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CONTENTS

EDITORIAL

Dragan Dankuc

HISTORY OF OTOSURGERY - THE 20 th CENTURY – HISTORY OF MASTOID SURGERY AND TYMPANOPLASTY.....	79-84
--	-------

ORIGINAL STUDIES

Radoslav Gajanin, Živorad Gajanin, Zoran Vujković, Vesna Gajanin, Zdenka Gojković and Vesna Ljubojević IMMUNOHISTOCHEMICAL EXPRESSION OF P16INK4a IN INFLAMMATORY, PRENEOPLASTIC AND NEOPLASTIC CERVICAL LESIONS.....	85-92
--	-------

Stanislav Škrkar, Milena Mikalački, Nebojša Čokorilo and Mirela Erić ANALYSIS OF DIFFERENCES IN BLOOD PRESSURE OF WOMEN BELONGING TO DIFFERENT AGE GROUPS.	93-97
---	-------

Milana Jaraković, Bojan Mihajlović, Snežana Čemerlić, Filip Ađić, Miroslava Sladojević and Bogoljub Mihajlović THE LEVEL OF GRAMMAR SCHOOL STUDENTS' KNOWLEDGE ON CARDIOVASCULAR DISEASE RISK FACTORS	98-102
--	--------

Aleksandar Knežević, Tatjana Salamon, Miroslav Milankov, Srđan Ninković, Milica Jeremić Knežević and Snežana Tomašević Todorović ASSESSMENT OF QUALITY OF LIFE IN PATIENTS AFTER LOWER LIMB AMPUTATION.....	103-108
--	---------

PROFESSIONAL ARTICLES

Katarina Nikoletić, Jasna Mihailović, Emil Matovina, Radmila Žeravica and Dolores Srbovan RELIABILITY OF POSITRON EMISSION TOMOGRAPHY-COMPUTED TOMOGRAPHY IN EVALUATION OF TESTICULAR CARCINOMA PATIENTS.....	109-115
--	---------

Srđan Ninković, Snežana Avramov, Vladimir Harhaji, Mirko Obradović, Miodrag Vranješ and Miroslav Milankov INFLUENCE OF DIFFERENT LEVELS OF SPORTS ACTIVITIES ON THE QUALITY OF LIFE AFTER THE RECONSTRUCTION OF ANTERIOR CRUCIATE LIGAMENT	116-121
---	---------

Ivana B. Hrnjaković Cvjetković, Dejan Cvjetković, Aleksandra Patić, Nataša Nikolić, Sandra Stefan Mikić and Vesna Milošević CHIKUNGUNYA – A SERIOUS THREAT FOR PUBLIC HEALTH.....	122-125
--	---------

CASE REPORTS

Vesna Janevska, Liljana Spasevska, Milan Samardziski, Violeta Nikodinovska, Julija Zhivadinovik and Elizabeta Trajkovska FROM ANEURYSMAL BONE CYST TO TELANGIECTATIC OSTEOSARCOMA WITH METASTASIS IN INGUINAL LYMPH NODES – CASE REPORT	127-132
--	---------

Milana Obradović Tomašev, Mladen Jovanović and Aleksandra Popović PYODERMA GANGRENOSUM IN BURNED PATIENT - CASE REPORT.....	133-136
--	---------

Ivica Lalić, Mirko Obradović, Mirka Lukić Šarkanović and Vladimir Đan DEFINITE MANAGEMENT OF BILATERAL LOWER LEG NONUNION FRACTURES BY ILIZAROV APPARATUS IN POLYTRAUMATIZED PATIENT – CASE REPORT	137-142
---	---------

SADRŽAJ

UVODNIK

Dragan Dankuc
ISTORIJA OTOHIRURGIJE – XX VEK - ISTORIJA MASTOIDNE HIRURGIJE I TIMPANOPLASTIKE..... 79-84

ORIGINALNI NAUČNI RADOVI

Radoslav Gajanin, Živorad Gajanin, Zoran Vujković, Vesna Gajanin, Zdenka Gojković i Vesna Ljubojević
IMUNOHISTOHEMIJSKA EKSPRESIJA p16INK4a U INFLAMACIJSKIM, PRENEOPLASTIČNIM I NEOPLASTIČNIM LEZIJA-
MA GRLIČA MATERICE 85-92

Stanislav Škrkar, Milena Mikalački, Nebojša Čokorilo i Mirela Erić
ANALIZA RAZLIKA U KRVNOM PRITISKU KOD ŽENA IZ RAZLIČITIH STAROSNIH GRUPA 93-97

Milana Jaraković, Bojan Mihajlović, Snežana Čemerlić, Filip Adić, Miroslava Sladojević i Bogoljub Mihajlović
NIVO ZNANJA UČENIKA GIMNAZIJE O FAKTORIMA RIZIKA ZA KARDIOVASKULARNE BOLESTI 98-102

Aleksandar Knežević, Tatjana Salamon, Miroslav Milankov, Srđan Ninković, Milica Jeremić Knežević i Snežana Tomašević Todorović
PROCENA KVALITETA ŽIVOTA PACIJENATA NAKON AMPUTACIJE DONJIH EKSTREMITETA 103-108

STRUČNI ČLANCI

Katarina Nikoletić, Jasna Mihailović, Emil Matovina, Radmila Žeravica i Dolores Srbovan
POUZDANOST POZITRONSKE EMISIONE TOMOGRAFIJE - KOMPJUTERIZOVANE TOMOGRAFIJE U EVALUACIJI OBOLE-
LIH OD KARCINOMA TESTISA 109-115

Srđan Ninković, Snežana Avramov, Vladimir Harhaji, Mirko Obradović, Miodrag Vranješ i Miroslav Milankov
UTICAJ RAZLIČITIH NIVOVA SPORTSKE AKTIVNOSTI NA KVALITET ŽIVOTA POSLE REKONSTRUKCIJE PREDNJEG UKR-
ŠTENOG LIGAMENTA 116-121

Ivana B. Hrnjaković Cvjetković, Dejan Cvjetković, Aleksandra Patić, Nataša Nikolić, Sandra Stefan Mikić i Vesna Milošević
ČIKUNGUNIJA - OZBILJNA PRETNJA ZA JAVNO ZDRAVLJE 122-125

PRIKAZI SLUČAJEVA

Vesna Janevska, Liljana Spasevska, Milan Samardžiski, Violeta Nikodinovska, Julija Zhivadínovik i Elizabeta Trajkovska
OD ANEURIZMALNE KOŠTANE CISTE DO TELANGIEKTATIČNOG OSTEOSARKOMA SA METASTAZOM U INGVINALNIM
LIMFNIM ČVOROVIMA – PRIKAZ SLUČAJA 127-132

Milana Obradović Tomašev, Mladen Jovanović i Aleksandra Popović
PIODERMA GANGRENOZUM KOD PACIJENATA SA OPEKOTINAMA – PRIKAZ SLUČAJA 133-136

Ivica Lalić, Mirko Obradović, Mirka Lukić Šarkanović i Vladimir Đan
DEFINITIVNO ZBRINJAVANJE BILATERALNIH NESRASLIH PRELOMA POTKOLENICA APARATOM PO ILIZAROVU KOD
PACIJENTA SA POLITRAUMOM – PRIKAZ SLUČAJA 137-142

EDITORIAL

UVODNIK

Clinical Centre of Vojvodina, Novi Sad
Department of Ear, Nose and Throat Diseases
University of Novi Sad, Faculty of Medicine

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HISTORY OF OTOSURGERY – THE 20th CENTURY HISTORY OF MASTOID SURGERY AND TYMPANOPLASTY

*ISTORIJAT OTOHIRURGIJE – XX VEK
ISTORIJAT MASTOIDNE HIRURGIJE I TIMPANOPLASTIKE*

Dragan DANKUC

Introduction

During the 20th century, otology became an essentially surgical specialty. The combination of antibiotics, asepsis, reliable anesthesia and the operating microscope heralded the modern era of microsurgery.

Mastoid Surgery

At the beginning of the 20th century, the definition of various types of mastoid surgery was described in relation to the encountered pathology, notably acute mastoiditis, cholesteatoma, and chronic otitis media. New approaches were presented in 1906. Charles Joseph Heath [1] (1856-1934), from London and William Sohler Bryant [2] (1861-1956), from New York published reports of "modified radical" operations independently, whereby they advocated the posterior meatal wall removal in order to establish an opening from the antrum to the meatus, and preservation of a "bridge" of the deep meatal and outer attic wall, thus preserving the eardrum and ossicles.

In 1910, Gustav Bondy [3] (1870-1954), an Austrian surgeon extended the bone excision to include the outer attic wall and bridge in order to exteriorize disease localized to the attic and antrum. His aim was to preserve at least the pars tensa and the ossicles if possible. After clearing the disease, he turned the meatal skin flaps to cover the exposed bodies of the incus and malleus, or if those had been removed because of disease, to close off the defect between epitympanum and mesotympanum. A meatoplasty was always performed. Bondy's operation was the forerunner of the contemporary concept of a modified radical mastoidectomy. It attracted little interest during the 1920s and 1930s but was revived by Julius Lempert [4] (1890-1968), New

York in 1938, by Alexis Tumarkin [5] (1900-1990), Liverpool as an "atticotomy" in 1948 and by Shirley Harold Baron [6] (1904-1979), San Francisco in



Figure 1. Sir Charles Ballance (1856-1936)
Slika 1. Ser Čarls Balans (1856-1936)

Abbreviations

TORP – total ossicular replacement prosthesis

PORP – partial ossicular replacement prosthesis

1949. Nowadays the operation of modified radical mastoidectomy is still frequently practiced; however, its conception is rarely related to the name of Gustav Bondy.

The incisions of early mastoid operations were mostly postaural. Fritz Thies [7, 8] (1873-1957), from Leipzig claimed in 1908 that the endaural approach to radical mastoidectomy was his invention but the earliest record of an endaural incision can be found in the writing of Johannes Kessel [9] from Jena in 1885.

In 1926, René Jacquot [10] from Lyon published his thesis about the history of trepanation of the mastoid apophysis and three years later William Mollison [1] from London reviewed the history of mastoid operation and summarized the contemporary treatment of the meatus and cavity. He noted that the narrowing of the meatus could be avoided by packing, tubes or the use of skin-grafts as pioneered by Sir Charles Ballance and that the bony cavity was filled with periosteal flaps, skin flaps, fat grafts or temporalis muscle turned down into the cavity.

At the same time when advances in surgery of the temporal bone were being made, attention was

successfully directed to the management of intracranial complications of mastoid infection. Meningitis and subdural abscess were lethal but brain abscess, extradural abscess and lateral sinus thrombosis were conditions that became amenable to surgery. Sir Charles Ballance (1856-1936) wrote about “suppuration in the temporal bone” in his two-volume set *Essays on the Surgery of the Temporal Bone*, published in 1919.

The surgery of brain abscess reached its zenith in 1922 when “*Brain Abscess, its Surgical Pathology and Operative Technique*” written by the American neurosurgeon Wells Phillips Egletion (1865-1946) was published. During the early part of the 20th century, there was some controversy as to whether intracranial pus should be drained via mastoidectomy or separately via craniotomy. In general, these abscesses were drained via the mastoid, the technique being to follow the discharging tract intracranially and to open the abscess cavity with a trocar and cannula.

By the 1930s the essential indications for mastoid surgery had been established. The next stage in the history of mastoid disease and surgery was associated with the radical change in the acute disease brought about by the introduction of antibiotics and the reappraisal of surgical techniques was made possible by the use of operating microscope and the



Figure 2. Julius Lempert (1890-1968)
Slika 2. Žulijus Lemper (1890-1968)

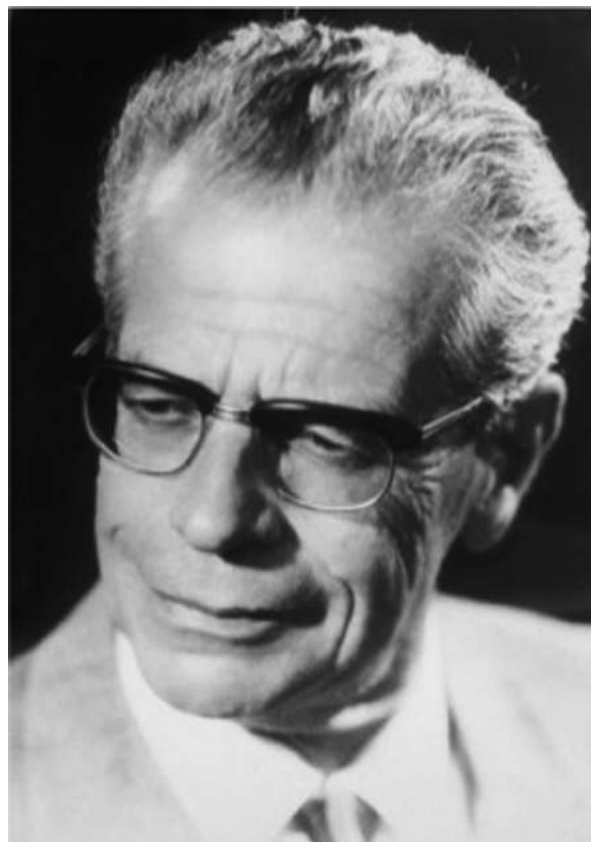


Figure 3. Fritz Zöllner (1901-1986)
Slika 3. Fric Celner (1901-1986)



Figure 4. Horst Wüllstein (1906-1986)
Slika 4. Horst Vilštajn (1906-1986)

general use of electrical drill in the 1950s. The American otologist William F. House [12] (1923-2012) from Los Angeles wrote that it had a revolutionary impact on mastoid surgery. In the 1960s, an air-driven hand piece replaced the conventional, belt-driven one, giving more flexibility in speed and allowing each step of mastoidectomy to be done with different sizes of drills.

Tympanoplasty

The term “tympanoplasty” was first used in 1952 by Horst Wüllstein [13] (1906-1986) from Würzburg to describe surgical reconstructions of the middle ear hearing mechanism, which had been impaired or destroyed by suppurative disease.

It is interesting that the principles of the middle ear sound-pressure transformer mechanism, as described by Hermann von Helmholtz, were not appreciated by those striving to restore hearing throughout the first half of the 20th century. The mechanics of the fenestrated ear remained obscure until the Hungarian Nobel Prize winner Gyorgy von Békésy (1899-1972) and Arthur L. Juers, from Louisville, Kentucky, began to study the problem. Arthur Juers [14] noted in 1948 that the drum membrane of the fenestrated ear had to be intact “to protect the round window somewhat from sound pressure”. In 1950, Hallowell Davis (1896-1992) and Theo E. Walsh [15] from St Louis, Missouri, defined the residue of unrestored conductive loss after successful fenestration as being “due to loss of the



Figure 5. Georges Portmann (1890-1986)
Slika 5. Džordž Portman (1890-1986)

impedance matching mechanism of the drum membrane, ossicular chain and the oval window”. Thus the two basic principles of tympanoplasty were defined, namely “sound protection for the round window” and “sound pressure transformation for the oval window”.

The next stage in the application of these principles came from Germany in 1950, when Walter Rudolf Moritz [16] first described the use of pedicled skin flaps to construct a closed middle ear cavity in cases of chronic suppuration in order to provide shielding of the round window. This idea was quickly taken up by Fritz Zöllner [17, 18] (1901-1986) from Freiburg in 1951 and Horst Wüllstein [19, 20] in 1952. The latter advocated the use of free skin grafts, either from the meatus or from postaural skin and described his famous five types of tympanoplasty. The grafts were used to repair the tympanic membrane and close the tympanum and were positioned as free “onlay” grafts over the membrane remnant or whatever elements of the ossicular chain remained after surgical excision of the disease (types 1-3). If only a mobile footplate remained, this was left exteriorized and the skin graft was positioned so as to create a round window baffle (type 4). If the stapes was fixed by tympanosclerosis or otosclerosis, a fenestration was performed (type 5).

Following the technique introduced by Siegfried Unterberger [21] (1893-1979) from Klagenfurt in 1954, Fritz Zöllner also used fascia lata in 1956 and Simson Hall recommended autologous cheek mu-

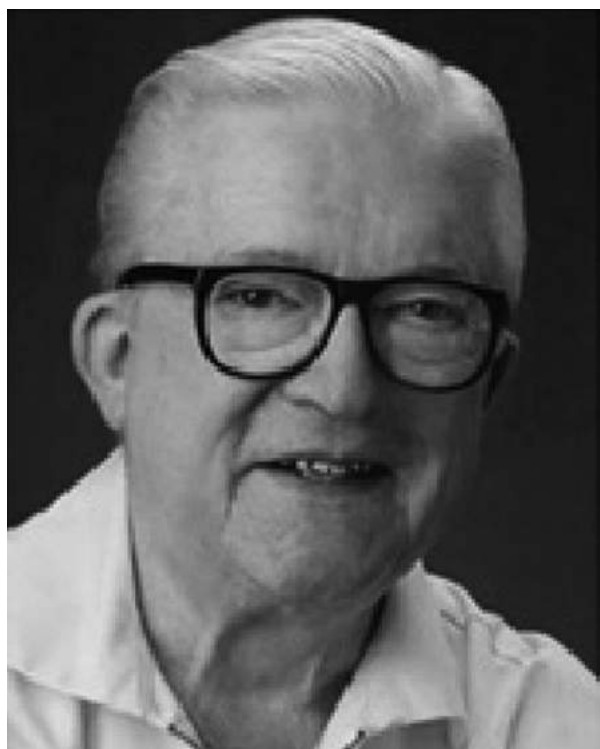


Figure 6. James L. Sheehy (1926-2006)
Slika 6. Džejs Šihi (1926-2006)

cosa in 1958. After the first attempt by Urban Örtengren [22, 23] (1921-1991) from Lund in 1958, Hans Heermann [24] (1900-1996) from Essen popularized the use of temporalis fascia in 1961, as did Lloyd A. **Stoors** from Lubbock, Texas. Claros Domenech from Barcelona and Ettore Bocca [25] (1914-2003) from Milan both introduced autologous periosteum in 1959. John Shea and Harold G. Tabb (1919-2004) from New Orleans described the use of vein graft independently in 1960. John Shea used the “underlay” technique for the first time. Jordan C. Ringenberg from Grand Rapids, Michigan, described the use of ear lobe fat in 1962. Claus Jansen [26] (1921-2001) from Gummersbach used cartilage and perichondrium in 1963. Victor Goodhill [27] (1911-1995) from Los Angeles promoted the use of tragal perichondrium in 1964. The best “take rates” reported were between 70 and 90 per cent and the most widely used grafting substance was temporalis fascia.

The first homograft tympanic membrane was inserted by Ned Chalat in Detroit in 1964 and the “en bloc” homograft tympanic membrane with ossicles was reported by Christian Betow in Berlin in the same year. The technique was popularized by Jean Marquet [28] (1928-1991) in Antwerp.

The reconstruction of the ossicular chain, often named “columellisatio” was close to the reconstruction of the tympanic membrane in tympanoplasties. Many different materials were proposed up to the end of the 20th century. Heinz Utech from Hamburg

used cartilage for ossicular reconstruction in 1960. Carlo Zini from Parma made homograft ossicles from teeth. Homograft ossicles results obtained in the large series were comparable to the use of other more readily obtainable tissues. However, the technique did not gain wide acceptance despite the availability of material through tissue banks created by Michel Portmann and Gay Lacher in Bordeaux, Dietrich Plester (b. 1922) in Tübingen, Gilbert Sedee (b. 1919) in Utrecht, Ugo Fisch in Zurich, Rodney Perkins in Palo Alto and Mansfield F. W. Smith (b. 1929) in San José.

Since the late 1950s attention has been directed towards the use of heterograft nasal cartilage and other implant materials, such as polyethylene, stainless steel wire, Teflon®, proplast and ceramic as a means of reconstructing the ossicular chain. The concept of total ossicular replacement prosthesis (TORP) to produce a columella from the tympanic membrane to the oval window and partial ossicular replacement prosthesis (PORP) from the stapes head to the tympanic membrane were introduced in the early 1970s. In 1971 David F. Austin [29] from Chicago presented non-absorbable plastic sheeting, such as polyethylene, Teflon® and Silastic® to prevent adhesions and to help maintaining of an aerated middle ear. Absorbable substances such as Gelfoam® and Gelfilm® were advocated originally by Horst Wüllstein in 1960.

In the surgical treatment of chronic suppurative otitis media, it became apparent that there was no single operative technique that could be applied in all cases. Each case needed a careful evaluation and decision as to whether the disease could be removed via the ear canal or whether some form of mastoidectomy together with tympanoplasty was required. If a mastoidectomy was considered necessary, two basic surgical techniques evolved: the “canal wall down – open technique” and the “canal wall up – closed technique” procedures. The former is an extension of the modified radical mastoidectomy developed by Gustav Bondy and the latter was introduced initially by Claus Jansen [30, 31] in 1957 and adapted by James L. Sheehy [32] (1926-2006) in Los Angeles in 1958. The canal wall up technique, or combined approach tympanoplasty was aimed at preserving the posterior bony external auditory canal wall and the tympanic sulcus, thus avoiding an exteriorized mastoid cavity. The critical area of the sinus tympani was approached via a “posterior tympanotomy“. In 1971 Gordon Smyth [33] (1929-1992) from Belfast presented a review of the techniques and a greater tendency to stage the operation, thus giving an opportunity to re-explore for residual disease after an interval of time.

This change resulted in a swing towards the canal wall down mastoidectomy with reconstruction techniques. Some large cavities became prone to recurrent infection due to incomplete epithelialization; therefore, cavity obliteration and posterior canal wall reconstruction techniques were redeveloped.

oped to avoid this problem. These techniques included the use of autologous cancellous iliac crest bone grafts, allogeneic femoral cortical bone chips and a variety of pedicled muscle-periosteal transposition and rotation flaps. The outer attic and posterior canal walls were repaired with autologous or allogeneic cartilage, mastoid bone, mastoid bone pate, and ceramic and titanium materials.

Numerous authors report on various methods of reconstruction of the posterior wall of the external auditory canal and its removal.

In 1959, Schüller and Singer performed the reconstruction by using an autologous iliac crest bone graft, and Wullstein used a homograft septal cartilage in 1961. Mahoney and Meuser suggested application of methacrylate in 1962, whereas Marquet and Pulec-Reams applied a homograft of the tympanic canal in 1966 and 1977, respectively. Lapidot described a technique applying muscle-periosteum-bone flap in 1968. Shea and Gardner used femoral head fragments obtained from a bone bank in 1970. Aboulker and Marquet, as well as Perkins performed reconstruction of the posterior wall of the auditory canal by applying an autologous mastoid bone powder in 1975.

The new era in reconstruction of the posterior bone wall of the meatus started in 1979 with S. Wullstein, who first applied bio-materials. Diverse materials have been used, such as porous plastic (*proplast® - plastiport®*, Johns, 1981, ceramics (*ceravital® - hydroxylapatite®*) used by Gersdorff, Lacher, Reck in 1983 and by Grote and Magnan in 1984. In 1990, Robier and Helms and Babighian applied *corail®* and *ionomer®*, respectively. Magnan applied *titane®* for reconstruction of the posterior bone wall of the external auditory canal in 1995.

F. R. Guilford [34] was the first to describe the technique of temporary removal of the posterior

bone wall of the external auditory canal en block, which is then put back into its anatomic place after elimination of pathological process in the mastoid and middle ear. Considering the advantages and disadvantages of open and closed technique tympanoplasty, many otosurgeons have been applying this method. Schnee [35–37] first applied the technique of displacement of the posterior bone wall of the external auditory canal (osteoplastic mastoidectomy) in 1963, 1964 and 1969. Lapidot and Brandow [38], Richards [39] and Lin [40] also applied mobilization and repositioning of the bone canal in 1966, 1972 and 1990, respectively. Temporary removal and subsequent reconstruction of the posterior bone wall of the external auditory canal was applied by numerous authors, such as Gerlach [41] in 1969, Portmann [42] in 1979, S. Wullstein [43–45] in 1972, 1973 and 1974, Feldmann [46, 47] in 1977 and 1978, Tange [48] in 1988, Mercke [49] in 1987 and Babighian [50] in 1992.

Tympanoplasty has been applied in order to eliminate the pathological process in the middle ear at the Department of Ear, Nose and Throat Diseases in Novi Sad for more than fifty-seven years, i.e. since 1957. This method was introduced at our Department by Radivoj Topolac [51]. The combined method, i.e. mobile-bridge tympanoplasty has been applied at our Department since 1998. This technique was introduced by Dragan Dankuc [52, 53]. This method was introduced as a result of increased incidence of recurrent cholesteatoma after closed technique tympanoplasty procedures and it is aimed at eliminating the pathological process in the middle ear and preserving the posterior wall of the external auditory canal. Furthermore, in case of posterior bone wall destruction or injury its reconstruction is performed using autologous mastoid cortical bone and cartilage graft.

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IMMUNOHISTOCHEMICAL EXPRESSION OF P16INK4a IN INFLAMMATORY, PRENEOPLASTIC AND NEOPLASTIC CERVICAL LESIONS

IMUNOHISTOHEMIJSKA EKSPRESIJA p16INK4a U INFLAMACIJSKIM, PRENEOPLASTIČNIM I NEOPLASTIČNIM LEZIJAMA GRLIĆA MATERICE

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Summary

Introduction. High-risk human papilloma viruses play a main role in the development of cervical dysplasias and carcinomas. p16INK4a can be considered as a surrogate marker of active high-risk human papillomavirus infection in dysplastic and neoplastic cells of the cervix. This study was aimed at determining the presence and level of p16INK4a expression in inflammatory, preneoplastic and neoplastic lesions of the cervix. **Material and Methods.** The study was performed on 109 samples of cervical biopsy. Cervical cancer was diagnosed in 36 patients, 34 patients had a preneoplastic change (dysplasia) in stratified squamous cervix epithelium and a nonspecific inflammatory process was found in 39 patients. In all samples, immunohistochemical analysis using antibodies to p16INK4a was performed. **Results.** The expression of p16INK4a was verified in all cases of cervical cancer (100%), in 67.65% of dysplastic cervical lesions and in 38.5% of inflammatory lesions. A statistically highly significant difference was found in the presence and level of expression among neoplastic, dysplastic and inflammatory lesions of the cervix ($\chi^2 = 76.02$, $p < 0.001$). The expression was more frequent and had a higher level in neoplastic and high grade dysplastic lesions compared to expression in inflammatory lesions and low grade dysplasias. **Conclusion.** The analysis of the presence of p16INK4a can differentiate non-neoplastic, high grade preneoplastic and neoplastic changes of the cervix. The use of p16INK4a in interpreting borderline lesions of the cervix can enable a rational therapeutic treatment of patients.

Key words: Squamous Intraepithelial Lesions of the Cervix; Uterine Cervical Neoplasms; Uterine Cervicitis; Immunohistochemistry; Cyclin-Dependent Kinase Inhibitor p16; Papillomaviridae; Papillomavirus Infections; Biological Markers.

Introduction

Cervical cancer is still at the top of the list of oncologic diseases. Morbidity and mortality are significantly reduced by introducing a program using

Sažetak

Uvod. Humani papiloma virusi visokog rizika imaju glavnu ulogu u nastanku displazije i karcinoma cerviksa. p16INK4a se može smatrati „surogat“ markerom prisustva aktivne infekcije humanim papiloma virusima visokog rizika u displastičnim i neoplastičnim ćelijama grlića materice. Cilj istraživanja bio je utvrđivanje prisustva i stepena ekspresije p16INK4a u inflamacijskim, preneoplastičnim i neoplastičnim lezijama grlića materice. **Materijal i metode.** Istraživanje je izvršeno na 109 biopsijskih uzoraka grlića materice. Kod 36 pacijentkinja dijagnostikovano je karcinom grlića materice; kod 34 pacijentkinje je utvrđena preneoplastična promena (displazija) u pločastoslojevitom epitelu grlića materice; a kod 39 utvrđen je nespecifični inflamacijski proces. Na svim uzorcima urađena je imunohistoheмиjska analiza upotrebom antitela na p16INK4a. **Rezultati.** Ekspresija p16INK4a je verifikovana u svim slučajevima kod pacijentkinja sa karcinomom cerviksa (100%); u 67,65% slučajeva u displastičnim lezijama grlića materice i 38,5% slučajeva u inflamacijskim lezijama. Statistički visoko značajna razlika je prisutna u prisustvu i stepenu ekspresije između neoplastičnih, displastičnih i inflamacijskih lezija grlića materice ($\chi^2 = 76,02$, $p < 0,001$). Ekspresija je češća i visokog je stepena u neoplastičnim i displastičnim lezijama, u odnosu na inflamacijske lezije i displazije niskog stepena. **Zaključak.** Analizom prisustva p16INK4a može se diferencirati neoplastična promena od preneoplastičnih promena visokog stepena i neoplastičnih promena na grliću materice. Upotrebom p16INK4a u interpretaciji graničnih lezija na cerviksu omogućava se racionalan terapijski tretman pacijentkinja. **Кljučне речи:** Skvamozne intraepitelijalne lezije cerviksa; Karcinomi grlića materice; Cervicitis; Imunohistohemija; P16INK4a; Papilomaviride; Infekcije papiloma virusom; Biološki markeri

cytological methods for making early diagnosis of changes. The number of newly diagnosed cases in the world is still significant and there are approximately 400,000 cases annually [1].

Abbreviations

HR-HPV	– high-risk human papilloma viruses
CIN	– cervical intraepithelial neoplasia
HPV	– human papilloma viruses
CDK	– cyclin-dependent kinase
RB	– retinoblastoma
PAPA	– Papanicolaou test
HE	– hematoxylin and eosin

The prevalence of preneoplastic lesions of the cervix - cervical intraepithelial neoplasia (CIN) is different and depends on the socioeconomic status, geographical factors, and exposure to risk factors and ranges from 1.05% to 13.7%. CIN is most commonly diagnosed in 20-year old women, carcinoma *in situ* in women aged 25–35 years, and invasive cervical cancer is diagnosed after the age of 40 [2, 3].

