Ilić M, Milošević Z, Vasić M, Bogdanović D, Šagrić Č. Level of job satisfaction among employees working at heathcare facilities in Nišava and Toplica district. Med Pregl. 2013:66(9-10):379-85. Serbian.

Rad je primljen 1. XI 2017.
Recenziran 20. XI 2017.
Prihvaćen za štampu 29. XI 2017.
BIBLID.0025-8105:(2018):LXXI:1-2:33-41.

22. Šantrić Miličević M, Vasić V, Terzić Šupić Z. Do health care workforce, population, and service provision significantly contribute to the total health expenditure? An econometric analysis of Serbia. Hum Resour Health. 2016;14(1):50.

23. Jakovljević M, Varjačić M. Commentary: Do health care workforce, population, and service provision significantly contribute to the total health expenditure? An econometric analysis of Serbia. Front Pharmacol. 2017;8:33.

24. The regulation on detailed conditions for the implementation of continuing education for healthcare workers and associate healthcare workers. Službeni glasnik RS. 2007;(130). Serbian.

25. Aščerić T. Motivation and behaviour modification of health workers [graduate/specialist thesis]. Banja Luka: Pan-European University "Apeiron"; 2008. Serbian.

26. Janus K, Amelung VE, Baker LC, Gaitanides M, Schwartz FW, Rundall TG. Job satisfaction and motivation among physicians in academic medical centers: insights from a cross-national study. J Health Polit Policy Law. 2008;33(6):1133-67.

27. Đurić P, editor. Introduction to scientific research work. Novi Sad: Faculty of Medicine; 2014. Serbian.

Clinical Center of Vojvodina, Clinic of Neurology, Novi Sad
University of Novi Sad, Faculty of Medicine Novi Sad

Professional article
Stručni rad
UDK 615.273.06:616.8-005.1
https://doi.org/10.2298/MPNS1802042P

EFFECT OF ANTICOAGULANT AND ANTIPLATELET THERAPY ON THE OCCURRENCE OF PRIMARY INTRACEREBRAL HEMORRHAGE

UTICAJ ANTIKOAGULANTNE I ANTITROMBOCITNE TERAPIJE NA POJAVU PRIMARNE INTRACEREBRALNE HEMORAGIJE

Aleksandra LUČIĆ PROKIN, Armin PAKOCI, Sanela POPOVIĆ and Arsen UVELIN

Summary

Introduction. The incidence of intracerebral hemorrhage related to oral anticoagulant and antiplatelet therapy has an increasing trend, thus it may be a potential indicator of unvaforable outcome of primary intracerebral hemorrhage. The aim of the study was to determine the effect of these therapies on the occurrence, localization and outcome of primary intracerebral hemorrhage. **Material and Methods**. A retrospective study included 246 patients with first time diagnosed primary intracerebral hemorrhage. Patients were divided into three groups, according to the drugs they have used. The incidence, anatomical distribution of primary intracerebral hemorrhage and survival/mortality rates were observed in all groups. **Results.** Antiplatelet therapy was used by 20.3% of patients, 8.2% received antocoagulant therapy, while the rest of 71.5% didn not take these drugs in the premorbid period. The most common risk factor was arterial hypertension (97.2%). In all groups, patients had a tendency for supratentorial hematomas. Only alcohol consumption had a significant impact on the localization of hemorrhage ($p < 0,05$). There was no statistically significant difference between groups in National Institutes of Health Stroke Scale score on admission and a modified Rankin Scale Score at discharge. Oral anticoagulant users presented with the highest mortality rate in the first 24 hours (odds ratio - 2.5). Patients in other two groups showed a significantly higher survival rate (odds ratio - 1.5). **Conclusion.** Oral anticoagulant users had significantly higher National Institutes of Health Stroke Scale score on admission with an increased risk for early death. A significantly higher percentage of survival was noted in other two groups. Approximately 2/3 of all patients had poor functional recovery.

Key words: Anticoagulants; Platelet Aggregation Inhibitors; Cerebral Hemorrhage; Incidence; Treatment Outcome; Recovery of Function; Drug-Related Side Effects and Adverse Reactions; Risk Factors

Sažetak

Uvod. Incidencija intracerebralnog krvarenja zbog upotrebe oralne antikoagulantne i antitrombocitne terapije ima tendenciju porasta, te bi ovaj vid terapije mogao da bude potencijalni indikator nepovoljnog ishoda primarne intracerebralne hemoragije. Cilj istraživanja bio je utvrđivanje uticaja antikoagulantne i antitrombocitne terapije na pojavu, lokalizaciju i ishod primarne intracerebralne hemoragije. **Materijal i metode.** Retrospektivno istraživanje obuhvatilo je 246 bolesnika sa prvi put doživljenom primarnom intracerebralnom hemoragijom. Bolesnici su podeljeni u tri grupe u zavisnosti od lekova koje su koristili. Praćene su učestalost pojavljivanja i anatomska distribucija primarne intracerebralne hemoragije, kao i preživljavanje/mortalitet među grupama. **Rezultati.** Antitrombocitnu terapiju koristilo je 20,3% bolesnika, 8,2% njih oralne antikoagulanse, dok ostalih 71,5% nije uzimalo nijedno od navedenog u periodu pre bolesti. Najčešće zastupljeni faktor rizika bila je arterijska hipertenzija (97,2%). Sve tri grupe imale su tendenciju ka supratentorijalnoj lokalizaciji hematoma. Jedino je alkohol imao značajan uticaj na lokalizaciju hemoragije ($p < 0,05$). Nije utvrđena statistički značajna razlika u na Skali moždanog udara Nacionalnog instituta za zdravlje skoru za težinu moždanog udara na prijemu i modifikovanom Rankin skoru na otpustu između grupa. Korisnici oralnih antikoagulanasa su imali najveći mortalitet u prvih 24 sata (OR = 2,5). Druge dve grupe su zabeležile značajnu sklonost ka preživljavanju (OR = 1,5). **Zaključak.** Korisnici oralne antikoagulantne terapije imaju veći skor moždanog udara na skali možanog udara Nacionalnog instituta za zdravlje na prijemu i povećan rizik za rani smrtni ishod. Druge dve grupe imaju veću sklonost ka preživljavanju. Oko 2/3 bolesnika imalo je loš funkcionalni oporavak.

Ključne reči: antikoagulanti; inhibitori agregacije trombocita; intracerebralno krvarenje; incidenca; ishod lečenja; oporavak funkcije; nuspojave i neželjene reakcije izazvane lekovima; faktori rizika

Introduction

Primary intracerebral hemorrhage (pICH) is a non-traumatic hemorrhage in the brain parenchyma and/or chamber system, most commonly from small blood vessels due to chronic hypertension or cerebral amyloid angiopathy [1, 2]. The most common risk factor (RF) is arterial hypertension (HTA), but oral anticoagulant therapy (OACT), as well as antithrombotic therapy (AT) are also significant RFs [2, 3]. Warfarin, as the most commonly used oral anticoagulant, is considered a significant RF for pICH [2, 4, 5]. The overall risk of stroke in persons using warfarin accounts for 0.3 – 3.7% per year, with an expo-

Corresponding Author: Doc. dr Aleksandra Lučić Prokin, Klinički centar Vojvodine, Klinika za neurologiju, 21000 Novi Sad, Hajduk Veljkova 1-7, E-mail: aleksandra.lucic-prokin@mf.uns.ac.rs

Abbreviations

pICH – primary intracerebral hemorrhage
HTA – arterial hypertension
RF – risk factor
OACT – oral anticoagulant therapy
AT – antithrombotic therapy
ASA – acetylsalicylic acid
IS – ischemic stroke
INR – International Normalized Ratio
NIHSS – National Institutes of Health Stroke Scale
mRS – Modified Rankin Score

nential growth rate with age, but the benefit of this drug still significantly outweighs the risk of bleeding [2, 6–11]. The AT comprises acetylsalicylic acid (ASA) and clopidogrel. The benefit of using low-dose ASA (75 – 100 mg) monotherapy by far exceeds the risk of potential adverse effects, while long-term use of high doses (> 175 mg/day and > 1225 mg/week) represents the potential RF for pICH. Although having a different mechanism of action, there was no difference in risk for pICH between patients who used ASA and clopidogrel [12]. The aim of this study was to determine the incidence of pICH in patients who used AT or OACT, anatomical distribution, and pICH outcome among the examined groups.

Material and Methods

A retrospective study included 246 patients with first-time pICH hospitalized at the Emergency Neurology Department of the Emergency Center and the Neurology Clinic of the Clinical Center of Vojvodina in Novi Sad, in the period from January 2014 to December 2015. The following clinical and demographic data were observed: age, sex, RF for pICH (hypertension, hyperlipidemia, alcohol consumption, use of AT and OACT), platelet count, anamnestic data of previous ischemic stroke (IS). The International Normalized Ratio (INR) value was recorded on admission. The normal INR value was < 1.3 for patients without prior use of OACT, while INR values 2 – 3 were taken as physiological for patients using OACT, and according to the recommended therapeutic range.

The initial pICH severity was reported, according to the National Institutes of Health Stroke Scale (NIHSS), a value derived from the clinical neurological examination on admission. The degree of functional dependence and disability was assessed based on the Modified Rankin Score (mRS) at discharge from the hospital. The positive functional outcome was defined by the values of mRS from 0 – 2, while unfavorable values of mRS were from 3 – 5. The patients' survival was monitored for 30 days, and mortality, depending on the time elapsed from admission: in the first 24 hours, from 24h to 7^{th} day, and from 8^{th} to 30^{th} day. Using available neuroradiological examinations, each patient was diagnosed with topographic localization of pICH: lobar, deep cerebral (basal ganglia, thalamus), brainstem or cerebellum.

Patients were divided into three groups: OACT users, AT users, and a group of patients who did not use any of these therapies (group without AT/OACT).

All data were obtained from the medical records, including medical histories and discharge letters. The exclusion criteria were as follows: data and neuroradiological findings on acute and chronic brain injuries and brain bleeding; existence of secondary bleeding in the brain tumor; hemorrhagic transformation of acute IS; presence of an aneurysm or a vascular brain malformation with earlier or existing intracranial bleeding; prehospital use of dual AT; prehospital use of a combination of OACT and AT.

Statistical data processing was done using Microsoft Excel 2007 and the Statistical Package for the Social Sciences. The numerical values were represented by mean values (arithmetic mean) and measure of variability (range of values, standard deviation). Attributive characteristics were represented using frequencies and percentages. A comparison of numerical values between the two groups was carried out using a Student's t-test. The testing of differences in the frequency of the attributes was performed using the χ^2 test. Fisher's Exact test was used in the analysis of the anatomical localization of pICH in all three groups of patients, since there were less than 5 patients for appropriate combination of categories within the analyzed parameters. The p value of < 0.05 was considered statistically significant.

Results

Out of a total of 246 patients, there were 157 (63.8%) males and 89 (36.2%) females. The mean age was 67.9 ± 11.8 years (range, 41 to 95 years). Most patients, 81 (32.9%) had pICH in the 7^{th} decade. The most prevalent RF was HTA, in 97.2% of patients. A statistically significant difference in the prevalence of the previous IS in the AT group was seen in relation to the other two groups ($p < 0.001$). The AT was used by 50 (20.3%) patients, 20 (8.2%) patients were using OACT, while the other 176 (71.5%) did not take any of the aforementioned therapy in the period before pICH. The distribution of patients and RFs among groups is shown in **Table 1**. Compared to the other two groups, the OACT group had a statistically significantly higher INR values ($p < 0.0001$). There was no significant difference in INR between the AT group and the group without OACT/AT **(Table 2)**. Patients from all three groups mostly presented with supratentorial localization of pICH (lobar or deep cerebral) ($p > 0.05$). The incidence of lobar localization of pICH was characteristic for the OACT group (60%), while deep cerebral localization was characteristic (50%) for the other two groups.

The analysis of RFs affecting the localization of pICH showed a statistically significant association only in alcohol consumption ($p = 0.03$) as follows: of the 75 patients who consumed alcohol, 44 (58.7%) had a deep cerebral localization of pICH. The effects of RFs on localization of pICH is shown in **Table 3**.

Table 1. Distribution of patients and risk factors among groups
Tabela 1. *Raspodela bolesnika i faktora rizika među grupama*

	Without OACT AT group/*Bez OAKT AT grupa*	AT group *AT grupa*	OACT group *OAKT grupa*	Total *Ukupno*	p (statistical significance)/*p (statistička značajnost)*
Age/*Starost*					
41-50	20 (95.2%)	1 (4.8%)	0	21 (8.5%)	
51-60	3 (75.6%)	9 (21.9%)	1 (2.5%)	41 (16.7%)	
61-70	58 (71.6%)	15 (18.5%)	8 (9.9%)	81 (32.9%)	
71-80	39 (60.9%)	16 (25%)	9 (14.1%)	64 (26%)	
≥ 81	28 (71.8%)	9 (23.1%)	2 (5.1%)	39 (15.8%)	
Sex/*Pol*					
Male/*Muški*	110 (70.1%)	33 (21%)	14 (8.9%)	157 (63.8%)	
Female/*Ženski*	66 (74.2%)	17 (19.1%)	6 (6.7%)	89 (36.2%)	
Previous IS/*Prethodni IMU*					
Yes/*Da*	7 (22.6%)	21 (67.7%)	3 (9.7%)	31 (12.6%)	<0.001
No/*Ne*	169 (78.6%)	29 (13.5%)	17 (7.9%)	215 (87.4%)	
Arterial hypertension/*Arterijska hipertenzija*					
Yes/*Da*	170 (71.1%)	49 (20.5%)	20 (8.4%)	239 (97.2%)	
No/*Ne*	6 (85.7%)	1 (14.3%)	0	7 (2.8%)	
Alcohol/*Alkohol*					
Yes/*Da*	59 (78.7%)	12 (16%)	4 (5.3%)	75 (30.5%)	
No/*Ne*	117 (68.4%)	38 (22.2%)	16 (9.4%)	171 (69.5%)	
Hyperlipidaemia/*Hiperlipidemija*					
Yes/*Da*	58 (61.7%)	28 (29.8%)	8 (8.5%)	94 (38.2%)	
No/*Ne*	118 (77.6%)	22 (14.5%)	12 (7.9%)	152 (61.8%)	
Platelet count/*Broj trombocita*					
<140	23 (76.7%)	5 (16.7%)	2 (6.7%)	30 (12.2%)	
140-400	149 (71.6%)	43 (20.7%)	16 (7.7%)	208 (84.5%)	
>400	4 (50%)	2 (25%)	2 (25%)	8 (3.3%)	
INR/*INO*					
Physiological/*Fiziološki*	160 (74.4%)	47 (21.9%)	8 (3.7%)	215 (87.3%)	
Pathological/*Patološki*	16 (51.6)	3 (9.7%)	12(38.7%)	31 (12.7%)	

Legend/*Legenda* :*OACT – oral anticoagulant therapy; **OAKT – oralna antikoagulantna terapija*; *AT – antiplatelet therapy/ *antitrombocitna terapija*; *IS – ischaemic stroke; **IMU – ishemijski moždani udar;* INR – international normalized ratio; *INO – internacionalni normalizovani odnos*

Table 2. Initial INR values
Tabela 2. *Inicijalne vrednosti INO*

$\bar{x}$ ± SD Range/*raspon*	±.0.2 0.82-2.75	p=0.88	1.09 ±.0.22 0.81-2.41	p<0.0001	2.47 ±.1.27 1.03–5.52

Legend/*Legenda*: INR- international normalized ratio; *INO – internacionalni normalizovani odnos*

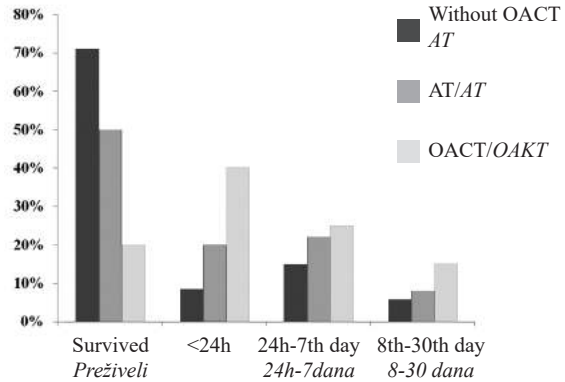
The largest number of patients in the OACT group (40%) had an initially severe clinical picture of pICH (NIHSS 14 – 20), while the other two groups about 30% of patients had a milder clinical picture (NIHSS 0 – 6). An estimated mRS was recorded in 154 (62.6%) patients. In approximately 2/3 of patients in all three groups, pICH had a poor functional outcome (mRS ≥ 3). There was no significant difference in the NIHSS on admission and mRS at discharge among the groups **(Table 4)**.

The OACT group had the highest mortality rate in the first 24 hours (40%), (OR = 2.5) compared to the other two groups. In the AT group (50%) and in the group without AT/OACT, (71%), a significantly higher survival rate (p <0.000), OR = 1.5 was established. These results point to a significant early mortality in the OACT group, while the other two groups presented with higher survival rates **(Graph 1)**.

Table 3. Risk factors for localization of pICH
Tabela 3. *Faktori rizika za lokalizaciju pIKH*

	Lobar *Lobarna*	Deep cerebral *Duboka cerebralna*	Cerebellum *Mali mozak*	Brainstem *Moždano stablo*	p (statistical significance)/*p (statistička značajnost)*
Age/*Starost*					
41-50	7 (33.3%)	9 (42.8%)	3 (14.3%)	2 (9.6%)	
51-60	18 (44%)	16 (39%)	5 (12.1%)	2 (4.9%)	
61-70	38 (46.9%)	35 (43.2%)	3 (3.7%)	5 (6.2%)	
71-80	28 (43.7%)	31 (48.4%)	2 (3.1%)	3 (4.6%)	
≥81	16 (41%)	22 (56.4%)	1 (2.6%)	0	
Sex/*Pol*					
Male/*Muški*	65 (41.4%)	77 (49%)	9 (5.7%)	6 (3.8%)	
Female/*Ženski*	42 (47.1%)	36 (40.4%)	5 (5.6%)	6 (6.7%)	
Previous IS/*Prethodni IMU*					
Yes/*Da*	14 (45.2%)	15 (48.3%)	1 (3.2%)	1 (3.2%)	
No/*Ne*	93 (43.2%)	98 (45.6%)	13 (6%)	11 (5.1%)	
Arterial Hypertension/*Arterijska hipertenzija*					
Yes/*Da*	105 (44%)	110 (46%)	13 (5.4%)	11 (4.6%)	
No/*Ne*	3 (42.8%)	3 (42.8%)	1 (14.3%)	0	
Alcohol/*Alkohol*					
Yes/*Da*	27 (36%)	44 (58.7%)	3 (4%)	1 (1.3%)	p=0.03
No/*Ne*	81 (47.4%)	68 (39.7%)	11 (6.4%)	11 (6.4%)	
Hyperlipidaemia/*Hiperlipidemija*					
Yes/*Da*	37 (39.3%)	48 (51%)	6 (6.4%)	3 (3.2%)	
No/*Ne*	69 (45.4%)	66 (43.4%)	8 (5.3%)	9 (5.9%)	
Platelet count/*Broj trombocita*					
< 140	12 (40%)	15 (50%)	3 (10%)	0	
140-400	92 (44.2%)	94 (45.2%)	11 (5.3%)	11(5.3%)	
>400	4 (50%)	3 (37.5%)	0	1 (12.5%)	
INR/*INO*					
Physiological/*Fiziološki*	88 (40.9%)	103 (47.9%)	13 (6%)	11 (5.1%)	
Pathological/*Patološki*	17 (54.8%)	11 (35.5%)	2 (6.4%)	1 (3.2%)	

Legend/*Legenda* : *IS – ischaemic stroke; **IMU – ishemijski moždani udar*; INR – international normalized ratio; *INO – internacionalni normalizovani odnos;* pICH – primary Intracranial Hemorhhage; *pJKH – primarna intreakranijalna hemoragija*



Graph 1. Analysis of survival in a one month period
Grafikon 1. *Analiza preživljavanja u periodu od mesec dana*
Legenda: AT – antitrombocitna terapija, OAKT – oralna antikoagulaciona terapija

Discussion

Our research confirmed that pICH is most frequent in males in the 7th decade of life with HTA as the dominant RF, which is in accordance with the known epidemiological data [2, 13–19]. It is believed that the susceptible hyaline degeneration and fibrinoid necrosis of the walls of small arteries and arterioles make HTA the main cause of pICH [20]. The previous IS treated with antithrombotic therapy represents a significant RF for pICH, as confirmed by the Seçil et al. [14]. The same authors established a significantly higher incidence of IS as a RF in the OACT group in regard to the group without OACT/AT, which is not the case in our study [14]. An acceptable explanation is based on the widespread use of AT in the prophylaxis of ischemic complications after IS in relation to OACT, in our area. The avail-

Table 4. Stroke severity on admission (NIHSS score) and functional outcome at discharge (mRS score)
Tabela 4. *Težina moždanog udara na prijemu (SMUNIZ skor) i funkcionalni oporavak na otpustu (mRS skor)*

	NIHSS score/ *SMUNIZ skor*					mRS score/*mRS skor*		
Group/*Grupa*	0-6	7-13	14-20	≥ 21	p =0.27	0-2	3-5	p=0.85
OACT/*OAKT*	1 (5%)	4 (20%)	8 (40%)	7 (35%)		1 (25%)	3 (75%)	
AT/*AT*	13 (26%)	12 (24%)	12 (24%)	13 (25%)		6 (24%)	19 (76%)	
Without OAKT/AT *Bez OAKT/ATT*	49 (27.8%)	43 (24.4%)	47 (26.7%)	37 (21%)		37 (29.6%)	88 (70.4%)	

Legend/*Legenda*: *OACT - oral anticoagulant therapy; **OAKT- oralna antikoagulantna terapija*; *AT - antiplatelet therapy/*antitrombocitna terapija*; *p - statistical significance/*statistička značajnost*; *NIHSS – The National Institutes of Health Stroke Scale/*SMUNIZ – Skala moždanog udara Nacionalnog instituta za zdravlje*; *mRS – modified Rankin Scale/*modifikovana Rankinova skala*

able literature data indicate that the initial INR value greater than 4.0 represents a significant RF for pICH [21]. Our research did not show a significant influence of INR on the occurrence of pICH. However, if groups with pathological values of INR (> 3.0) are observed, about 40% of them were from the OACT group, and 50% were from the group without AT/OACT. In the second case, we assume that some other factors could have been important, perhaps liver dysfunction.

Lobar localization was a characteristic of pICH [22], and it was confirmed by our research. The most common was the supratentorial localization, which was most frequent in the OACT group (60%), while deep cerebral (50%) was mostly found in the AT group and the group without AT/OACT. The potential explanation lies in the following: cerebral amyloid angiopathy represents the pathohistological basis of the lobar pICH, while vasculopathy of deep perforated arteries is a characteristic of the deep cerebral localization [23, 24]. It is assumed that there are different sensitivities of these two in the AT and OACT group [25]. It is also unavoidable that HTA primarily increases the risk of the non-lobar pICH, while warfarin is a RF predominantly for lobar pICH [14].

Several studies have shown the cause-effect relationship between alcohol consumption and lobar localization of pICH [26–28], while there are also opposite views [29]. Our results indicate a more significant incidence of deep cerebral pICH in alcoholics. Our hypothesis implies selective effects of alcohol on individual brain structures, in this case deep cerebral.

The constant increase rates and an aging population with a higher prevalence of hypertension and cerebral amyloid angiopathy, with a widespread use of AT and OACT, have an impact on the clinical picture and mortality [30, 31]. In our study, most patients in the OACT group (40%), had a more severe clinical picture, with NIHSS 14–20, unlike the other two groups, which had a milder clinical picture (NIHSS 0 – 6). However, no significant difference was noted. Similarly, other authors have confirmed the dominance of NIHSS 7 – 18 in all groups of patients [17, 32].

It is known that pICH has poor functional recovery; a very small number of patients recover without severe residual disability, and only 20% have a good functional outcome observed in a six-month period [11, 32]. More than 2/3 of patients in this study, in all three observed groups, had mRS ≥ 3, that is a poor functional recovery at discharge.

Different factors cause these devastating results. Early mortality in the first 24 hours is associated with the OACT group, which is affected by greater hematoma volumes, multiplicity, and their expansion in the first hours of illness [11]. Some authors report a 2.5 times higher relative risk of early mortality in the AT group than in patients who did not use AT, explaining it by possible early expansion of hematoma and association with other RFs, primarily age, diabetes, and previous IS [33]. Our results point to higher prevalence of survival in the AT group and the group without AT/OACT. This may be explained by the following hypothesis: the use of AT leads to delaying the onset of edema and the expected "mass" effect of hematoma, which prolongs the time of action in the "therapeutic window" in which antiedematous measures may have a positive effect [17].

The benefits of OACT and AT are well known in many primary and secondary indications. Considering that new oral anticoagulants are used in the daily clinical practice [34], warfarin may not be the most commonly used oral anticoagulant any longer. However, intracranial bleeding associated with the use of OACT remains a significant clinical problem. The review of the current therapy, warning patients about an increased risk of bleeding, and prevention of proven RFs for pICH, must be an imperative in everyday clinical practice. This would contribute to further evaluation of the mechanisms of intracerebral hemorrhage and determination of the prognosis.

Knowledge on the following facts could have made our research more complex: the length and dosage of AT and OACT may have an impact on the localization, size and volume of pICH; Glascow coma scale and Intracerebral hemorrhage score on admission are the best predictors of pICH outcome.

Conclusion

Patients using antithrombotic therapy who had a previous ischemic stroke are at a higher risk for primary intracerebral hemorrhage. Alcohol consumption often leads to a deep cerebral localization, while use of warfarin is associated with early mortality of patients with primary intracerebral hemorrhage.

References

1. Qureshi AI, Tuhrim S, Broderick JP, Batjer HH, Hondo H, Hanley DF. Spontaneous intracerebral hemorrhage. N Engl J Med. 2001;344(19):1450–60.

2. Raičević R, Kostić V, urednici. Hemoragijski moždani udar: etiologija, patofiziologija, dijagnostika i terapija. Beograd: VMA i Medicinski fakultet Univerziteta u Beogradu; 2005.

3. Lević Z. Osnovi savremene neurologije. Beograd: Zavod za udžbenike i nastavna sredstva; 2000.

4. Ikram MA, Wieberdink RG, Koudstaal PJ. International epidemiology of intracerebral hemorrhage. Curr Atheroscler Rep. 2012;14(4):300-6.

5. Hart RG, Boop BS, Anderson DC. Oral anticoagulants and intracranial hemorrhage. Facts and hypotheses. Stroke. 1995;26(8):1471–7.

6. Steiner T, Al-Shahi Salman R, Beer R, Christensen H, Cordonnier C, Csiba L, et al. European Stroke Organisation (ESO) guidelines for the management of spontaneous intracerebral hemorrhage. Int J Stroke. 2014;9(7):840–55.