Epidemiological risk factors for the development of CIN are similar to those for the development of cervical cancer: multiple sexual partners, early onset of sexual activity, high risk sexual partners, infection with human papilloma viruses (HPV), neoplasm of lower genital tract, previous sexual contacts with persons who had cancer of genital organs, exposure to sexually transmitted diseases, smoking, human immunodeficiency virus (HIV) infection, other forms of immunosuppression, multiparity and long-term use of oral contraceptives [4].

HPVs are the main etiological factor in the occurrence of CIN and cervical cancer. Most of the above mentioned social factors are insignificant in comparison with HPV infection. The analyses have confirmed the presence of HPV in more than 80% of cases in CIN lesions and 99.7% of all invasive cervical cancers. HPV infection is very common and varies depending on the age of the patient.

There are more than 120 types of HPV and half of them cause infections of the anogenital epithelium. Because of their malignant potential, HPVs are classified in low-risk and high-risk categories. In the category of low-risk HPVs, types 6, 11, 42, 43, 44 are present and are associated with genital warts and mild dysplasia (CIN 1). The category of high risk HPVs includes types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82, which are associated with the development of severe grade of dysplasias (CIN 2 and CIN 3) and invasive cervical cancer [5].

Different markers and their expression in dysplastic epithelial cells were examined in cytological and histological materials (Ki 67, p53, Cdc6, Mcm5, MN) [6, 7].

Most studies emphasize the major role of HPV in the development of dysplasia and cervical cancer. High-risk HPV, mostly 16 and 18, have been identified in 99% of cervical cancers. Two viral oncogenes, E6 and E7, are expressed in the HPV infected cells. Products of viral oncogenes are involved in the mechanism of genesis of malignant cells. Oncogenic activity of the products of viral genes is based on the interaction with specific cellular proteins. E6 viral protein causes premature degradation of the p53 tumor suppressor protein. Similarly, E7 binds retino-

blastoma (RB) protein and leads to the release of the E2F transcription factor. Functionally, E7 leads to the inactivation of the RB protein. Inactivation of the RB protein (due to E7 or mutations, deletion of the gene) leads to enhanced phosphorylation and expression of cyclin-dependent kinase (CDK) (CDK4 and CDK6). In a normal cell cycle, the activity of CDK4 and CDK6 is tightly regulated by a number of CDKI, including p16INK4a. If the RB protein is inactivated directly, the affected cells release large amounts of growth inhibitor including CDKI (p16INK4a). The cells proliferate despite high levels of p16INK4a. The nature of expression of P16INK4a represents a negative feedback control of RB protein loss. Thus, the loss of function of RB protein results in increased p16INK4a. Inactivation of the RB protein with E7 viral protein leads to increased expression of p16INK4a, which can be a sensitive and significant biomarker of active expression of HPV oncogenes. Expression of p16INK4a may be considered as a marker of viral E7 protein activity in the cell. Therefore, p16INK4a may be considered as a “surrogate” marker for the presence of active high-risk human papilloma viruses (HR-HPV) infection in dysplastic and neoplastic cells of the cervix [1, 8–10].

The importance of p16INK4a expression in cervical tissue samples has been shown in many studies [11–13]. In cytology, the analysis of p16INK4a expression is done to assess the benefits in terms of triage of patients whose findings on routine cytology are interpreted as boundary cases. In most studies, the sensitivity is similar to HPV testing, but there is a significantly higher rate of specificity of p16INK4a expression in cytologic materials [13–15].

The aim of this study was to determine the presence of p16INK4a expression in inflammatory, preneoplastic (dysplasias) and neoplastic lesions of the cervix, and to determine whether the presence and level of expression of p16INK4a depend on the type of pathological processes in the cervix, the grade of dysplasia of the cervical epithelium and histological type and the grade of invasive cervical cancer.

Material and Methods

The study was performed on biopsy specimens of patients who were biopted due to pathological Papanicolaou (PAPA) test or colposcopic findings at the Department of Gynecology and Obstetrics, Clinical Center of Banja Luka.

The patients' age was taken from the outpatients' protocols and referrals for histopathologic analysis. Analysis of biopsy tissue samples was performed at the Department of Pathology, Clinical Center of Banja Luka. The material obtained during biopsy was fixed in 10% formalin. After the routine processing, paraffin tissue blocks were made and cut on a microtome in serial sections and stained with hematoxylin and eosin (HE) staining method. The analysis was performed by a binocular micros-

cope *Leica* (objectives 10x, 25x, 35x, 40x; 10x eye-pieces) with the width of the visual field of 1.4 mm.

The nature of changes in the mucosa of vaginal portions was determined (mucosa without changes, inflammation, dysplasia in stratified squamous epithelium, invasive carcinoma of the cervix) for all patients. The classification was made according to the recommendations of the World Health Organization (WHO) [16].

Changes were classified into three groups:

- group I - 36 bioptic samples - in which histological analysis revealed the diagnosis of a malignant epithelial tumor of the cervix. Cytological diagnosis and biopsy of changes localized in the cervix were used in the diagnostic procedure before surgical intervention. After the diagnosis had been confirmed and clinical stage of disease determined, the patients underwent surgery. After surgery, histopathological analysis of the removed material was performed, and definitive diagnosis and pathological stage of the disease were determined.

- group II - 34 bioptic samples - in which the histological analysis confirmed the diagnosis of preneoplastic lesions (dysplasia) in the epithelium of the cervix.

- group III - 39 bioptic samples - in which histological analysis revealed the diagnosis of inflammatory lesions in the cervical epithelium (non-specific inflammation).

In all patients in group I, the following morphological details were defined: type of material in which the definitive histological diagnosis was determined, histological type of the tumor, tumor grade according to the International Federation of Gynecology and Obstetrics (FIGO), depth of stromal invasion and the level of expression of p16^{INK4a}.

The type and grade of dysplasia were determined in all subjects of group II.

The presence and type of benign lesions in the mucous membrane of the cervix were determined in all subjects of group III.

In all samples (from group I, II and III), immunohistochemical analysis was performed using antibody to p16^{INK4a} (Dako, Denmark).

The anti-human p16^{INK4a} monoclonal antibody (clone E6 mtm laboratories AG, Heidelberg, Germany) was used for immunohistochemical analysis. The tissue samples were cut in the microtome (*Leica* 2000) to the thickness of 2-4 microns and subsequently deparaffinized. Antigen unmasking was performed in a citrate buffer, 0.007M for 40 minutes at 97 degrees Centigrade, pH 6.0. Unblocking was done with 1.5% hydrogen peroxidase for 15 minutes. The slides were stained with primary monoclonal antibody (1:1000 overnight at 4 degrees Centigrade), and visualization was performed with avidin-biotin kit (Ultra Vision DetectionSystem).

The presence of p16^{INK4a} expression was assessed in the following way:

The level of p16^{INK4a} expression was evaluated semiquantitatively according to the percentage of

epithelial cells showing the expression. Positive reaction involves intensive nuclear and cytoplasmic immunoreaction (coloration).

The level of expression was evaluated to be:

Score 0- <1% of positive epithelial cells;

Score 1- 1-5 % of positive epithelial cells;

Score 2- 5-25 % of positive epithelial cells;

Score 3- >25% of positive epithelial cells.

Statistical analysis was performed using SPSS, version 17.0. The descriptive statistical analysis (average value) was used to describe the overall sample as well as individual groups. The differences between the groups were analyzed by using the following tests: Chi square, Wilcoxon signed-rank test and Mann Whitney test.

Results

Our study included bioptic material of 109 patients, who had been operated at the Clinical Center of Banja Luka, Department of Obstetrics and Gynecology. The analysis was done at the Department of Pathology and the definitive histopathological diagnosis was established. The study was performed during the period from January 2006 to December 2013.

The first group consisted of materials of 36 patients, who were operated due to invasive squamous cell cervical carcinoma.

The second group consisted of materials of 34 patients who were diagnosed with preneoplastic lesion in cervical samples.

The third group consisted of materials of 39 patients in whom morphologically nonspecific inflammation in the samples of the cervix was diagnosed.

The youngest patient was 23 years old and the oldest 86 years old.

The average age of all patients was 50 years. The youngest patients were in group II and group I, whose average age was 37 and 50 years, respectively. The average age in group III was 61 years (**Table 1**).

The patients in group III were the oldest and they had the diagnosis of nonspecific inflammation. The patients in group III were operated mainly due to the prolapse of uterus, which is most commonly diagnosed in postmenopausal women, thus explaining the age structure of this group of patients. The average age of patients in group II was 37 years and they were diagnosed to have dysplasia of the squamous epithelium of the vaginal portion. The average age of patients in group I, with the diagnosis of squamous cell carcinoma, was 50.78 years.

Mann-Whitney U test was used to determine whether there was a difference in the age of patients having the confirmed diagnosis of invasive squamous cell cervical cancer and preneoplastic lesions. The test showed a statistically significant difference, being $U = 214.00$, $p < 0.005$.

The average age of patients with invasive squamous cell carcinoma of the cervix was higher (46.56 years) compared to the patients with preneoplastic lesion (23.79 years).

Table 1. Characteristics of patients
Tabela 1. Karakteristike pacijentkinja

Characteristics of patients <i>Karakteristike pacijentkinja</i>		Group I <i>Grupa I</i>	Group II <i>Grupa II</i>	Group III <i>Grupa III</i>
Number (n)/Broj (n)		36	34	39
The average age <i>Prosečna starost</i>		50	37	61
Type of the material <i>Vrsta materijala</i>	Uterus/ <i>Uterus</i>	33 (91.67%)	/	39 (100%)
	Conical section <i>Koničan isečak</i>	3 (8.33%)	34 (100%)	/
Histological diagnosis <i>Histološka dijagnoza</i>	Squamous cell carcinoma/ <i>Skvamozni karcinom</i>	35 (97.22%)	Dysplasia in squamous epithelium/ <i>Displazija u skvamoznom epitelu</i>	Chronic non specific inflammation/ <i>Hronično zapaljenje</i>
	Adenosquamous carcinoma <i>Adenoskvamozni karcinom</i>	1 (2.78%)	34 (100%)	39 (100%)
Grade/ <i>Stepen</i>	I	2 (5.56%)	7 (20.59%)	/
	II	27 (75%)	10 (29.41%)	/
	III	7 (19.44%)	17 (50%)	/
Average depth of invasion <i>Prosečna dubina invazija</i>		14.43 mm (range 2 – 66 mm/ <i>raspon 2 - 66 mm</i>)	/	/
Expression of p16INK4a <i>Ekspresija p16INK4a</i>		36 (100%)	23 (67.65%)	15 (38.46%)

In most patients in group I, the uterus, as surgical material, was used for the analysis in 33 (91.67%) cases, whereas a conical section of vaginal portion was used in only 3 (8.33%) patients. In all patients of group II (n = 34), surgical material on which the analysis was done was a conical section of the vaginal portion of the cervix. In all 39 patients in group III, surgical material for the analysis was the uterus (**Table 1**).

Squamous cell carcinoma was diagnosed in 35 (97.22%) patients from group I, and only one case (2.78%) of adenosquamous carcinoma was found. As for group II, the diagnosis of severe grade of dysplasia of squamous epithelium (CIN 3) was made in 17 (50%) patients, a moderate dysplasia of squamous epithelium (CIN 2) and mild dysplasia of squamous epithelium (CIN 1) was found in 10 (29.41%) and 7 (20.59%) cases, respectively. Histological diagnosis of chronic nonspecific inflammation of the cervix was made in all 39 (100%) patients from group III (**Table 1**).

The tumors in samples of group I were most frequently moderately differentiated. A medium grade of differentiation was present in 27 (75%) patients. Grade III was verified in 7 (19.44%) patients, while grade I was seen in only 2 (5.56%) cases (**Table 1**).

The depth of invasion in the analyzed material ranged from 2 mm to 66 mm. The average depth of invasion in all patients in group I was 14.43 mm. Most of the patients, i.e. 32 (88.9%), had invasion of cervical stroma with cancer tissue larger than 5 mm (**Table 1**).

Expression of p16INK4a in materials of group I was present in 36 (100%) cases. In all samples, diffuse cytoplasmic and nuclear positivity (high level of expression) was determined, more than 25% of tumor cells were positive (**Figure 1**). A diffuse, high expression was found, which was semiquantitatively graded as score 3 in all samples. The level of expression in this group did not depend on the histological type and grade of the tumor. Expression of p16INK4a in the materials of group II was present in 23 (67.65%) cases. The expression ranged from focal, low expression which was semiquantitatively graded as 1 in 5 (14.7%) cases to high expression which was graded as 3 in 16 (47.06%) cases. Expression was not present in 11 (32.35%) cases (**Table 1, Figure 1**). Expression of p16INK4a in the materials of group III was present in 15 (38.46%) cases. Expression had a low grade in 13 (33.33%) cases, whereas medium grade was present only in 2 (5.13%) cases. Expression of p16INK4a was not observed in 24 (61.54%) cases (**Table 1, Figure 2**).

The analysis showed that high expression of p16INK4a was verified in materials diagnosed to have a severe grade dysplasia of squamous cervical epithelium or CIN 3. The absence or low expression was associated with a low or moderate grade of dysplasia, or CIN 1 and CIN 2 (**Table 2**).

There was a statistically significant difference in the presence and level of expression of p16INK4a among the patients from group II with different grades of dysplasia in squamous epithelium. Expression of p16INK4a was rarely present in materials of group II

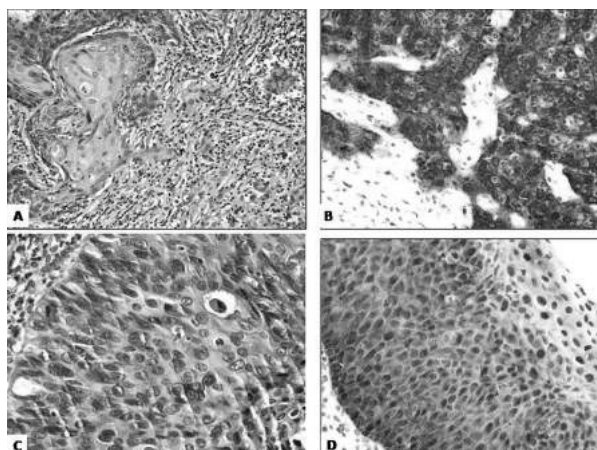


Figure 1. A. Squamous cell carcinoma, HE x 200; B. Squamous cell carcinoma, high level of expression of p16INK4a, anti-p16 x 200; C. Dysplasia in the squamous epithelium of the cervix of high grade (CIN 3), HE x 200; D. Dysplasia in the squamous epithelium of the cervix of high grade (CIN 3), high level of expression of p16INK4a anti-p16 x 200.

Slika 1. A. Carcinoma squamosum, HE x 200; B. Carcinoma squamosum cervicis - visok stepen ekspresije p16INK4a, anti p16 x 200; C. Displazija u skvamoznom epitelu cerviksa visokog stepena (CIN 3), HE x 200. D. Displazija u skvamoznom epitelu visokog stepena (CIN 3) - visok stepen ekspresije p16INK4a u displastičnom epitelu grlića materice, anti-p16 x 200.

with a low grade of dysplasia (CIN 1), and if it was present, it had a lower level in the patients who were diagnosed with moderate and severe grade of dysplasia (CIN 2 and CIN 3), $\chi^2 (3, N = 34) = 11.09, p < 0.05$ (Table 2).

There was a difference in the presence and level of expression of p16INK4a among three examined groups, $\chi^2 (6, N = 109) = 76.02, p < .001$. The patients with neoplastic and preneoplastic changes had expres-

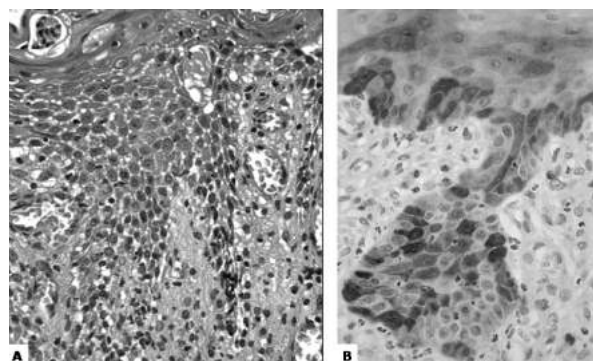


Figure 2. A. Nonspecific chronic cervicitis, HE x 200; B. Nonspecific chronic cervicitis, the image of expression of p16INK4a (score 2), anti p16 x 200.

Slika 2. A. Cervicitis chronica non specifica, HE x 200; B. Cervicitis chronica non specifica - prikaz ekspresije p16INK4a (skor 2), anti p16 x 200.

sion of p16INK4a more frequently and more intensely than the patients with nonspecific inflammation. The difference in the presence and level of expression of p16INK4a was found between groups I and II, $\chi^2 (3, N = 70) = 25.65, p < .001$. The patients with preneoplastic lesions rarely had p16INK4a expression, and the level of expression was lower in the patients with invasive cervical squamous cell carcinoma (Table 2).

By examining the differences in the level of expression of p16INK4a between group I and group II, with the grade of dysplasia CIN 1, a statistically significant difference, $\chi^2 (2, N = 43) = 43.00, p < .001$ was found. The patients with preneoplastic lesion and grade of dysplasia CIN 1 had a lower level of expression of p16INK4a than those patients with invasive squamous cell carcinoma of the cervix (Table 2).

There was a statistically significant difference in the level of expression of p16INK4a, $\chi^2 (1, N = 63) = 14.00, p < .001$ between group I and II, with grades of dysplasia CIN 2 and CIN 3. The patients with

Table 2. Level of expression of p16INK4a in samples in groups I, II and III

Tabela 2. Stepen ekspresije p16INK4a u uzorcima grupe I, II i III

		Level of expression of p16INK4a/Stepen ekspresije p16INK4a				Total/Ukupno
		0	1	2	3	
Group I %	N	0	0	0	36	36
Grupa I %	.0%	.0%	.0%	100.0%	100.0%	
CIN 1	N	4	3	0	0	7
	%	57.1%	42.9%	.0%	.0%	100.0%
Group II Grupa II	CIN 2	N	6	2	2	11
	%	54.5%	18.2%	18.2%	9.1%	100.0%
CIN 3	N	1	0	0	15	16
	%	6.3%	.0%	.0%	93.8%	100.0%
Total % Ukupno %	N	11	5	2	16	34
	32.4%	14.7%	5.9%	47%	100.0%	
Group III Grupa III	N	24	13	2	0	39
	61.5%	33.3%	5.2%	.0%	100.0%	

preneoplastic lesion and grade of dysplasia CIN 2 and CIN 3 had a lower level of expression of p16INK4a than patients with invasive squamous cell carcinoma of the cervix (**Table 2**).

No significant difference in the presence and level of expression of p16INK4a, $\chi^2(1, N=53) = 1.16$, $p < .142$ was found between group I and II with the grade of dysplasia CIN 3 (**Table 2**).

There was a statistically significant difference in the level of expression of p16INK4a, $\chi^2(3, N=75) = 75.00$, $p < .001$ between group I and III. The patients with invasive squamous cell carcinoma of the cervix had a higher level of expression of p16INK4a compared to the patients with nonspecific inflammation (**Table 2**).

A statistically significant difference in the presence and level of expression of p16INK4a, $\chi^2(3, N=73) = 24.16$, $p < .001$ was found between groups II and III. The patients with nonspecific inflammation rarely had p16INK4a expression, and the expression level was lower than in patients with preneoplastic lesions (**Table 2**).

No statistically significant difference, $\chi^2(1, N=46) = 0.37$, $p = .54$ in the level of expression of p16INK4a was found between group III and group II, who were diagnosed with a low grade of dysplasia (CIN 1) (**Table 2**).

A statistically significant difference, $\chi^2(3, N = 66) = 32.27$, $p < .001$, was found in the level of expression of p16INK4a between group III and group II (CIN 2 and CIN 3). The patients with nonspecific inflammation had a lower level of expression of p16INK4a compared with patients with preneoplastic lesion and grade of dysplasia CIN 2 and CIN 3 (**Table 2**).

Discussion

Cervical cancer incidence has remained unchanged for years in many poor countries without organized screening programs, while in developed countries, including the United States, the number of cases and deaths is constantly decreasing, now being below 10 per 100 000 women. This decrease in mortality and morbidity rate of this cancer in the second half of the last century can be explained by the organized screening program in most developed countries [1, 16]. The incidence of this cancer in the Republic of Srpska is 19.1 according to the data of the Institute of Public Health [17]. The incidence of cervical cancer ranges from 4 to 14 per 100 000 women in developed countries, where the screening program is developed and funded by the state, while the incidence of this disease is up to 10 times higher in developing countries.

Nevertheless, there are difficulties due to the costs associated with screening programs, limited accuracy of cytology and complications associated with unnecessary treatments, which prompted the research and development of new, more effective preventive tests for the diagnosis of changes that precede cervical cancer. During the last two dec-

ades there has been a considerable progress in understanding HPV infection and advances in molecular technology (diagnostics). New methods (techniques) have been developed and used in screening, alone or together with cytological techniques. Some of these tests are HPV deoxyribonucleic acid (DNA) screening tests that have been used successfully for triage and are more reliable than cytological techniques [18].

Morphological interpretation of lesions in the cervix is not completely reliable. The lack of morphological interpretations is obvious at borderline lesions (in cytology and histology), where the differences are significant. It is often impossible to predict the further evolution with morphological interpretation [14, 15].

Klaes et al. have developed an analysis of interpreting bioptic samples in HE treated samples, followed by interpretation of samples treated with HE and p16INK4a. The following diagnoses were determined on cervical samples: normal findings (without dysplasia - non-dysplastic lesions/NDL/), cervical intraepithelial neoplasia (CIN 1, CIN 2, CIN 3) and cervical cancer. One of the pathologists who analyzed the material frequently used the NDL category (15%). Two pathologists rarely used the diagnosis of CIN 3 (<20%), and they used diagnosis of CIN 2 more frequently than other pathologists. The concordance in diagnosing invasive carcinoma was high (94%), while the concordance in interpreting CIN lesions ranged from 35% (CIN 2) to 72% (CIN3) on materials processed only with HE method [19]. The application of immunohistochemical techniques and p16INK4a decreased the difference in interpretation between pathologists. The concordance in all categories was above 90%. They concluded that the use of p16INK4a reduced the difference in interpreting lesions of the cervix between pathologists [19]. Similar results were reached by other authors [20–22].

New biomarker, p16INK4a, is an inhibitor of cyclin-dependent kinases, whose increased expression is present in cancer and precancerous lesions of the cervix. The increased presence of p16INK4a can be demonstrated in different ways: by immunocytochemical and immunohistochemical methods and the enzyme-linked immunosorbent assay (ELISA) [1, 5, 23]. The use of liquid cytology together with immunocytochemical examination of the presence of p16INK4a expression is very effective in detecting precancerous and cancerous lesions of the cervix [11, 24]. The sensitivity ranged from 0.59 to 0.96 and specificity between 0.41 and 0.96 in detecting dysplastic lesions [25, 26].

The expression of p16INK4a in the materials of the group I was present in all 36 (100%) samples. In all samples, a high level of expression (more than 25% of tumor cells positive) was found. The expression was high, cytoplasmic and nuclear.

Sano et al. demonstrated the expression of p16INK4a in all carcinomas of the cervix, and it

was high in 97% of cases. Expression of p16INK4a in CIN lesions ranged from 53% in CIN 1 lesions up to 100% in CIN 3 lesions [27].

In our study, expression of p16INK4a was present in 67.65% of patients with CIN lesions. It should also be noted that expression was not present in most cases of CIN 1 lesions (57.14%), and, in cases where it was verified, the expression had low level, i.e. expression in <5% of positive epithelial cells (42.86%). A similar expression of p16INK4a was found in CIN 2 lesions; it was absent in 60% of cases, low expression was found in 20% of cases, and a moderate level of expression (5 to 25% of the cells showed expression) was seen in 20% of cases. Expression of p16INK4a in CIN 3 lesions was verified in 94.12% of cases. Expression was not observed in one case only. Our results differ from the results reached by Sano et al., who have verified the expression of p16INK4a in a high percentage of CIN 1 (54% of samples) and CIN 2 (94% of samples) [28]. Our results are consistent with the results of Izadi-Mood et al. [1].

Statistical testing found that there was a statistically significant difference in the expression of p16INK4a in relation to the grade of dysplasia. There was a significant difference in expression of p16INK4a in the patients who were diagnosed with cancer in relation to all dysplastic lesions (considered together). The difference in expression was not present only when groups with cancer and severe grade dysplasias were compared. In our study, a statistically significant difference was found in expression and the level of expression of p16INK4a between the patients with the diagnosis of inflammation (cervicitis) and the patients who were diagnosed with invasive cancer or dysplasia. In addition, a statistically significant difference was not found in expression of p16INK4a among the patients with established inflammation and those who had a low grade of dysplasia or CIN I. The results are similar to those found in the work of Agoff et al. They proved that the level of expression of p16INK4a and Ki-67 highly correlated with the grade of dysplasia in the cervical epithelium and HR-HPV infection [28].

Thus, for lesions which were in cytological materials interpreted as atypical squamous cells of undetermined significance (ASC-US), those lesions interpreted as atypical squamous cells when it is not possible to exclude high-grade squamous intraepithelial lesion (ASC-H) and squamous intraepithelial lesions of low grade (L-SIL) should also be processed with immunocytochemical techniques, and the level of expression of p16INK4a should be assessed. Lesions morphologically interpreted as a low (CIN

1) and moderate (CIN 2) grade of dysplasia should be processed by immunohistochemical techniques in histological materials as well, and the level of expression of p16INK4a should be assessed before planning any treatment of the patient [10].

Our study can contribute to a more reliable differentiation of "borderline cases" of lesions in the cervical epithelium, both on cytological and histological materials. The application of immunocytochemical and/or immunohistochemical analysis with an antibody to p16INK4a can help to differentiate lesions which are cytologically interpreted as atypical squamous cells of undetermined significance or atypical squamous cells and histologically interpreted as preneoplastic lesions (CIN 1, CIN 2). In addition, false diagnosis of reactive changes and their classification as preneoplastic lesions in histological materials can be prevented. Unnecessary treatment (usually surgical) can also be prevented. And vice versa, it will help to prevent classification of dysplastic lesions as non dysplastic lesions, and therefore enable timely and appropriate treatment [10].

Our study, as well as many others, has proved that the determination of expression of p16INK4a is a sensitive and specific method for identification of dysplastic and tumor cells in histological samples.

Conclusion

Expression of p16INK4a in inflammatory lesions of the cervix is rarely present; it is focal and has a low intensity.

Expression of p16INK4a in preneoplastic lesions of the cervix is often present. The presence and level of expression of p16INK4a depend on the grade of dysplasia. Expression of p16INK4a in severe grade of dysplasia is present, diffuse and has a high intensity, while in low grade dysplasia it is rarely present, focal and has a low intensity.

Expression of p16INK4a in neoplastic lesions of the cervix is present, diffuse and has a high intensity.

Expression of p16INK4a in neoplastic cervical lesions does not depend on the histological type and grade of the tumor.

Inflammatory changes may be differentiated from preneoplastic changes of severe grade and neoplastic changes in the cervix by analyzing the presence of p16INK4a.

The use of p16INK4a in interpreting borderline lesions on the cervix allows adequate therapeutic treatment of patients.

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ANALYSIS OF DIFFERENCES IN BLOOD PRESSURE OF WOMEN BELONGING TO DIFFERENT AGE GROUPS

ANALIZA RAZLIKA U KRVNOM PRITISKU KOD ŽENA IZ RAZLIČITIH STAROSNIH GRUPA

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Summary

Introduction. One of the risk factors for the occurrence of arteriosclerosis and coronary heart diseases is physical inactivity. Together with hypokinesia, excessive feeding, age and other factors, make a multifactorial cause of cardiovascular disease. Positive effects of physical activities have been proved in the primary, secondary and tertiary prevention of coronary heart diseases. **Material and Methods.** This study included 119 women from 20 to 76 years of age. All subjects were nonsmokers who did not have a cardiovascular disease, and were divided into five different age groups. Systolic and diastolic blood pressure was measured by the digital blood pressure measuring device with cuff OMRON M4-1. The evaluation of blood pressure was performed at the Faculty of Sport and Physical Education in Novi Sad. The data processing was done by the statistical package SPSS 20.0. **Results.** According to the obtained data it can be concluded that there are statistically significant differences in both individual and general system of the observed variables in different age groups. In addition, there are statistically significant differences between pairs of groups, which were observed when comparing with the oldest age group. **Conclusion.** The programmes of prevention and control of cardiovascular diseases should decrease the influence of risk factors and improve diagnostics and therapy of cardiovascular diseases.