7. Juvela S. Prevalence of risk factors in spontaneous intracerebral hemorrhage and aneurysmal subarachnoid hemorrhage. Arch Neurol. 1996;53(8):734-40.

8. Schulman S, Beyth RJ, Kearon C, Levine MN. Hemorrhagic complications of anticoagulant and thrombolytic treatment: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest. 2008;133(6 Suppl):257S-98S.

9. Paciaroni M, Agnelli G. Should oral anticoagulants be restarted after warfarin-associated cerebral haemorrhage in patients with atrial fibrillation? Thromb Haemost. 2014;111(1):14–8.

10. Levine MN, Raskob G, Landefeld S, Kearon C. Hemorrhagic complications of anticoagulant treatment. Chest. 2001;119(1 Suppl):108S–21S.

11. Flaherty ML. Anticoagulant-associated intracerebral hemorrhage. Semin Neurol. 2010;30(5):565-72.

12. Hankey GJ, Sudlow CL, Dunbabin DW. Thienopyridines or aspirin to prevent stroke and other serious vascular events in patients at high risk of vascular disease? A systematic review of the evidence from randomized trials. Stroke. 2000;31(7):1779–84.

13. Boden-Albala B, Sacco RL. Stroke. In: Nelson LM, Tanner CM, Vanden Eeden SK, McGuire VM, editors. Neuroepidemiology. From principles to practice. Oxford, New York: Oxford University Press; 2004. p. 223-53.

14. Seçil Y, Çiftçi Y, Tokuçoğlu F, Beckmann Y. Intracranial hemorrhages related with warfarin use and comparison of warfarin and acetylsalicylic acid. J Stroke Cerebrovasc Dis. 2014;23(2):321-6.

15. Ma M, Meretoja A, Churilov L, Sharma GJ, Christensen S, Liu X, et al. Warfarin-associated intracerebral hemorrhage: volume, anticoagulation intensity and location. J Neurol Sci. 2013;332(1):75-9.

16. Zubkov AY, Mandrekar JN, Claassen DO, Manno EM, Wijdicks EF, Rabinstein AA. Predictors of outcome in warfarin-related intracerebral hemorrhage. Arch Neurol. 2008;65(10):1320-5.

17. Stead LG, Jain A, Bellolio MF, Odufuye AO, Dhillon RK, Manivannan V, et al. Effect of anticoagulant and antiplatelet therapy in patients with spontaneous intra-cerebral hemorrhage: does medication use predict worse outcome? Clin Neurol Neurosurg. 2010;112(4):275-81.

18. Saloheimo P, Ahonen M, Juvela S, Pyhtinen J, Savolainen ER, Hillbom M. Regular aspirin use preceding the onset of primary intracerebral hemorrhage is an independent predictor for death. Stroke. 2006;37(1):129-33.

19. Lučić Prokin A, Čuzdi A, Živanović Ž, Šekarić J, Kokai Zekić T, Popović N, et al. Dyslipidemia as a risk factor for primary intracerebral hemorrhage. Med Glas (Zenica). 2014;11(1):31-6.

20. Mehta SR, Yusuf S, Peters RJ, Bertrand ME, Lewis BS, Natarajan MK, et al. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. Lancet. 2001;358(9281):527-33.

21. Hart RG, Pearce LA, Aguilar MI. Meta-analysis: antithrombotic therapy to prevent stroke in patients who have nonvalvular atrial fibrillation. Ann Intern Med. 2007;146(12):857-67.

22. Saulle MF, Schambra HM. Recovery and rehabilitation after intracerebral hemorrhage. Semin Neurol. 2016;36(3):306-12.

23. Al-Shahi Salman R, Labovitz DL, Stapf C. Spontaneous intracerebral haemorrhage. BMJ. 2009;339:b2586.

24. Labovitz DL, Boden-Albala B, Hauser WA, Sacco RL. Lacunar infarct or deep intracerebral hemorrhage: who gets which? The northern Manhattan study. Neurology. 2007;68(8):606–8.

25. Lee SM, Park HS, Choi JH, Huh JT. Location and characteristics of warfarin associated intracranial hemorrhage. J Cerebrovasc Endovasc Neurosurg. 2014;16(3):184-92.

26. Monforte R, Estruch R, Graus F, Nicolas JM, Urbano-Marquez A. High ethanol consumption as risk factor for intracerebral hemorrhage in young and middle-aged people. Stroke. 1990;21(11):1529-32.

27. Woo D, Sauerbeck LR, Kissela BM, Khoury JC, Szaflarski JP, Gebel J, et al. Genetic and environmental risk factors for intracerebral hemorrhage. Stroke. 2002;33(5):1190–6.

28. Thrift AG, Donnan GA, McNeil JJ. Heavy drinking, but not moderate or intermediate drinking, increases the risk of intracerebral hemorrhage. Epidemiology. 1999;10(3):307–12.

29. Labovitz DL, Halim A, Boden-Albala B, Hauser WA, Sacco RL. The incidence of deep and lobar intracerebral hemorrhage in whites, blacks, and Hispanics. Neurology. 2005;65(4):518-22.

30. Van Asch CJ, Luitse MJ, Rinkel GJ, van der Tweel I, Algra A, Klijn CJ. Incidence, case fatality, and functional outcome of intracerebral haemorrhage overtime, according to age, sex, and ethnic origin: a systematic review and meta-analysis. Lancet Neurol. 2010;9(2):167–76.

31. Lovelock CE, Molyneux AJ, Rothwell PM, Oxford Vascular Study. Change incidence and aetiology of intracerebral haemorrhage in Oxfordshire, UK, between 1981 and 2006: a population-based study. Lancet Neurol. 2007;6(6):487–93.

32. Sansing LH, Messe SR, Cucchiara BL, Cohen SN, Lyden PD, Kasner SE, et al. Prior antiplatelet use does not affect hemorrhage growth or outcome after ICH. Neurology. 2009;72(16):1397-402.

33. Saloheimo P, Ahonen M, Juvela S, Pyhtinen J, Savolainen ER, Hillbom M. Regular aspirin-use preceding the onset of primary intracerebral hemorrhage is an independent predictor for death. Stroke. 2006;37(1):129–33.

34. Rosenow F, Hojer C, Meyer-Lohmann C, Hilgers RD, Mühlhofer H, Kleindienst A, et al. Spontaneous intracerebral hemorrhage. Prognostic factors in 896 cases. Acta Neurol Scand. 1997;96(3):174-82.

Rad je primljen 31. X 2017.
Recenziran 5. XII 2017.
Prihvaćen za štampu 28. XI 2017.
BIBLID.0025-8105:(2018):LXXI:1-2:42-48.

CASE REPORTS
PRIKAZI SLUČAJEVA

General Hospital Vršac, Department of Surgery, Vršac[1]
Institute of Pulmonary Diseases of Vojvodina, Sremska Kamenica
Clinic of Thoracic Surgery[2]
University of Novi Sad, Faculty of Medicine Novi Sad[3]

Case report
Prikaz slučaja
UDK 616.411-007.6
https://doi.org/10.2298/MPNS1802049P

DELAYED SPLENIC RUPTURE IN MASSIVE SPLENOMEGALY – A CASE REPORT

ODLOŽENA RUPTURA SLEZINE KOD MASIVNE SPLENOMEGALIJE – PRIKAZ SLUČAJA

Srđan S. PUTNIK[1] and Miroslav D. ILIĆ[2,3]

Summary
Introduction. We present a patient with a delayed rupture of the spleen following a mild abdominal trauma. For years, the patient was treated for hereditary massive splenomegaly with thrombocytopenia, without established etiology. **Case Report**. The initial non-operative treatment lasted seven days, after which the patient was readmitted to the Emergency Department with signs of intra-abdominal hemorrhage and underwent emergency open splenectomy. Even after pathohistological examination, the etiology of massive splenomegaly remained unknown. **Conclusion.** Conservative treatment is not recommended in cases of massive splenomegaly; thorough surgical observation in a tertiary care hospital with interventional radiology and a good multidisciplinary team is necessary, while splenectomy is a surgery of choice.
Key words: Splenic Rupture; Splenomegaly; Abdominal Injuries; Delayed Diagnosis; Wounds, Nonpenetrating; Hemorrhage; Splenectomy; Thrombocytopenia; Risk Factors; Diagnosis

Sažetak
Uvod. Prikazan je slučaj pacijenta sa naslednom splenomegalijom praćenom trombocitopenijom nerazjašnjene etiologije i odloženom rupturom slezine nakon blage tupe traume abdomena. **Prikaz slučaja.** Kod pacijenta je primenjeno konzervativno lečenje nakon inicijalne dijagnostike traume, a sedam dana je kasnije pacijent rehospitalizovan sa znacima masivnog intraabdominalnog krvarenja kada je podvrgnut otvorenoj splenektomiji, sa uspešnim ishodom. Nakon patohistološkog ispitivanja, etiologija masivne splenomegalije ostaje nepoznata. **Zaključak.** Kod masivne splenomegalije konzervativno lečenje abdominalne traume se ne preporučuje zbog povećanog rizika od rupture organa. Ukoliko je moguće pacijenta treba uputiti u tercijarnu ustanovu sa mogućnošću interventne embolizacije organa i dobrim multidisciplinarnim timom. Hirurška splenektomija jeste metoda izbora kada dođe do znaka masivne intraabdominalne hemoragije.
Ključne reči: ruptura slezine; splenomegalija; povrede abdomena; odložena dijagnoza; nepenetrirajuće rane; krvarenje; splenektomija; trombocitopenija; faktori rizika; dijagnoza

Introduction

According to Poulin, enlarged spleen weighing over 1,000 grams is defined as massive splenomegaly, and "severe" if the largest dimension is greater than 20 cm [1]. Splenomegaly is associated with many diseases, but the etiology requires hematological studies on immune response work hypertrophy, red blood cell (RBC) destruction work hypertrophy, congestive (splenic vein thrombosis or portal hypertension), myeloproliferative, infiltrative (benign or malignant) or purely neoplastic diseases. Differential diagnosis of massive splenomegaly includes several hereditary diseases like Niemann-Pick disease, acid sphingomyelinase deficiency, Gaucher disease, hereditary spherocytosis, thalassemias, and mucopolysaccharidosis. Massive splenomegaly is associated with thrombocytopenia, but also spontaneous infarction and rupture [2]. It is well known that patients with splenomegaly must take additional measures in order to prevent blunt traumas to the abdomen and decrease the risk of splenic rupture [3].

Case Presentation

We present a 46-year-old Caucasian male with a documented medical history of hereditary splenomegaly and thrombocytopenia since childhood. Five years prior to the injury, ultrasound showed the largest diameter of the spleen of 24 cm **(Figure 1)**, and bone marrow aspiration failed to determine the etiology of the disease. However, the tests did reveal pseudo-Gaucher cells and elevated levels of alpha 1 globulin and chitotriosidase, with normal enzyme activity of acid β-glucosidase. The patient was admitted to the surgery department of a rural hospital with symptoms

Corresponding Author: Dr Srđan Putnik, Opšta bolnica Vršac, Hirurško odeljenje, 26300 Vršac. Abraševićeva bb, E-mail: putniksrdjan@outlook.com

Abbreviations
RBC – red blood cell
CT – computerized tomography
FAST – focused assessment with sonography for trauma

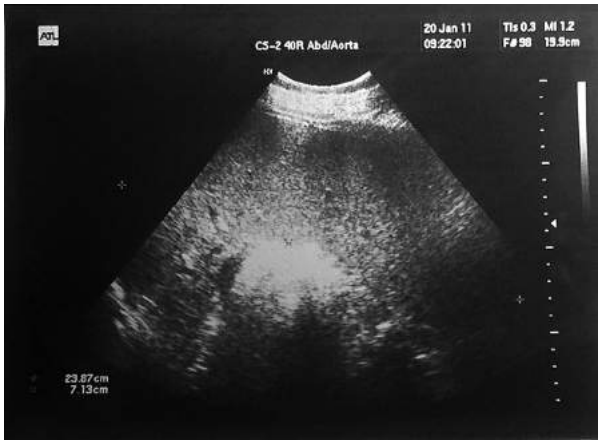


Figure 1. Splenomegaly five years prior to the injury – ultrasound examination
Slika 1. *Splenomegalija pet godina pre povrede – ultrazvučni pregled*

of abdominal pain seven days after a mild lower chest trauma. The initial treatment was conservative. The patient was stable; RBC and hematocrit were normal, as well as the blood pressure over the brachial artery, while the ultrasound showed a small amount of fluid above the enlarged spleen (28 x 10 cm) without any intraparenchymal pathological morphology. The patient was discharged and advised to visit a hematologist. Seven days after the initial conservative treatment and two weeks after the trauma, the patient was admitted, this time with hypotension, low hematocrit, and anemia with signs of hemorrhagic shock and increased bilirubin levels. The ultrasound confirmed a large amount of free fluid in the entire peritoneal cavity a large subcapsular hematoma of 20 x 10 cm **(Figure 2)**, and a smaller intraparenchymal hematoma. Emergency laparotomy and splenectomy were performed **(Figure 3)**. No other changes were found during the intervention, especially on the liver. The patient received two units of whole blood. The postoperative period was without complications. The patient was discharged without thrombocytopenia, and received a pneumococcal polyvalent vaccine. The pathology examination revealed a spleen weighing 1,800 grams with several intraparenchymal hematomas from 0.5 to 3 cm, while the largest subcapsular hematoma was 18 x 13 cm **(Figure 4)**. Subsequent histological examination failed to determine the etiology of this massive splenomegaly.

Discussion

Neither the performed splenectomy nor the hematological investigation of our patient with previously known massive splenomegaly succeeded in determining the etiology of the splenomegaly and thrombocytopenia. The patient received no specific therapy for the splenomegaly, apart from being warned not to use nonsteroidal anti-inflammatory drugs. Recent publications do not advise splenectomy for hereditary massive splenomegaly not only due to adverse effects of sepsis but also osteolytic activity [4]. Splenectomy is indicated only in certain circumstances, mainly in patients with severe anemia and life-threatening thrombocytopenia, of which our patient had none. However, he did have a minor trauma for which splenectomy could be indicated [2].

Non-operative management of blunt injuries to the spleen is the treatment of choice for a normal spleen, as well as computerized tomography (CT), provided the patient is hemodynamically stable [5, 6]. In rural hospitals without a CT scanner, the available diagnostic tool is ultrasound, which can also be simply performed as bedside ultrasound. Focused assessment with sonography for trauma (FAST) is indicated in intraperitoneal hemorrhages and intraparenchymal morphology of the spleen [7, 8]. However, special attention must be paid to cases of massive splenomegaly with abdominal pain even after a mild trauma. Upon first admission, our patient had only massive splenomegaly and a small amount of intraperitoneal fluid. He was hemodynamically stable and with normal RBC count. No intraparenchymal or subcapsular hematomas were detected. An attempt was made with conservative treatment. What else can be done for a patient with massive splenomegaly? The first method of choice may include interventional procedure such as splenic artery embolization in a hospital with interventional radiology. The success rate in salvaging the spleen that shows no pathological changes is high, over 90%, but it depends on the severity of injuries [9]. Upon second admission, the ultrasound examination revealed a subcapsular bleeding hematoma, namely grade III, according to the organ injury scale [10]. It is uncertain if splenic artery embolization would be successful in massive splenomegaly and intraparenchymal or subcapsular grade III hematoma. Our hospital has no in-

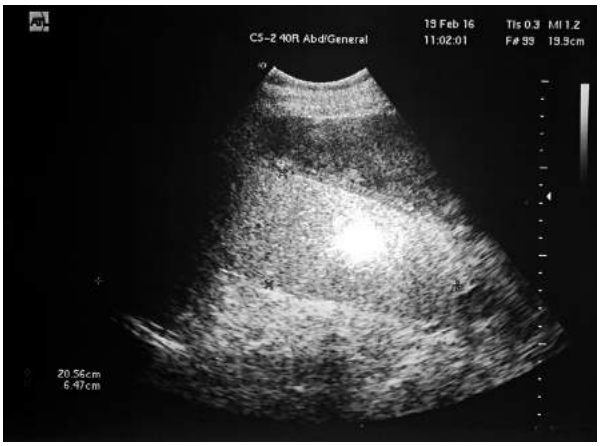


Figure 2. Subcapsular haematoma of the spleen
Slika 2. *Supkapsularni hematom slezine*

Figure 3. Ruptured subcapsular haematoma of the spleen
Slika 3. *Rupturirani supkapsularni hematom slezine*

terventional radiology or a CT scanner, so the first conclusion is that splenic trauma and massive splenomegaly cases should not be monitored in small hospitals.

Another issue is whether the organ injury scale can be applied in patients with massive splenomegaly. We believe that the standard spleen injury scale is not applicable in cases of massive splenomegaly, whereas non-operative treatment of splenic trauma is not a preferable method [10]. However, each case of a mild trauma in massive splenomegaly should be treated in a hospital not only equipped for conducting interventional and laparoscopic procedures but also has surgeons with experience in splenic resection and a proper hematological support. Delayed rupture of a traumatic hematoma in massive splenomegaly is expected. Upon discharge following splenectomy, our patient had a normal platelet count. The etiology of his

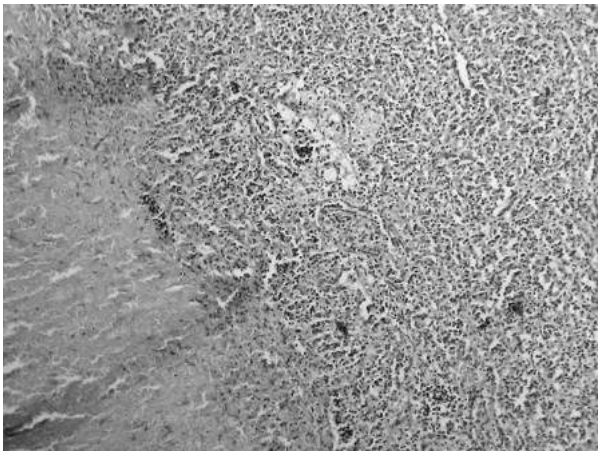


Figure 4. Histology image of the splenic rupture
Slika 4. *Histološka slika rupture slezine*

disease remains unknown even after histopathological examination.

Clinical follow-up will explain the role of splenectomy in this case of massive splenomegaly of unknown etiology.

Conclusion

Conservative treatment is not recommended in cases of massive splenomegaly; careful surgical observation in a hospital with interventional radiology and a good multidisciplinary team is necessary, while splenectomy is a surgery of choice.

References

1. Poulin EC, Mamazza J, Schlachta CM. Splenic artery embolization before laparoscopic splenectomy. An update. Surg Endosc. 1998;12(6):870-5.

2. Weinreb NJ, Rosenbloom BE. Splenomegaly, hypersplenism, and hereditary disorders with splenomegaly. Open J Genet. 2013;3:24–43.

3. Brady RR, Bandari M, Kerssens JJ, Paterson-Brown S, Parks RW. Splenic trauma in Scotland: demographics and outcomes. World J Surg. 2007;31(11):2111-6.

4. Deegan PB, Pavlova E, Tindall J, Stein PE, Bearcroft P, Mehta A, et al. Osseous manifestations of adult Gaucher disease in the era of enzyme replacement therapy. Medicine (Baltimore). 2011;90(1):52-60.

5. El-Matbouly M, Jabbour G, El-Menyar A, Peralta R, Abdelrahman H, Zarour A, et al. Blunt splenic trauma: assessment, management and outcomes. Surgeon. 2016;14(1):52-8.

6. Stassen NA, Bhullar I, Cheng JD, Crandall ML, Friese RS, Guillamondegui OD, et al. Selective nonoperative management of blunt splenic injury: an Eastern Association for the Surgery of Trauma practice management guideline. J Trauma Acute Care Surg. 2012;73(5 Suppl 4):S294-300.

7. American Institute of Ultrasound in Medicine, American College of Emergency Physicians. AIUM practice guideline for the performance of the focused assessment with sonography for trauma (FAST) examination. J Ultrasound Med. 2014;33(11):2047-56.

8. Matsushima K, Frankel HL. Beyond focused assessment with sonography for trauma: ultrasound creep in the trauma resuscitation area and beyond. Curr Opin Crit Care. 2011;17(6):606-12.

9. Frandon J, Rodiere M, Arvieux C, Vendrell A, Boussat B, Sengel C, et al. Blunt splenic injury: are early adverse events related to trauma, nonoperative management, or surgery? Diagn Interv Radiol. 2015;21(4):327-33.

10. Moore EE, Cogbill TH, Jurkovich GJ, Shackford SR, Malangoni MA, Champion HR. Organ injury scaling: spleen and liver (1994 revision). J Trauma. 1995;38(3):323-4.

Rad je primljen 15. V 2017.
Recenziran 14. IX 2017.
Prihvaćen za štampu 26. IX 2017.
BIBLID.0025-8105:(2018):LXXI:1-2:49-51.

SEMINAR FOR PHYSICIANS
SEMINAR ZA LEKARE U PRAKSI

Clinical Centre of Vojvodina, Novi Sad
Department of Obstetrics and Gynecology[1]
University of Novi Sad, Faculty of Medicine Novi Sad[2]

Seminar for physicians
Seminar za lekare u praksi
UDK 616-006.6-08-052:618.177-089.888.11
UDK 616-006.6-08-052:602.1
https://doi.org/10.2298/MPNS1802053B

FERTILITY PRESERVATION IN CANCER PATIENTS

OČUVANJE FERTILITETA KOD ONKOLOŠKIH PACIJENATA

Artur BJELICA[1,2], Aleksandra TRNINIĆ PJEVIĆ[1,2], Ivana JAGODIĆ[1] and Jelena VUKOSAVLJEVIĆ[1]

Summary

Introduction. Progress in the field of cancer treatment has led to increased survival rate of cancer patients. Chemotherapy and surgical treatment may be the consequence of decreased fertility in both genders. **Fertility Preservation.** Some of the prominent techniques for fertility preservation, cryopreservation of gametes and embryos, are efficient and safe techniques in clinical practice, while cryopreservation of gonadal tissue and cells is considered experimental, and it is not used in everyday practice yet. **Conclusion.** Timely and complete information about the effects of cancer treatment on fertility, as well as information about the potential techniques for fertility preservation, should be available to all patients.

Key words: Fertility Preservation; Neoplasms; Cryopreservation; Tissue Preservation; Antineoplastic Agents; Reproductive Techniques, Assisted; Oocytes; Drug Related Side Effects and Adverse Reactions

Sažetak

Uvod. Napredak na polju lečenja karcinoma doveo je do povećanja stope preživljavanja kod onkoloških pacijenata. Posledica primene radio i hemioterapije kao i hiruškog lečenja je smanjenje fertilne sposobnosti kod oba pola nakon sprovedenog tretmana lečenja. **Očuvanje fertiliteta.** Među dostupnim tehnikama za očuvanje fertiliteta izdvajaju se krioprezervacija gameta i embriona kao efikasne i bezbedne za primenu u kliničkoj praksi dok se krioprezervacija tkiva gonada smatra eksperimentalnom metodom i za sada se ne primenjuje u rutinskoj praksi. **Zaključak**. Blagovremene i kompletne informacije o uticaju onkološke terapije na fertilitet, kao i informacije o mogućim tehnikama za očuvanje fertiliteta moraju da budu dostupne svim pacijentima.

Ključne reči: očuvanje fertiliteta; karcinomi; krioprezervacija; prezervacija tkiva; antineoplastički agensi; asistirane reproduktivne tehnike; oociti; nuspojave i neželjene reakcije izazvane lekovima

Introduction

The progress in the field of early diagnosis and cancer treatment in the past few decades has led to a significant decrease in mortality rate of cancer patients, whereas the quality of their lives has significantly deteriorated. Taking into consideration that cancer treatment requires aggressive methods, which are exceptionally difficult for patients and affect their social and emotional lives and behavior, the focus is on preserving the overall integrity of the person, rehabilitation and return to their families and social life [1].

Despite the increase of long-term survival of cancer patients, cancer treatment, including chemotherapy, radiotherapy and surgical treatment methods, can contribute to a decrease of fertility in both genders. Considering the increasing number of surviving young patients, there is a need to introduce the term - oncofertility, which means preservation of fertility in cancer patients after treatment [2].

Infertility induced by cancer treatment decreases the quality of life of surviving patients in the reproductive age, and leads to a long-term emotional stress, especially among patients who did not get relevant information about the possibilities and ways of fertility preservation before treatment initiation [3].

The aim of this paper is to present current options and techniques for fertility preservation in cancer patients.

The Effects of Cancer Treatment on Fertility in Women

Cancer treatment may affect the reproductive potential of women through various mechanisms. In order to get pregnant naturally, women must have a normal ovarian reserve, a functioning hypotha-

Corresponding Author: Prof. dr Artur Bjelica, Klinički centar Vojvodine, Klinika za ginekologiju i akušerstvo, 21000 Novi Sad, Branimira Ćosića 37, E-mail: artur.bjelica@mf.uns.ac.rs

Abbreviations

FSH – follicle-stimulating hormone
DNA – deoxyribonucleic acid
MOPP – mechlorethamine, oncovin, procarbazine, prednisone
AC – adriamycin, cyclophosphamide
GnRH – gonadotropin-releasing hormone
COS – controlled ovarian stimulation
OHSS – ovarian hyperstimulation syndrome
cIVF – conventional *in vitro* fertilization
ICSI – intacytoplasmic sperm injection
ASRM – American Society for Reproductive Medicine

lamic-pituitary-gonadal axis, a functional uterus capable of fetal implantation and development, as well as the capacity of other organs and the cardiovascular system to respond to the changes caused by pregnancy. A malfunction of any of the mentioned systems after cancer treatment can render natural conception and pregnancy impossible.

An accurate assessment of the effects of cancer and its treatment on fertility in women cannot be determined in a simple manner, considering that it depends on: the type and stage of the disease, treatment modality, and patient age, number of treatments and cumulative dose of radiation and chemotherapy, basal ovarian reserve and many other factors [4].

The Effects of Chemotherapy

The mechanism of impairment of the ovarian function caused by chemotherapy involves the decay of primordial follicles, disruption in the maturation of recruited follicles, or a combination of these two mechanisms, depending on the class of drug used. The degree of impairment of the ovarian function depends on the drug dose, and considering that most agents disrupt cell division, the damage occurs to the oocytes in the stage of maturation, and to somatic granulosa cells in the stage of cell growth and proliferation [5]. The impaired ovarian function is characterized by higher levels of follicle-stimulating hormone (FSH) (≥ 20 IU/L) and estradiol (>75 pg/ml), while the levels of inhibin B and anti-Mullerian hormone are decreased. Ultrasonography shows a decrease in the antral follicle count and ovarian volume itself [6].