Key words: Women's Health; Age Factors; Blood Pressure; Hypertension; Cardiovascular Disases; Risk Factors

Introduction

One of the risk factors for the occurrence of arteriosclerosis and coronary heart diseases is physical inactivity, as well as hypokinesia, overeating, age and other factors. Positive effects of physical activities have been proved in the primary, secondary and tertiary prevention of coronary heart diseases. It has also been accepted that physical activity is a significant factor in preservation of normal physical and mental health. Meyer et al. have shown that those who are in good shape have a twice lower risk of getting a cardiovascular disease even in the presence of main risk factors, such as hypertension, diabetes, smoking, etc. In addition, there has been

Sažetak

Uvod. Jedan od faktora rizika za nastanak ateroskleroze i srčanih oboljenja je fizička neaktivnost. Zajedno sa hipokinezijom, prekomernom ishranom, starosti i drugim faktorima, čine multifaktorijalni uzrok kardiovaskularnih bolesti. Pozitivni efekti fizičke aktivnosti su dokazani u primarnoj, sekundarnoj i tercijarnoj prevenciji srčanih oboljenja. **Materijal i metode.** Ova studija je obuhvatila 119 žena starosti od 20 do 76 godina. Sve ispitanice su bile nepušači i nisu imale kardiovaskularna oboljenja. Podeljene su u pet različitih starosnih grupa. Sistolni i dijastolni krvni pritisak je meren digitalnom uređajem sa manžetnom OMRON M4-1. Merenje krvnog pritiska je izvedeno na Fakultetu sporta i fizičkog vaspitanja u Novom Sadu. Obrada podataka urađena je statističkim paketom SPSS 20.0. **Rezultati.** Prema dobijenim podacima može se primetiti da postoje statistički značajne razlike u individualnom i grupnom sistemu posmatranih varijabli kod različitih starosnih grupa. Takođe postoje statistički značajne razlike između parova grupa, gde smo zabeležili primetne razlike prilikom poređenja sa najstarijom starosnom grupom. **Zaključak.** Programi prevencije i kontrole kardiovaskularnih bolesti treba da smanje uticaj faktora rizika i unaprede dijagnostiku i terapiju kardiovaskularnih bolesti.

Glavne reči: Zdravlje žene; Starosna dob; Krvni pritisak; Hipertenzija; Kardiovaskularna oboljenja; Faktori rizika

a significant decrease of people's physical activity in the modern civilization [1]. The trend of sedentary life-style has resulted from the occurrence and improvement of technological developments. The necessity to use physical force while performing various tasks tends to disappear very quickly and human work is being replaced by machines and new technological achievements, so people's energy loss during a working day has been reduced to its minimum. Such inactive lifestyle has added to the development of a range of associated diseases which mostly include chronic diseases of locomotor system and deterioration of the general body resistance, especially the cardio-respiratory system. One of the leading diseases of such inactive lifestyle is in-

Table 1. Multivariate and univariate analyses of variance for the differences in blood pressure.**Tabela 1.** Multivarijantne i univarijantne analize varijanse za razlike u krvnom pritisku

Variable/Varijabila	Groups/Grupe	N	AM	SD	f	p
SBP	20-29	16	107.18	8.93	5,96	0.000
	30-39	23	107.17	11.16		
	40-49	29	112.41	13.60		
	50-59	28	110.17	15.24		
	60+	23	125.86	19.86		
DBP	20-29	16	69.37	7.50	3.22	0.015
	30-39	23	71.08	9.64		
	40-49	29	70.17	7.13		
	50-59	28	73.21	10.38		
	60+	23	78.04	9.01		
		F=3.81			P=0.000	

Legend: SBP – Systolic blood pressure, DBP – Diastolic blood pressure, AM – arithmetic mean, SD- standard deviation, f – the value of f-test of the univariate analysis of the variance, p – the level of significance of the univariate analysis, F- the value of f-test of multivariate analysis of variance P- the level of significance of the multivariate analysis.

Legenda: SBP – sistolni krvni pritisak, DBP – dijastolni krvni pritisak, AM – aritmetrička sredina, SD – standardna devijacija, f – vrednost f-testa univarijantne analize varijanse, p – nivo signifikantnosti univarijantne analize, F – vrednost f-testa multivarijantne analize varijanse, P – nivo signifikantnosti multivarijantne analize

creased blood pressure. High blood pressure or hypertension increases the pressure on circulatory and excretion organs, simultaneously increasing the risk of heart attack, stroke and kidney diseases [2]. In some instances, high blood pressure causes a

headache, ear tingling, dizziness, feeling of tension in chests and short breath, nausea, sight obstructions. However, the largest numbers of people with hypertension have no symptoms at all and do not know that they have high blood pressure since they

Table 2. T-test for independent samples of systolic and diastolic blood pressure between the pairs of all age groups.**Tabela 2.** T-test za nezavisne uzorke sistolnog i dijastolnog krvnog pritiska između parova svih starosnih grupa

Variable/Varijabila	Pairs/Parovi	t	p
Systolic B.P./Sistolni krvni pritisak	20-29 – 30-39	0,00	0,997
	20-29 – 40-49	-1,37	0,175
	20-29 – 50-59	-0,31	0,478
	20-29 – 60+	-3,40	0,002
	30-39 – 40-49	-1,49	0,142
	30-39 – 50-59	0,78	0,435
	30-39 – 60+	-3,76	0,001
	40-49 – 50-59	0,38	0,561
	40-49 – 60+	-2,64	0,012
	50-59 – 60+	-2,98	0,005
Diastolic B.P./Dijastolni krvni pritisak	20-29 – 30-39	-0,59	0,556
	20-29 – 40-49	0,35	0,726
	20-29 – 50-59	-1,29	0,002
	20-29 – 60+	-3,05	0,004
	30-39 – 40-49	0,39	0,696
	30-39 – 50-59	-0,75	0,456
	30-39 – 60+	-2,44	0,019
	40-49 – 50-59	-1,28	0,205
	40-49 – 60+	-3,28	0,002
	50-59 – 60+	-1,68	0,099

Legend: t – value of t-test, p – the level of significance of t-test

Legenda: t – vrednost t-testa, p – nivo signifikantnosti t-testa

Table 3. Categorization of patients according to the values of their blood pressure (based on European Society for Hypertension and European Association of Cardiologists, 2007)*Tabela 3.* Kategorizacija pacijenata prema vrednostima njihovog krvnog pritiska (na osnovu Evropskog društva za hipertenziju i Evropske asocijacije kardiologa, 2007)

Category/Kategorija	Systolic (mmHg)/Sistolni (mmHg)	Diastolic (mmHg)/Dijastolni (mmHg)
Optimal/Optimalne	< 120	< 80
Normal/Normalne	120 – 129	80 – 84
Increased/Povišene	130 – 139	85 – 89
Level 1 hypertension/Hipertenzija 1. nivoa	140 – 159	90 – 99
Level 2 hypertension/Hipertenzija 2. nivoa	160 – 179	100 – 109
Level 3 hypertension/Hipertenzija 3. nivoa	≥ 180	≥ 110

do not have regular medical check-ups. Besides, the majority of people do not know that hypertension can be successfully controlled by having a healthy lifestyle [3].

Cardiovascular diseases are the leading cause of premature deaths in Serbia. During 2008, heart and blood vessel diseases accounted for over half of all causes of death (55.8%); 57,343 people died, while this group of diseases occurred more often in women (54.9%) than in men (45.1%). In the period from 2002 to 2008, death rates for this disease increased by 5.5% in women and 0.2% in men. Risk factors for the occurrence of this disease are present in a high percentage in all citizens of Serbia. In addition, blood pressure is increased in 46.5% of adults. It is important to mention the fact that 67.7% of citizens of Serbia are inactive, which is very disturbing. There are over 4,500 cardiac interventions, while the number of interventions on blood vessels is significantly higher [4]. The aim of this study is to determine whether there are differences between different categories of females in blood pressure and to emphasize the importance of aging and its negative impact on cardiovascular system.

Material and Methods

The study sample consisted of 119 women from Novi Sad, aged 20-76. Regarding their education, 93 had high school certificate, 21 graduated from a faculty and 5 completed postgraduate studies (master or doctor of science and the majority of them were employed). Of 119 women, 75 had one or more births and 43 women were in menopause. The patients included in the study sample did not have a cardiovascular disease, and were not active smokers. The data obtained were used for the scientific research project called "The Anthropological Status and Physical Activity of the Citizens of Vojvodina" which was carried out by the Faculty of Sport and Physical Education in Novi Sad and financed by the Regional Secretariat for Science and Technological Development.

Systolic and diastolic blood pressure was measured with the digital blood pressure measuring device with cuff OMRON M4-1 (Omron Healthcare Europe BV, the Netherlands). The cuff was placed

firmly on the upper arm 2-3 cm above the elbow. During the measurement, the subjects were sitting for 15 minutes. Blood pressure was taken three times. The values of blood pressure varied at each measurement, being highest at the first measurement, and lowest at the third one. Since the measured values did not differ for more than 4.5 mmHg for diastolic pressure, the mean value was the average of all three measurements, not the average of the second and third measurement, as recommended by the World Health Organization (WHO, 2005) in order to increase the level of freedom for the mean values. The results are given in mmHg.

The evaluation of blood pressure was performed at the Faculty of Sport and Physical Education in Novi Sad. During the process of testing, all rules were followed. Transversal measurement was also performed.

The univariate and multivariate analyses of variance were applied in order to show the differences on the quantitative level. For all statistical analyses, the level of statistical significance was 0.05. The data processing was done by the statistical package „SPSS 20.0“.

Results

The obtained data show that in the sample of women belonging to various age groups there were statistically significant differences in both individual and general system of the observed variables (**Table 1**).

Besides, there are statistically significant differences between pairs of groups, which can be observed when comparing with the oldest age group (**Table 2**).

If the values of arithmetic means of systolic and diastolic blood pressure from the first four age groups are taken into account and compared with the values from the **Table 3** [5], it can be noticed that they belong to the category of optimal blood pressure, while the fifth group (aged 60+) has normal blood pressure. It is also important to say that differences between the pairs of groups only occurred when comparing younger groups with the oldest groups, 50-59 and 60+. The differences in the obtained values can be explained by a number of rea-

sons, such as inactivity, the type of regular diet, social status and many more.

Discussion

Hypertension is a disease with increased risk in older and obese population [6, 7]. The fact that physical activities can normalize blood pressure was confirmed in the research [8] where the decreased values in the final measurement after the experimental treatment were the confirmation that the exercising by using Nordic poles influenced the stability of heart rate during inactivity, as well as systolic and diastolic blood pressure.

According to Czech authors [9] there is a relationship between obesity, age and blood pressure. Their study included the sample of middle-aged men and women. They concluded that obesity was a reliable parameter of influence on blood pressure, but, the age could be a good predictor of high blood pressure as well.

In the introduction to this article we can notice that large numbers of countries in the world, including Serbia, have a high rate of cardiovascular diseases, one of the most frequent being hypertension. In the developed countries, the programs of prevention and control over cardiovascular diseases are mainly aimed at decreasing the influence of risk factors, as well as the improvement of diagnostics and therapy of cardiovascular diseases. It has been established that 46.5% of adults have increased blood pressure [4]. Prevention of cardiovascular diseases includes education of people aimed at increasing the level of awareness and knowledge about risk factors and diseases related to heart and blood vessels, systematic medical check-ups for the people aged above 40, early detection of cardiovascular diseases and risk factors, implementation of contemporary diagnostic methods, therapy and control

over cardiovascular diseases, etc. The programs of prevention and control of cardiovascular diseases ought to decrease the influence of risk factors and improve diagnostics and therapy of cardiovascular diseases [10]. One of the ways is taking up various physical exercises, especially those which contain natural movements and are therefore adequate for all age groups, especially the elderly [11].

The Ministry of Health of the Republic of Serbia has adopted the "National Program of Prevention and Control of Cardiovascular Diseases in the Republic of Serbia until 2020", with the purpose to decrease the number of premature deaths and reduce the diseases related to heart and blood vessels by taking integrated actions in order to improve the quality of life and prolong the life expectancy in the population in Serbia [12]. The program included a number of objectives and principles, among which the most important are the early recognition of cardiovascular diseases, better diagnostics, reduced mortality and disability, improved quality of life of patients. One of the frames of the program is the prevention of risk factors primarily addressing the objective of this research which is to point out the significance of the consequences of aging on the cardiovascular system [13].

Conclusion

The results of our study confirmed presence of differences in blood pressure between the women of different age groups.

In addition to the efforts to prolong the life expectancy within the Program of Prevention and Control of Cardiovascular Diseases, it is important to follow the recommendations of the World Health Organization regarding the prevention of increased blood pressure.

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THE LEVEL OF GRAMMAR SCHOOL STUDENTS' KNOWLEDGE ON CARDIOVASCULAR DISEASE RISK FACTORS

NIVO ZNANJA UČENIKA GIMNAZIJE O FAKTORIMA RIZIKA ZA KARDIOVASKULARNE BOLESTI

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Summary

Introduction. Cardiovascular diseases are one of the leading causes of mortality and morbidity worldwide. The atherosclerotic process in the aorta starts in childhood, while atherosclerotic changes of coronary heart vessels start in adolescence. The aim of the study was to evaluate the knowledge of the students attending all four grades of grammar school about the risk factors for cardiovascular disease, with special attention to the risk factors that can be influenced by modification of life-style. **Material and Methods.** Data from the entrance and exit tests were collected from 197 students attending a grammar school in Novi Sad. Chi-square test and Student T-test or Mann-Whitney U test were used to examine the statistical difference between categorized variables and the continuous variables, respectively. **Results.** The difference between the number of correct answers for all the students on the entrance test and exit test was statistically significant ($p < 0.0005$) and the overall knowledge level after lectures was increased by 29.4%. The lowest level of knowledge on the entrance tests was noted among the students of the third grade of grammar school and after the lectures, the student's knowledge level was increased by 82.3% ($p < 0.0005$). **Conclusion.** Children and adolescents from Vojvodina and Serbia should be well informed about the cardiovascular disease risk factors and their prevention with special attention paid to the risk factors that can be influenced by changing lifestyle habits.

Key words: Cardiovascular Diseases; Risk Factors; Adolescent; Students; Life Style; Risk Reduction Behavior; Health Knowledge, Attitudes, Practice

Introduction

Cardiovascular diseases (CVD) are one of the leading causes of mortality and morbidity worldwide. In 2001, approximately one third of all mortality causes in the world were attributable to CVDs and they are predicted to become the leading cause of death in developed countries. In the United States of America (USA), CVD accounts for 35.2% of mortality in comparison with 48% in Europe [1]. According to the data of the Institute for Public Health of Vojvodina from 2011, the number of patients in Vojvo-

Sažetak

Uvod. Kardiovaskularne bolesti predstavljaju jedan od vodećih uzroka mortaliteta i morbiditeta širom sveta. Aterosklerotski proces na aorti počinje u detinjstvu, dok aterosklerotske promene koronarnih krvnih sudova počinju u adolescenciji. Cilj studije je bila evaluacija znanja učenika od prvog do četvrtog razreda gimnazije o faktorima rizika za kardiovaskularne bolesti, sa posebnim osvrtom na faktore na koje se može uticati promenom stila života. **Materijal i metode.** Prikupljeni su podaci sa ulaznih i izlaznih testova za 197 učenika od prvog do četvrtog razreda jedne novosadske gimnazije. Za ispitivanje razlika između kategorisanih varijabli korišćen je χ^2 test, a između kontinuiranih Studentov T test ili Mann-Whitney U test. **Rezultati.** Prema broju tačnih odgovora svih učenika na izlaznom u odnosu na ulazni test dokazana je statistički značajna razlika ($p < 0,0005$) i ukupni nivo znanja nakon edukacije podignut je za 29,4%. Među učenicima trećeg razreda gimnazije zapažen je najniži nivo znanja na ulaznim testovima, a nakon učinjenih predavanja, postignuto je najveće ukupno poboljšanje nivoa znanja od 82,3% ($p < 0,0005$). **Zaključak.** Deca i omladina koja žive u Vojvodini treba da budu dobro informisana o faktorima rizika za nastanak kardiovaskularnih bolesti i njihovoj prevenciji, pre svih o faktorima rizika na koje se može uticati promenom stila života.

Ključne reči: Kardiovaskularna oboljenja; Faktori rizika; Adolescent; Studenti; Životni stil; Smanjenje rizičnog ponašanja; Znanje o zdravlju stavovi, praksa

dina with a registered diagnosis of CVD in Departments of General Medicine, Occupational Health and Inpatient Institutions was greater than 500 000 [2].

Although the average age of patients suffering from CVD has been increasing in recent years, even a greater number of young people get ill from CVD [3–7].

Coronary heart disease (CHD) develops slowly, insensibly and without any symptoms over the years, so that it is often diagnosed at an advanced stage.

The development of a CHD depends on the presence of risk factors that can be divided into two

Abbreviations

CVD	– Cardiovascular disease
CHD	– Coronary heart disease
USA	– United States of America

groups. The first group consists of the risk factors that cannot be influenced and they include age, gender and inheritance. Particularly significant is the other group, which includes the risk factors that can be influenced by changing habits and lifestyle. These factors are: hypertension, diabetes, obesity, high serum lipoprotein level, alcohol, smoking, physical inactivity and stress. Risk factors for CVD can be individual, but they are mostly combined. The probability of developing a CVD tends to be greater with a higher number of associated risk factors.

Epidemiological studies conducted so far have shown that there is a high level of correlation between morbidity and mortality of CVD in adulthood and early atherosclerotic changes and the presence of risk factors among children and youth.

McGill and McMahan have proved in their studies that atherosclerosis is a "pediatric problem". The atherosclerotic process starts in the aorta in childhood, while atherosclerotic changes of coronary heart vessels start in adolescence. It has also been proved that these changes appear earlier in the populations where the morbidity rate of CHD is higher. The changes on coronary heart vessels are reversible until the twenties [8–10].

Wissler has shown in his multicenter study, published in 1991, that risk factors contribute to the faster progression of the CHD, especially if they are cumulated. The cumulated effect of risk factors increases the risk of CHD as early as fifteen years of age. It has also been confirmed that most of the factors that are present in childhood have tendency to persist during adulthood, that being called a persistent phenomenon [11].

According to the data of the Institute for Public Health of Vojvodina from 2011, every eighth child in Vojvodina is overweight and every twentieth child is obese. It has been observed that 24.7% of those aged 15–19 do not have regular breakfast. In their free time, only 28.7% of the respondents have some daily sports activities. Most of them go to sleep late and thus, they have insufficient number of sleep hours during the night. In addition, 20% of those aged 15–19 smoke periodically or every day and only one out of five wants to quit that habit [2, 12–15].

These results point to the fact that it is necessary to stop the development of bad habits among children and adolescents and start the primary prevention of risk factors for CVD before the age of fifteen.

The aim of the study was to evaluate the knowledge of the students from 1st to 4th grade of grammar school about the risk factors for CVD, with special attention paid to the risk factors that can be influenced by changing life-style.

Material and Methods

During 2013, a pilot study was conducted in a grammar school in Novi Sad. More than 200 students from the 1st to the 4th grade were included in the study. All the students completed entrance tests consisting of 20 questions, and the results were used to determine the level of knowledge pertaining to the risk factors for the development of CVD. After the entrance tests, lectures were given by medical doctors, with special attention paid to the risk factors for the development of CVD which can be influenced by changing the lifestyle. After the lectures, the same students completed the exit test containing the same set of questions as the entrance tests. By analyzing the results of the exit tests, it was concluded that the knowledge of the students from the 1st to 4th grade of grammar school was at the lowest level regarding the risk factors for CVD that can be influenced, primarily obesity, high serum lipoprotein levels, alcohol, smoking and physical inactivity.

Based on the results of the conducted pilot study, the same study was conducted in the same grammar school in Novi Sad in 2014, this time including 197 students from the 1st to the 4th grade.

The data were analyzed statistically by the following standard descriptive methods: mean value, standard deviation, absolute and relative incidence. Chi-square test and Student T-test or Mann-Whitney U test were used to examine the statistical difference between categorized variables, and the continuous variables, respectively.

For all the tests, the level of statistical significance was analyzed and a p-value lower than 0.05 was considered statistically significant. All the data were collected in the specially created database and statistical analyses were performed using SPSS software. The results are shown in tables with a text comment.

Results

The difference between the number of correct answers for all the students on the entrance test and exit test was statistically significant ($p < 0.0005$). The average students' knowledge level was 37.1% before the lectures and 48% after them. The overall knowledge level was increased by 29.4% (**Table 1**). The students attending the 1st grade of grammar school showed the highest level of knowledge before the educational lectures were given (48.2%), as well as the highest level of knowledge after the lectures (52.6%), thus their knowledge level was increased by 9.1% ($p = 0.024$). Among the students of the 2nd grade, the level of knowledge was 35.4% and the percent of its increase after the lectures was only 8.5%, thus being the lowest one.

When comparing number of the correct answers between the grades, there was a statistically significant difference ($p < 0.0005$) between the number of correct answers on the exit test compared with the number of correct answers on the entrance test

Table 1. Level of overall student's knowledge on the entrance and exit test
Tabela 1. Nivo ukupnog znanja učenika na ulaznom i izlaznom testu

	Entrance test (%) <i>Ulazni test (%)</i>	Exit test (%) <i>Izlazni test (%)</i>	P-value <i>P-vrednost</i>	Improvement (%) <i>Poboljšanje (%)</i>
Total/ <i>Ukupno</i>	37.1	48.0	< 0.0005	29.4%
1st grade/ <i>1. razred</i>	48.2	52.6	0.024	9.1%
2nd grade/ <i>2. razred</i>	35.4	38.4	0.236	8.5%
3rd grade/ <i>3. razred</i>	28.3	51.6	< 0.0005	82.3%
4th grade/ <i>4. razred</i>	36.8	50.1	< 0.0005	36.1%

among the students of 3rd and 4th grade. The lowest level of knowledge on the entrance tests was noted among the 3rd grade students (28.3%). After the lectures, the students' knowledge level was 51.6%, so that the knowledge level among the 3rd grade students was increased by 82.3%, which was the highest increase in knowledge among all the respondents. The 4th grade students answered correctly to 36.8% of all the asked questions on the entrance test, while 50.1% of them gave the correct answers to the same questions on the exit test, which led to the 36.1% increase in the knowledge level (**Table 1**).

As for the risk factors that can be influenced, the students showed the highest level of knowledge on the entrance test related to the questions connected with eating habits (55%) (**Table 2**). The highest number of the correct answers on the exit test was also connected to the eating habits (70%), which led to the overall improvement in the knowledge level of 27.3%, that being statistically highly significant ($p < 0.0005$). In addition, the students showed a high knowledge level related to the questions concerning obesity. On the entrance test, 50% of examined students gave the correct answer about this risk factor. On the exit test, 62% of the examined students gave

the correct answer regarding obesity, which led to the overall improvement in knowledge level by 24%, that also being statistically highly significant ($p < 0.0005$). As for cigarette smoking habit, the students' knowledge level on the entrance test was 32.5%, while on the exit test, the level of knowledge was 52.5%, that meaning that the knowledge was increased by 61.5% ($p < 0.0005$). When the students were asked about everyday physical activity, 35% of them gave the correct answer on the entrance test, while 42.5% gave the correct answer on the exit test, which led to the 21.4% improvement in the knowledge level ($p = 0.007$). Regarding the students' knowledge about elevated serum lipid level, high blood pressure and diabetes, there was no improvement in the level of knowledge on the exit test compared with the entrance test (**Table 2**).

On the entrance test, 32.5% of the students answered correctly the questions concerning the change of lifestyle in order to lower the risk of CVD. During the lectures, special attention was paid to the significance of everyday life habits in the primary prevention of CVD. On the exit test, 42.5% of the students gave the correct answer, which made the overall improvement of the knowledge level of 30.7% ($p = 0.059$) (**Table 2**).

Table 2. Student's level of knowledge for the individual risk factors for cardiovascular disease
Tabela 2. Nivo znanja učenika u odnosu na pojedinačne faktore rizika za nastanak kardiovaskularnih bolesti

	Tests/ <i>Testovi</i>		P-value <i>P-vrednost</i>	Improvement (%) <i>Poboljšanje (%)</i>
	Entrance test (%)/ <i>Ulazni test (%)</i>	Exit test (%)/ <i>Izlazni test (%)</i>		
Obesity <i>Gojaznost</i>	50.0	62.0	< 0.0005	24
Physical activity <i>Fizička aktivnost</i>	35.0	42.5	0.007	21.4
Smoking/ <i>Pušenje</i>	32.5	52.5	< 0.0005	61.5
Eating habits <i>Ishrana</i>	55.0	70.0	< 0.0005	27.3
Diabetes <i>Šećerna bolest</i>	14.2	19.0	0.275	33.8
Hypertension <i>Hipertenzija</i>	36.0	21.3	0.003	- 40
Serum lipid levels <i>Nivo masnoća u krvi</i>	38.1	36.2	0.792	- 5
Life style/ <i>Stil života</i>	32.5	42.5	0.059	30.7
Total/ <i>Ukupno</i>	42.1	47.4	< 0.0005	12.6

Discussion

According to the contemporary recommendations regarding the CVD, special attention should be paid to the prevention of risk factors development (primordial prevention), as well as to the prevention of future CVD by effective management of identified risk factors (primary prevention) [16].

No multi-decade, population-based, longitudinal studies have been conducted that would link absolute levels of risk factors in childhood to incident clinical cardiovascular events in adult life. Moreover, no randomized clinical trials have demonstrated that reduction of risk factor levels in childhood prevents cardiovascular events in adult life. Such studies are difficult to undertake because large sample sizes are needed, and multi-decade follow-up period is necessary. In addition, costs of long-term interventions and monitoring would be rather high. Large cohort studies are possible and much is expected from the National Children's Study that started in 2011 in the USA that will examine the effect of environment and genetics on the growth, development and health of children across the USA. The study will follow the cohort from before birth until 21 years of age and it is expected to contribute to better understanding of the role played by various factors on health and disease [3].

Histopathological studies have shown that both the presence and extent of atherosclerotic lesions at autopsy after unexpected death of children and young adults correlate positively and significantly with the established risk factors, namely low density lipoprotein cholesterol, triglycerides, systolic and diastolic blood pressure, body mass index, and cigarette smoking habit. Multiple epidemiological studies have demonstrated a disturbing increase in the prevalence of obesity beginning in childhood, with at least 22% of 6- to 17-year-old subjects diagnosed as overweight. This is a cause for particular concern because of the strong association between obesity and hypertension, dyslipidemia, and type II diabetes mellitus beginning in childhood [17].

The Bogalusa study findings indicate that the number of cardiovascular risk factors increases, the amount of pathological evidence for atherosclerosis in the aorta and coronary arteries beginning in early childhood. Electron beam computed tomography of coronary artery calcium and increased carotid artery intima-media thickness, an ultrasound measure of carotid artery atherosclerosis, have been evaluated in 29- to 39-year-old subjects monitored from the age of 4 years. Significant risk predictors for coronary artery calcium were obesity and elevated blood pressure in childhood and increased body mass index and dyslipidemia in young adults [18].

According to the results of our study, it was concluded that the knowledge of the students attending

the chosen grammar school in Novi Sad was the least about CVD risk factors that could be influenced by changing the lifestyle, including obesity, unhealthy pattern of eating, high blood serum lipoprotein level, physical inactivity, smoking, alcohol use and stress. As a result, it has been concluded that it is necessary to repeat lectures and analysis of the entrance and exit tests among students of the same grammar school.

The study conducted among grammar school students by comparing the results from the tests done before and after the lectures showed a statistically significant increase in the students' knowledge about CVD risk factors. Particularly good results were achieved by the 3rd grade students of this grammar school, who showed the lowest knowledge level on the entrance test, but a very high level of knowledge about the CVD risk factors, especially those factors that can be influenced by the change of the lifestyle on the exit test with the increase in the overall knowledge level by more than 80% ($p < 0.0005$).

The change of the lifestyle among children and youth by reducing or eliminating risk factors that can be influenced would include the following: an overall healthy eating pattern, reduced intake of salt, alcohol and fatty substances, appropriate body weight, desirable lipid profile, desirable blood pressure, control of fasting plasma glucose level, no new initiation of cigarette smoking, no exposure to environmental tobacco smoke, complete cessation for those who smoke, being physically active every day, reduced sedentary time (television watching, computer, video games, or time on the phone), avoidance of stressful situations whenever possible.

The study included a relatively small number of participants from a single high school in Novi Sad. Further research should obtain results and conclusions that could be extrapolated to wider population.

Conclusion

By analyzing the results of the performed study, we came to the conclusion that healthy lifestyle should be popularized among children and adolescents not only from Novi Sad, but also from Vojvodina and Serbia. Children and youth should be well informed about the cardiovascular disease risk factors. Lectures on the prevention of risk factors development, as well as the prevention of future cardiovascular disease by effective management of identified risk factors should be given. Special attention should be paid to the risk factors that can be influenced by changing the life style habits. That could be a way to have healthier adult population and the morbidity and mortality from cardiovascular diseases would be lowered.

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ASSESSMENT OF QUALITY OF LIFE IN PATIENTS AFTER LOWER LIMB AMPUTATION

PROCENA KVALITETA ŽIVOTA PACIJENATA NAKON AMPUTACIJE DONJIH EKSTREMITETA

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Summary

Introduction. Lower extremity amputation is a surgical procedure resulting in important anatomical, functional, psychological, and social consequences that can influence the quality of life of these patients. The aim of this research was to compare the quality of life of patients with lower extremity amputation and people without amputation taking into account gender differences as well as the amputation level. **Material and Methods.** The study was designed as a cross-sectional study which included 56 subjects. The patients from the experimental group underwent prosthetic rehabilitation treatment at the Department of Medical Rehabilitation, Clinical Centre of Vojvodina. The experimental group included 28 patients (21 male, 7 female) with lower extremity amputation, their average age being 65.36±13.64. The control group consisted of 28 age and gender matching subjects without amputation. **Research ANd Development (RAND) 36 – Item Health Survey 1.0 (SF – 36)** was used to measure the quality of life. **Results.** The results showed that patients with lower extremity amputation scored lower than the control group on all SF- 36 variables ($p < 0.05$). None of the SF-36 variables differed between the genders ($p > 0.05$). Seventeen (61%) patients were with transfemoral, and 11 (39%) with transtibial level of amputation. The patients with transtibial amputations scored higher on physical functioning and general health status variables ($p < 0.05$). **Conclusion.** The patients with lower extremity amputations have numerous limitations compared to the control group, regardless of gender, while the patients with lower level of amputation have a higher level of physical functioning.

Key words: Quality of Life; Lower Extremity; Amputation; Questionnaires; Sex Characteristics; Motor Activity; Adaptation, Psychological

Introduction

Amputation is a surgical method by which a part or the whole extremity is being removed. Anatomical loss is also manifested by the consequential loss of the function, change of the distribution of body mass, coordination disorder and psychosocial dis-

Sažetak

Uvod. Amputacija donjih ekstremiteta predstavlja hiruršku metodu koja dovodi do značajnih anatomskih, funkcionalnih, psiholoških i socijalnih posledica koje mogu da utiču na kvalitet života ovih pacijenata. Cilj studije je procena kvaliteta života osoba sa unilateranom amputacijom donjih ekstremiteta u odnosu na osobe sa intaktnim donjim ekstremitetima kao i u odnosu na pol i nivo amputacije. **Materijal i metode.** Ispitivanje je koncipirano kao studija preseka i sprovedeno je nakon protetičke faze rehabilitacije na Klinici za medicinsku rehabilitaciju Kliničkog centra Vojvodine. Istraživanje je uključilo 56 osoba. Eksperimentalnu grupu je činilo 28 pacijenata (21 muškarac, 7 žena) sa jednostranom amputacijom donjih ekstremiteta, prosečne starosti 65,36 ± 13,64 godina. Kontrolnu grupu činilo je 28 osoba bez amputacije, homogenih po polu i starosti. Za procenu kvaliteta života korišćen je upitnik *Research ANd Development 36 - Item Health Survey 1.0 (SF-36)* preveden na srpski jezik. **Rezultati.** Dobijeni rezultati ukazuju da pacijenti sa amputacijom donjih ekstremiteta imaju statistički značajno umanjen kvalitet života u odnosu na kontrolnu grupu prema svim varijablama SF-36 ($p < 0,05$). Ne postoji statistički značajna razlika između vrednosti varijabli SF-36 kod različitih polova u ekperimentalnoj grupi ($p > 0,05$). Bilo je 17 pacijenata (61%) sa transfemoralnim i 11 (39%) sa transtibijalnim nivoom amputacije. Pacijenti sa transtibijalnim nivoom amputacije postižu značajno više vrednosti kada su u pitanju varijable fizičko funkcionisanje i opšte zdravstveno stanje ($p < 0,05$). **Zaključak.** Pacijenti sa amputacijom donjih ekstremiteta imaju brojna ograničenja usled emocionalnih problema i lošije fizičko funkcionisanje u odnosu na kontrolnu grupu, nezavisno od pola, dok viši nivo fizičkih sposobnosti imaju pacijenti sa nižim nivoom amputacije.

Glavne reči: Kvalitet života; Donji ekstremiteti; Amputacija; Upitnici; Polne karakteristike; Fizička aktivnost; Psihološka adaptacija

orders. The most common causes of surgical amputations are the complications caused by diabetes (diabetic foot) including a number of vascular complications in the form of ischemia and peripheral artery disease [1–3]. Different types of prostheses and good training to use them properly enable the

lower extremity amputees to walk normally and carry out their daily activities independently.

The World Health Organization defines the quality of life as one's own perception of their own life in the context of the culture and value systems in which they live, but also in relation to their own goals, expectations, standards and interests. The quality of life is a broad concept and consists of physical, mental and social health of an individual, his/her financial independence, i.e. level of independence and the personal attitude towards important developments in the society [4–7]. The highest number of lower extremity amputations is performed due to complications caused by a vascular disease in the old age, often followed by more comorbidities which further complicate the rehabilitation treatment and impede the normal functioning of a patient [8]. Additional problems that amputees are faced with are phantom pain, stump pain and numerous infections [9]. Proper personal hygiene, every day activities, getting in and out of cars and normal functioning are often difficult or impossible because the patients are faced with the loss of independence and need to depend on others, which considerably contributes to a poor physical, psychological, social and financial aspect of their lives [10]. The aim of this study is to assess the quality of the life of the patients with lower extremity amputation in relation to the control group, as well as to examine their quality of life, depending on the gender and the level of amputation.

Material and Methods

The study was designed as a cross sectional study and it included 56 subjects, aged 30 to 83 years. The experimental group consisted of 28 patients, their average age being 65.36 ± 13.64 , with unilateral amputation of lower extremities, while the control group consisted of 28 people (average age 63 ± 13.74) with intact lower extremities. After amputation, the patients went through prosthetic rehabilitation phase at the Department of Medical Rehabilitation, Clinical Center of Vojvodina. The

criteria for inclusion of the patients into the study were: unilateral transtibial or transfemoral amputation of lower extremities, the ability to walk with the help of prosthesis with or without aids, period of ≥ 6 months after the completion of rehabilitation treatment at the Department of Medical Rehabilitation to the time of questionnaire completion. The inclusion criteria for the control group included the presence of lower extremities, ability to walk independently, homogeneity in relation to age and gender in the experimental group and having two chronic diseases at most.

The quality of life was assessed with Research ANd Development (RAND) *36-Item Health Survey 1.0* (SF - 36) - the version translated into Serbian. The SF-36 version consists of 36 questions with multiple choice answers relating to 8 different domains (divided into two groups: the physical health and mental health component): physical functioning, limitations of their usual roles due to the physical health, pain, general health, limitations of their usual roles due to the emotional problems, energy/fatigue, emotional well-being and social functioning. The score for each scale ranges from 0 to 100, the higher score indicating a higher level of functioning or a higher level of well-being [11].

Statistical analysis of the data was performed by the statistical program STATISTICA 5.5. The results are presented using standard statistical measures of central tendency and range of results. T-test was used to test the statistical significance of independent samples.

Results

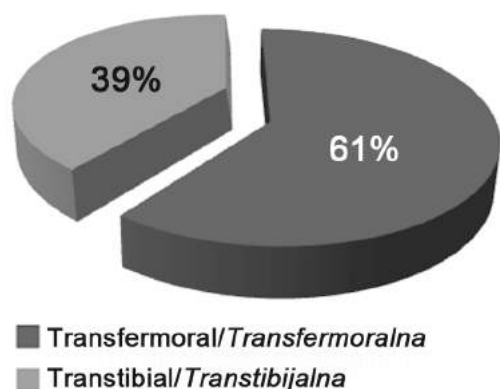
The conducted study included 28 patients: 21 (75%) men and 7 (25%) women) with unilateral transtibial or transfemoral amputation of lower extremities. Seventeen patients (11 men and 6 women) had transfemoral and 11 patients (10 men and 1 woman) had transtibial amputation (**Graph 1**).

The control group consisted of 28 subjects without amputations, homogenous regarding gender and

Table 1. The SF-36 questionnaire results (experimental and control group)

Tabela 1. Rezultati SF-36 upitnika

SF-36 questionnaire/Group <i>SF-36 Upitnik/Grupa</i>	Experimental group <i>Eksperimentalna grupa</i>	Control group <i>Kontrolna grupa</i>	p	T
Physical functioning/ <i>Fizičko funkcionisanje</i>	40.18 \pm 24.47	84.10 \pm 17.9	0.00	-7.66
Role limitation due to physical health <i>Ograničenje usled fizičkog zdravlja</i>	25.89 \pm 39.37	75.89 \pm 35.01	0.00	-5.02
Pain/ <i>Bol</i>	49.46 \pm 22.99	62.03 \pm 14.63	0.017	-2.44
General health status/ <i>Opšte zdravstveno stanje</i>	50.14 \pm 23.89	69.28 \pm 18.49	0.001	-3.35
Role limitation due to emotional problems <i>Ograničenje usled emocionalnih problema</i>	38.08 \pm 41.29	86.90 \pm 31.87	0.00	-4.95
Vitality/ <i>Energija/Umor</i>	48.57 \pm 23.37	70.71 \pm 14.95	0.00	-4.22
Emotional wellbeing/ <i>Emocionalno blagostanje</i>	56.92 \pm 26.43	76.60 \pm 18.82	0.001	-3.34
Social relations/ <i>Socijalno funkcionisanje</i>	62.05 \pm 26.67	89.28 \pm 18.23	0.00	-4.46



Graph 1. Amputation level
Grafikon 1. Nivo amputacije

age ($t = 0.809$, $p = 0.421$) in relation to the experimental group.

The youngest subjects in both groups were 30 and the oldest were 83 years old.

The obtained results have shown a statistically significant difference in the quality of life of the patients with lower extremity amputations compared to the quality of life of the control group, present in both domains of research: the physical component of quality of life consisting of questions about their physical state, activities and pain and the mental component of their quality of life, which is explicit regarding the social functioning, vitality and emotions of the subjects (**Table 1**).

When compared to the men, the women in the experimental group attained lower scores in all tested variables, except in the variable of physical functioning of SF-36 questionnaire. This difference, however, did not reach a statistical significance (**Table 2**).

The results of the SF-36 questionnaire pertaining to different levels of amputations are shown in **Table 3**. The patients with below-knee amputations have less limitations in physical functioning (52.27 ± 3.25) compared to the patients with above-knee amputations (32.35 ± 21.29) ($p < 0.05$). A statistically significant difference is present in the domain of general health where below-knee amputees

achieve better results when their physical functioning and their own perception of their health are taken into consideration (**Table 3**).

Discussion

Amputation brings a major change in an individual's life, whose image of their own body is changed; movement activities and self-care are made more difficult; the psycho-social status of the patient's life is changed as well and the performance of professional and other activities are significantly affected. The most affected function is walking, especially in different terrains and slopes [10].

Lower extremities amputations are more often done on men, usually at the age between their fifties and seventies [12, 13]. In our research, the experimental and control group did not differ demographically. There were more men than women (3 times more) in both groups, and the average age of the patients with amputation was 65 years.

The biggest difference in the obtained results of the SF-36 questionnaire was in the domain of physical functioning; however, the tested subjects achieved significantly lower scores of the SF-36 questionnaire in all tested variables when compared to the control group. These results could be expected, although some authors did not find that the patients with lower extremity amputations had such deteriorated quality of life [14]. The reason for this discrepancy can probably be found in the fact that transfemoral amputations in these studies are represented in a smaller number (16%), compared to our 61% [14]. It is very likely that the ability to move has the greatest impact on a group of variables related to physical health component. We must bear in mind that a large number of factors affect these people's ability to move, as well as their overall quality of life [8, 15, 16]. Patients with lower extremity amputations usually have associated chronic diseases, such as diabetes and cardiovascular disease. The reason for such worse scores of the SF-36 questionnaire can be found in the limitations resulting from these diseases [17-23].

Table 2. Comparison of the SF-36 questionnaire results between men and women

Tabela 2. Rezultati SF-36 upitnika u odnosu na pol pacijenata

SF-36 questionnaire/Gender/SF- 36 upitnik/Pol	Men/Muškarci	Women/Žene	p	T
Physical functioning/Fizičko funkcionisanje	40 ± 25	40.7 ± 24.73	0.94	-0.66
Role limitation due to physical health Ograničenje usled fizičkog zdravlja	29.76 ± 42.29	14.28 ± 28.34	0.38	0.89
Pain/Bol	50.48 ± 24.97	46.43 ± 16.88	0.69	0.39
General health status/Opšte zdravstveno stanje	53.80 ± 25.09	39.14 ± 16.79	0.16	1.43
Role limitation due to emotional problems Ograničenje usled emocionalnih problema	44.43 ± 43.8	19.02 ± 26.2	0.16	1.44
Vitality/Energija/umor	52.38 ± 23.48	37.14 ± 20.38	0.14	1.53
Emotional wellbeing Emocionalno blagostanje	59.47 ± 26.61	48.85 ± 26.17	0.36	0.91
Social relations/Socijalno funkcionisanje	63.09 ± 25.45	58.92 ± 32.04	0.73	0.35

Table 3. Comparison of the SF-36 questionnaire results between transtibial and transfemoral amputation level
Tabela 3. Rezultati SF-36 upitnika u odnosu na nivo amputacije

SF-36 questionnaire/Level of amputation <i>SF -36 upitnik/Nivo amputacije</i>	Transtibial <i>Transtibijalne l</i>	Transfemoral <i>Transfemoralne</i>	p	T
Physical functioning/ <i>Fizičko funkcionisanje</i>	52.27 ± 25.03	32.35 ± 21.29	0.03	2.26
Role limitation due to physical health <i>Ograničenje usled fizičkog zdravlja</i>	40.90 ± 47.79	16.17 ± 30.54	0.1	1.68
Pain/ <i>Bol</i>	57.95 ± 26.36	43.97 ± 19.38	0.11	1.62
General health status/ <i>Opšte zdravstveno stanje</i>	61.36 ± 25	42.88 ± 20.73	0.04	2.12
Role limitation due to emotional problems <i>Ograničenje usled emocionalnih problema</i>	54.54 ± 45.39	27.43 ± 35.8	0.08	1.76
Vitality/ <i>Energija/umor</i>	56.36 ± 29.94	43.53 ± 19.10	0.16	1.44
Emotional wellbeing/ <i>Emocionalno blagostanje</i>	60.73 ± 29.05	54.29 ± 25.18	0.54	0.62
Social relations/ <i>Socijalno funkcionisanje</i>	67.77 ± 30.52	60.29 ± 24.69	0.67	0.42

Compared to the control group, the patients with lower extremities amputations achieve significantly lower scores in the part of the SF-36 questionnaire pertaining to the pain variable. Such results are expected, given that the amputees experience phantom pain, pain in residual and intact extremities and back pain [9]. From this point of view, the study results of Kazemi et al. are very interesting as they found there was a greater degree of anxiety and depression in the patients with chronic pain than in the patients with phantom pain [24]. When we compared the values of variables SF-36 in the patients with lower extremity amputations in our study with the values of the same variables of the patients with rheumatoid arthritis or lumbar radiculopathy, we discovered some interesting facts [25, 26]. Some variables related to the physical component of the quality of life in our experimental group had higher values than the same variables in the patients with radiculopathy at the beginning of treatment [26]. The reason for this probably lies in the fact that these patients were examined in the acute phase of radicular disease when the symptoms (especially pain) are most prominent. Due to the existence of numerous emotional problems such as the feeling of guilt, anxiety and depressive behavior, patients with lower extremity amputations have numerous limitations caused by their emotional state [14, 27–29]. The results of the questionnaire SF-36 for assessing the quality of life in the domain of mental health indicate numerous emotional and social problems the amputees are faced with. Of these problems, the lack of energy and depression particularly stand out as they significantly contribute to the reduction in physical functioning besides the existing limitations caused by amputation [30, 31].

Literature offers scarce data concerning the differences between functional enabling of men and women after lower extremities amputation [32]. In our study, the women had lower scores of the SF-36 questionnaire in all tested variables (except the physical functioning), although this difference does

not have a statistical significance. One of the possible reasons why the women in the experimental group had lower results may be a higher number of transfemoral amputations when compared to the men. Lafévre et al. also reported a higher number of transfemoral level of amputations in women in their research [33]. Hirsh et al. found no significant difference in the presence of pain between the genders in their study [13]. Frlan-Vrgoč et al. found no significant difference between men and women regarding their ability to walk, while Singh et al. found that the prosthetic rehabilitation was rarely successful in women when compared to men [32, 34].

The patients with transtibial amputation level are much more mobile than the patients with transfemoral amputation level [8, 35–37]. This is probably one of the reasons why the results of individual domains of the SF-36 had significantly higher values (in physical functioning and general health) in the people with transtibial amputations compared to the ones with transfemoral level of amputation. When compared to the people suffering from chronic rheumatic disease such as rheumatoid arthritis, the patients with above-knee amputations achieved lower functional status, while the patients with below-knee amputations were much more functional [25].

Conclusion

The quality of life of patients with lower extremity amputations is significantly reduced compared to the control group, despite quite successful and satisfactory restitution of walking function and relative independence in everyday activities. In the overall physical and mental function there is no significant difference between the genders, while the patients with different levels of amputation vary in the domain of physical function and general health. The patients with transtibial level of amputations are more functional and are of better general health compared to the patients with transfemoral amputation level.

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RELIABILITY OF POSITRON EMISSION TOMOGRAPHY-COMPUTED TOMOGRAPHY IN EVALUATION OF TESTICULAR CARCINOMA PATIENTS

POUZDANOST POZITRONSKE EMISIONE TOMOGRAFIJE - KOMPJUTERIZOVANE TOMOGRAFIJE U EVALUACIJI OBOLELIH OD KARCINOMA TESTISA

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Summary

Introduction. The study was aimed at assessing the reliability of 18F-fluorodeoxyglucose positron emission tomography-computed tomography scan in evaluation of testicular carcinoma patients. **Material and Methods.** The study sample consisted of 26 scans performed in 23 patients with testicular carcinoma. According to the pathohistological finding, 14 patients had seminomas, 7 had nonseminomas and 2 patients had a mixed histological type. In 17 patients, the initial treatment was orchiectomy+chemotherapy, 2 patients had orchiectomy+chemotherapy+retroperitoneal lymph node dissection, 3 patients had orchiectomy only and one patient was treated with chemotherapy only. Abnormal computed tomography was the main cause for the oncologist to refer the patient to positron emission tomography-computed tomography scan (in 19 scans), magnetic resonance imaging abnormalities in 1 scan, high level of tumor markers in 3 and 3 scans were performed for follow-up. Positron emission tomography-computed tomography imaging results were compared with histological results, other imaging modalities or the clinical follow-up of the patients. **Results.** Positron emission tomography-computed tomography scans were positive in 6 and negative in 20 patients. In two patients, positron emission tomography-computed tomography was false positive. There were 20 negative positron emission tomography-computed tomography scans performed in 18 patients, one patient was lost for data analysis. Clinically stable disease was confirmed in 18 follow-up scans performed in 16 patients. The values of sensitivity, specificity, accuracy, and positive- and negative predictive value were 60%, 95%, 75%, 88% and 90.5%, respectively. **Conclusion.** A high negative predictive value obtained in our study (90.5%) suggests that there is a small possibility for a patient to have future relapse after normal positron emission tomography-computed tomography study. However, since the sensitivity and positive predictive value of the study are rather low, there are limitations of positive positron emission tomography-computed tomography scan to suggest persistent disease.

Key words: Testicular Neoplasms; Positron Emission Tomography; Fluorodeoxyglucose 18F; Diagnostic Imaging; Predictive Value of Tests; Sensitivity and Specificity

Sažetak

Uvod. Cilj rada bio je utvrđivanje pouzdanosti pozitronske emisione tomografije - kompjuterizovane tomografije primenom 18F-fluorodeoksigluoze u evaluaciji obolelih od karcinoma testisa. **Materijal i metode.** Istraživanjem je obuhvaćeno ukupno 26 skenova načinjenih kod 23 pacijenta obolela od karcinoma testisa. Prema patohistološkom nalazu bilo je 14 seminoma, 7 neseminoma a dva pacijenta imala su mešoviti histološki tip karcinoma. Kod 17 obolelih inicijalni tretman je podrazumevao orhiektomiju + hemioterapiju; kod dva pacijenta načinjena je orhiektomija + hemioterapija + disekcija retroperitonealnih limfnih čvorova; kod 3 pacijenta urađena je samo orhiektomija, dok je jedan pacijent tretiran samo hemioterapijom. Patološki nalaz kompjuterizovane tomografije bio je najčešća indikacija za upućivanje na pozitronsku emisionu tomografiju - kompjuterizovanu tomografiju (kod 19 skenova), patološki nalaz magnetne rezonance kod jednog skena, povišene vrednosti tumorskih markera kod 3 skena dok je kod 3 skena indikacija za snimanje bilo praćenje. Nalaz pozitronsku emisionu tomografiju - kompjuterizovanu tomografiju poreden je sa patohistološkim nalazom, drugim dijagnostičkim metodama ili kliničkim praćenjem obolelih. **Rezultati.** Positronska emisiona tomografija - kompjuterizovana tomografija bila je pozitivna kod 6 i negativna kod 20 pacijenata. Kod 2 pacijenta nalaz je bio lažno pozitivan. Bilo je 20 negativnih nalaza pozitronske emisione tomografije - kompjuterizovane tomografije načinjenih kod 18 pacijenata, jedan pacijent izgubljen je u daljem praćenju zbog čega je analizirano preostalih 19 skenova kod 17 pacijenata. Kod 18 skenova (načinjenih kod 16 pacijenata) u daljem praćenju (6–36 meseci) registrovana je klinički stabilna bolest. Senzitivnost studije bila je 60%, specifičnost 95%, tačnost 75%, pozitivna prediktivna vrednost 88%, a negativna prediktivna vrednost 90,5%. **Zaključak.** Visoka negativna prediktivna vrednost naše studije (90,5%) ukazuje na malu verovatnoću da kod pacijenta sa urednom pozitronskom emisionom tomografijom - kompjuterizovanom tomografijom nastane relaps oboljenja. Međutim, imajući u vidu relativno niske vrednosti senzitivnosti i pozitivne prediktivne vrednosti, zaključak je da su mogućnosti ove metode u detekciji perzistentog oboljenja ograničene. **Ključne reči:** Karcinomi testisa; PET-CT; Fluorodeoksigluoza 18F; Dijagnostički imidžing; Prediktivna vrednost testova; Senzitivnost i specifičnost

Abbreviations

AFP	– α -fetoprotein
HCG	– human chorionic gonadotropin
LDH	– lactate dehydrogenase
MRI	– magnetic resonance imaging
CT	– computed tomography
MIBI	– methoxyisobutyl isonitrile
FDG	– fluorodeoxyglucose
PET-CT	– positron emission tomography - computed tomography
PH	– pathohistology
RPLND	– retroperitoneal lymph node dissection

Introduction

Testicular carcinoma represents 1% of all cancers in men and it is most common in the age group from 15 to 35 years. Although it is a significant cause of death in this age population, testicular carcinoma is considered curable in more than 90% of all cases [1]. The incidence of testicular cancer has doubled in the last 40 years, with the highest rates in developed countries especially affecting population of white Caucasians [2, 3]. In Europe, the highest incidence has been observed in Denmark with 9.2/100,000 [4].

Generally, all testicular carcinomas are divided into seminomas and nonseminomas. The accurate classification is important because it determines the type of treatment [5]. Seminomas originate from immature reproductive cells; they are usually well differentiated and most commonly seen in the fourth decade of life. Nonseminomas are developed from mature reproductive cells, usually seen in the third decade of life and histologically they represent a mixed type of tumors, such as embryonal cell carcinoma, choriocarcinoma, yolk sac tumor and teratoma. Teratomas are further divided into mature and immature teratomas [5, 6].

Alfa-fetoprotein (AFP), human chorionic gonadotropin (HCG) and lactate dehydrogenase (LDH) are routinely used tumor markers to make diagnosis, determine prognosis and follow up testicular cancer. AFP is synthesized in the liver cells and the gastrointestinal tract of the fetus. High values of this marker in adults raise suspicion of nonseminoma (AFP is elevated in 65% of patients with nonseminoma), although it can be elevated in seminoma with some elements of nonseminoma [7]. AFP is usually not elevated in pure seminomatous tumors. Placental trophoblast produces HCG immediately after the implantation of a fertilized egg into the uterine wall. HCG is a more specific tumor marker for nonseminomas (elevated in 60% of patients with advanced nonseminoma), but is also raised in 10-20% of seminomas [8, 9]. Although LDH is a less specific marker, its level reflects the growth rate and tumor burden. A high level of LDH is seen in most patients with the advanced stage of seminoma or nonseminoma [10].

In addition to the levels of AFP, HCG and LDH, physical examination of the patient and testicular ultra-

sound are necessary to diagnose testicular cancer. It has been reported that magnetic resonance imaging (MRI) can distinguish between seminomas and nonseminomas; however, this is not clinically relevant since orchiectomy is performed as primary treatment [11, 12].

Computed tomography (CT) is still the imaging modality of choice in determining the stage of testicular cancer. The main pattern of metastatic spread for testicular cancer is through the lymphatic system from testicular lymph vessels to the retroperitoneal lymph nodes. The size of metastatic lymph node can be from 1 cm up to the large retroperitoneal masses. Huge retroperitoneal masses are easily seen and diagnosed by CT scan, but the determination of a small single lymph node suspicious for metastatic disease is usually extremely difficult. This problem is critical in situations when further patient management depends on a distinction like this, such as distinguishing of stage I from stage II. In the patients with stage I disease, only surveillance is recommended, but the patients with stage II usually need chemotherapy. A great number of studies have reported that between 25% and 30% of patients with testicular cancer have occult metastatic lymph node disease that cannot be detected by CT [13–15].

Rare testicular neoplasms, such as testicular lymphoma, could be evaluated using technetium-99m methoxyisobutyl isonitrile (^{99m}Tc -MIBI). This radiopharmaceutical, known for its role in myocardial perfusion scintigraphy, has demonstrated an increased uptake in the skeletal/bone marrow metastases as diffuse and/or focal increased uptake, especially focal/tubular MIBI activity of the femoral marrow [16, 17].

New imaging modalities, such as fluoro-deoxyglucose (^{18}F -FDG) positron emission tomography-computed tomography (PET-CT) may overcome these limitations of CT in detecting small lymph node metastases. Although ^{18}F -FDG is inferior to other tracers for primary staging of cancers such as testicular, prostate and bladder one, it may be useful in the selected patients with suspected high-grade cancer [18]. The main advantage of ^{18}F -FDG PET-CT compared to CT is that it is a functional imaging thus providing information about metabolically active lymph nodes. A potential advantage is the ability of ^{18}F -FDG PET-CT to detect small lymph node metastases that are not identified as enlarged on CT, which could influence the future clinical decision.

The aim of this study was to assess if ^{18}F -FDG PET-CT scan is reliable in evaluation of testicular carcinoma patients. The study represents a retrospective review of ^{18}F -FDG PET-CT scans which were recommended by oncologists to be performed in the patients with testicular cancer.

Material and Methods

This retrospective study included the patients with testicular carcinoma referred for PET-CT scan to the Oncology Institute of Vojvodina in a 3-year

period (from 2011–2014). A total of 1090 ^{18}F -FDG PET-CT scans were performed in 850 patients, among them were 26 studies in 23 patients (aged 20-54, their mean age being 35.5 years) with testicular carcinoma. PET-CT was performed following the injection of 241-370 megabecquerel (MBq) of ^{18}F -FDG. The patients were prepared according to the instructions for fasting for at least 6 hours before the injection, with glucose level from 2.9 – 8.2 mmol/l at the time of injection. The patients were scanned according to the principles of previously described methodology [19]. All PET-CT scans were performed on 64-slice hybrid PET-CT scanner (Biograph, Siemens) 60-120 minutes after the injection. A 3-dimensional PET scan and low-dose unenhanced CT scan were acquired from the base of the skull to the proximal parts of the femur. CT, PET (attenuation-corrected) and combined PET-CT images were displayed for analysis and interpreted by two nuclear medicine physicians.

The clinical history data were examined in order to compare ^{18}F -FDG PET-CT results with the pathohistology (PH) reports, clinical examination, CT or any other imaging modality for a minimum of 6 months after ^{18}F -FDG PET-CT scanning. If there were no data of pathohistology, a stable disease was assumed if CT scan showed no progression of previously known abnormality or if the serum tumor markers were not elevated (AFP, HCG or LDH). Progressive disease was assumed in the patients with an enlargement of previously known CT abnormality, detection of new lesions on any imaging modality or elevation of tumor markers.

The sensitivity, specificity, accuracy, positive and negative predictive values were calculated to determine the diagnostic significance of ^{18}F -FDG PET-CT in the evaluation of testicular carcinoma.

Results

Clinical data were collected for 23 patients; only one patient was lost for follow up. In most patients, the initial treatment prior to PET-CT was orchiectomy + chemotherapy (17 patients; 73.9%), 2 patients (8.7%) had orchiectomy + chemotherapy + retroperitoneal lymph nodes dissection (RPLND), 3 patients (13.1%) had orchiectomy only (followed by chemotherapy after PET-CT) and only one patient (4.3%) was treated with chemotherapy only (extragonadal seminoma). The pathohistology type of cancer included seminoma (14 patients; 60.9%), nonseminoma (7 patients; 30.4%) and a mixed histological type (2 patients; 8.7%).

The total number of scans performed in 23 patients was 26. The main cause for having been referred to ^{18}F -FDG PET-CT scan by an oncologist was an abnormality registered on CT (in 19 scans; 73.1%), MRI abnormalities in 1 scan (3.8%), high level of tumor markers in 3 scans (11.5%) and 3 scans (11.5%) were performed for follow-up. Tumor markers were elevated in 3 patients, and two of them

had elevated both AFP and HCG (mixed type, and in nonseminoma) and only AFP was elevated in one patient (seminoma). One patient with elevated tumor markers had positive PET scan, he was operated and pathohistology (PH) results confirmed metastases of testicular carcinoma. Two patients had negative scans, one was later assumed to have progressive disease according to much higher levels of tumor markers than at the time of the scan and one was clinically stable.

The results showed positive ^{18}F -FDG PET-CT scan in 6 (23.1%) and normal in 20 (76.9%) patients. A true positive scan was found in 4 (66.7%), and PET-CT was false positive in two patients (33.3%). True positive ^{18}F -FDG PET-CT findings were confirmed pathohistologically in two patients (one with seminoma and the other one with nonseminoma), and two patients (both seminoma) were treated with chemotherapy with a significant reduction in the size of retroperitoneal lymph nodes (**Figures 1 and 2**). The sites of ^{18}F -FDG accumulation were retroperitoneal, inguinal lymph nodes and a bone (a rib).

A false positive ^{18}F -FDG PET-CT scan (a patient with embryonal cell carcinoma) registered ^{18}F -

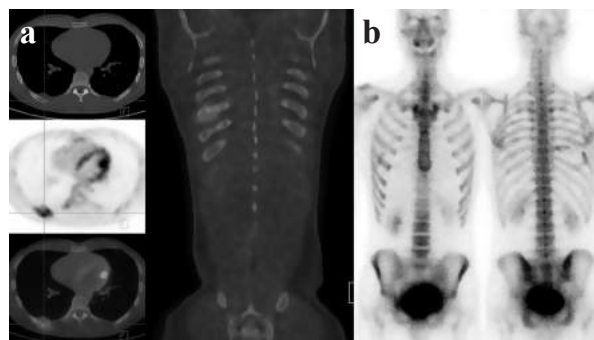


Figure 1. A 30-year-old man with right-sided nonseminoma (PH: mature teratoma+immature teratoma+embryonal cell carcinoma with elements of Yolk sac differentiation+trophoblastic cells). Initial treatment – right orchiectomy followed by chemotherapy. Referred to PET for evaluation of elevated tumor markers. a) ^{18}F -FDG PET-CT scan revealed FDG-avid focus in the posterior part of the right 8th rib with local thickening of the pleura (SUV max 4.8). b) bone scintigraphy with $^{99\text{m}}\text{Tc}$ -diphosphonate shows osteolytic lesion of the same rib. He was operated (resection of the rib), PH: Mature teratoma with elements of immature teratoma.