Alkylating agents are a class of drugs used in chemotherapy and their gonadotoxicity has been well-studied. Their effect is related to deoxyribonucleic acid (DNA) damage which leads to breaking the process of DNA transcription and replication, later manifested through cell cycle arrest. Alkylating agents also lead to vascular damage and ovarian cortical fibrosis, which is another mechanism that leads to ovarian dysfunction. Most agents used in chemotherapy are not used independently, but as a part of joint protocols, so one of the approaches in assessing the gonadotoxic effect of these agents is to analyze the effect of a comprehensive treatment protocol on the ovarian function. The mechlorethamine, oncovin, procarbazine, prednisone (MOPP) therapy, used in lymphoma treatment, causes amenorrhea in up to 80% of female patients and acute ovarian failure in 39–46% of young adults [7]. Newer regimens such as doxorubicin, cyclophosphamide (AC) have recorded a significantly lower amenorrhea rates, in 55% patients [8]. The effects of biological agents (tamoxifen, herceptin, etc.) on fertility, as a relatively new class of cancer drugs, which are directed towards specific receptors, growth factors or signalling pathways, are still insufficiently tested.

The Effects of Radiotherapy

The degree of ovarian damage is directly associated with the proximity of the ovary to the radiation field, the basal ovarian reserve, and the dose of radiation. Also, certain high doses of radiation lead to a higher degree of ovarian damage compared to fractionated, lower doses, even when the cumulative radiation dose is the same.

Ovarian failure has been reported in 97% of girls after total body irradiation with 20–30 Gy, while in adults, doses of 10–15.75 Gy cause the same effect in 90% of cases [9]. There are numerous consequences of radiation on the uterus, which can lead to infertility. A direct exposition of the uterus leads to reduced vascularization, endometrial insufficiency, and even fibrosis of the myometrium with consequential disruptions of implantation and gestation. A radiation dose higher than 25 Gy, used in children, induces irreversible damage to the uterus in treated patients. Reulen et al. reported an increased rate of miscarriages, premature births and intrauterine growth restriction after abdominal and pelvic radiation treatment [10]. The use of high doses of cranial radiation can also induce hypogonadism by disrupting the function of the hypothalamus and pituitary gland. Unlike the previously mentioned damage to the ovaries and uterus, which is irreversible, infertility occurring as a consequence of that type of radiation can be successfully treated with hormone substitution therapy [11].

Surgical treatment can jeopardise the anatomic functionality of the female genital tract by injuring or removing the reproductive organs. Lately, there is a tendency to use less radical surgical approaches in patients of reproductive age [12].

Fertility Preservation Techniques in Women

The following techniques of fertility preservation are nowadays used in female cancer patients: cryopreservation of embryos and ova, cryopreservation of ovarian tissue, protective hormonal suppression of ovaries, gonadotropin-releasing hormone (GnRH) analogues and antagonists, and ovarian transposition in radiation therapy. When choosing the technique for fertility preservation, the matters to be considered include the age of patients, whether they have a partner, and the time available before the initiation of therapy.

Cryopreservation of Embryos

Cancer patients of reproductive age who need to be submitted to some treatment procedures which

can lead to early ovarian dysfunction and decline of fertility, are candidates for cryopreservation of ova and embryos.

As a technique routinely used in assisted reproductive technologies, cryopreservation is a widespread method of fertility preservation in cancer patients. After the first successful pregnancy in 1983 [13], the perfection of cryopreservation technology has today approximately equalized the rate of live births after frozen embryo transfer and embryo transfer in fresh *in vitro* fertilization (IVF) cycle [14].

The rate of embryo viability after thawing, the rate of implantation, and the rate of clinical pregnancies after frozen embryo transfers is not significantly different among cancer patients compared to the cycles of patients who are not facing cancer. In a retrospective study, Cardozo et al. compared the pregnancy rate in cancer patients after frozen embryo transfer with the pregnancy rate in patients with tubal factor infertility in the IVF process. The cumulative pregnancy rate after frozen embryo transfer in cancer patients was 37%, and 43% in the control group. The rate of births per embryo transfer also showed no significant deviations, 30% compared to 32% in the control group [15].

The process of cryopreservation of embryos implies the existence of a partner or a sperm donor sample depending on the legislation of the state in question. Also, this procedure is reserved only for the patients whose medical state allows for a safe implementation of ovarian stimulation. Ovarian stimulation requires certain time for follicles to develop, and it is the doctor's duty to assess the safety of postponing the start of treatment of the given patient. The process involves a controlled ovarian stimulation, aspiration of oocytes, IVF and freezing of embryos.

Protocols with GnRH antagonists are most often used for controlled ovarian stimulation (COS) considering that this type of protocol requires less time and bears a lower risk for the development of the ovarian hyper-stimulation syndrome (OHSS). The protocol starts on the second or third day of the menstrual cycle by administration of gonadotropin, while the dose depends on the ovarian reserve. The development of follicles is followed by a series of transvaginal ultrasound folliculometry procedures, and when follicles achieve the desired size, GnRH antagonists are administered in order to prevent a premature peak of the luteinizing hormone. After administration of the human chorionic gonadotropin or synthetic luteinizing hormone, the final maturation of oocytes is initiated, and transvaginal aspiration of oocytes is conducted in 35 – 36 hours [14]. The fertilization of oocytes is conducted in IVF conditions using the method of conventional in vitro fertilization (cIVF) or via the intracytoplasmic sperm injection (ICSI). After the embryo formation, it is further cultivated in controlled laboratory conditions, followed by cryopreservation and storage. Embryo cryopreservation is performed using the slow freezing or the vitrification technique.

If the treatment of the primary disease cannot be postponed, modified protocols are used with administration of GnRH antagonists, regardless of the phase of the menstrual cycle [16]. Antiestrogens (tamoxifen and letrozole) are induced to stimulate ovulation in patients with estrogen-dependent cancers, considering that they decrease the risk of exposure to high estrogen concentrations.

Cryopreservation of Oocytes

The cryopreservation of oocytes is a convenient method for fertility preservation in sexually mature women, women without a partner, or women who refuse embryo cryopreservation because of ethical, religious or other reasons.

The first successful pregnancy resulting from an IVF cycle with frozen and then thawed oocytes by using the slow freezing method was achieved in 1986 [17]. In the following decades, achievements in the field of cryobiology have enabled significant improvements in the field of cryopreservation of oocytes. The introduction of the vitrification technique, which includes the process of solidifying liquid to a noncrystalline, "glassy" phase, which is achieved through rapidly lowering the temperature below the temperature of glass transition, while at the same time increasing the viscosity in order to prevent intracellular formation of ice crystals, enables a safe freezing of the very sensitive oocyte structure. A combination of enhanced cryoprotectants and fertilization via the ICSI method has led to more than 1,500 children being born in cycles with cryopreserved oocytes [18].

Studies have shown that there is no increased risk of aneuploid embryos after oocyte cryopreservation, nor an increased rate of children born with congenital anomalies [19, 20]. The oocyte survival rate after thawing is 90–95%, while the rate of pregnancies per embryo transfer is 38% in a cycle with frozen oocytes. The percentage of pregnancies in cycles with fresh embryos is 45%, according to the same study [21]. Owing to this progress, according to the American Society for Reproductive Medicine (ASRM), cryopreservation of oocytes is no longer considered experimental, but used as a standard method of fertility preservation in cancer patients [22]. The protocols of ovary stimulation are no different than protocols used when it comes to embryo cryopreservation, while oocytes in metaphase II stage are subjected to cryopreservation in laboratory conditions.

Cryopreservation of Ovarian Tissue

Cryopreservation of ovarian tissue is a technique of freezing the ovarian cortex, which is rich in primordial follicles. This technique of fertility preservation is advised for prepubertal girls as well as adult patients who need to start their treatment right after being diagnosed with cancer. The advantage of this technique is that it does not require a partner, like embryo cryopreservation, the start of treatment and ovarian stimulation are not postponed, a big pool of primordial follicles can be preserved, and

after the transplantation, ovarian function can be re-established [23].

Ovarian tissue needed for cryopreservation does not require previous ovarian stimulation, and it can be provided soon after cancer has been laparoscopically diagnosed. The obtained tissue is cryopreserved after dissection in small fragments, either through slow freezing technique or the vitrification technique in laboratory conditions [23].

When the cancer treatment is finished, after thawing the ovarian cortex tissue, an orthotopic or heterotopic transplantation is possible. Orthotopic transplantation involves transplantation in the pelvis area, while in heterotopic transplantation the tissue is transplanted outside the pelvis area, most often on the forearm or in the abdominal region. Orthotopic transplantation is a successful way of re-establishing fertility, and numerous cases of live births were recorded after spontaneous pregnancy and after hormonal ovary stimulation and IVF [24]. The first pregnancy after heterotopic transplantation of ovarian tissue was achieved in 2013 by Stern and associates [25].

Apart from the above mentioned advantages, ovarian tissue cryopreservation also bears risks of reintroducing cancer cells after autotransplantation. Many types of cancer do not metastasize in the area of ovaries, but autologous transplantation is contraindicated if there is a possibility of cryopreserved tissue containing cancer cells, as with hematological malignancies.

According to current recommendations of the ASRM, this method of fertility preservation remains experimental, and is not routinely used in practice [26].

Ovarian Transposition

Ovarian transposition is a surgical relocation of the ovary outside the radiation field in case radiotherapy is used directly in the area of the pelvis [4]. The ovarian transposition procedure can be done laparoscopically, or, if needed, through laparotomy. During the selection of patients for this procedure, it should be considered if the uterus will be exposed to high radiation doses (14–30 Gy), because such doses cause high and permanent damage to the uterus function [9]. Since this procedure cannot reduce the gonadotoxic effect of chemotherapy, its use should be limited to patients whose treatment protocols do not require a combination of radiotherapy and chemotherapy. Ovarian transposition may hamper the transvaginal approach during oocyte aspiration in the process of IVF, and if transabdominal oocyte aspiration is not an option, the ovary needs to be repositioned before using some of the methods of assisted reproduction.

Immature Oocyte Retrieval and In-Vitro Oocyte Maturation

This method involves aspiration of immature oocytes without controlled ovarian stimulation, or with minimal stimulation. It is used in patients in whom postponing cancer treatment would negatively affect the success of treatment, and for prepubertal patients. After *in vitro* maturation of oocytes, the mature cells are cryopreserved or fertilized, and then the embryo is cryopreserved through previously described techniques. In addition to transvaginal aspiration, oocytes can also be collected from ovarian tissue, which was removed in order to cryopreserve it. *In vitro* maturation of oocytes is nowadays successfully used in patients who are diagnosed with polycystic ovary syndrome and in patients who are candidates for developing the OHSS in IVF programs with a high rate of clinical pregnancies [27].

Hormonal Ovarian Function Suppression

Ovarian suppression by GnRH analogues or antagonists is used before or during the administration of gonadotoxic chemotherapy in order to reduce the risks of premature ovarian function failure. GnRH-agonists cause a suppression of the pituitary function and secretion of gonadotropins, and decrease utero-ovarian perfusion, so it is considered that these mechanisms can protect the deterioration of ovarian reserve. The ovarian suppression is debatable because there are not enough random studies to confirm the efficiency of this method for the preservation of ovarian reserve and fertility. Even if some studies do show a much higher percentage of establishing the menstrual cycle in patients co-treated with GnRH-agonists during chemotherapy, there is no evidence that the pregnancy rate has increased, which is the ultimate goal of successful fertility preservation after treatment [28].

The results of recent researches on using GnRH analogues in women with cancer are controversial, and there is no definitive consensus on their use.

Surgery and Fertility Preservation

Less radical surgical cancer treatment techniques, intended to preserve reproductive organs, are contemporary and mostly still being developed. In young patients diagnosed with early-stage cervical cancer, a radical trachelectomy can be conducted instead of standard radical hysterectomy. Complications were recorded after this procedure, such as cervical stenosis and premature birth, but increased cancer recurrence rate was not recorded [12]. In early-stage endometrial cancer, a hormonal progestogen treatment is used, but the recurrence rate is high, and the patient requires frequent controls with endometrial biopsy, and definitive treatment after giving birth [29].

The Effects of Cancer Treatment on Fertility in Men

The etiology of male infertility after cancer treatment involves direct and indirect effects of cancer, as well as damages occurring after surgical treatment, radiotherapy and chemotherapy. The treatment itself involves a combination of the mentioned methods, and there is a decrease or complete multifactorial lack of fertility in conjunction with the type, dose and duration of the therapy, as well as the fertile capacity of the patient before the treatment [4].

Affected semen analysis parameters are often found in cancer patients as a primary issue, before therapy is conducted. The tumor secretion of metabolically active substances, such as cytokines, can cause direct damage to the germinal epithelium, while hormonally active tumors can have an indirect effect on spermatogenesis by disrupting the interaction between the hypothalamus and pituitary gland [30]. Among the various types of cancer (leukemia, lymphoma, and testicular cancer) which affect spermatogenesis as the primary illness, the prominent ones are the testicular germinal epithelium tumors, having the greatest negative effects on spermatogenesis and male fertility [31].

The Effects of Chemotherapy

The primary effect of chemotherapy on fertility is the direct damage to the spermatogonia, which is the most sensitive stage of spermatogenesis. Sertoli cells and Leydig cells show a lower degree of sensitivity, and the gonadotoxic effect of chemotherapy is reflected in a low sperm count, low motility, distortion of morphology and DNA integrity, while the production of testosterone may remain preserved after treatment [32]. The extent of damage depends on the type and dose of drugs, manner of administration (oral or intravenous), duration of treatment and the patient's age. Among chemotherapeutics, the drugs with the highest gonadotoxic effect are alkylating agents (cyclophosphamide, chlorambucil, procarbazine, busulfan) and platinum agents (cisplatin and carboplatin) [33].