Slika 1. 30-godišnji pacijent sa desnostranim nonseminomom (PH: mature teratoma+immature teratoma+karcinom embrionalnih ćelija sa elementima Yolk sac diferencijacije+trofoblastične ćelije). Početna terapija – desna orhiektomija praćena hemioterapijom. Upućen na PET radi evaluacije eleviranih tumorskih markera. a) ^{18}F -FDG PET-CT ukazuje na FDG-avidan fokus na posteriornom delu VIII-og rebra desno uz evidentno lokalno zadebljanje pleure (SUV max 4,8). b) scintigrafija skeleta sa $^{99\text{m}}\text{Tc}$ -difosfonatom ukazuje na osteolitičke lezije istog rebra. Operisan (resekcija rebra). PH: Mature teratoma sa elementima immature teratoma.

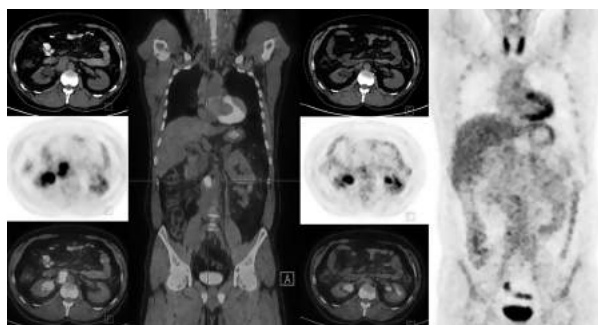


Figure 2. A 42-year-old man with stage IIC disease from right-sided seminoma. Initial treatment – right orchiectomy followed by chemotherapy. Referred to PET for evaluation of CT abnormality. a) First ^{18}F -FDG PET-CT scan (left part of the figure) revealed FDG-avid aortocaval lymph nodes (SUV max 11.3). b) Second ^{18}F -FDG PET-CT scan (right side of the figure), performed after chemotherapy, was normal suggesting complete response to therapy.

Slika 2. 42-godišnji pacijent sa desnostranim seminomom u stadijumu IIC. Početna terapija – desna orhiektomija praćena hemioterapijom. Upućen na PET radi evaluacije patološkog CT nalaza. a) Prvi ^{18}F -FDG PET-CT (leva strana slike) ukazuje na FDG-avidan aortokavalni limfni čvor (SUV max 11,3). b) Drugi ^{18}F -FDG PET-CT (desna strana slike), načinjen nakon aplikovane hemioterapije, uredan je i ukazuje na kompletan odgovor na primljenu terapiju

FDG-avid retroperitoneal lymph nodes, after PET-CT RPLND was performed and there was no evidence of tumor cells (PH: necrosis) in the PH report (**Figure 3**). Another false positive ^{18}F -FDG PET-CT scan reported ^{18}F -FDG-avid mediastinal lymph nodes, mediastinoscopy of the paratracheal lymph nodes was performed and PH confirmed lymphadenitis granulomatosa non necroticans.

Twenty negative PET-CT scans were performed in 18 patients, one patient was lost for data analysis and the remaining 19 scans in 17 patients were further analyzed. The further follow up (6-36 months) showed clinically stable disease in 18 scans (performed in 16 patients) (88.9%) and those scans were considered to be true negative. Pathohistological proof of a ^{18}F -FDG non-avid CT abnormality was found in one patient. Following a biopsy using bronchoscopy the histopathology showed no evidence of tumor (**Figure 4**). The patient with false negative ^{18}F -FDG PET-CT scan (PH: yolk sac choriocarcinoma+mature teratoma) was referred to ^{18}F -FDG PET-CT because he had elevated tumor markers (HCG, 12 mU/mL; AFT, 14 IU/mL; LDH was normal). During the 13-month follow-up, tumor markers significantly increased: AFP was reported to be five times higher and HCG 150 times higher.

After ^{18}F -FDG PET-CT, one out of 23 patients was lost for follow-up and the data were collected for 22 patients. When there was regression or no progression on CT abnormality and no elevation of

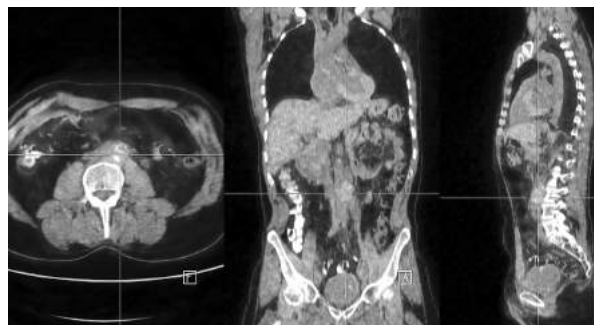


Figure 3. A 36-year-old man with stage IV disease from left-sided nonseminoma (embryonal cell carcinoma). Initial treatment: right orchiectomy + chemotherapy + RPLND. Referred to PET for evaluation of CT abnormality. ^{18}F -FDG PET-CT scan revealed FDG-avid left paraaortic lymph node (SUV max 5.5). He was operated (RPLND), PH: Necrosis.

Slika 3. 36-godišnji pacijent sa levostranim nonseminomom (karcinom embrionalnih ćelija) u stadijumu IV. Početna terapija: desna orhiektomija + hemioterapija + RPLND. Upućen na PET radi evaluacije patološkog CT nalaza. ^{18}F -FDG PET-CT ukazuje na FDG-avidan limfni čvor paraaortalno levo (SUV max 5,5). Operisan (RPLND), PH: Nekroza.

tumor markers, the disease was clinically stable (14 patients; 63.6%). One of patients from this group was with negative ^{18}F -FDG PET-CT scan but treated with chemotherapy after the scan because he was referred to chemotherapy by his urologist. If tumor markers continued to rise (1 patient; 4.5%) or there was evidence of progression previously diagnosed or newly detected abnormality on any imaging modality (none of the patients), the patient was considered to have clinically progressive disease. When the patient received chemotherapy after PET-CT with significant regression of tumor mass on any imaging modality, he was considered to have clinical response to therapy (2 patients after chemotherapy; 9.1%). In five patients (22.7%), PH was obtained as a result of operation (3) or invasive diagnostic procedures (2) (**Table 1**). The sensitivity, specificity, accuracy, positive and negative predictive values were 60%, 95%, 75%, 88% and 90.5%, respectively.

Discussion

The main way of metastatic spread of testicular carcinoma is through the lymphatic vessels. Most frequently, right-sided tumors spread to the aortocaval nodes, precaval nodes and right paracaval and retrocaval nodes, while left-sided tumors usually spread to the left paraaortic nodes and preaortic nodes. CT is still the main imaging modality for staging, evaluation and follow-up of testicular carcinoma patients [20, 21]. However, there are some limitations in the ability of CT to diagnose small volume metastatic disease [22, 23]. Further develop-



Figure 4. A 30-year-old man with right-sided nonseminoma (embryonal cell carcinoma). Initial treatment – right orchiectomy followed by chemotherapy. Referred to PET because of CT abnormality. ¹⁸F-FDG PET-CT scan showed FDG-nonavid pulmonary node and non-avid left inguinal lymph node. Bronchoscopy was performed, PH: no presence of tumor cells; in follow-up period inguinal lymph node clinically decreased.

Slika 4. 30-godišnji pacijent sa desnostranim nonseminomom (karcinom embrionalnih ćelija). Početna terapija – desna orhiektomija praćena hemioterapijom. Upućen na PET radi evaluacije patološkog CT nalaza. ¹⁸F-FDG PET-CT ukazuje na FDG-neavidan plućni čvor i FDG-neavidan ingvinalni limfni čvor levo. Načinjena je bronhoskopija, PH: nema tumorskih ćelija; tokom kliničkog praćenja evidentira se smanjenje ingvinalnog limfnog čvora levo.

ment in imaging methods may overcome these limitations, specially in the possibility to diagnose small metastases in retroperitoneal lymph nodes, such as ¹⁸F-FDG PET-CT and MRI with lymphotropic nanoparticles. An important advantage of ¹⁸F-FDG PET-CT as a functional imaging over CT

is the possibility to identify metabolically active disease. Albers et al. compared ¹⁸F-FDG PET-CT with CT in staging testicular carcinoma and have suggested that ¹⁸F-FDG PET-CT is useful for detecting viable tumor in lesions detected with CT and it may prevent false-positive diagnosis in stage II [24]. In the patients with testicular cancer in stage I, the same study has shown that PET does not improve staging over the CT [24].

The results of our study have shown that two patients with positive ¹⁸F-FDG PET-CT scan (both seminoma) were successfully treated with chemotherapy after PET-CT scan. Seminoma is very sensitive to chemotherapy, and after the completion of treatment, residual mass (necrosis, fibrosis) is sometimes present. ¹⁸F-FDG PET-CT has shown to be the best imaging modality in differentiation of such residual mass [25]. De Santis et al. performed PET scan in this group of patients with PH verification after the scan and reported no false-positive findings, suggesting that PET scan is highly specific for tumor viability [25]. In our group of patients, there were two cases with false positive result, when PH confirmed necrotic or granulomatous tissue in ¹⁸F-FDG-avid lymph nodes of retroperitoneum and mediastinum. In the study of 24 PET scans performed in 19 patients, Lewis et al. reported that out of 12 positive scans there were 4 false positive scans that led to surgical resections of residual masses revealing only fibrosis, necrosis or inflammation [26]. Muggia et al. reported a case of false positive mediastinal finding in a patient with seminoma [27]. Many investigators have reported a certain number of false positive PET-CT findings in the follow-up of seminoma cancer patients [26, 28, 29]. In our study, there were 18 normal ¹⁸F-FDG PET-CT scans performed in 16 patients, and most of them (15; 93.75%) were true negative, which suggests that normal PET scan was a good predictor of future stable disease. A high negative predictive

Table 1. Characteristics of patients with testicular carcinoma (S=seminoma, NS=nonseminoma, M=mixed type) according to type of follow up.

Tabela 1. Karakteristike pacijenata sa testikularnim karcinomom (S = seminom, NS = nonseminom, M = mešoviti tip) prema vrsti praćenja

Patients Pacijenti (22)	PH tumor type PH tip tumora			Type of follow up Vrsta praćenja	Follow up (months) Praćenje (meseci)	Diagnostic significance Dijagnostička signifikantnost
	S	NS	M			
5	2	3	-	PH verification PH verifikacija	6-28	TP=2 FP=2 TN=1
1	-	1	-	Clinical progression Klinička progresija	13	FN
14	10	2	2	Clinically stable disease Klinički stabilna bolset	6-36	TN
2	2	-	-	Clinical response to therapy Klinički odgovor na terapiju	10-15	TP

PH - patohistološki

value of our study (90.5%) suggests that there is a very small possibility for a patient to have future relapse of the disease after negative PET-CT study. Many studies recommend surveillance as a method of choice in the follow-up of patients with negative PET-CT scan because they are unlikely to relapse and do not require any further treatment [30, 31]. However, there are other studies, such as the study performed by Huddart et al, that report a high relapse rate in this group of patients [32]. In our study group, only one false negative ^{18}F -FDG PET-CT scan was noted in a patient of nonseminoma who was referred to PET-CT because he had elevated tumor markers. In the 13-month follow-up period, his tumor markers significantly increased since the time of ^{18}F -FDG PET-CT scan, thus suggesting that the scan was false negative. However, we did not have a chance to perform a follow-up scan, which is why this false negative scan should be understood tentatively. Hain et al. investigated 23 scans of testicular carcinoma with elevated tumor markers, and found that a subsequent PET-CT was positive in three out of five false negative cases and PET-CT was the first imaging technique to identify the site of recurrence [33]. Therefore, when tumor markers are elevated and imaging findings are normal (including negative PET-CT scan), the most appropriate follow-up imaging may be to repeat PET-CT.

Since patients suffering from testicular carcinoma are mainly young, they should be spared radiation burden as much as possible which is why it is impor-

tant to choose those imaging techniques providing most appropriate information for future treatment options. Therefore, although CT is still a crucial technique, PET-CT can be helpful in certain clinical situations.

The major limitation of our investigation is a small number of patients as a result of not so frequent pathology among those who are referred to PET-CT. Another limitation is the fact that in some cases etiology was obtained with clinical follow-up instead of pathology report. We will try to overcome these limitations in our additional future research work.

Conclusion

The results of our study indicate that ^{18}F -fluorodeoxyglucose positron emission tomography-computed tomography scan is recommended in the follow-up of patients with testicular carcinoma particularly due to the high probability of normal scan to assure oncologist of low likelihood of relapse of the disease. However, since the sensitivity and positive predictive values of the study are rather low, there are limitations of positive ^{18}F -fluorodeoxyglucose positron emission tomography-computed tomography scan to suggest persistent disease. Therefore, the oncologist should not rely only on the results of the positron emission tomography-computed tomography scan in making decisions about future treatment of patients with positive positron emission tomography-computed tomography findings.

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INFLUENCE OF DIFFERENT LEVELS OF SPORTS ACTIVITIES ON THE QUALITY OF LIFE AFTER THE RECONSTRUCTION OF ANTERIOR CRUCIATE LIGAMENT

UTICAJ RAZLIČITIH NIVOVA SPORTSKE AKTIVNOSTI NA KVALITET ŽIVOTA POSLE REKONSTRUKCIJE PREDNJEG UKRŠTENOG LIGAMENTA

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Summary

Introduction. The goal of this study was to examine the nature and presence of influence of different levels of sports activity on the life quality of the patients a year after the reconstruction of anterior cruciate ligament. **Material and Methods.** The study included 185 patients operated at the Department of Orthopedic Surgery and Traumatology of the Clinical Centre of Vojvodina, who were followed for twelve months. Data were collected using the modified Knee Injury and Osteoarthritis Outcome Score questionnaire which included the Lysholm scale. **Results.** This study included 146 male and 39 female subjects. The reconstruction of anterior cruciate ligament was equally successful in both gender groups. In relation to different types of sports activity, there were no differences in the overall life quality measured by the questionnaire and its subscales, regardless of the level (professional or recreational). However, regarding the level of sports activities, there were differences among the subjects engaged in sports activities at the national level as compared with those going in for sports activities at the recreational level, and particularly in comparison with physically inactive population. A significant correlation was not found by examining the aforementioned relationship between sports activities. **Conclusions.** This study has shown that the overall life quality a year after the reconstruction of the anterior cruciate ligament does not differ in relation to either the gender of the subjects or the type of sports activity, while the level of sports activity does have some influence on the quality of life. Professional athletes have proved to train significantly more intensively after this reconstruction than those going in for sports recreationally.

Key words: Sports; Quality of Life; Anterior Cruciate Ligament; Anterior Cruciate Ligament Reconstruction; Recreation; Athletes; Sex Characteristics

Introduction

Sports has become a global game in the modern society and the number of sports injuries is increasing as a result of the imposed requirement to achieve top sports results often not regarding the psychological and physical possibilities of an individual

Sažetak

Uvod. Cilj ove studije bio je ispitivanje prirode i postojanja uticaja različitih nivoa sportske aktivnosti na kvalitet života pacijenata, godinu dana nakon rekonstrukcije prednjeg ukrštenog ligamenta. **Materijal i metode.** Studija je obuhvatila 185 pacijenata Klinike za ortopedsku hirurgiju i traumatologiju Kliničkog centra Vojvodine, praćenih tokom perioda od 12 meseci. Podaci su prikupljeni pomoću modifikovanog *Knee Injury and Osteoarthritis Outcome Score* upitnika koji sadrži Lišolmovu skalu. **Rezultati.** Studija je obuhvatila 146 muških i 39 ženskih ispitanika. Rekonstrukcija prednjeg ukrštenog ligamenta bila je podjednako uspešna u obe populacije. U odnosu na različite tipove sportskih aktivnosti, nije bilo razlika u ukupnom kvalitetu života merenim upitnikom i njegovim supskalama, nezavisno od nivoa (profesionalni ili rekreativni). Bolji kvalitet života u odnosu na nivo sportskih aktivnosti, pokazali su ispitanici na nacionalnom nivou u poređenju sa onima na rekreativnom nivou, a svi nivoi sportskih aktivnosti poređeni su sa neaktivnima. Značajna korelacija nije pronađena ispitivanjem navedenih odnosa između sportskih aktivnosti. **Zaključak.** Sprovedenjem ove studije pokazano je da se ukupni kvalitet života godinu dana nakon rekonstrukcije prednjeg ukrštenog ligamenta ne razlikuje u odnosu na pol ispitanika ili tip sportske aktivnosti, dok nivo sportskih aktivnosti ima neki uticaj na kvalitet života. Značajno intenzivnije treniraju profesionalni sportisti u odnosu na rekreativne nakon rekonstrukcije. **Ključne reči:** Sport; Kvalitet života; Prednji ukršteni ligament; Rekonstrukcija prednjeg ukrštenog ligamenta; Rekreacija; Sportisti; Polne karakteristike

[1]. The most frequent injuries are injuries of the knee ligaments, especially of the anterior cruciate ligament (Ligamentum cruciatum anterius – LCA). Anterior cruciate ligament (ACL) maintains the stability of the knee and prevents anterior sliding of the tibia relative to femur thus contributing to normal function of the knee [2, 3] Injuries of the ante-

Abbreviations

ACL	– anterior cruciate ligament
KOOS	– Knee Injury and Osteoarthritis Outcome Score
BTB	– bone-to-bone

rior cruciate ligament require special attention in training and competition of athletes because they induce serious consequences and are one of the leading risk factors for early development of degenerative processes in the knee joint [4, 5]. They lead to difficulties in walking, running and jumping especially during the change of direction and thus reduce the quality of patients' life. The level of activities which the injured athlete will regain after the therapy is the most significant. The aim of the ACL reconstruction is to regain the knee stability, increase the quality of life, maintain the range of motion and thus protect the knee from further damage. A successful ACL reconstruction is the "quality of life" operation which enables young people to return to their professional, life and sports activities [1].

According to the World Health Organization (WHO), the quality of life presents a personal observation of one's own position in life in the cultural value system in which an individual lives related to his own aims, expectations, standards and interests [6]. It is made of physical health of an individual, one's psychological status, financial independence, social relations and their relations to significant characteristics of the environment [7]. In the contemporary clinical practice, the concept of quality of life is gaining a great significance in analysis of problems in various clinical situations [8]. The evaluation of quality of life can be subjective or personal and objective or an evaluation done by an observer. The scales are designed to measure the range in which a person is satisfied by the quality of life.

The aim of our study was to measure the quality of life of professional and recreational athletes as well as to determine whether different levels of sports activities influence the quality of life a year after the ACL reconstruction.

Material and Methods

A registry was introduced at the Department of Orthopedic Surgery and Traumatology, Clinical Center of Vojvodina in Novi Sad in 2012 in order to

provide the insight into the quality of life after the ACL reconstruction. The quality of life questionnaire provides an objective image a year after the surgery and the patients express their opinion and evaluation of the treatment results and the quality of life.

The research was approved by the Ethics Committee of the Clinical Center of Vojvodina in Novi Sad and it was conducted as a cross-sectional study. The study provided the insight into the quality of life of professional and recreational athletes at different levels of sports activity. The data were collected from the registry of patients operated at the Department of Orthopedic Surgery and Traumatology and the questionnaire was sent to the patients by e-mail during the period from January 1st, 2013 to December 31st, 2013. The exclusion criteria were the patient's wish not to participate and not replying to e-mail or telephone call.

The variables were operationalized through the questionnaire which consisted of three segments - after general information, the first part included questions about closer description of the injury characteristics, the second part was the Knee Injury and Osteoarthritis Outcome Score (KOOS) questionnaire with some added questions relevant for the research, and the third part was the Lysholm score.

The KOOS questionnaire [9] is used for injuries of the knee which may lead to posttraumatic osteoarthritis - ACL, meniscal and cartilage injuries. It consists of 43 questions in five subscales. It is constructed as a Likert type multilevel scale where lower score means better or higher result. The questionnaire is divided into five sections. The first section refers to the quality of life after the ACL reconstruction, the second section refers to data regarding the pain during various activities, the third section refers to daily activities, the fourth section refers to the level of physical activity and the fifth section refers to the patient's own awareness about the quality of life and the way he perceives his injury. The term "during the past week" is the time on which the participant has to focus.

The Lysholm scale is one-dimensional and consists of eight questions which are scored by the previously determined system. In this case, a higher score means a higher result. The score spans from 0 to 100 and has four grades: poor (<65), fair (66-83), good (84-90), and excellent (>90).

Table 1. Gender differences in the KOOS questionnaire
Tabela 1. Polna razlika u KOOS upitniku

GENDER (male/female)/POL (muški/ženski)	t-test	p
Life quality/Kvalitet života	-0,79	0.46 (>0.05)
Pain intensity/Intenzitet bola	1,09	0.27 (>0.05)
Usual activities/Uobičajene aktivnosti	-0,05	0.95 (>0.05)
Sports activities/Sportske aktivnosti	-0,6	0.53 (>0.05)
Life quality awareness/Svest o kvalitetu života	-1,08	0.27 (>0.05)
KOOS	-0,60	0.54 (>0.05)
Lysholm score/Lišolmov skor	1,89	0.06 (>0.05)

Table 2. Differences in the KOOS questionnaire score according to the type of sports activities and intensity of training**Tabela 2.** Razlika u rezultatu KOOS upitnika prema tipu sportskih aktivnosti i intenzitetu treninga

Sports activity (professional/amateur athletes) <i>Sportske aktivnosti</i> (profesionalni sportisti/amateri)	AA		SD	
	Professional athletes <i>Profesionalni sportisti</i>	Amateur athletes <i>Amateri</i>	Professional athletes <i>Profesionalni sportisti</i>	Amateur athletes <i>Amateri</i>
Intensity of training <i>Intenzitet treninga</i>	2.13	2.52	0.91	0.97
KOOS	112.37	107.16	17.96	19.16
Lysholm score/ <i>Lišolmov skor</i>	90.27	89.39	8.52	8.25

Out of 185 patients included in the study, 146 were males and 39 females. The average age of the whole group was 26.1 (16-55), being 26.9 for males and 23.3 for females. The ACL reconstruction was performed between January 1st, 2012 and December 31st, 2012. The follow-up period lasted 12 months. The left knee was injured in 73 and the right knee in 112 cases. The injuries occurred during recreational sports activities (95), professional sports activities (84), walking (4), training (1) and one participant injured his knee in a traffic accident. According to the level of sports activity, all participants were graded to international (26), state (43), regional (28), recreational level (82) and amateurs (6). In almost all cases (99%), the bone-to-bone (BTB) surgical technique was performed and the "Hamstring" technique was performed in 1% of the cases.

The first assignment, i.e. the display of the sample structure and relevant characteristics which caused the nature of the variables in this research, was carried out by descriptive statistics. The t-test for independent samples was used for the second assignment, i.e. determination of differences in questionnaire and its subscales of measured life quality related to gender, sports activity and training intensity prior to surgery. F-test was used for the third assignment, i.e. determining the differences of life quality related to the level of sports activity. The correlation (Spearman's Rho) was used for the fourth assignment, i.e. determining the correlation of the aforementioned relationship between sports activities. The data were processed by the software IBM SPSS Statistics 21.0 of the statistical software package IBM SPSS (Statistical Package for the Social Sciences) and are displayed by tables and graphs.

Results

The overall level of reliability of the complete questionnaire is very satisfactory. There were no differences in the KOOS questionnaire score related to the gender, while the difference on the Lysholm scale occurred due to the unevenness of the gender distribution of the sample (**Table 1**). The male population prevailed in this study sample. When the subjects were compared according to the type of sports activity, there was no difference in the questionnaire subscales nor in the Lysholm scale, no matter whether they were professional or recreational athletes (**Tables 2 and 3**).

A year after the reconstruction, the average value of the training intensity by the groups was AA=2.13 for professional athletes and AA=2.52 for recreational athletes (**Table 2**) according to the scale 1-4 (where 1 represents higher training intensity after the reconstruction, 2 represents the same intensity, 3 represents the poorer intensity and 4 represents the absence of training activity). There was a difference in the training intensity compared to the period prior to the surgery ($p < 0.05$) meaning that the professional athletes train significantly more intensively than recreational athletes after the surgery (**Table 3**).

As for the level of sports activity, this study included the subjects on international, national, regional and recreational level as well as amateurs. F test showed statistically significant difference ($p < 0.05$) in the KOOS questionnaire score between the amateurs and all other groups as well as a difference in life quality between the state level athletes and recreational athletes (**Graph 1**). Lysholm scale did not show any difference. The Sports activities subscale showed the difference between the amateurs and the state level athletes as well as be-

Table 3. Differences in the KOOS questionnaire score according to the type of sports activities and intensity of training**Tabela 3.** Razlika u rezultatu KOOS upitnika prema tipu sportskih aktivnosti i intenzitetu treninga

Sport activity (professional/amateur athletes) <i>Sportske aktivnosti (profesionalni sportisti/amateri)</i>	t-test	p value <i>p vrednost</i>
Intensity of training/ <i>Intenzitet treninga</i>	2.78	0.005 (<0.05)
KOOS	-1.86	0.06 (>0.05)
Lysholm score/ <i>Lišolmov skor</i>	-0.70	0.48(>0.05)

Table 4. Correlation of the KOOS questionnaire score and the Lysholm score with type and level of sports activity
Tabela 4. Korelacija rezultata KOOS upitnika i Lysholm skora sa tipom i nivoom sportske aktivnosti

Correlations/Korelacije		Spearman Rho <i>Spearmanov Rho</i>	p - value <i>p - vrednost</i> * >0.05
KOOS	Sports activity/ <i>Sportska aktivnost</i>	0.102	0.16*
	Level of sports activity/ <i>Nivo sportske aktivnosti</i>	0.083	0.26*
Lysholm score <i>Lisholmov skor</i>	Sports activity/ <i>Sportska aktivnost</i>	0.055	0.45*
	Level of sports activity/ <i>Nivo sportske aktivnosti</i>	0.086	0.24 *

tween the amateurs and the international level athletes (**Graph 2**). All other subscales as well as the Lysholm scale did not show any differences.

The examination of correlation between the quality of life and the type and level of sports activity as well as the correlation of the Lysholm score and the mentioned relations was not statistically significant in this study sample (**Table 4**).

Discussion

The main reason for the ACL reconstruction is to regain the ability of going in for sports activities at the same level without limitations, to return to daily activities as well as to prevent secondary degenerative changes [10] Sports activities have a positive effect on health of an individual indicating the importance of the level of sports activity and the participation in an organized sports training [11].

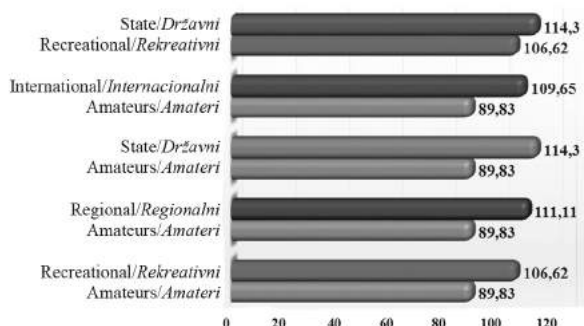
During the last few years, the measurements of the "health-related quality of life" are introduced into the overall outcome of orthopedic surgical procedures. These measurements provide the patient's perspective and serve as an addition to other measurements of the outcome of knee injuries and contribute to make a more complete picture of how an injury or an illness affects all spheres of life [12, 13], thus providing an opportunity to compare the outcome in the patients with different injuries or illnesses which affect the musculoskeletal system.

The male population prevails in this as well as in some other studies performed worldwide [14, 15]. This

can be contributed to the fact that men more often participate in professional sports as well as in contact sports where forces which are exerted to the joints are extremely strong. In the study done by Ott SM et al. [16], where the follow-up period was 5 years, (74 male and 77 female participants), women had a much higher Lysholm score. As opposed to that, other gender-related differences were not identified.

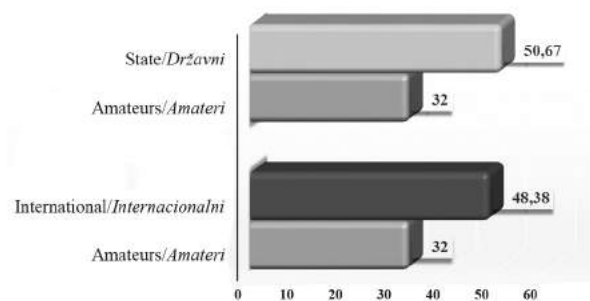
The quality of life evaluation was done by the KOOS questionnaire which was developed in the 1990s as an instrument for evaluating the patient's opinion about his/her knee and related problems. It consists of five subscales and the term "during the last week" is the time on which the participant has to focus while answering the questions [9]. In the study performed by Swirtun L.R and Renstro P. [17], the average follow-up was 5.6 years and women had lower score on the Sports activity subscale of the KOOS questionnaire than men while there were no significant differences on other subscales.

Participation in sports activities in the modern society is growing into a game on the global scale. In that social climate, sports is distinguished as a recreational activity which has the purpose to establish and preserve psychological and physical balance of an individual, and professional sports which starts at ever younger age and with an increasing number of trainings with its essential feature – providing evidence of the relativity of the final frontier of man's psychological and physical abilities [1]. The absence of difference in life quality related to the type of sports activity in our sample can be



Graph 1. Differences in the KOOS questionnaire score according to the level of sports activity

Grafikon 1. Razlike u rezultatu KOOS upitnika prema nivou sportskih aktivnosti



Graph 2. Differences on the subscale of sports activity in the KOOS questionnaire according to the level of sports activity

Grafikon 2. Razlike na supskali sportskih aktivnosti u KOOS upitniku prema nivou sportskih aktivnosti

explained by the performance of the same surgical technique (99% BTB) and the same and well-conducted physical rehabilitation program.

One of the most important reasons of the ACL reconstruction is to enable the patient to regain his sports activity to the level prior to injury. A statistically significant difference in the KOOS questionnaire score was observed in this study between the amateurs and all other groups as well as differences in the life quality of state and recreational level athletes. The state level athletes had better life quality compared to the recreational athletes, and that can be explained by bigger muscle mass before the surgery, earlier diagnosis and reconstruction as well as the more intense rehabilitation program because state level athletes are supposed to get back to full activity as soon as possible. One more reason for these results could be that some recreational athletes ceased to train sports after the ACL reconstruction. The study done by Kostogiannis et al. [18] showed a statistically significant difference on Lysholm scale 15 years after the injury which was not the case in our one-year follow-up. In their study, the lower life quality score in the KOOS questionnaire was reported among the subjects who had injured their knee in sports involving contact than those subjects who had injured their knee in a sport without contact. Our study did not include the evaluation of life quality related to the type of sports but it may certainly be one of the aims of our future studies.

On the Sports activities subscale, the statistically significant difference ($p < 0.05$) is between amateurs (AA=32) and international level athletes (AA=48.38), as well as between amateurs (AA=32) and state level athletes (50.67). In our sample, there was a high unevenness in the number of amateurs and athletes and that may produce these results. Regarding the fact that the Sports activity scale included the questions regarding the level of sports activity (How much time passed from surgery to sports activity? How intensive is your training now? Do you have problems with the operated leg during standing, running, etc.), there is a possibility that amateurs did not answer these questions or answered them inadequately, which may be one more reason for these results. International and state athletes showed satisfactory results on this subscale.

By going through different phases from the moment of injury to the moment of healing, which include the proper medical intervention and rehabilitation, an athlete goes through different emotional reactions. Emotions such as fear, anger or depression may cause an athlete to stop training as well as to develop chronic fatigue and muscle tension. All of that can affect the injury in terms of intensifying experience of pain and prolonging the recovery period [19]. The ability of athletes to cope with stress shows a result in recovery and advancement through the rehabilitation program [20]. Mal-adjusted psychological mechanisms may be harmful

and have negative effect on the athlete's ability to regain the level of sports activity and may also affect the quality of sports performances thus increasing the risk of re-injury [21].

The difference in training intensity related to the preoperative period showed a statistically significant difference in our study sample. Professional athletes train significantly more intensively than recreational athletes after surgery. We believe that the information on the frequency of level of training is an important descriptor of the sample because it indicates the level of success of the ACL reconstruction and requires a follow-up because Arden et al. [22] have stated in their study that persons who regain their level of sports activity in the first 12 months cannot retain the same level during the later follow-up of seven and more years. Therefore, the 12-month period is considered to be too short for assessing the precise level of activity and the quality of life.

The anterior cruciate ligament reconstruction is a successful surgical procedure with rare complications, which enables patients to return to their daily and work activities more quickly, lowers the overall expenses and shortens the time of healing [23, 24]. The general goal is to restore the biomechanics of the knee, allow activities that were possible prior to the injury and optimize the quality of life related to health [25]. No statistically significant correlation was found between the quality of life and the Lysholm score with the type and level of sports activity in our 1-year follow-up. As far as we know, other authors have not examined the existence of correlations.

Having had access to the current world literature, we can conclude that future studies should be conducted for five or more years and expand the sample which will provide an insight into the possible existence of differences in the quality of life as well as the difference in the quality of life related to whether the subjects injured their knee in a sport with contact or in a sport without contact, or related to a period of returning to competition after the ACL reconstruction which may be one more reason for further follow-up. One-year follow-up is considered to be too short for the precise evaluation of quality of life.

Conclusion

By analyzing the data we collected in this research, we have found that the quality of life a year after the reconstruction does not differ regarding the gender and the type of sports activity. Different level of sports activity affects the quality of life. There is a difference between training intensity before and after the surgery – professional athletes train much more intensively after surgery than recreational athletes. The examination of correlation of quality of life and the Lysholm score with type and level of sports activity is not statistically significant in our sample.

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CHIKUNGUNYA – A SERIOUS THREAT FOR PUBLIC HEALTH

ČIKUNGUNIJA VIRUS – OZBILJNA PRETNJA ZA JAVNO ZDRAVLJE

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Summary

Introduction. Chikungunya is a contagious disease caused by Chikungunya virus, an arbovirus from the *Togaviridae* family. This infection is mostly spread by mosquitoes from the genus *Aedes*, especially *Aedes albopictus*, which have spread from Asia to America and Europe including some countries surrounding Serbia. **Epidemiologic Features.** The outbreak of epidemics has been reported in Philippines, Sumatra, Java, Indonesia, West Africa region (from Senegal to Cameroon), Congo, Nigeria, Angola, Uganda, Guinea, Malawi, Central African Republic, Burundi, South Africa and India. At the beginning of the 21st century, large outbreaks were recorded on the island of Réunion. During 2006, 1.400.000 cases of chikungunya infection were recorded in India. Local transmission of infection in continental Europe was reported from Northeast Italy (254 suspected and 78 laboratory confirmed cases in Emilia-Romagna region) and France (two cases in 2010). From December 2013 to June 2014, 5.294 confirmed cases and more than 180.000 suspected cases of chikungunya were reported in the Caribbean. **Clinical Findings.** The disease presents suddenly with fever, rash and arthralgia. In general, chikungunya is a mild self-limited disease. Less often, it may be presented with signs of meningoencephalitis or fulminant hepatitis, sometimes with fatal outcome. **Conclusion.** Fast developing international traffic and booming tourism as well as the vector spreading from its homeland make chikungunya a real threat to our country.

Key words: Chikungunya Fever; Chikungunya virus; Serbia; Signs and Symptoms; Diagnosis; Epidemiology; Risk Factors; Public Health; *Aedes*

Introduction

Chikungunya is a disease of viral origin, and until recently it has been geographically connected with tropical areas of Africa, Southeast Asia and

Sažetak

Uvod. Čikungunija je zarazna bolest izazvana arbovirusom čikungunija iz porodice *Togaviridae*. U širenju infekcije presudna je uloga vektora komarca *Aedes albopictus* koji se iz postojbine Azije proširio i na Ameriku i Evropu, uključujući i zemlje u našem okruženju. **Epidemiološke karakteristike.** Epidemijska pojava bolesti registrovana je na Filipinima, Sumatri, Javi, Timoru, u Indoneziji, Zapadnoj Africi od Senegala do Kameruna, Kongu, Nigeriji, Angoli, Ugandi, Gvineji, Malaviju, Centralnoj Afričkoj Republici, Burundiju, Južnoj Africi i Indiji. Početkom 21. veka registrovane su velike epidemije na ostrvu Reunion (*Le Réunion*). U Indiji je registrovano 1 400 000 slučajeva čikungunije tokom 2006. godine. U nekoliko evropskih zemalja registrovani su importovani slučajevi ovoga oboljenja. U severoistočnoj Italiji u oblasti Emilija-Romanja registrovana je prva epidemija u umerenom klimatskom pojasu sa 254 suspektna i 78 laboratorijski potvrđenih slučajeva. Dva slučaja lokalne transmisije registrovana su i u Francuskoj 2010. godine. Od decembra 2013. do juna 2014. godine, 5 294 potvrđena slučaja i 180 000 verovatnih slučajeva čikungunije registrovano je na Karibima. **Klinički nalazi.** Bolest se klinički manifestuje akutnim početkom s groznicom, osipom po koži i artralgijsama. Generalno, čikungunija je blaga bolest koja prolazi sama, bez komplikacija. Registrovani su i slučajevi meningoencefalitisa i fulminantnog hepatitisa. Smrtni ishod je redak. **Zaključak.** Sve intenzivniji saobraćaj i razvoj turizma, uz širenje vektora iz njegove postojbine, čine ovu bolest realnom pretnjom i za našu zemlju.

KLjučne reči: Čikungunija groznica; Čikungunija virus; Srbija; Znaci i simptomi; Dijagnoza; Epidemiologija; Faktori rizika; Javno zdravlje; Komarac *Aedes albopictus*

the region of the Indian Ocean [1]. Chikungunya presents with an abrupt onset, fever, skin rash and heavy arthralgia. The term originates from the word which in Swahili and Maconde language means "the one that is folded". Chikungunya has become the subject of increased interest at the beginning of the 21st century due to a number of large outbreaks with a lot of severe clinical forms of disease in India and on the islands in the Indian Ocean. Until recently, there were no outbreaks of chikungunya in Europe and America. However, an outbreak of chikungunya in Northeast Italy, expansion of

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Abbreviations

RT-PCR	– Real-time polymerase chain reaction
ELISA	– enzyme linked immunosorbent assay
IgM	– immunoglobulin class M
IgG	– immunoglobulin class G
MRC-5	– human lung fibroblast cell culture
DNK	– deoxyribonucleic acid
ECDC	– European Centre for Disease Prevention and Control
USA	– United States of America

chikungunya virus infection in the Caribbean and in South America in addition to fast international transportation and developed tourism requires from the Serbian health professionals to be aware of the possibility of professional exposure to chikungunya virus [2, 3]. Chikungunya virus is an arbovirus, a member of the *Alphavirus* genus in the family *Togaviridae*, 60-70 nm in diameter, with icosahedral symmetry and single-stranded ribonucleic acid – positive genome of 11 – 12 kilobases. Nucleocapsid is enveloped by phospholipid layer with glycoprotein antigens E1 and E2 inserted onto this layer. Those glycoproteins play a role in virus adsorption to the cell membrane receptor; they are responsible for the production of neutralizing antibodies. According to antigenic structure, genus *Alphavirus* can be divided into 7 serocomplexes. Chikungunya virus belongs to antigenic complex IV (Semliki Forest complex). The virus was discovered in a serum sample of a patient in Tanzania in 1953. Since then, two viral lines have been registered: Central-South-East-African line and West-African line including Asian genotype [4].

Epidemiologic Features

The primates are the natural reservoir of chikungunya virus. The *Aedes mosquitoes* transmit this virus to humans. The *Aedes aegypti* and the *Aedes albopictus* are the vectors of infection in Africa and Asia [5, 6]. It is believed that the *Aedes albopictus* is transmitted from Asia to other continents by importing used tires contaminated with mosquito eggs. Due to remarkable adaptability to urban life conditions, the *Aedes albopictus* has settled Belgium, Bosnia, Croatia, France, Greece, the Netherlands, Spain, Switzerland as well as Central America, Brazil and the United States of America (USA) [7]. In addition to mutations in viral genome, which increase contagiousness of virus for *Aedes albopictus*, vector expansion represents main factors that contribute to spreading this virus worldwide [8].

In geographic areas where the reservoirs and the vectors are widely distributed and human population possesses protective immunity to a high degree, sporadic cases and small outbreaks occur. Massive outbreaks occur in population with no protective immunity. Epidemic appearance of disease was reported in the Philippines, Sumatra, Java, Timor, Indonesia, West Africa from Senegal to Cameroon, Congo, Nigeria, Angola, Uganda, Guinea, Malawi,

Central African Republic, Burundi, South Africa and India [9]. More than 1.400.000 cases of chikungunya virus infection were recorded in India in 2006 [10]. Reunion, an island in the Indian ocean, was hit by a chikungunya outbreak in 2005; the estimated incidence of 40.000 cases per week was reached there in 2006 [11].

In the first decade of the 21st century, imported cases of chikungunya among passengers arriving from areas with a high incidence of chikungunya virus infection were reported in Europe and the USA. The appearance of imported cases in areas where the vectors are maintained concomitantly with the presence of specific climate features of environment creates the conditions for the occurrence of local autochthonous cases [12]. The first outbreak with autochthonous cases in Europe was recorded in Northeast Italy, Emilia-Romagna region, in July 2007 with a total of 254 suspected and 78 laboratory confirmed chikungunya cases [13]. Two autochthonous cases of chikungunya infection were recorded in southeastern part of France in 2010 [14]. The first autochthonous viral transmission in America was recorded on December 6, 2013, when two cases of chikungunya infection were confirmed in laboratory on Saint Martin island (located in the Caribbean). In the next few months, chikungunya infection spread across the majority of Caribbean islands and South America. More than 100.000 probable cases and 4.182 confirmed cases of disease were reported in June 2014 [15]. Experts from the European Center of Control and Prevention of Diseases have estimated that there is a considerable risk of disease spreading across the continental America where the *Aedes aegypti* is endemic, as well as to European continent where the *Aedes albopictus* exists and to the island of Madeira where there is a high risk to get infected by chikungunya virus. Two factors which contribute to virus spreading on the island of Madeira are the native presence of vector and appropriate climate features for its growth [3]. Is it a repetition of epidemiologic scenario already seen during the expansion of the West Nile virus that has been only an exotic member of arboviruses for a long time which has subsequently spread and become endemic in America and Europe provoking several outbreaks, two of which occurred in Serbia in 2012 and 2013 [16].

Clinical Findings

Incubation period ranges from 1 to 12 days (2-4 days on average). Asymptomatic infection may occur, but its incidence is still unknown. Clinically manifested disease begins abruptly with fever, headache, back pains, myalgia and arthralgia. The most common sites of pains are ankles, wrists and interphalangeal joints of hand; big joints may be involved less often. Skin rash is present in half of the cases. In adults, rash may be maculopapular, pruriginous and most dense on the thorax. In children, rash most often has bullous presentation, followed by

crusting. Facial edema may appear as well as localized petechiae and gingival hemorrhages [17, 18]. The analysis of 504 patients in the outbreak on Reunion island showed that fever and arthralgias were present in all of them, as well as headache (70%), myalgia (60%) and rash (39%). In the outbreak in Malaysia in 1998, all of 51 patients were febrile, a half of them manifested rash, myalgia, headache and back pains, 78% of patients presented with small joint arthralgias, whereas 18% had large joint arthralgias. The patients' complaints resolved within 7–10 days except rigidity and arthralgias, which lasted for a longer period of time [19]. The main characteristic of chikungunya is the presence of recurrent arthralgia and arthritis, which persist in 73–80% of cases with serologic confirmation of disease. In one-third of cases the duration of arthralgia/arthritis is 4 months, whereas in 10% of cases it lasts from 3 to 5 years. Pathogenesis of joint manifestations is unclear. Radiographs of affected joints are normal, while biologic inflammatory markers are normal or show moderately elevated values [20].

Chikungunya is most often associated with favorable prognosis *quoad vitam*. During the outbreak of chikungunya on Reunion islands, 3.9% of patients were admitted to hospital [21]. Meningoencephalitis cases from this outbreak confirm neurotropism of chikungunya virus. Those cases were recorded in the outbreak of chikungunya on Reunion islands at a rate of 1.7/1000 cases of chikungunya [21].

Infection of pregnant women is possible during the whole pregnancy. Transmission of infection during the first trimester of pregnancy may be ended by miscarriage. Viral transmission from the mother to her fetus usually occurs through maternal viremia during the delivery. Transplacental and perinatal transmission of infection was first described in relation to the outbreak on Reunion island. In 159 pregnant women with clinically manifested disease, etiology was confirmed by serologic testing or real-time polymerase chain reaction (RT-PCR). Out of 35 pregnant women who were ill at the time of delivery, 30 gave birth to an infected child. The disease in pregnant women is not more severe than in non-pregnant ones. Neonatal chikungunya is severe disease and clinically resembles neonatal dengue virus infection [9]. So far there has been no prospective study to examine the risk for embryopathies, fetopathies and late sequelae in infected children.

Laboratory Diagnosis

RT-PCR with reversal transcription performed on serum sample of patient is suitable to make the diagnosis early (the first week of disease) [3]. Among serologic tests, hemagglutination inhibition reaction, complement fixation test, fluorescent antibody test and enzyme linked immunosorbent assay (ELISA) could be applied. Immunoglobulin M (IgM) chikungunya antibodies can be proved as early as on the second day of disease and persist for 3 months. In all imported cases, IgM and IgG

chikungunya antibodies were proved after the 5th day since the onset of disease [22]. IgM antibodies persist for a number of years. Evaluation of serological results should not ignore cross-reactivity to dengue virus and o'nyong-nyong virus. The diagnosis can also be made either by virus isolation on suitable cell cultures or in mice. Cell cultures of mammals are suitable. The virus may be isolated by intracerebral inoculation into baby mice [4].

Immunity and Vaccination

After recovering from natural chikungunya virus infection, humans acquire solid immunity. There is no commercial vaccine for humans. However, studies on live vaccine developed in United States Army Medical Research Institute of Infectious Diseases were conducted until 2000; as vaccinia virus, the "15561" virus isolated in a Thai patient and then attenuated by serial passages in MRC-5 cells was used. Since the 1970s, the investigational efforts on developing inactivated vaccine have been made. Inactivated by formaldehyde and tested on volunteers, this dead vaccine has been shown as efficient and safe [23]. In an experimental work with mice, a solid humoral and cellular immune response has been achieved by use of a deoxyribonucleic acid (DNA) vaccine. In order to get this vaccine, gene sequences encoding capsid and envelope glycoprotein E1 and E2 have been used [24].

Prevention

Vector control and individual protection from mosquitoes in the absence of active immunization represent major measures for prevention of chikungunya infection. A network of research laboratories capable of prompt diagnosing of arbovirus infections should be developed worldwide wherever *Aedes albopictus* exists in order to control chikungunya.

Treatment

Treatment of chikungunya is symptomatic. Bed rest, rehydration and analgesics (among which ibuprofen, naproxen or acetaminophen are recommended) are needed. Acetylsalicylic acid should be avoided. Combination of alpha-interferon and ribavirin appears to have synergistic effect [25]. Chloroquine has successfully helped with chronic arthralgia caused by chikungunya virus, but has not been useful for acute arthralgia [4].

Conclusion

Considering expansion of chikungunya virus in the Caribbean and in South America, growing transportation, commercial and touristic connections of Serbian population and the presence of vector in the surrounding countries alerts to potential risk of chikungunya appearance in our country.

Serbian health professionals must be educated on this arbovirus infection and its significance for public health. In addition, it may be useful to design programs for monitoring the vector and virus ac-

tivities, on the basis of which a program of disease control and prevention should be created. This would be a multidisciplinary task for physicians, veterinarians, biologists and entomologists.

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PRIKAZI SLUČAJEVA CASE REPORTS

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Case report
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FROM ANEURYSMAL BONE CYST TO TELANGIECTATIC OSTEOSARCOMA WITH METASTASIS IN INGUINAL LYMPH NODES – CASE REPORT

*OD ANEURIZMALNE KOŠTANE CISTE DO TELANGIEKTATIČNOG OSTEOSARKOMA SA
METASTAZOM U INGVINALNIM LIMFNIM ČVOROVIMA – PRIKAZ SLUČAJA*

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Summary

Introduction. Aneurysmal bone cyst is a benign bone lesion composed of blood filled cystic cavities lined by fibrous septa. Its malignant transformation of is a rare event. **Case report.** We report a case of a lesion in the second metatarsal bone in a 29-year-old male, presented as a slight swelling of the right foot. After the curettage had been done, the diagnosis of aneurysmal bone cyst was made but the recurrence occurred 4 years later. The biopsy of the recurrent tumor showed compact neoplastic tissue consistent with diagnosis of giant cell tumor with malignancy. The malignant component was recognized as a high grade sarcoma with osteoid production. A tumor mass with the whole II metatarsal bone was extirpated and a resected part of fibula was transplanted. A year later, another recurrence occurred, an amputation was performed and a telangiectatic osteosarcoma with inguinal lymph nodes metastases was diagnosed. No other tumor mass was confirmed, either clinically or by imaging techniques at the time of his third operation. He died 4 months later with multiple pulmonary metastases. **Conclusion.** We emphasize the importance of team work in order to achieve the accurate diagnosis, highlighting careful radiological examinations, good sampling and awareness of unusual cases in bone tumor pathology.

Key words: Bone Cysts, Aneurysmal; Osteosarcoma; Neoplasm Metastasis; Lymph Nodes; Adult; Giant Cell Tumors; Metatarsal Bones; Diagnosis

Introduction

Aneurysmal bone cyst (ABC) is a benign bone lesion composed of blood filled cystic cavities lined by fibrous septa. The septa are composed of mesenchymal tissue containing fibroblasts, multinucleated giant cells and osteoid or immature trabeculae of reactive

Sažetak

Uvod. Aneurizmalna koštana cista je benigna lezija sastavljena od cističnih šupljina razdvojenih fibroznim pregradama. Veoma je retka njena maligna transformacija. **Prikaz slučaja.** Prikazujemo slučaj lezije u drugoj metatarsalnoj kosti kod muškarca starog 29 godina u vidu blagog otoka na desnom stopalu. Posle urađene kiretaže, postavljena je dijagnoza aneurizmalne koštane ciste i recidiv se javio četiri godine kasnije. Biopsija recidivnog tumora je pokazala kompaktno neoplastično tkivo koje odgovara dijagnozi tumora džinovskih ćelija sa malignitetom. Maligna komponenta je prepoznata kao sarkom visokog stepena sa stvaranjem osteoida. Tumorska masa sa celom drugom metatarsalnom kosti je izvađena i deo fibule je transplantiran. Godinu dana kasnije, došlo je do još jednog recidiva, izvedena je amputacija i dijagnostikovao je telangiektatični osteosarkom sa metastazama u ingvinalnim limfnim čvorovima. Tokom treće operacije, nije potvrđena neka druga tumorska masa, bilo klinički bilo nekim tehnikama snimanja. Pacijent je preminuo četiri meseca kasnije sa multiplim metastazama u plućima. **Zaključak.** Naglašen je značaj timskog rada u cilju dobijanja tačne dijagnoze, važnost pažljivog radiološkog pregleda, pravilnog uzimanja uzorka i saznanja o postojanju neuobičajenih slučajeva tumora kostiju.

Ključne reči: Aneurizmalna koštana cista; Osteosarkom; Metastaze; Limfni čvorovi; Odrasli; Tumori gigantskih ćelija; Metaatarzalne kosti; Dijagnoza

bone. It may be a primary or secondary lesion developing into other benign and malignant tumors [1–3].

Malignant transformation of ABC is a rare event and has been described as a result of previous treatment, more often radiation than curettage. Malignant tumors developed from ABC are recognized as malignant fibrous histiocytoma or osteosarcoma [4–6].

Abbreviations

- ABC – aneurysmal bone cyst
 GCT – giant cell tumor
 COSS – Cooperative Osteosarcoma Study Group

Metastatic dissemination in osteosarcoma occurs haematogenously, though the regional lymph node involvement is rarely reported [7].

We present a patient with aneurysmal bone cyst of foot who developed a telangiectatic osteosarcoma with metastases in the inguinal lymph nodes four years later.

Case Report

A 29-year-old male was admitted to the hospital because of the pain lasting for a few months and a slight swelling of his right foot. Radiography showed a lytic lesion with benign radiographic characteristics in the proximal part of the second metatarsal bone. The differential diagnosis, based on radiographic examination, was giant cell tumor (GCT) or aneurysmal bone cyst (Figure 1). Curettage was performed and the histological analysis confirmed aneurysmal bone cyst (Figure 2).

Four years later in October 2008, the patient was admitted to the same hospital because of a local recurrence manifested as a large swelling of the foot, followed by severe pain during walking. Imaging studies showed a lytic lesion that expanded the bone and destroyed the cortex (Figure 3).



Figure 1. Lytic lesion with benign radiographic characteristics consistent with ABC

Slika 1. Litična lezija sa benignim radiografskim karakteristikama koje odgovaraju aneurizmalnoj koštanoj cisti

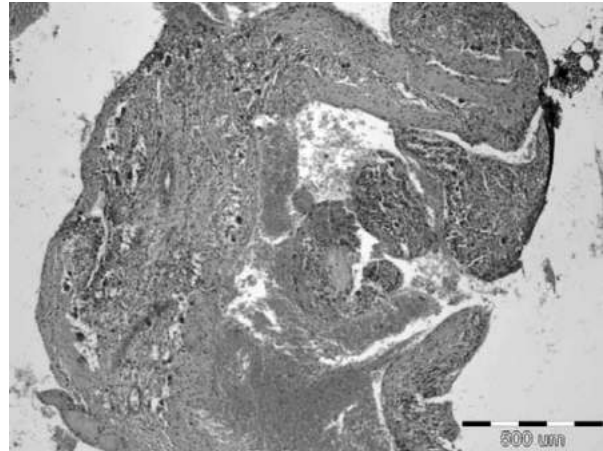


Figure 2. Microphotograph of ABC (H.E. 10 x 10)

Slika 2. Mikrofotografija aneurizmalne koštane ciste (H.E. 10 x 10)

After a needle biopsy had been performed and malignant cytology confirmed, a surgical intervention was done. A tumor mass with the whole II metatarsal bone was extirpated and a resected part of fibula was transplanted (Figure 4). The microscopic analysis confirmed giant cell tumor with malignancy (Figure 5).

The patient refused any other therapy except surgery and was disease-free till 2009.

Recurrent tumor mass was found in October 2009, affecting the first and third metatarsal bone as well. An inguinal lymph node enlargement was present at the same side. The amputation and lymphadenectomy were performed and the histological examina-



Figure 3. Computed tomography of the first recurrence; a lytic lesion that expanded the bone and destroyed the cortex
Slika 3. Kompjuterizovana tomografija prvog recidiva; litična lezija koja je uvećala kost i uništila korteks

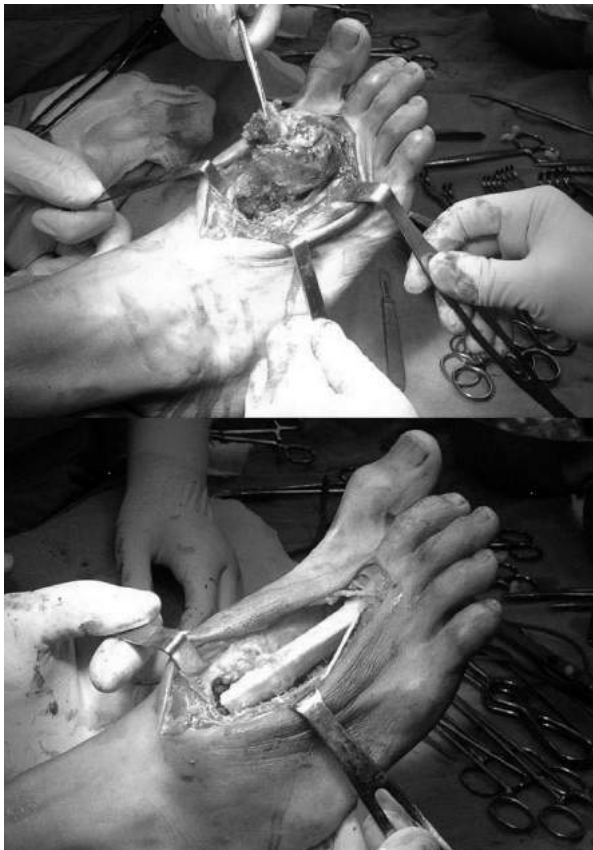


Figure 4. a) Extirpation of the tumor b) fibula transplant
Slika 4. a) Ekstirpacija tumora, b) transplantacija dela fibule

tion confirmed telangiectatic osteosarcoma with lymph node metastases (**Figure 6**).

No other tumor mass was confirmed, either clinically or by imaging techniques at the time of his third operation. He died 4 months later with multiple pulmonary metastases. Autopsy was not performed.

Histological Examination

Tissue samples were taken from the operative material, fixed in formalin and cut in 5 microns thin sections for routine light microscopy.

Pathologic Findings

The first operation: The curettage material was composed of numerous bone and soft tissue fragments, with different shapes merged in coagulated blood.

Microscopically, the tumor tissue was composed of multiple fibrous septa containing capillary vessels, fibroblasts, myofibroblasts, giant cells, some hemosiderin loaded macrophages, osteoblasts and osteoid. They enclosed the vascular spaces filled with blood.

The second operation: The operative material was composed of 10 tissue fragments (cut by the surgeon), which were recognized as parts of metatarsal bone measuring 9x4x3.7 cm and meaty tumor tis-

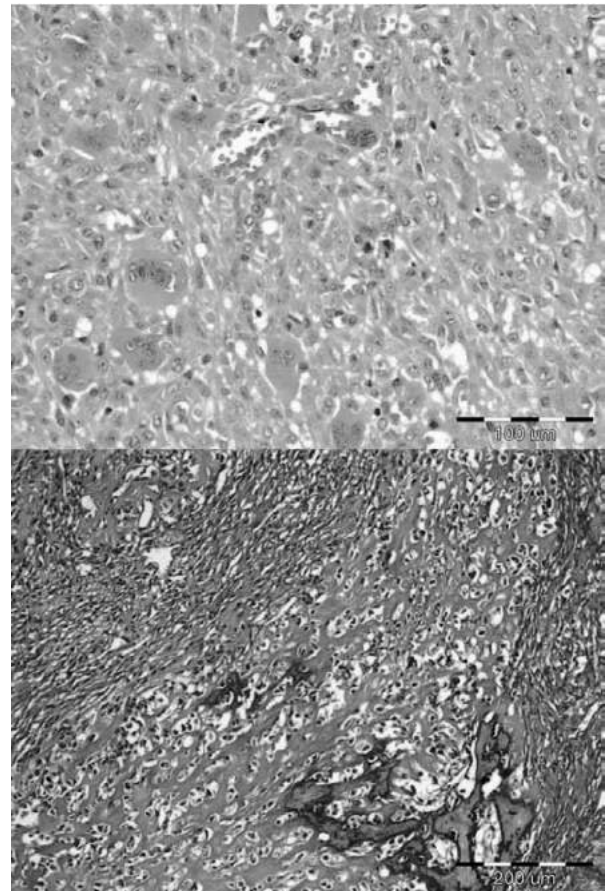


Figure 5. a) Areas with morphological features of GCT (H.E 10 x 20), b) Malignancy in GCT (the same tumor) with morphological features of osteosarcoma (H.E. 10 x 10)
Slika 5. a) Površine sa morfološkim odlikama tumora džinovskih ćelija (H.E 10 x 20), b) Malignitet u istom tumoru sa morfološkim odlikama osteosarkoma (H.E 10 x 10)

sue having reddish-gray color, firm consistency merged with soft yellow areas and areas of hemorrhage.