The Effects of Radiotherapy

The effects of radiotherapy on male fertility also depend on the dose. A dose of 0.15 Gy, used on the testicles, leads to a decrease in the sperm count, while a dose of 2.5 Gy can cause prolonged or permanent azoospermia. If the radiation dose exceeds 20 Gy, the germinal epithelium is completely destroyed and consequently the patient becomes permanently sterile [34]. It should be noted that, in addition to direct radiation of testicular tissue, the indirect exposure of testicles to radiation is also fatal to spermatogenesis. Due to cranial radiation with doses exceeding 35 Gy, the function of the pituitary gland is disrupted, which can lead to secondary hypogonadism [35]. Spermatogenesis arrest can be reversible, but radiotherapy causes DNA damage, which should be considered if the patient will be subjected to some of the assisted reproduction techniques.

Surgical treatment of cancer can lead to damaging the secretory canals of the genital tract, and it can also lead to erectile dysfunction. Unilateral orchiectomy of testicular cancer can decrease sperm production, while bilateral orchiectomy leads to permanent male infertility [4].

Fertility Preservation Techniques in Men

Semen Cryopreservation

Semen cryopreservation is the most commonly used technique for fertility preservation in pubertal and post-pubertal males. Due to the progress in the field of assisted reproduction and to the introduction of intracytoplasmic sperm injection, sperm cryopreservation, as a method of fertility preservation is especially important for cancer patients because only one sperm is enough to fertilize an egg and the patient may become a biological father.

The manner of collecting the sample for cryopreservation depends on the level of integrity of the reproductive tract of the given patient. If there is no anatomical or neurological disparity, a sample of ejaculate is provided by ejaculating after 2 – 5 days of abstinence. Two or three ejaculate samples are collected, mostly because of low referential values of proper semen caused by the cancer. The samples need to be cryopreserved before the treatment, in order to preserve DNA integrity and sperm quality [36]. Cryopreservation is done by vitrification method, or slow freezing method, after which the samples are stored until further use. Due to the urgency of treatment, after the patient is diagnosed with cancer, cryopreservation can be conducted during the treatment, considering that the lowest sperm count in the ejaculate is recorded 4 – 6 months after the therapy ends [35].

If the patient has anatomical, neurological or other changes which disrupt ejaculation, the sperm sample can be collected via: vibrostimulation, electroejaculation, catheterization and by isolating sperm cells from the urinary bladder in the case of retrograde ejaculation, as well as aspiration or surgical extraction from the testicles or epididymis if spermatogenesis is preserved [37].

Hormonal Suppression

The effects of hormonal gonadal suppression during cancer treatment have not been studied enough. Recent studies have not shown positive effects of hormonal suppression on lowering the risk of infertility after cancer treatment. Improvements in re-establishing spermatogenesis after chemotherapy have also not been recorded [36].

Cryopreservation of Spermatogonia and Testicular Tissue

Currently available fertility preservation techniques are only used in pubertal and postpubertal young men in whom spermatogenesis has started. When it comes to boys in whom there was no initiation of spermatogenesis, the possibilities of fertility preservation are limited to cryopreservation of testicular tissue. The cryopreservation of testicular tissue is still considered experimental, considering that cryopreserved testicular tissue cannot be used in clinical practice at present [4]. Initial studies on animals showed encouraging results, and there is hope that in the future, as technology in the field of reproductive medicine advances, cryopreserved testicular tissue will find its use in assisted reproductive technologies [38]. Excluding the technical limitations for obtaining mature, functional sperm cells from cryopreserved, immature testicular tissue, there is also fear of reintroducing malignant cells to the body after the transplantation.

Conditions for Implementing Fertility Preservation Programs in Cancer Patients

According to the ASRM, a comprehensive program for fertility preservation includes:

– *Quick access* of patients to doctors.

– *Interdisciplinary medical team* comprising an oncologist, a reproductive endocrinologist, urologist, and a specially trained surgeon for conducting surgical techniques for fertility preservation.

– *Laboratory requirements* in fertility preservation programs include all techniques for fertility preservation, with an emphasis on successfully established programs of cryopreservation of gametes and embryos, which are constantly available. In addition, the program should provide adequate counselling for prepubertal patients, and offer the possibility of performing testicular and ovarian tissue cryopreservation.

– *A clinic* with experts in the field of genetics, mental health and finance, considering that many fertility preservation techniques are not covered by health insurance.

– *Interdisciplinary cooperation* between oncologists, surgeons, reproductive endocrinologists and urologists is of crucial importance. The oncologist is obliged to inform the patient about the risks cancer treatment poses to future fertility, while the reproductive endocrinologist and urologist should provide information about the options for fertility preservation. Interdisciplinary cooperation is crucial for determining the optimal strategy and the length of using fertility preservation techniques, with mandatory individual approach to each patient.

Patients in the fertility preservation programs should be informed about all available techniques for preserving their own gametes and embryos, about programs of donating gametes, embryos, surrogacy and adopting children in accordance with the legislation in cases when the use of mentioned techniques is not possible due to the primary disease.

In patients who decide to cryopreserve gametes, embryos and gonadal tissue, options of further use or destruction of the reproductive material should be considered in case of death, and these decisions should be appropriately documented.

Ideally, if the time from making the diagnosis to cancer treatment allows, the patient should talk to and get advice from medical experts in the mentioned fields on several occasions in order to choose the appropriate technique for fertility preservation. This approach allows a comprehensive evaluation and understanding of psychosocial and medical needs of each patient [4].

Conclusion

Progress in the field of cancer treatment has led to a long-term survival of patients, and fertility preservation in such patients is an imperative. Considering the influence of the possibility of having children on the quality of life of reproductive age patients, all cancer patients should be informed about the available techniques for fertility preservation and about the potential effects of cancer treatment on their fertility before the treatment protocol starts. Raising the awareness about the importance of preserving fertility requires a multidisciplinary approach.

References

1. Kovač A, Popović-Petrović S, Nedeljković M, Kojić M, Tomić S. Stanje pacijentkinja obolelih od karcinoma dojke u postoperativnom periodu sa psihoonkološkog aspekta - preliminarni rezultati. Med Pregl. 2014;67(1-2):43-8.

2. Redig AJ, Brannigan R, Stryker SJ, Woodruff TK, Jeruss JS. Incorporating fertility preservation into the care of young oncology patients. Cancer. 2011;117(1):4-10.

3. Letourneau JM, Ebbel EE, Katz PP, Katz A, Ai WZ, Chien AJ, et al. Pretreatment fertility counseling and fertility preservation improve quality of life in reproductive age woman with cancer. Cancer. 2012;118(6):1710-7.

4. Practice Committee of the American Society for Reproductive Medicine. Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a commitete opinion. Fertil Steril. 2014;100(5):1214-23.

5. Familiari G, Caggiati A, Nottola SA, Ermini M, Di Benedetto MR, Motta PM. Ultrastructure of human ovarian primordial follicles after combination chemotherapy for Hodgkin's disease. Hum Reprod. 1993;8(12):2080-7.

6. Su HI. Measuring ovarian function in young cancer survivors. Minerva Endocrinol. 2010;35(4):259–70.

7. Behringer K, Breuer K, Reineke T, May M, Nogova L, Klimm B, et al. Secondary amenorrhea after Hodgkin's lymphoma is influenced by age at treatment, stage of disease, chemotherapy regimen, and the use of oral contraceptives during therapy: a report from the German Hodgkin's Lymphoma Study Group. J Clin Oncol. 2005;23(30):7555–64.

8. Tham YL, Sexton K, Weiss H, Elledge R, Friedman LC, Kramer R. The rates of chemotherapy-induced amenorrhea in patients treated with adjuvant doxorubicin and cyclophosphamide followed by a taxane. Am J Clin Oncol. 2007;30(2):126–32.

9. Wallace WH, Thomson AB, Saran F, Kelsey TW. Predicting age of ovarian failure after radiation to a field that includes the ovaries. Int J Radiat Oncol Biol Phys. 2005;62(3):738-44.

10. Reulen RC, Zeegers MP,Wallace WH, Frobisher C, Taylor AJ, Lancashire ER, et al. Pregnancy outcomes among adult survivors of childhood cancer in the British Childhood Cancer Survivor Study. Cancer Epidemiol Biomarkers Prev. 2009;18(8):2239-47.

11. Bath LE, Anderson RA, Critchley HO, Kelnar CJ, Wallace WH. Hypothalamic-pituitary-ovarian dysfunction after prepubertal chemotherapy and cranial irradiation for acute leukaemia. Hum Reprod. 2001;16(9):1838–44.

12. Abu Rustum NR, Sonoda Y. Fertility sparing surgery in early stage cervical cancer: indications and applications. J Natl Compr Canc Netw. 2010;8(12):1435-8.

13. Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight cell embryo. Nature. 1983;305(5936):707-9.

14. Basirat Z, Adib Rad H, Esmailzadeh S, Jorsaraei SG, Hajian-Tilaki K, Pasha H, et al. Comparison of pregnancy rate

between fresh embryo transfers and frozen-thawed embryo transfers following ICSI treatment. Int J Reprod Biomed (Yazd). 2016;14(1):39-46.

15. Cardozo ER, Thomson AP, Karmon AE, Dickinson KA, Wright DL, Sabatini ME. Ovarian stimulation and in-vitro fertilization outcomes of cancer patients undergoing fertility preservation compared to age matched controls: a 17-year experience. J Assist Reprod Genet. 2015;32(4):587-96.

16. Cakmak H, Katz A, Cedars MI, Rosen MP. Effective method for emergency fertility preservation: random start controlled ovarian stimulation. Fertil Steril. 2013;100(6):1673-80.

17. Chen C. Pregnancy after human oocyte cryopreservation. Lancet. 1986;1(8486):884–6.

18. Konc J, Kanyó K, Kriston R, Somoskői B, Cseh S. Cryopreservation of embryos and oocytes in human assisted reproduction. Biomed Res Int. 2014;2014:307268.

19. Cobo A, Rubio C, Gerli S, Ruiz A, Pellicer A, Remohi J. Use of fluorescence in situ hybridization to assess the chromosomal status of embryos obtained from cryopreserved oocytes. Fertil Steril. 2001;75(2):354-60.

20. Noyes N, Porcu E, Borini A. Over 900 oocyte cryopreservation babies born with no apparent increase in congenital anomalies. Reprod Biomed Online. 2009;18(6):769-76.

21. Rienzi L, Romano S, Albricci L, Maggiulli R, Capalbo A, Baroni E, et al. Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. Hum Reprod. 2010;25(1):66-73.

22. Practice Committee of the American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. Mature oocyte cryopreservation: a guideline. Fertil Steril. 2013;99(1):37–43.

23. Donnez J, Jadoul P, Squifflet J, Van Langendonckt A, Donnez O, Van Eyck AS, et al. Ovarian tissue cryopreservation and transplantation in cancer patients. Best Pract Res Clin Obstet Gynaecol. 2010;24(1):87-100.

24. Donnez J, Silber S, Andersen CY, Demeestere I, Piver P, Meirow D, et al. Children born after autotransplantation of cryopreserved ovarian tissue. A rewiev of 13 live births. Ann Med. 2011;43(6):437-50.

25. Stern CJ, Gook D, Hale LG, Agresta F, Oldham J, Rozen G, et al. First reported clinical pregnancy following heterotopic grafting of cryopreserved ovarian tissue in a woman after a bilateral oophorectomy. Hum Reprod. 2013;28(11):2996-9.

Rad je primljen 4. IX 2017.
Recenziran 4. IX 2017.
Prihvaćen za štampu 7. X 2017.
BIBLID.0025-8105:(2018):LXXI:1-2:53-59.

26. Practice Committee of the American Society for Reproductive Medicine. Ovarian tissue cryopreservation: a committee opinion. Fertil Steril. 2014;101(5):1237–43.

27. Son WY, Lee SY, Yoon SH, Lim JH. Pregnancies and deliveries after transfer of human blastocysts derived from in vitro matured oocytes in in vitro maturation cycles. Fertil Steril. 2007;87(6):1491–3.

28. Chen H, Li J, Cui T, Hu L. Adjuvant gonadotropin-releasing hormone analogues for the prevention of chemotherapy induced premature ovarian failure in premenopausal women. Cochrane Database Syst Rev. 2011;9(11):CD008018.

29. Dorais J, Dodson M, Calvert J, Mize B, Travarelli JM, Jasperson K, et al. Fertility sparing management of endometrial adenocarcinoma. Obstet Gynecol Surv. 2011;66(7):443-51.

30. Sabanegh ES Jr, Ragheb AM. Male fertility after cancer. Urology. 2009;73(2):1049-57.

31. Berthelsen JG, Skakkebaek NE. Gonadal function in men with testis cancer. Fertil Steril. 1983;39(1):68-75.

32. van Casteren NJ, van der Linden GH, Hakvoort-Cammel FG, Hählen K, Dohle GR, van den Heuvel-Eibrink MM. Effect of childhood cancer treatment on fertility markers in adult male long-term survivors. Pediatr Blood Cancer. 2009;52(1):108-12.

33. Howell S, Shalet S. Gonadal damage from chemotherapy and radiotherapy. Endocrinol Metab Clin North Am. 1998;27(4):927-43.

34. Trottmann M, Becker AJ, Stadler T, Straub J, Soljanik I, Schlenker B, et al. Semen quality in men with malignant diseases before and after therapy and the role of cryopreservation. Eur Urol. 2007;52(2):355-67.

35. Levine J, Canada A, Stern CJ. Fertility preservation in adolescents and young adults with cancer. J Clin Oncol. 2010;28(32):4831-41.

36. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol. 2006;24(18):2917–31.

37. Müller J, Sønksen J, Sommer P, Schmiegelow M, Petersen PM, Heilman C, et al. Cryopreservation of semen from pubertal boys with cancer. Med Pediatr Oncol. 2000;34(3):191-4.

38. Hermann BP, Sukhwani M, Winkler F, Pascarella JN, Peters KA, Sheng Y, et al. Spermatogonial stem cell transplantation into rhesus testes regenerates spermatogenesis producing functional sperm. Cell Stem Cell. 2012;11(5):715-26.

Medical Faculty, Priština, Kosovska Mitrovica
Department of Physiology[1]
Department of Histology[2]
General Hospital Požarevac, Department of Cardiology[3]

Seminar for physicians
Seminar za lekare u praksi
UDK 616.12-008-092
UDK 611.12/.13.018.7
https://doi.org/10.2298/MPNS1802060S

ENDOCARDIAL ENDOTHELIUM AS A BLOOD-HEART BARRIER

ENDOKARDNI ENDOTEL KAO KRVNO-SRČANA BARIJERA

Sonja SMILJIĆ[1], Slađana SAVIĆ[2], Zvezdan MILANOVIĆ[1] and Goran GRUJIĆ[3]

Summary

Introduction. Endocardial endothelium is formed from a single layer of closely related cells with complex interrelationships and extensive overlap at the junctional edges. **Morphological characteristics of blood-heart barrier.** Endocardium is composed of three layers: endocardial endothelium, subendothelial loose connective tissue and subendocardium. The fibrous component of the subendothelium consists of small amount of collagen and elastic fibers. Several cell types are present in subendocardium: telocytes, fibroblasts and nerve endings. **Intercellular bonds between the endocardial endothelial cells.** Endocardial endothelial cells are attached to one another via sets of binding proteins forming solid, adherent and communicating connections. Communicating connections form transmembrane channels between the neighboring cells, while solid and adherent connections form pericellular structures like stitches. The maintenance of the presumed transendocardial electrochemical potential difference provides a high gradient for certain ions as well as a selective boundary barrier, basal lamina, preventing ionic leakage. The negatively charged glycocalyx also modulates endothelial permeability. **Electrophysiological characteristics of heart-blood barrier.** Electrophysiological studies have shown the existence of a large number of membrane ion channels in the endocardial endothelial cells: inward rectifying K^+ channels, Ca^{2+} dependent K^+channels, voltage-dependent Cl^- channels, volume-activated Cl^- channels, stretch-activated cation channels and one carrier mediated transport mechanism – Na^+K^+adenosine triphosphatase. **Conclusion.** Numerous diseases of the cardiovascular system may be a consequence, but also the cause of the endocardial endothelium dysfunction. Selective damage to the endocardial endothelium and subendocardium is found in arrhythmia, atrial fibrillation, ischemia/reperfusion injury and heart failure. Typical lesions of endocardial and microvascular endothelium have also been described in sepsis, myocardial infarction, inflammation and thrombosis. The result of endothelial dysfunction is the weakening of the endothelial barrier regulation and electrolyte imbalance of the subendocardial interstitium.

Key words: Endothelium; Endothelial Cells; Intercellular Junctions; Electrophysiology; Cardiac Electrophysiology; Morphological and Microscopic Findings; Heart Failure; Cardiovascular Diseases

Sažetak

Uvod. Endokardni endotel formira jedan sloj tesno povezanih ćelija sa kompleksnim međuodnosima i opsežnim preklapanjem susednih ivica. **Morfološke karakteristike krvno-srčane barijere.** Endokard čine tri sloja: endokardni endotel, subendotelno rastresito vezivno tkivo i subendokard. Vlaknasta komponenta subendotela se sastoji od male količine kolagenih i elastičnih vlakana. U subendokardu su prisutni telociti, fibroblasti i nervni završeci. **Međućelijske veze između endokardnih endotelnih ćelija.** Endotelne ćelije su povezane jedna sa drugom putem skupova vezujućih proteina koji čine čvrste veze, adherentne veze i komunikantne veze. Komunikantne veze formiraju transmembranske kanale između kontaktnih ćelija, a tesne i adherentne veze formiraju pericelularne strukture slične „šavu“. Održavanje pretpostavljene transendokardne elektrohemijske potencijalne razlike obezbeđuje visok gradijent za izvesne jone dok selektivna granična barijera, bazalna lamina, prevenira jonsko curenje. Negativno naelektrisani glikokaliks, takođe, modulira endotelnu permeabilnost. **Elektrofizihološke karakteristike krvno-srčane barijere.** Elektrofiziološke studije su ukazale na postojanje velikog broja membranskih jonskih kanala: ulazno-ispravljački K^+ kanali, Ca^{2+} zavisni K^+ kanali, voltažno-zavisni Cl^- kanali, volumen-aktivni Cl^- kanali, rastezanjem aktivisani katjonski kanali i jedan nosačem posredovani transport, Na^+K^+ adenozin trifosfataza. Zaključak. Bolesti kardiovaskularnog sistema mogu biti posledica ali i uzrok disfunkcije endokardnog endotela. Selektivno oštećenje endokardnog endotela i subendokarda dešava se u aritmiji, atrijalnoj fibrilaciji, ishemijsko/reperefuzijskim oštećenjima i srčanoj insuficijenciji. Tipične lezije endokardnog endotela opisane su u sepsi, akutnom infarktu miokarda, zapaljenju i trombozi. Rezultat endotelne disfunkcije je slabljenje barijerne uloge endokardnog endotela i elektrolitskog disbalansa subendokardnog intersticijuma.

Ključne reči: endotel; endotelne ćelije; intercelularne veze; elektrofiziologija; kardiološka elektrofiziologija; morfološki i mikroskopski nalazi; otkazivanje srca; kardiovaskularna oboljenja

Corresponding Author: Prof. dr Sonja Smiljić, Univerzitet u Prištini, Kosovska Mitrovica, Medicinski fakultet, Institut za fiziologiju, 38220 Kosovska Mitrovica, Anri Dinana bb, E-mail: sonja.smiljic@med.pr.ac.rs

Abbreviations

EE – endocardial endothelium
PECAM-1 – platelet-endothelial adhesion molecule-1
TRP – transient receptor potential
ATPase – adenosine triphosphatase

Introduction

The cardiovascular system is lined with endothelium, a continuous single-layer flaked epithelium, forming a cobblestone-like layer on the surface of the tunica intima of the blood vessels and the endocardial layer of the heart. It has a total surface area of several hundred square meters and the endothelium of a person weighing 70 kg covers about 700 m^2 [1]. The vascular endothelium has long been thought of as just a layer lining the blood vessels like a cellophane wrapper, without a significant functional role. It is now recognized as a massive, regionally specific multi-functional organ. Out of all the layers of the blood vessel wall, endothelium is the most exposed to mechanical forces and the pressure that blood exerts on it.

Given the strategic anatomical position of endothelium between the circulating blood components on one side and a vascular component of smooth muscle and cardiac muscle on the other, it represents a physiologically significant organ whose dysfunction is seen as a critical factor for the development of various diseases [2]. Before the initial research on the effects of endocardial endothelium (EE) on the contractility, rhythm and remodeling of the adjacent myocardium, information about any physiological roles of EE on the function of the heart were rare [3]. However, studies have shown that myocardial contractility depends on the presence of EE and the degree of its damage regardless of the technique employed to remove the endothelium: immersion in 1% Triton X-100, mechanical peeling, exposure to a high-frequency ultrasound, or flow rate of dry air. Fully removing or partially damaging EE cells directly affect contractile cardiac performance causing a lower contractility of cardiomyocytes [4, 5]. The inotropic effect of EE is achieved through the synthesis and release of endothelial mediators, the sensory ability to detect changes in blood plasma and the quality of blood-heart barrier to control transendothelial transport. Adding a mediator of endothelial origin will not restore or prevent the loss of heart contractility. The EE can affect the establishment of transcellular physicochemical gradient across the endocardium, and thereby affects the cardiac function [6].

The removal of EE is reflected in the acute disorder of subendocardial ionic environment. It took time and numerous studies to support the hypothesis of EE as the blood-heart barrier and to compare its importance to the one of the blood-brain barrier. In the last 20 years, following the initial studies of Paul Fransen (1995), the analogy of EE barrier with the concept of the blood-brain barrier has strengthened [7]. The blood-brain barrier is the best studied endothelial barrier. Highly excitable tissues such as neurons have a larger concentration of Na^+ ions in the interstitium, which enables a faster rise of action potential and less interstitial K^+, thereby increasing the membrane excitability. In the heart, the sub-endocardial network of terminal Purkinje fibers and the surrounding myocardial cells consist of highly excitable cells making the ion homeostasis the essence of the vitality of the heart function. Changes in the extracellular concentration of Ca^{++}, K^+, Na^+, Mg^{++}, Cl^- i HCO_3^- ions have significant effects on the rhythm and the mechanical properties of the cardiac muscle.

The maintenance of the assumed trans-endocardial electrochemical potential differences provides a high gradient for certain ions while the selective barrier, basal lamina, prevents ionic leakage. The negatively charged glycocalyx also modulates endothelial permeability.

Morphological Characteristics of the Blood-Heart Barrier

The heart wall consists of three layers, epicardium, myocardium and endocardium. Each layer is distinctive in thickness, cellular composition and the specific role. From a histological point of view, endocardium consists of three layers: the endothelium, the subendothelial loose connective tissue and subendocardium.

The fibrous component of the subendocardium consists of a small amount of collagen and elastic fibers [8]. Electron microscope images clearly show the existence of typical telocytes within the subendothelial layer. Telocytes appear as the main interstitial cells in the loose connective tissue of the subendothelial endocardium. The subendocardium consists of telocytes, fibroblasts and nerve endings. About one third of endothelial cells are underlined by telocytes. Subendocardial telocytes have small oval-shaped cellular bodies with very long, thin cellular processes named telopodes. Telocytes are in close vicinity with nerve endings and they establish contacts with other interstitial cells in the subendocardial space. Telocytes create a tridimensional cardiac network at the interstitial level and integrate all constitutive layers of heart via their telopodes. Since endocardium is considered a blood-heart barrier, it is obvious that telocytes which constitute the main population in the subendothelial layer of endocardium may be of importance in creating this blood-heart barrier [9].

The EE forms a single layer of closely related cells with complex interrelations and extensive overlapping of adjacent edges. This structure allows its unique permeable properties. Tight junctions are located at the luminal side of intercellular gaps between endothelial cells. The EE cells exhibit characteristic morphological asymmetry.

Electrophysiological measurements of properties and electric currents, as well as fluorometric measurements, have demonstrated that EE cells form an interconnecting network through electrical synapses, such as gap junctions. Gap junction channels

of EE cells are composed of different connexin types (Cx43, Cx40, Cx37) and are located at the borders of the intercellular areas and at the overlapping areas and the EE cells [10].

Gap junctions have intercellular pores that facilitate the passage of ions (mostly Ca^{2+}), secondary messenger molecules (inositol-1,4,5 trisphosphate) and small metabolites which pass without pausing between adjacent EE cells, ensuring that the entire EE acts as a single unit with electrical and diffusive continuity [11]. We examine the EE as a syncytium with electrochemical connections between neighboring EE cells. Gap junction connections between heart endothelial cells and cardiomyocytes are not identified. The absence of morphological connections between EE and cardiomyocytes does not exclude the electrotonic current propagation or the effects of EE syncytium on the excitability and conductivity to the adjacent cardiomyocytes.

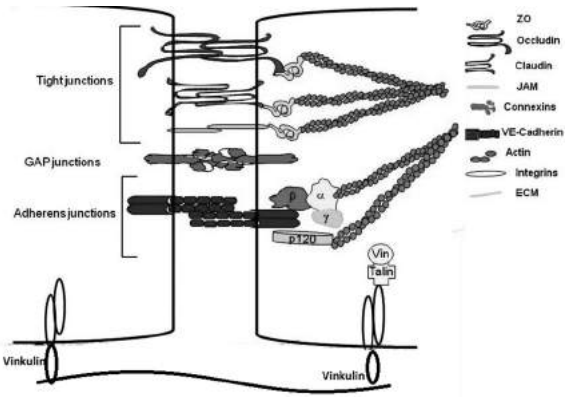
Endocardial endothelium syncytium can be functionally represented as a large cell, with a very large membrane area, which is connected to smaller groups of Purkinje fibers and subendocardial nerve plexuses. Electrical phenomena arising from the high conductive power of cardiomyocyte syncytium may propagate to the Purkinje network, nerve plexus and EE.

There is no experimental evidence to support a conclusion that EE cells are "electrically silent" in the sense that they do not show regular action potentials. However, they depolarize and repolarize following the action potentials of adjacent cardiomyocytes [12]. Syncytial character of EE is essential to establish the barrier properties in the transcellular transport of ions. Moreover, it is possible that it enables an increased synthesis and the release of endothelial paracrine mediators.

Intercellular Junctions between EE Cells

Endothelial cells are connected by a complex set of junctional proteins that comprise tight junctions, adherens junctions and gap junctions. Gap junctions form transmembrane channels between contiguous cells. Tight junctions and adherens junctions form pericellular zipper like structures along the cell border through their transmembrane homophilic interactions.

The cell adhesion necessary to maintain the integrity of all tissues, including the endothelium, involves two groups of the adhesion proteins, in addition to intercellular junctions, hemidesmosomes, focal adhesions and extracellular matrix proteins. One group of adhesion proteins is responsible for the adhesion of cells with the extracellular matrix (integrins), while the other group participates in intercellular adhesion of endothelial cells. One very important adhesion protein is platelet-endothelial adhesion molecule-1 (PECAM-1) that is expressed on the surfaces of the interendothelial contacts. PECAM-1 is uniformly distributed along the juncture giving it stability and is one of the main components of the endothelial junctions. Identification of PECAM-1 enables visualization of intercellular junctions within endocardial cells. In the EE, PECAM-1 staining is typically confined to the border zone of the EE cells corresponding to the zone of cellular overlap and intercellular clefts.