Microscopically the tumor tissue contained 2 different components. The first was recognized as GCT and the second as a high grade sarcoma with osteoid production. The first type of tissue was composed of round and elongated mononuclear cells mixed with multinuclear osteoclast-like giant cells. The nuclei of mononuclear and giant cells were identical. Cytological and nuclear atypia were mild and there were no atypical mitoses, although some mitotic activity was present. Spindle cell rich areas with fascicular and storiform pattern and areas of hemorrhage were also found, but no cystic spaces or any other certain morphological signs of preexisting ABC was found. The second type of tissue, juxtaposed to GTC, was recognized as a malignant neoplasm with the characteristics of osteosarcoma with areas of heavy deposition of osteoid. There were some giant cells in the sarcomatous stroma.

The third operation: The amputated right lower extremity had a large tumor mass at the dorsal site

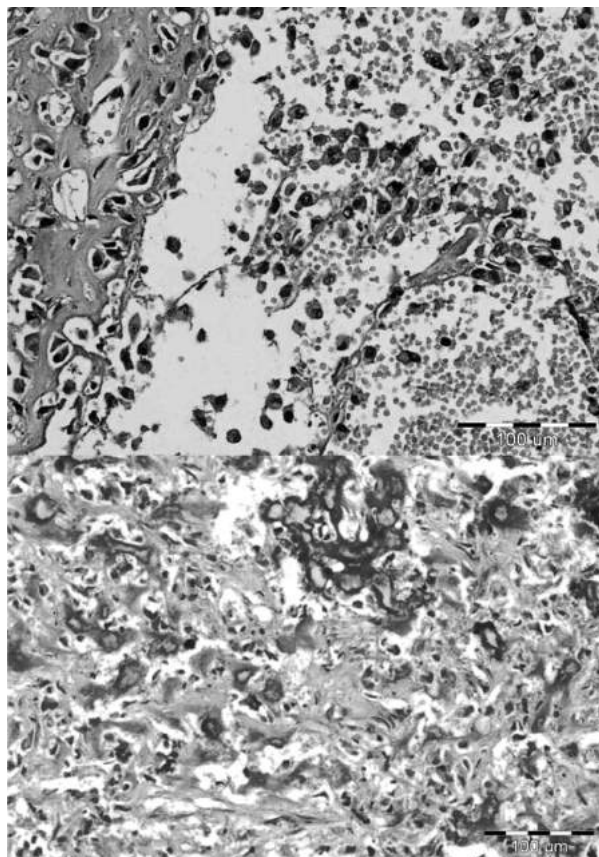


Figure 6. a) Typical morphology of telangiectatic osteosarcoma in the second recurrence (H.E. 10 x 20) b) lymph node metastasis (H.E. 10 x 20)

Slika 6. a) Tipična morfologija teleangiektatičnog osteosarkoma u drugom recidivu (H.E. 10 x 20), b) metastaza u limfnom čvoru (H.E. 10 x 20)

of the foot measuring 18 x 10 cm. There were 3 ulcerations of the skin due to tumor infiltration and a 20 cm long scar from an old surgical incision over it. After the dissection of the soft tissues, a highly vascular tumor mass was found. The blood-filled cyst at the centre and some more solid tissue at the periphery with necrosis were macroscopically recognized.

Microscopically large part of the tumor was composed of blood and necrotic tissue, in which some malignant cells could be found. Small amount of the tumor tissue from the periphery contained blood-filled spaces separated by thin cell rich septa and areas of more solid tissue. The septa contained benign looking giant cells and mononuclear cells with malignant cytological characteristics. Deposition of osteoid was found in some tumor areas.

Two lymph nodes extirpated from the right inguinal region were enlarged, measuring 6 x 3 and 1.5 x 1.5 cm. Gray-white tumor tissue was present at the cut surface in both of them

A metastatic osteosarcoma was confirmed microscopically in both lymph nodes.

Discussion

Aneurysmal bone cyst is a benign, locally destructive lesion of bone that was first described as a distinct entity in 1942 by Jaffe and Lichtenstein [1]. In about 79% of cases, it develops as a primary tumor without any recognized precursor bone lesion or, in about 30% of cases, as a secondary lesion when a preexisting osseous lesion can be identified [5]. ABC of a metatarsal is relatively uncommon, despite the predilection of this lesion for long bones [1, 8, 9].

The natural course of ABC involves a continued local growth and destruction, although the tumor is not considered to be a premalignant lesion [1, 10]. From a diagnostic standpoint, ABC is widely confused with other giant cell containing tumors of the bone. The differential diagnosis includes other more prevalent giant cell tumors of the bone such as giant cell tumor, giant cell reparative granuloma, brown tumor of hyperparathyroidism, and the less common but ominous telangiectatic osteosarcoma [1, 11].

There is a strong association of ABC and GTC [3].

Giant cell tumor is a neoplasm composed of mononuclear, plump, round, oval or spindle – shaped stromal cells and osteoclast-like multinucleated giant cells. The tumor has unpredictable potential of growth with possibility of recurrence and metastases [4, 12, 13]. GTC of bone accounts for about 5% of all bone tumors and 20% of all benign tumors. It most frequently occurs in the epiphysis of long bones around the knee, and in distal radius and proximal humerus. It rarely occurs in other bones.

GCT usually affects skeletally mature young adults and adults between the third and fourth decade of life, predominantly women. Rare examples of multifocal GCT have also been reported [4].

Radiographically, GCT is a lytic lesion with relatively well defined margins, eccentrically located within the bone. The bone is often expanded and the cortex thinned. There is a little or no periosteal reaction. There are no specific radiological features that predict malignancy in GCT [4].

The malignant transformation of GCT of bone is a relatively rare phenomenon. Malignant GCTs are divided into primary and secondary forms. Primary malignant GCTs are those with malignant sarcomatous components that are present de novo in conjunction with a giant cell tumor of bone and are exceedingly rare [5]. The term 'dedifferentiated GCT' is also used to describe these tumors [5]. Secondary malignant GCTs are high-grade sarcomas occurring at the sites of previously treated GCT of bone. Most malignancies in GCTs fall into the latter category and occur several years after radiation therapy or, much less frequently, after surgery [5, 14, 15].

Histologically, malignancy in giant cell tumors is recognized as malignant fibrous histiocytoma, fibrosarcoma or osteosarcoma [4–6, 16]. Heffernan et al. summarize the frequency at which each of these has been encountered in published cases of malignant transformation of GCT. Osteosarcoma occurred

most frequently, both primarily and secondarily, and was seen in 23 of the 42 cases reviewed. Fibrosarcoma and malignant fibrous histiocytoma were found in 10 and 7 of the 42 cases, respectively [5].

Osteosarcoma is a primary malignant bone tumour that affects both adults and children [7]. Metastatic dissemination in osteosarcoma usually occurs hematogenously, the lung and bones being the most common metastatic sites [17–20].

Regional lymph node involvement has been reported in patients with osteosarcoma in multiple case reports and case series, and is thought to be rare, with incidence rates varying from <1% to 10% [7]. Tobias et al. reported an incidence of regional lymph node involvement of 2.3% in their patients (4/176) [7]. They found that the patients with lymph node involvement did not differ by race, sex or age. The presence of regional lymph node involvement was found to be a poor prognostic factor. In their study, overall survival was poor for the patients, with median survival of only 8.5 months after diagnosis, which was similar to the patients with distant metastatic disease in their series. Thampi et al. reported the incidence rate of lymph node involvement to be 2.7%, with no significant difference between histologic subtypes. The Cooperative Osteosarcoma Study Group (COSS) reported the incidence of lymph node involvement in osteosarcoma of 0.8% (15/1702). In addition, the COSS found that the patients with the lymph node involvement had osteoblastic osteosarcoma subtype [7]. Case reports of regional node involvement have also been described in patients with osteoblastic osteosarcoma [7].

We present this case as a rare case of aneurysmal bone cyst transformation into telangiectatic osteosarcoma passing through a solid tumor phase in which the neoplastic tissue had the morphological features of giant cell tumor with malignancy having the morphological features of osteosarcoma. The second rarity of this case was the inguinal lymph node metastasis from the secondary developed osteosarcoma.

Another possible aspect of this case is worth considering.

Is there a possibility of a different order of events? It can be assumed that the primary lesion was underdiagnosed due to an extraordinary localization for osteosarcoma or scant curettage material. Since the second recurrence of the primary lesion was a clear and undoubted telangiectatic osteosarcoma, one can assume that the primary lesion was not ABC but osteosarcoma.

In this situation the first relapse becomes interesting for discussing the histopathological point of view. The diagnosis of malignant GCT was made at an institution other than the first one where the pathologist could not get the insight into the slides from the first lesion.

We revised the archived radiological data and all our histological slides. During the repeated analysis of the first lesion diagnosed as ABC we did not find any solid areas suggesting a preexisting giant cell tumor. There were no other morphological features except those consistent with ABC.

Immunohistochemical stains against CD68 and actin revealed CD68 positive giant cells and some actin positive fusiform cells in the septa.

Careful examination of the slides by three independent pathologists did not reveal any morphological features of malignancy or even a field that would be suspected for telangiectatic osteosarcoma.

We made a review of the radiologic findings: the lesion was intraosseous, the osteolysis was of geographic type, there was no periosteal reaction, the cortex was intact, and there was a slight osteosclerotic rim at one site - all the features characteristic for benign lesion. Computed tomography was also consistent with benign lesion.

The revision of slides of the second lesion did not reveal any certain signs of preexisting ABC or typical telangiectatic osteosarcoma. The neoplasm consisted mostly of solid tumor parts. The greatest percentage of tumor tissue had features of a GCT which interfered with a lower percentage of high grade sarcoma with osteoid production.

From here it seems logical that GCT with malignancy is diagnosed, although it seems that a diagnosis of osteosarcoma would be more appropriate.

The parts of osteosarcoma were quite evident, but without certain morphological signs of telangiectatic type. Careful examination of the osteosarcomatous areas showed the presence of giant cells. It is not an unexpected finding of giant cells in this situation to be attributed to the GCT component instead to a giant cell rich osteosarcoma. In most areas, osteosarcomatous tissue was composed of small and atypical osteoblasts embedded in abundant osteoid. There were parts of tumor tissue in which hemorrhages were present; however, they were not identified as blood field cysts but as an ordinary hemorrhage.

It can be assumed that if the pathologist who diagnosed GCT with malignancy had had the access to the first diagnosis, he should have made an effort to make abundant sampling in order to prove telangiectatic osteosarcoma.

The revision of radiological findings showed the characteristics for malignant bone tumor. The cortex was destroyed and tumor spreading to the soft tissue was evident. No fluid levels were found.

The revision of the slides from the second recurrence (third lesion) showed the presence of tissue typical of telangiectatic osteosarcoma in most parts of the tumor. The infiltrating parts of tumor in the dermis showed abundant osteoid deposition. Atypical, predominantly small osteoblasts were embedded in the osteoid; a tissue very similar to that which was found in the previous lesion diagnosed as GCT with malignancy.

Basic precondition for the accurate diagnosis of bone tumors is the team approach which requires close cooperation between the pathologist, orthopedic surgeon and radiologist.

Clinical presentations of most malignant bone tumors are rather unspecific, but some of them, such as localization and age, together with the in-

sight into the radiographic findings, provide the pathologist some rational differential diagnostic possibilities. Radiographic findings of bone tumors should be an integral part of histological examination and diagnosis of these lesions.

A serious problem may appear in diagnosing telangiectatic osteosarcoma and giant cell rich osteosarcoma because of their similarity to the aneurysmal bone cyst and giant cell tumor. Extensive sampling should provide a correct diagnosis especially in cases in which radiography suggests malignancy.

In our case, radiologic findings in the first and second relapse correspond to the histopathological diagnosis.

The radiological characteristics of the primary lesion are consistent with benign tumor.

Is there a possibility that there are rare osteosarcomas presented with unusual radiological features and is the order of events that we propose possible?

Supported by X-ray findings, the primary lesion in our case seems to have been ABC that underwent malignant transformation.

Malignant transformation of ABC is a rare event and has been described as a result of previous treatment, more often radiation than curettage; in our case the previous treatment was curettage.

We report this case as an interesting example of different possibilities and as a case in which many unusual events happened: a) a malignant transformation of ABC or possible non representative tissue sample, b) a solid tumor phase in which the neoplastic tissue had morphological features of giant cell tumor with malignancy or osteosarcoma c) inguinal lymph node metastasis from the secondary developed osteosarcoma or late lymph node metastasis from the first misdiagnosed lesion instead a pulmonary one.

Conclusion

We emphasize the importance of team work in order to achieve the accurate diagnosis, highlighting careful radiological examinations, good sampling and awareness of unusual cases in bone tumor pathology.

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Case report
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PYODERMA GANGRENOSUM IN BURNED PATIENT - CASE REPORT

PIODERMA GANGRENOZUM KOD PACIJENTA SA OPEKOTINAMA - PRIKAZ SLUČAJA

Milana OBRADOVIĆ TOMAŠEV^{1,2}, Mladen JOVANOVIĆ^{1,2} and Aleksandra POPOVIĆ¹

Summary

Introduction. Pyoderma gangrenosum is a rare, chronic, destructive, ulcerating skin disease of uncertain etiology. It develops most frequently in patients between 25-45 years of age and affects both sexes equally. **Case report.** We present a case of pyoderma gangrenosum in a young female patient who sustained a burn injury of 40% total body surface area. She underwent four operations. She developed a wound infection and urinary infection during her hospital stay. By the end of hospitalization, the papules followed with coalesce of ulcerations formed on the previously epithelized areas of her legs. The patient complained of the intensive pain localized on these surfaces. Since pyoderma gangrenosum was suspected, a dermatologist was included in treatment. Therapy was initiated (methylprednisolone 60 mg per day intravenously) with gradual reduction of the dosage. The patient was discharged from hospital two weeks later with almost fully complete cicatrization and epithelization. **Conclusion.** Pyoderma gangrenosum is still difficult to be diagnosed in the absence of specific and sensitive diagnostic methods; however, it is crucial to be suspected as early as possible and to start treatment immediately. Multidisciplinary approach is essential for optimal results.

Key words: Pyoderma Gangrenosum; Burns; Wounds and Injuries; Skin Transplantation; Pain; Signs and Symptoms; Therapeutics; Early Diagnosis

Introduction

Pyoderma gangrenosum was first described and named by Brunsting, Goeckman and O'Leary in 1930. They believed that streptococcal infection was responsible for secondary cutaneous gangrene and hence the name pyoderma gangrenosum was given. Today it is known that it is a misnomer [1].

It is a rare and serious disease. It develops most frequently in patients between 25 and 45 years of age and affects both sexes equally. Diagnosis is set empirically by the method of exclusion.

Case Report

A 24-year old female was admitted with a severe burn injury of 40% total body surface area (TBSA).

Sažetak

Uvod. Pioderma gangrenozum je retka, hronična, destruktivna, ulcerozna bolest kože nepoznate etiologije. Najčešće se javlja u grupi pacijenata između 25 i 45 godina starosti i podjednako je zastupljena kod oba pola. **Prikaz slučaja.** Prikazujemo slučaj pioderme gangrenozum kod mlade pacijentkinje koja je pretrpela opekotine od 40% telesne površine. Operisana je četiri puta. Tokom boravka u bolnici došlo je do razvoja lokalne infekcije i urinarnе infekcije. Pri kraju hospitalizacije, na prethodno epitelizovanim regijama na donjim ekstremitetima, došlo je do formiranja papula koje su se potom stapale u ulceracije. Pacijentkinja se žalila na intenzivne bolove lokalizovane na tim površinama. Postavljena je sumnja na piodermu gangrenozum, te je dermatolog bio uključen u lečenje. Započeta je terapija (metilprednizolon 60 mg dnevno intravenozno) uz postupno smanjivanje doze. Pacijentkinja je otpuštena iz bolnice nakon dve nedelje s gotovo potpunom cikatrizacijom i epitelizacijom ranjavih površina. **Zaključak.** Postavljajući dijagnozu pioderme gangrenozum je i dalje teško zbog nepostojanja specifičnih i senzitivnih dijagnostičkih metoda. Presudno je da se na vreme posumnja na ovo oboljenje i da se odmah započne s terapijom. Multidisciplinarni pristup je od suštinskog značaja da bi se postigli optimalni rezultati lečenja.

Ključne reči: Pioderma gangrenozum; Opekotine; Rane i povrede; Transplantacija kože; Bol; Znaci i simptomi; Terapija; Rana dijagnoza

The burn was a combination of full thickness and partial thickness depth of the skin. She sustained it during a seizure in the bath-tub with hot water. On admittance, the primary surgical revision of burns was done and appropriate therapy initiated. During the 92-day hospital stay, she had three more operations in order to cover all burned skin with split thickness skin grafts. Laboratory analyses revealed anemia and hypoproteinemia which were in correlation with the depth and extent of burns. After the first necrectomy of 15% TBS burned area (on the sixth day of hospital care) due to profound coagulation disturbance (which was resolved with recombinant coagulation factor VIIa), an autoimmune disorder was suspected. Testing was done (anti nuclear antibodies, anti-mitochondrial antibodies, anti-parietal antibodies, anti-smooth muscle antibodies and anti-

Abbreviations

TBSA – total body surface area
Ig – immunoglobulin

nuclear antibodies on HEP-2 cells) and results came out negative. Graft acceptance was low due to a poor contact with the underlying tissue. Because of the excessive bleeding after the first operation, low thrombocyte level (resistant to therapy) continuation of the necrectomy of the residual subdermal and deep dermal burns was postponed. The patient's general condition was very bad, with periods of intermittent fever, high procalcitonine level, C reactive protein level and alkaline phosphatase values. Other blood analyses were within the normal range. The patient was treated with topical application of silver sulfadiazine until the granulation tissue was formed. The staged excochleation of granulation tissue and skin grafting was done (the first one a month later and the second one two months later). The wound swabs were performed twice a week and *coagulase-negative staphylococci*, *Staphylococcus aureus* and *Acinetobacter spp.* were isolated. Urine cultures and blood cultures were negative during entire hospitalization. At the beginning of the third month of hospital stay, the swabs were negative. At about that time, the papules turning into the ulcers started to appear on the areas of previously fully accepted and consolidated skin grafts and healed donor sites of legs. The ulcers kept on confluating, forming larger and larger surfaces of skin defects (circumscribed defects in total of 2% TBSA) (**Figures 1 and 2**). In consultation with a dermatologist, a new immunological testing was done (anti-nuclear antibodies, immunoglobulin (IgG, IgM, and IgA) and the results were within the normal limits. A skin biopsy was performed. Histopathological analysis revealed no specific changes (some signs of inflammatory reaction with neutrophils were found). All of the above led to clinical suspicion of pyoderma gangrenosum. Therefore, any surgical therapy was ruled out. Thera-

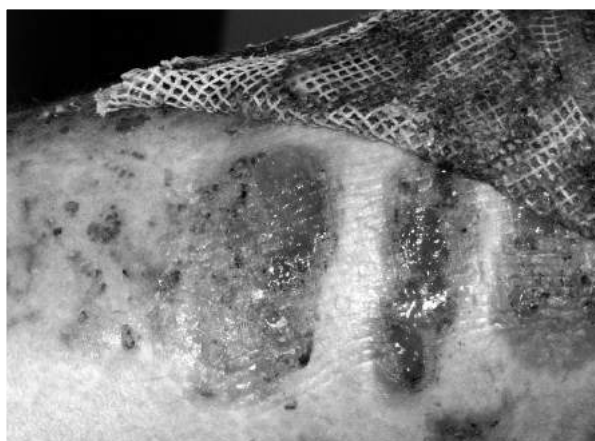


Figure 1. Ulcers on previously healed donor site - two months after injury

Slika 1. Ulceracije na prethodno zaraslim davajućim regijama – dva meseca nakon povrede

py with intravenous methylprednisolone was introduced, the dose being 60 mg per day during three days; afterwards it was reduced to 40 mg per day during the next 10 days. Oral administration of methylprednisolone was continued with gradual dose reduction. The ulcers were simultaneously treated with greasy gauze and compresses with ethacridine lactate. The local status was considerably better, cicatrization and epithelization from the edges was almost complete and the patient was discharged and advised about out-patient care.

Discussion

Pyoderma gangrenosum is a chronic, destructive, ulcerating skin disease. Its incidence is very low, being approximately one person per 100.000. It can be associated with other diseases such as inflammatory bowel diseases (15%), arthritis (37%) and hematological malignances. In 50% of cases, it appears as isolated skin conditions [2]. It may involve other organ systems, where it manifests as sterile neutrophilic infiltrates. Most common extracutaneous localizations are the lungs, heart, central nervous system, gastrointestinal system, eyes, liver, spleen, bones and lymph nodes [3].

Pyoderma gangrenosum is a disease of uncertain etiology, but there are a few hypotheses concerning its development such as genetic factors (it has been suggested to be autosomal recessive disorder), immunological factors (where abnormalities in both humoral and cell-mediated immunity are responsible for its occurrence), and vascular factors (which suggest that pyoderma gangrenosum may represent a type of vascular disorder) [2].

Pyoderma gangrenosum is classified into four varieties: ulcerative (classic form), pustular type (in which pustules do not evolve into ulcers), bullous (mostly in patients with myeloproliferative disease)

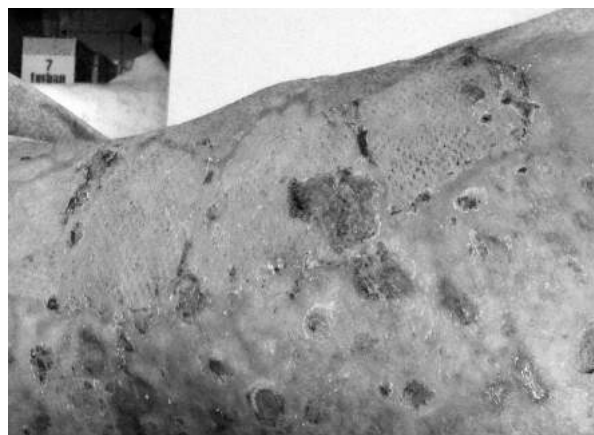


Figure 2. Ulcers on area of previously accepted skin grafts - two months after injury

Slika 2. Ulceracije na mestima gde su prethodno primljeni kožni transplantati – dva meseca nakon povrede

and vegetative type (lesions are chronic and limited, non aggressive variant) [1].

Classic pyoderma gangrenosum, most frequently occurring as a variant, is typically localized on the legs (in 75% of cases) [2]. It begins as a small papule or a collection of papules, and when they break down, an ulcer is formed. The ulcers coalesce with necrosis in the central area. Pyoderma gangrenosum presents as a deep ulcer with the defined border violet or blue in color. The edges are often worn and damaged and the surrounding skin is erythematous and indurated [4]. Its appearance can be accompanied by pain and deterioration of general condition with fever, malaise, arthralgia and myalgia. The characteristic feature of pyoderma gangrenosum is a pathergy reaction but it is present in about 25% of cases [2].

Histopathology of pyoderma gangrenosum depends on the timing and site of biopsy [4]. Massive neutrophilic infiltration in the absence of vasculitis and granuloma formation may be considered suggestive of pyoderma gangrenosum [1].

Diagnosis of pyoderma gangrenosum is based upon the morphology of lesions, clinical course and the tentative presence of underlying medical condition associated with its higher occurrence. There are no pathognomonic laboratory tests, diagnostic methods or histopathological finding [5]. Pyoderma gangrenosum is often a diagnosis of exclusion, and thus presents many clinical challenges; therefore it is frequently misdiagnosed [6].

There are numerous skin conditions which can mimic pyoderma gangrenosum such as infections (ecthyma, herpes virus ulcers, deep mycoses, etc), necrotizing systemic vasculitis (Wegener's granulomatosis, polyarteritis nodosa, rheumatoid arthritis, etc.), proliferative processes, reaction to drugs (warfarin, iodine, etc.), autoimmune diseases and exogenous tissue injury [5].

Treatment of pyoderma gangrenosum requires multiple modalities in order to reduce inflammation and create optimal conditions for wound healing and pain control [6, 7]. There is no gold standard in treatment of pyoderma gangrenosum [8]. It is essential to exclude other infectious disease before it starts because corticosteroids and immunosuppressant drugs are therapy of choice [1]. Most treatments are empirical and based on small series or local experience. Immunosuppression is the main goal in treatment and it is usually achieved with corticosteroids and cyclosporine. Prednisolone is a drug of choice and it is introduced with high dosage (60–120 mg). Cyclosporine is mainly used to reduce the dependence on corticosteroids or in situations when corticosteroids fail. Other immuno-

suppressant, such as azathioprine, tacrolimus and anti-tumor necrosis factor α agents, can also be used [3].

Topical therapy can sometimes be sufficient for early and mild manifestations. It comprises treatment with wet compresses, hydrophilic occlusive dressings, antimicrobial agents and topical corticosteroids [2]. Gentle debridement with Burrow's solution, silver nitrate or potassium permanganate baths are important in local treatment. Aggressive surgical therapy and skin grafting should be avoided. It could be performed if the patient is on systemic corticosteroid therapy until both the donor and recipient area are healed [1].

The prognosis of pyoderma gangrenosum is generally good. It must be emphasized that it is prone to recurrence and as a consequence leaves a residual scarring. Death from pyoderma gangrenosum is rare but may occur due to underlying medical conditions or as a result of the therapy [3].

In this case, pyoderma gangrenosum was observed in a young female patient, a burn victim. The changes appeared on her legs, where the skin grafts were previously fully accepted and the donor sites healed. They were in form of papules evolving into ulcers which were merging, and the defect was getting bigger every day in spite of everyday topical treatment. The patient was complaining of pain in this region.

Extensive burns lead to disturbance in the cell mediated as well as in humoral immunity which can be an explanation for the development of pyoderma gangrenosum in our patient. Growing defects in the form of ulcers preceded by papules, resistant to any form of topical therapy, on typical localization, which was also a site of surgical intervention, should arouse a suspicion of pyoderma gangrenosum. It was empirically confirmed that the patient suffered of pyoderma gangrenosum when the positive response was obtained with parenteral and afterwards per oral therapy with corticosteroids. Laboratory and histopathological analyses showed no signs which could help to make the diagnosis of pyoderma gangrenosum. It was made by excluding other conditions which might lead to similar clinical presentation.

Conclusion

Each growing ulcer localized on the site of surgical intervention, which does not heal or react to topical treatment, should arouse suspicion of pyoderma gangrenosum, particularly if the patient is complaining of pain and worsening of general condition and has some of medical conditions associated with pyoderma gangrenosum.

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Case report
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DEFINITE MANAGEMENT OF BILATERAL LOWER LEG NONUNION FRACTURES BY ILIZAROV APPARATUS IN POLYTRAUMATIZED PATIENT – CASE REPORT

DEFINITIVNO ZBRINJAVANJE BILATERALNIH NESRASLIH PRELOMA POTKOLENICA APARATOM PO ILIZAROVU KOD PACIJENTA SA POLITRAUMOM – PRIKAZ SLUČAJA

Ivica LALIĆ¹, Mirko OBRADOVIĆ¹, Mirka LUKIĆ ŠARKANOVIĆ² and Vladimir ĐAN³

Summary

Introduction. Nonunion of long bones may often be associated with significant function loss of affected extremity, joint stiffness, and even extremity amputation or systemic manifestations in the case of infection. The aim of this case report is to highlight the possibilities of Ilizarov apparatus in the treatment of fracture nonunions of both lower legs treated by different operative methods and to show that it is not necessary to remove osteosynthetic material (intramedullary nail) in every case when nonunion occurs to achieve its recovery. **Case Report.** A 62 year-old man was injured in a traffic accident as a pedestrian in April 2012, when he experienced polytrauma, including shaft fracture of the right femur, and segmental open fractures of the right (Gustillo-Anderson grade I) and left (Gustillo-Anderson grade II) lower leg. The fractures of right femur and right tibia were stabilized initially with intramedullary nails, while the left lower leg fracture was treated by unilateral external fixator. After 5 months, there were no clinical and radiographic signs of union on lower legs, therefore the patient underwent re-surgery. Ilizarov apparatus was applied on both lower legs. The patient was early verticalized and both apparatus were removed after 4 months. According to the modified protocol of the Association for the Study and Application of Methods of Ilizarov, the lower leg bony results were good and excellent, and the functional results were excellent on both sides. **Conclusion.** Nonunion fracture of the right lower leg initially treated by the method of intramedullary osteosynthesis and afterwards by placing Ilizarov apparatus shows that in some cases it is not indicated to remove fixative material in order to achieve full recovery of fracture, thus eliminating the danger of all negative effects resulting from the classical extensive surgical treatment.