Schema 1. Structural organization of intercellular endothelial and matrix cells interactions
Shema 1. *Strukturna organizacija intercelularnih i matričnih interakcija endotelnih ćelija*
Legend: ZO - Zonula occludens; JAM - junctional adhesion molecule; VE cadherin - vascular endothelial cadherin; ECM - extracellular matrix
Legenda: ZO - Zonula occludens; JAM - adhezivni (vezivni) molekul; VE cadherin - vaskularni endotelijalni kadherin; ECM - ekstracelularno matriks

Transendothelial transport (transcytosis) is mediated by diffusion through intracellular clefts by vesicular transport, or focal adhesion contracts (gap junctions). Thus, trans-EE-permeability is predominantly controlled through intracellular clefts, mediated through the extent and structurally complex paracellular space which is partially lined by an electrically charged glycocalyx, the presence of one or more tight junctions (zonula occludens) and the presence of well-organized zonula adherens. The presence of actin and nonmuscle myosin in EE cells suggests that the trans-endocardial permeability may be mediated by contraction or retraction of EE cells by phosphorylation of actin-binding proteins, such as vinculin and a-catenin, but also by activation of actin-myosin interactions.

Endothelial cells are interconnected via sets of binding proteins which form occludent, tight junctions (zonula occludens), adherens junctions (zonula adherens) and communicative junctions (gap junctions), whereas gap junctions form transmembrane channels between the adjacent cells, tight junctions and adherens junctions form pericellular zipper-like structures along the cell border through their transmembrane homophilic adhesion **(Schema 1).**

Tight junctions are located at the luminal side of the lateral membranes between adjacent EE cells and represent about 20% of total junctional complexes present in endothelial cells. They are composed of claudins, occludin, and junctional adhesion molecules. These junctions are mostly responsible for the

restriction of the passage of water, electrolytes and small molecules through the endothelium. The role of tight junctions in regulating endothelial permeability remains incompletely understood. The expression level of occluding was found to correlate with enhanced endothelial barrier properties. Thus, occluding through its interaction with ZO-1 and the actin cytoskeleton stabilizes tight junctions [13].

Adherens junctions lie just below the tight junctions and they secure the junction between adjacent cells. Within the gap about 15 – 20 nm between the two cells, there is a cell membrane glycoprotein-cadherin. The cadherins from adjacent cells interact to 'zipper' up the two cells together. Within the cells, actin filaments (microfilaments) achieve their adhesive role and tend to be arranged circumferentially around the cell, into what is called a 'marginal' band. This marginal band may contract and also deform the shape of cells held together. Thus, adherens junctions position cells within the endothelium. The stability of these junctions depends on the concentration of calcium ions, in the absence of which the junctions break [14].

Numerous communicating gap junctions, 2 – 3 μm in size, interconnect the cytoplasm of multiple cells. Permeable junctions in the EE provide the characteristics of a 'functional syncytium' because they coordinate the function of a set of cells. Connexins are gap junction proteins and represent a protein family with high homology in their amino acid sequences. Connexins aggregate to form hexamers. Hexamers of two adjacent cells form a connexon gap junction. Permeable junctions allow the passage of molecules up to 1 kDa in size and a rapid exchange of information in the form of molecules of low molecular weight, secondary messengers, Ca^{2+} and inositol triphosphatate between the adjoining cells [12, 15].

Connexins have several regulatory sites on the cytoplasmic side that regulate the state of permeable junctions. The functional state of permeable junctions is influenced by voltage, pH Ca^{2+} ions, calmodulin, phosphorylation, and G proteins [16]. The binding of a ligand to the receptor at one EE cell leads to cascade signalization amplifying cellular response. Alterations in both the amount and cellular distribution of gap junctions have been reported in many types of cardiac disease, and it has been suggested that these changes may cause arrhythmias and/or sudden cardiac death. Cardiac diseases are often associated with a reduced and/or heterogeneous expression of connexins. In atherosclerosis, expression of Cx37, Cx40, and Cx43 varies throughout the progression of the disease and connexins play different roles in plaque development [17].

Electrophysiological Characteristics of the Blood-Heart Barrier

The EE cells are electrically highly active, similarly to the brain capillary endothelial cells. Electrophysiological studies have demonstrated the existence of a large number of membrane ion channels: inward rectifier K^+ channels (I_{kr}), Ca^{2+} dependent K^+ channels (K_{Ca}), voltage-dependent Cl^- channels, the volume activated Cl - channels, stretch activated cation channels and a carrier-mediated transport, Na^+K^+ adenosine tripzhosphatase (ATPase) [18]. The asymmetric nature of the luminal compared to abluminal localization of ion channels and Na^+K^+ ATPase suggests that the net transcellular ionic transport from the blood to the cardiomyocyte interstitium occurs via a passive diffusion through the ion channels and through the active, carrier dependent transport.

Transendocardial electrical resistance in the endothelial cells of the right ventricle is two to five times higher than in other endothelial membranes (6–25 ohm/cm^2) [19]. This is consistent with the assumption that EE functions as an active barrier between the circulating blood and cardiomyocyte interstitium. By combining the applicable electrophysiological techniques, Western blot and real-time polymerase chain reaction, in the EE cell culture we depicted a significant presence of Na^+K^+-ATPase, predominantly of alpha-1-type, typically associated with the luminal membrane of EE cells [20, 21]. This asymmetric configuration can explain the net Na^+ transport from the heart into the blood and K^+ transport from the blood to the heart. A lower interstitial sodium level in the heart is more favorable for ensuring electrical stability, while a higher level of interstitial sodium causes the necessary excitability.The atrial volume reflex arc is an important contributor to the maintenance of bodily homeostasis, primarily responding to blood volume changes. Atrial volume receptors located in the endocardium of the atrial wall undergo mechanical deformation as blood is returned to the atria of the heart. The mechanosensitive channel(s) responsible for regulating plasma are the transient receptor potential (TRP) channel family members TRPC1 and TRPV4, expressed in sensory nerve endings in the atrial endocardium [22].

Conclusion

All endocardial endothelial cells act together in the organization of the endocardium as a functional syncytium to achieve a complex, well-organized, auto- and paracrine mediated physicochemical barrier between the circulating blood and subjacent heart tissue. Endothelial cells are able to dynamically regulate paracellular and transcellular transport of dissolved particles and water.

Paracellular permeability is determined by complex structures of junctions and intercellular adhesive strengths balanced with the contra-adhesive properties generated by the molecular mechanism of actin-myosin. The intact endothelial barrier limits the transport primarily by closing the interendothelial junctions. Through their receptor sites, integrins are related to the extracellular matrix, thus contributing to the stabilization of the barrier func-

tion of sealed intercellular spaces. While binding to their receptors, inflammatory mediators, thrombin, bradykinin, histamine, vascular endothelial growth factor and others, disturb the organization of inter-endothelial junctions and the integrin-extracellular matrix complex formation, thus creating the junctions that constitute the barrier.

Numerous diseases of the cardiovascular system can be a consequence but also the cause of endocardial endothelial dysfunction. Selective damage to the endocardial endothelium and subendocardium occurs in arrhythmia, atrial fibrillation, ischemia/reperfusion injury, cardiac hypertrophy and heart failure. Typical lesions of endocardial and microvascular endothelium have also been described in sepsis, myocardial infarction, inflammation, thrombosis, and in hypertensive patients. The result of endothelial dysfunction is the weakening of the endothelial barrier regulation and the electrolyte imbalance of the subendocardial interstitium.

References

1. Boulanger CM. Endothelium. Arterioscler Thromb Vasc Biol. 2016;36(4):e26–31.

2. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation. 2007;115(10):1285–95.

3. Brutsaert DL, Fransen P, Andries LJ, De Keulenaer GW, Sys SU. Cardiac endothelium and myocardial function. Cardiovasc Res. 1998;38(2):281–90.

4. Brutsaert DL. Cardiac endothelial-myocardial signaling: role in cardiac growth, contractile performance, and rhythmicy. Physiol Rev. 2003;83(1):59–115.

5. Smiljić S, Radović D, Miletić M, Nestorović V, Trjaković G, Savić S. Influence of metabolism modifiers of cyclic nucleotides on contractility of right ventricle of rat heart with intact and removed endocardial endothelium. Srp Arh Celok Lek. 2010;138(9-10):577–83.

6. Smiljić S, Radović D, Nestorović V, Milanović Z, Biševac B. Endothelins as mediators in the modulation of cardiac performance. Praxis medica. 2014;43(4):79–84.

7. Fransen P, Andries LJ, Van Bedaf D, Demolder M, Sys SU. Cell coupling in cultured endocardial endothelium. Pflugers Arch. 1996;431(Suppl 6):R323.

8. Ishibashi-Ueda H, Matsuyama TA, Ohta-Ogo K, Ikeda Y. Significance and value of endomyocardial biopsy based on our own experience. Circ J. 2017;81(4):417–26.

9. Gherghiceanu M, Manole CG, Popescu LM. Telocytes in endocardium: electron microscope evidence. J Cell Mol Med. 2010;14(9):2330–4.

10. Nishii K, Seki A, Kumai M, Morimoto S, Miwa T, Hagiwara N, et al. Connexin45 contributes to global cardiovascular development by establishing myocardial impulse propagation. Mech Dev. 2016;140:41–52.

11. Bény JL. Information networks in the arterial wall. New Physiol Sci. 1999;14:68–73.

12. Brutsaert DL, Andries LJ. The endocardial endothelium. Am J Physiol. 1992;263(4 Pt 2):H985–1002.

13. Kevil CG, Okayama N, Trocha SD, Kalogeris TJ, Coe LL, Specian RD, et al. Expression of zonula occludens and adherens junctional proteins in human venous and arterial endothelial cells: role of occludin in endothelial solute barriers. Microcirculation. 1998;5(2-3):197–210.

14. Noireaud J, Andriantsitohaina R. Recent insights in the paracrine modulation of cardiomyocyte contractility by cardiac endothelial cells. Biomed Res Int. 2014;2014:923805.

15. Brutsaert DL. Cardiac endothelial-myocardial signaling: role in cardiac growth, contractile performance, and rhythmicy. Physiol Rev. 2003;83(1):59–115.

16. Nielsen MS, Axelsen LN, Sorgen PL, Verma V, Delmar M, Holstein-Rathlou NH. Gap junctions. Compr Physiol. 2012;2(3):1981–2035.

17. Kwak BR, Mulhaupt F, Veillard N, Gros DB, Mach F. Altered pattern of vascular connexin expression in atherosclerotic plaques. Arterioscler Thromb Vasc Biol. 2002;22:225–30.

18. Guo D, Yu M, Liu Q, Cox RH, Liu T, Yan GX. Ventricular hypertrophy amplifies transmural dispersion of repolarization by preferentially increasing the late sodium current in endocardium. J Electrocardiol. 2014;47(5):642–8.

19. Bannon PG, Kim MJ, Dean RT, Dawes J. Augmentation of vascular endothelial barrier function by heparin and low molecular weight heparin. Thromb Haemost. 1995;73(4):706–12.

20. Fransen P, Hendrickx J, Brutsaert DL, Sys SU. Distribution and role of Na+/K+ ATPase in endocardial endothelium. Cardiovasc Res. 2001;52(3):487–99.

21. Fransen P, Sys SU. K+ and Cl- contribute to resting membrane conductance of cultured porcine endocardial endothelial cells. Am J Physiol. 1997;272(4 Pt 2): H1770-9.

22. Shenton FC, Pyner S. Expression of transient receptor potential channels TRPC1 and TRPV4 in venoatrial endocardium of the rat heart. Neuroscience. 2014;267:195–204.

Rad je primljen 6. V 2017.
Recenziran 28. IX 2017.
Prihvaćen za štampu 12. X 2017.
BIBLID.0025-8105:(2018):LXXI:1-2:60-64.

REGISTAR ZA 2017. GODINU

INDEKS AUTORA

INDEX KEY WORDS

INDEKS KLJUČNIH REČI

UPUTSTVO ZA AUTORE

Časopis ***Medicinski pregled*** objavljuje radove koji prethodno nisu objavljeni niti poslati u drugi časopis. U Časopisu mogu biti objavljeni radovi iz različitih oblasti biomedicine, koji su namenjeni lekarima različitih specijalnosti.

Od 1. januara 2013. godine *Medicinski pregled* je počeo da koristi usluge *e-Ur* − Elektronskog uređivanja časopisa. Svi korisnici sistema − autori, recenzenti i urednici, moraju biti registrovani korisnici sa jednom elektronskom adresom.

Korisnici časopisa treba da se registruju na adresi:
http://aseestant.ceon.rs/index.php/medpreg/user/register

Prijava rada treba da se učini na adresi:
http://aseestant.ceon.rs/index.php/medpreg/

U postupku prijave neophodno je da se pošalje saglasnost i izjava autora i svih koautora da rad nije delimično ili u celini objavljen ili prihvaćen za štampu u drugom časopisu.

Elektronsko uređivanje časopisa obezbeđuje korišćenje sistema *CrossCheck*, koji prijavljene radove automatski proverava na plagijarizam i autoplagijarizam. Autori ne bi smeli da pošalju isti rad u više časopisa istovremeno. Ukoliko se to desi, glavni urednik časopisa *Medicinski pregled* ima pravo da rad vrati autorima bez prethodnog slanja rada na recenziju; da odbije štampanje rada; da se obrati urednicima drugih časopisa u koje je rad poslat ili da se obrati direktoru ustanove u kojoj su autori rada zaposleni.

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.