Key words: Tibial Fractures; Fractures, Ununited; Ilizarov Technique; Clinical Protocol; Multiple Trauma; Treatment Outcome

Introduction

Fractures of long bones have become the most frequent skeletal trauma in modern times [1]. There is also a notable incidence raise of long bone nonunions, which is a significant problem in modern orthopedic surgery and traumatology [2]. Nonunion

Sažetak

Uvod. Nesrastanje preloma kostiju potkolenice često može biti udruženo sa značajnim gubitkom funkcije povređenog ekstremiteta, ukočenošću zglobova pa čak i sa amputacijom ekstremiteta ili sistemskim manifestacijama u slučaju infekcije. Cilj ovog prikaza slučaja je da ukaže na mogućnosti primene Ilizarovljevog aparata u lečenju nesraslih preloma obe potkolenice tretiranih različitim operativnim metodama i da pokaže da nije potrebno u svakom slučaju odstraniti osteosintetski materijal (intramedularni klin) kod nesraslog preloma da bi došlo do njegove sanacije. **Prikaz slučaja.** Muškarac starosti 62 godine, povređen u saobraćajnoj nesreći, doživeo je politraumu, gde je između ostalih povreda zadobio prelom tela desne butne kosti, segmentne otvorene prelome desne (Gustiljo-Anderson, gradus I) i leve (Gustiljo-Anderson gradus II) potkolenice. Prelomi desne butne kosti i desne tibije inicijalno su stabilizovani intramedularnim klinovima, dok je prelom leve potkolenice zbrinut unilateralnim spoljašnjim fiksatorom. Nakon 5 meseci nismo uočili kliničke i radiografske znake srastanja kostiju potkolenice, pa je pacijent podvrgnut ponovnom operativnom zahvatu. Postavljen je Ilizarovljev aparat na obe potkolenice. Pacijent je rano „vertikalizovan” i aparati su odstranjeni nakon 4 meseca. Prema modifikovanom protokolu Udruženja za izučavanje i primenu metoda po Ilizarovu (eng. *Association for the Study and Application of Methods of Ilizarov*), rezultat na potkolenicama bio je dobar i odličan, dok su funkcionalni rezultati obostrano bili odlični. **Zaključak.** Nesrastao prelom desne potkolenice inicijalno tretiran metodom intramedularne osteosinteze gde je lečenje nastavljeno postavkom aparata po Ilizarovu pokazuje da u pojedinim slučajevima nije indikovano odstranjenje fiksacionog materijala da bi došlo do pune sanacije preloma, čime smo otklonili opasnost od svih negativnih efekata koje podrazumeva klasični ekstenzivni hiruški tretman.

Glavne reči: Frakture potkolenice; Nesrasli prelomi; Tehnika po Ilizarovu; Klinički protokoli; Politrauma; Ishod lečenja

of long bones is often associated with a significant loss of function of affected extremity, joint stiffness, muscular atrophy, diffuse osteopenia, and even extremity amputation or systemic manifestations in the case of infection. Indications for an appropriate treatment method are often unclear [3]. Because of the intense pain, blood loss, prolonged secretion of

Abbreviations

ISS – injury severity score

inflammatory mediators and loss of mobility of the injured patient, with all the negative, resulting consequences (aggravated management of the injured patient, venous thrombosis and hypostatic pneumonia due to inactivity and prolonged lying), treatment of polytraumatized patients with fractures of the tibia is a very complex problem [4]. Tibial shaft fractures are the most frequent type of long bone fractures treated by orthopedic surgeons. The incidence in general population is approximately 26 fractures per 100.000 [5]. The introduction of dynamic compression plates by Association for the Study of Internal Fixation (AO) led to popularization of open reduction and internal fixation (ORIF) [6]. In recent years, plates with less periosteal contact have been developed [7]. Intramedullary locking nails represented a revolution in the treatment of tibial shaft fractures [8]. One of the advantages of Ilizarov external fixation is the possibility of early weight bearing on the operated limb. Ilizarov method has also proved to be a successful choice in treatment of tibial fracture nonunion, especially after failure of other methods [9]. Intramedullary nailing combined with grafting and the Ilizarov apparatus compression and distraction techniques were used for the treatment of nonunions, pseudoarthrosis and bone defects. A similar technique was reported by Giebel [10] and popularized by Salis de Gauzag et al. [11] although we did not use grafting and distraction. This case report is aimed at highlighting the possibilities of Ilizarov apparatus in the treatment of nonunion fractures of both lower legs treated by different surgical methods. In addition, it should be said that it is not always necessary to remove osteosynthetic material (intramedullary nail) when nonunion occurs to accomplish the recovery of fracture.

Case Report

A 62-year-old man was injured in a traffic accident as a pedestrian in April 2012. Having been given first aid and immobilized, he was transported to the emergency room. Clinical examination, laboratory, radiographic and computed tomography findings indicated the following injuries: multiple lacero-contusions of body, seven fractured teeth, commotion syndrome, the right femur shaft fracture, segmental open fractures of the right (Gustillo-Anderson grade I) and the left (Gustillo-Anderson grade II) lower leg (**Figure 1**), liver rupture, gallbladder hematoma, intra-abdominal bleeding. Injury severity score (ISS) was 29. After extensive preoperative preparations, the first surgery, performed in general anesthesia, was focused on intra-abdominal bleeding and drainage of abdominal cavity as well as primary surgical treatment of wounds. For all fixation procedures, an x-ray image amplifier (“C-arm”) was used. After orthopedic

reduction of the right femur and intramedullary fixation with retrograde nail (S2™, Stryker, Montreux, Switzerland), segmental fracture of right lower leg was stabilized with intramedullary nail (Versanail™, DePuy, Leeds, England). Finally, stabilization of segmental fracture of left lower leg was performed after orthopedic reduction and fixation using external fixator by Mitkovic (Trafix®, Niš) with two convergently set nails in each tibial segment (six in total). A good position of fragments of segmental fracture on the left lower leg was achieved; however, it was seen that the right leg tibia was shorter by 1 cm (shortness of tibial length of 1 cm was observed on the right leg), noticeably passing of proximal fibula with preservation of tibial axis which was also verified by standard radiography postoperatively (**Figure 2**). The patient was then transferred into the intensive care unit. After stabilization of vital parameters, the patient was transferred to the Department of Orthopedic Surgery and Traumatology. All measures of early rehabilitation were performed. The patient was discharged from hospital in good general condition and advised on the wound management and verticalization. However, control radiographs within the outpatient department checkups showed healing of the fracture on the right femur, but not union of both, left and right lower leg fractures (**Figure 3**). A free bone fragment, which raised the skin not allowing interfragmentary contact of proximal and distal part of tibia, was visible on the right lower leg. Dislocation in proximal segment occurred on the left lower leg despite the corrections of external fixator. The patient’s verticalization was practically impossible and any attempt of weight bearing on lower extremities was very painful. Limitation of movement was present on both sides in the knee joint. Having been informed on the possible surgical treatment on lower legs, the patient gave his consent and was hospitalized again in September 2012. After preoperative preparation, the first external fixator was removed

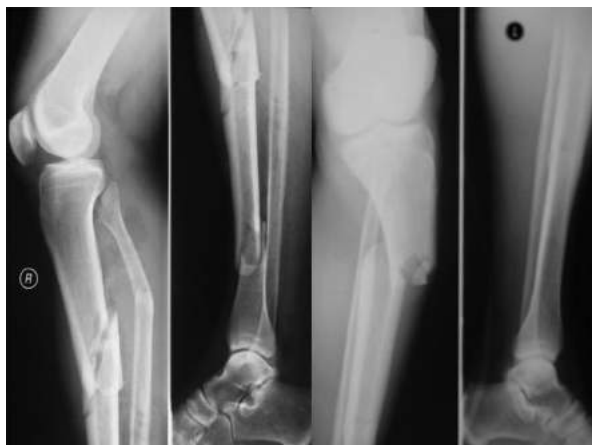


Figure 1. Open segmental fractures of right and left lower leg

Slika 1. Otvoreni segmentni prelomi desne i leve potkoljenice



Figure 2. External fixation of left tibia and intramedullary osteosynthesis of right tibia

Slika 2. Spoljašnja fiksacija leve golenjače i intramedularna osteosinteza desne golenjače

from the left lower leg, osteotomy of proximal fibula was performed and Ilizarov apparatus was applied with four frames attached to each other by threaded rods. Olive pins were used only. A free bony fragment from anteromedial part of right tibia, irregularly shaped, sized 1x2 cm, which in transverse plane made up to the 1/3 of tibial circumference, was placed between the nail and the proximal tibia, and since it did not allow the proper contact between the fragments, it was removed. The proximal transfixation nail screw was removed, osteotomy of proximal fibula was performed and Ilizarov apparatus was set with four frames attached to each other with threaded rods. Olive pins were introduced transcortically near the intramedullar nail (**Figure 4**). Interfragmentary compression was performed on the second postoperative day on both legs, and remained for a total of 10 days with dynamic of 1 mm per day. The patient underwent intensive early rehabilitation program and verticalization started immediately. The full weight-bearing was allowed on the second postoperative day, while the full weight-bearing on the left leg was allowed 10 days after surgery (**Figure 5**). No signs of pin tract infection were noticed; however, four pin site infections developed, which were successfully treated with oral antibiotics and wound management. During his stay in hospital, the patient underwent intensive kinesiotherapy and magnetic therapy. After discharge, the patient came for regular checkups and the healing of both lower legs was monitored radiographically. Outpatient physical procedures were applied to achieve the rapid recovery of both tibias. The apparatus was



Figure 3. Right lower leg fracture nonunion with loose fragment, and left lower leg fracture nonunion

Slika 3. Nezarastanje preloma desne potkolenice sa slobodnim ulomkom i nezarastanje preloma leve potkolenice

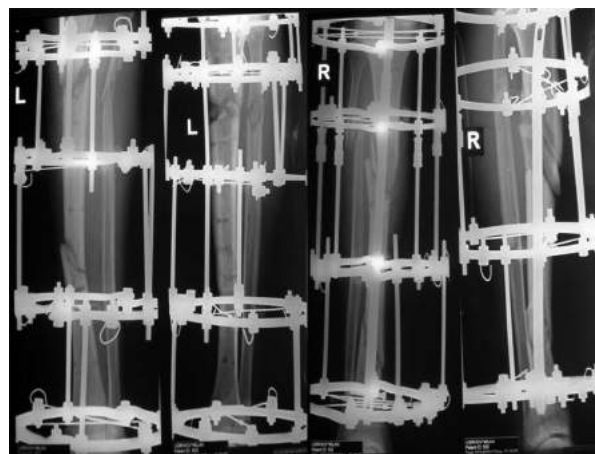


Figure 4. Left and right tibia osteosynthesis using Ilizarov apparatus

Slika 4. Osteosinteza leve i desne golenjače pomoću Ilizarovljevog aparata

removed from the right lower leg in December 2012 and the full weight bearing was allowed. In February 2013, the apparatus was removed from the left lower leg and a protective upper leg cast was set and the patient wore it for two weeks. The full weight bearing on the left leg was allowed four weeks after the removal of Ilizarov apparatus. After this, the patient was treated in a spa for two months. On the outpatient department checkup in December 2013, the complete healing of segmental fractures of both lower legs was radiographically confirmed (**Figure 6**). The range of knee and ankle joints motions was measured: the left knee joint flexion was 130 degrees, the right knee flexion was 120 degrees; the right ankle – plantar flexion was 40 degrees, the dorsal flexion was 15 degrees; the left ankle – plantar flexion was 35 degrees, and the dorsal flexion was 20 degrees. Neither any signs of infection nor any lesions of fibular nerves were observed. The right lower leg was measured to be shorter by 1.5 cm and the patient compensates it by wearing an insert in the right shoe. Valgus position of left lower leg of 10 degrees did not affect normal gait and occurrence of pain in ankle joint. The patient was followed for two years in total. He returned to his life and work activities fully recovered.

Discussion

In our case, the risk for nonunion occurrence was high as correctly determined by ISS which was 28. In polytraumatized patients with $ISS \geq 16$, fractures are often treated by methods inappropriate for that type of fracture, which can further worsen prognosis of these fractures, particularly if they are accompanied by a significant soft tissue lesion [12].

Treatment of atrophic nonunions is aimed at restoring osteogenic activity at the fracture site, which can be achieved by resection of fibrous tissue within the fracture site and implantation of an autograft, and if there is instability, osteosynthesis revision



Figure 5. Full weight bearing on both lower extremities
Slika 5. Pun oslonac na oba donja ekstremiteta

should be made [13]. Surgery for nonunion is often very difficult and many complications may occur. Available definitions of nonunion are various, inconsistent and arbitrary and can be interpreted subjectively. There are several time points that have been recognized as parameter of nonunion, ranging from 20 to 26 weeks [14]. Food and Drug Administration (FDA) of the United States of America defined nonunion as the absence of radiographic healing 9 months after injury [15]. We have decided to accept a new definition, which describes nonunion as the absence of progressive signs of healing on radiographs for 3 consecutive months [16]. Because no clinical or radiographic evidence of healing was recorded, we have decided to perform reosteosynthesis. Partial fibulectomy was described as a useful part of tibial nonunion surgery [17]. This procedure is usually performed on a level different from nonunion to avoid tibial destabilization [18]. We performed bilateral partial fibulectomy above the levels of nonunion. As the Ilizarov frame disabled translational



Figure 6. Complete union of right and left lower leg fracture
Slika 6. Potpuna sanacija preloma desne i leve potkoljenice

movement of fragments, combination of axial compression and partial fibulectomy proved to be a great tool for nonunion treatment. There are numerous Ilizarov methods for high-energy tibial fracture treatment [19] in order to achieve union, deformity correction, soft tissue healing and limb alignment. Whatever method is used, nonunion and infection are still frequently recorded [20].

The vascularity of the nonunion is one of determining factors in bony healing because vascular or hypertrophic nonunions present a smaller treatment problem than atrophic nonunions [21]. The common aims of treatment are to correct axial or rotational malalignment, equalize extremity lengths, prevent or treat infection, and allow functional restoration of the limb [22]. Generally, good results have been seen in the treatment of tibial nonunions with small-wire circular frame devices. Paley et al. [23] reported a 100% union rate in 25 patients treated with the Ilizarov apparatus for tibial nonunions with segmental bone loss. In 197 patients with various types of open tibial fractures, treatment was initially performed using unilateral external fixation. Healing was achieved in 61% of cases. The remaining 39% of patients were treated with intramedullary nail (17%), Ilizarov apparatus (13%) and Sarmiento cast (9%), which eventually led to the complete union [24]. Stoiljković et al. used internal fixation with self-dynamisable internal fixator as a re-osteosynthesis method in 6 polytraumatized patients, thereby achieving union in 83% of patients [25]. Revision osteosynthesis in our case was done by Ilizarov external circular fixator, which resulted in fracture healing. Ebraheim et al. [26] found 89% union rate in nine patients treated for tibial nonunions with angular deformity. Laursen et al. [27] reported a 94% union rate in 16 patients treated with the Ilizarov fixator for complex tibial nonunions, with limb-length discrepancy reduced to within 1.5 cm of the contralateral leg. According to the Association for the Study and Application of Methods of Ilizarov (ASAMI) classification, excellent bone and functional results were achieved despite shortening of the

right leg, while on the left leg, excellent bone and functional results were observed despite angular valgus deformity of 10 degrees. Sanders et al. [28] reported ankle pain as a major disability after the application of the Ilizarov fixator for tibial nonunion. We have not had this problem when applying the Ilizarov method. In a series of 390 patients with fractures of the tibial shaft treated with intramedullary osteosynthesis, of which 79% were closed, and the remaining 21% open, union was achieved in 98% of cases, while in the remaining 2%, nonunion or pseudoarthrosis was recorded. In 5 fracture nonunion revisions, intramedullary nailing was performed, while pseudoarthrosis was treated by Ilizarov transosseus osteosynthesis, which resulted in complete union [29]. Gulabi et al. reported five patients with nonunions, which had previously been treated with different methods. His treatment of choice was intramedullary nailing combined with bone resection, compression and distraction of the Ilizarov apparatus and autografting at the docking site. The grafts were harvested from the iliac crest [30]. In our case, the nail was already placed inside the bone. We removed the loose bone fragment and the proximal transfixational screw. We then placed the Ilizarov apparatus in order to apply compression and achieve rotational stability of the tibia. In our case, there was no pin-site and pin-track infection and we removed the Ilizarov apparatus after three months. Early removal of the external fixator reduces the risk of pin-site infection and allows earlier rehabilitation of the patient [31]. So far, no studies or case reports have been published on this subject in the Serbian Citation Index. International literature offers articles that describe the combination of intramedullary nails and apparatus but only in cases of treatment of congenital pseudoarthrosis of the tibia [32] and lengthening of the tibia in infected fracture nonunions and segmental defects [33]. Transcortical placing of pins without nail removal has reduced the risk of infection, new distribution of intramedullary vascularity, and by removing proximal transfixation nail screw and continuous compressive effect on bone, we achieved the complete bone recovery and the full weight-bearing which is important in process of recovery and re-

habilitation. The stability of apparatus was not disturbed by transcortical introducing of pins which is not characteristic for this (transosseus) method of treatment. The Ilizarov techniques have shown to be useful in the management of difficult fractures and nonunions of the tibia but practicing surgeons call attention to their main drawbacks such as the long time the patients have to spend with the fixator on, much discomfort, and pin tract infections [34, 35]. Therefore, the techniques that combine the external device with intramedullary nailing have been advocated to avoid these problems. The combined techniques resulted in the reduction of the usual Ilizarov external fixation duration and good union rates [34–36]. Popkov et al. [37] conducted a study which was aimed at revealing the differences in radiographic and histological outcomes of bone repair by using flexible intramedullary nailing combined with the Ilizarov external fixation versus the Ilizarov external fixation alone on a canine model of an open diaphyseal tibial fracture. They have concluded that their experimental study proves that the combination of the Ilizarov apparatus and flexible intramedullary nailing augments fixation stability of bone fragments, accelerates the repair of tibial shaft fractures, and can be used in clinical settings. This combined technique does not contradict the biological principles of the Ilizarov method.

Conclusion

In our case, the polytraumatized patient had fractures of both lower legs, which later led to nonunion, so Ilizarov external fixation method proved to be a method of choice as it enabled early weight-bearing and rehabilitation. Treatment of tibial nonunion by using the technique that combined intramedullary nailing and Ilizarov apparatus proved to minimize angulation and malalignment, also allowing further mobilization and return to daily life. We think that the presentation of this case may be of use for orthopedic surgeons – traumatologists by suggesting how to solve complicated fractures of extremities without removal of hardware as well as providing full weight bearing on both extremities, which contributes to the rapid recovery.

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UPUTSTVO AUTORIMA

Časopis objavljuje sledeće kategorije radova:

1. Uvodnici (editorijali) – do 5 stranica. Sadržje mišljenje ili diskusiju o nekoj temi važnoj za Časopis. Uobičajeno ih piše jedan autor *po pozivu*.

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3. Pregledni članci – do 10 strana. Predstavljaju sažet, celovit i kritički pregled nekog problema na osnovu već publikovanog materijala koji se analizira i raspravlja, ilustrujući trenutno stanje u jednoj oblasti istraživanja. Radovi ovog tipa biće prihvaćeni samo ukoliko autori navođenjem najmanje 5 *autocitata* potvrde da su eksperti u oblasti o kojoj pišu.

4. Prethodna saopštenja – do 4 stranice. Sadržje naučne rezultate čiji karakter zahteva hitno objavljivanje, ali ne mora da omogući i ponavljanje iznetih rezultata. Donosi nove naučne podatke bez detaljnijeg obrazlaganja metodologije i rezultata. Sadržje sve delove originalnog naučnog rada u skraćenom obliku.

5. Stručni članci – do 10 stranica. Odnose se na proveru ili reprodukciju poznatih istraživanja i predstavljaju koristan materijal u širenju znanja i prilagođavanja izvornih istraživanja potrebama nauke i prakse.

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8. Druge vrste publikacija (feljtoni, prikazi knjiga, izvodi iz strane literature, izveštaji sa kongresa i stručnih sastanaka, saopštenja o radu pojedinih zdravstvenih ustanova, podružnica i sekcija, saopštenja Uredništva, pisma Uredništvu, novine u medicini, pitanja i odgovori, stručne i staleške vesti i *In memoriam*).

Priprema rukopisa

Propratno pismo

– Mora da sadrži svedočanstvo autora da rad predstavlja originalno delo, kao i da nije objavljivao u drugim časopisima, niti se razmatra za objavljivanje u drugim časopisima.

– Potvrditi da svi autori ispunjavaju kriterijume za autorstvo nad radom, da su potpuno saglasni sa tekstom rada, kao i da ne postoji sukob interesa.

– Navesti u koju kategoriju spada rad koji se šalje (originalni naučni rad, pregledni članak, prethodno saopštenje, stručni članak, prikaz slučaja, istorija medicine).

Rukopis

Za pisanje teksta koristiti *Microsoft Word for Windows*. Tekst treba otkucati koristeći font *Times New Roman*, na stranici formata A4, preredom od 1,5 (i u tabelama), sa marginama od 2,5 cm i veličinom slova od 12 pt. Rukopis treba da sadrži sledeće elemente:

1. Naslovna strana. Naslovna strana treba da sadrži kratak i jasan naslov rada, bez skraćenica, zatim kratki naslov (do 40 karaktera), puna imena i prezimena autora (najviše 6 autora) indeksirana brojkama koje odgovaraju onima kojim se u zaglavlju navode uz pun naziv i mesta ustanova u kojima autori rade. Na dnu ove stranice navesti titulu, punu adresu, e-mail i broj telefona ili faksa autora zaduženog za korespondenciju.

2. Sažetak. Sažetak treba da sadrži do 250 reči, bez skraćenica, sa preciznim prikazom problematike, ciljeva, metodologije, glavnih rezultata i zaključaka. Sažetak treba da ima sledeću strukturu:

– originalni naučni radovi: uvod (sa ciljem rada), materijal i metode, rezultati i zaključak;

– prikaz slučaja: uvod, prikaz slučaja i zaključak;

– pregled rada: uvod, odgovarajući podnaslovi koji odgovaraju onima u tekstu rada i zaključak.

U nastavku navesti do deset ključnih reči iz spiska medicinskih predmetnih naziva (*Medical Subjects Headings, MeSH*) Američke nacionalne medicinske biblioteke.

3. Sažetak na engleskom jeziku. Sažetak na engleskom jeziku treba da bude prevod sažetka na srpskom jeziku, da ima istu strukturu i da sadrži do 250 reči, bez upotrebe skraćenica.

4. Tekst rada

– Tekst originalnih članaka mora da sadrži sledeće celine:

Uvod (sa jasno definisanim ciljem rada), Materijal i metode, Rezultati, Diskusija, Zaključak, spisak skraćenica (ukoliko su korišćene u tekstu) i eventualna zahvalnost autora onima koji su pomogli u istraživanju i izradi rada.

– Tekst prikaza slučaja treba da sadrži sledeće celine: Uvod (sa jasno definisanim ciljem rada), Prikaz slučaja, Diskusija i Zaključak.

– Tekst treba da bude napisan u duhu srpskog jezika, oslobođen suvišnih skraćenica, čija prva upotreba zahteva navođenje punog naziva. Skraćenice ne upotrebljavati u naslovu, sažetku i zaključku. Koristiti samo opšte prihvaćene skraćenice (npr. DNA, MRI, NMR, HIV,...). Spisak skraćenice koje se navode u radu, zajedno sa objašnjenjem njihovog značenja, dostaviti na poslednjoj stranici rukopisa.

– Koristiti mere metričkog sistema prema Internacionalnom sistemu mera (*International System Units – SI*). Temperaturu izražavati u Celzijusovim stepenima (°C), a pritisak u milimetrima živinog stuba (mmHg).

– Ne navoditi imena bolesnika, inicijale ili brojeve istorija bolesti.

Uvod sadrži precizno definisan problem kojim se bavi studija (njegova priroda i značaj), uz navođenje relevantne literature i sa jasno definisanim ciljem istraživanja i hipotezom.

Materijal i metode treba da sadrže podatke o načinu dizajniranja studije (prospektivna/retrospektivna, kriterijumi za uključivanje i isključivanje, trajanje, demografski podaci, dužina praćenja). Statističke metode koje se koriste treba da budu jasne i detaljno opisane.

Rezultati predstavljaju detaljan prikaz podataka dobijenih tokom studije. Sve tabele, grafikoni, sheme i slike moraju da budu citirani u tekstu, a njihova

numeracija treba da odgovara redosledu pominjanja u tekstu.

Diskusija treba da bude koncizna i jasna, sa interpretacijom osnovnih nalaza studije u poređenju sa rezultatima relevantnih studija publikovanim u svetskoj i domaćoj literaturi. Navesti da li je hipoteza istraživanja potvrđena ili opovrgnuta. Izneti prednosti i ograničenja studije.

Zaključak u kratkim crtama mora da odbaci ili potvrdi pogled na problem koji je naveden u Uvodu. Zaključci treba da proizilaze samo iz vlastitih rezultata i da ih čvrsto podržavaju. Uzdržati se uopštenih i nepotrebnih zaključivanja. Zaključci u tekstu moraju suštinski odgovarati onima u Sažetku.

5. Literatura. Literatura se u tekstu označava arapskim brojevima u uglastim zagrada, prema redosledu pojavljivanja. Izbegavati veliki broj citata u tekstu. Za naslove koristiti skraćenice prema *Index Medicus*-u (<http://www.nlm.nih.gov/tsd/serials/lji.html>). U popisu citirane literature koristiti Vankuverska pravila koja precizno određuju redosled podataka i znake interpunkcije kojima se oni odvajaju, kako je u nastavku dato pojedinim primerima. Navode se svi autori, a ukoliko ih je preko šest, navesti prvih šest i dati et al.

Članci u časopisima:

* *Standardni članak*

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.

* *Nisu navedena imena 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 anaphylaxis. *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 gondii* [abstract]. *Clin Res* 1987;35:475A.

Knjige i druge monografije:

* *Jedan ili više autora*

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

* *Urednik(ci) kao autor*

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.

* *Rad u zborniku radova*

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.

* *Disertacije i teze*

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

Elektronski materijal

* *Članak u Časopisu u elektronskoj formi*

Aboud 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>

* *Monografije u elektronskoj formi*

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

* *Kompjuterski dokument (file)*

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

6. Prilozi (tabele, grafikoni, sheme i fotografije).

Dozvoljeno je najviše šest priloga!

– Tabele, grafikoni, sheme i fotografije dostavljaju se na kraju teksta rukopisa, kao posebni dokumenti na posebnim stranicama.

– Tabele i grafikone pripremiti u formatu koji je kompatibilan sa programom *Microsoft Word for Windows*.

– Slike pripremiti u JPG, GIF TIFF, EPS i sl. formatu

– Svaki prilog numerisati arapskim brojevima, prema redosledu njihovog pojavljivanja u tekstu.

– Naslov, tekst u tabelama, grafikonima, shemama i legendama navesti na srpskom i na engleskom jeziku.

– Objasniti sve nestandardne skraćenice u fusnotama koristeći sledeće simbole: *, †, ‡, §, ||, ¶, **, ††, ‡‡, §§.

– U legendama mikrofotografija navesti korišćenu vrstu bojenja i uvećanje na mikroskopu. Mikrofotografije treba da sadrže merne skale.

– Ukoliko se koriste tabele, grafikoni, sheme ili fotografije koji su ranije već objavljeni, u naslovu navesti izvor i poslati potpisanu izjavu autora o saglasnosti za objavljivanje.

– Svi prilozi biće štampani u crno-belom tehnici. Ukoliko autori žele štampanje u boji potrebno je da snose troškove štampe.

7. Slanje rukopisa

Prijem rukopisa vrši se u elektronskoj formi na stranici: aseestant.ceon.rs/index.php/medpreg/. Da biste prijavili rad morate se prethodno registrovati. Ako ste već registrovani korisnik, možete odmah da se prijavite i započnete proces prijave priloga u pet koraka.

8. Dodatne obaveze

Ukoliko autor i svi koautori nisu uplatili članarinu za Medicinski pregled, rad neće biti štampan. Radovi koji nisu napisani u skladu sa pravilima Medicinskog pregleda, neće biti razmatrani. Recenzija će biti obavljena najkasnije u roku od 6 nedelja od prijema rada. Uredništvo zadržava pravo da i pored pozitivne recenzije donese odluku o štampanju rada u skladu sa politikom Medicinskog pregleda. Za sva dodatna obaveštenja obratiti se tehničkom sekretaru:

Društvo lekara Vojvodine

Vase Stajića 9

21000 Novi Sad

Tel. 021/521 096; 063/81 33 875

E-mail: dlv@neobee.net

INFORMATION FOR AUTHORS

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This journal 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.

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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 auto-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 practise.

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– 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)

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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), borders of 2.5 cm and font size 12pt. The manuscript should contain the following elements:

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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:

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– 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. It is to be followed by up to 10 Key Words from the list of Medical Subject Headings, MeSH of the American National Medical Library.

3. The summary in Serbian language. The summary in Serbian should be the translation of the summary in English, it should be structured in the same way as the English summary, containing up to 250 words, without any abbreviations.

4. The text of the paper. The text of original studies must contain the following: introduction (with the clearly defined objective of the study), material 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.

– The text should be written in the spirit of Serbian language, without unnecessary abbreviations, whose first mentioning must be explained by the full term they stand for. Abbreviations should not be used in the title, summary and conclusion. Only commonly accepted abbreviations (such as DNA, MRI, NMR, HIV...) should be used. The list of abbreviations used in the text, together with the explanation of their meaning, is to be submitted at the last page of the manuscript.

– 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.

– No names, initials or case history numbers should be given.

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.

Material and methods should contain data on design of the study (prospective/retrospective, eligibili-

ty 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 problem mentioned in the introduction. Conclusions must be based solely on the author's own results, corroborating them. Avoid generalised and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

5. References. 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 organisation 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 anaphylaxis. *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 gondii* [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*

Aboud 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]. Reeves 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.

6. Attachments (tables, graphs, schemes and photographs). The maximum number of attachments allowed is six!

– Tables, graphs, schemes and photographs are to be submitted at the end of the manuscript, 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

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

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