Editor-in-Chief
Slobodan Janković

Co-Editors
Nebojša Arsenijević, Miodrag Lukić, Miodrag Stojković, Milovan Matović, Slobodan Arsenijević, Nedeljko Manojlović, Vladimir Jakovljević, Mirjana Vukićević

Board of Editors
Ljiljana Vučković-Dekić, Institute for Oncology and Radiology of Serbia, Belgrade, Serbia
Dragić Banković, Faculty for Natural Sciences and Mathematics, University of Kragujevac, Kragujevac, Serbia
Zoran Stošić, Medical Faculty, University of Novi Sad, Novi Sad, Serbia
Petar Vuleković, Medical Faculty, University of Novi Sad, Novi Sad, Serbia
Philip Grammaticos, Professor Emeritus of Nuclear Medicine, Ermou 51, 546 23, Thessaloniki, Macedonia, Greece
Stanislav Dubnička, Inst. of Physics Slovak Acad. Of Sci., Dubravska cesta 9, SK-84511 Bratislava, Slovak Republic
Luca Rosi, SAC Istituto Superiore di Sanita, Vaile Regina Elena 299-00161 Roma, Italy
Richard Gryglewski, Jagiellonian University, Department of Pharmacology, Krakow, Poland
Lawrence Tierney, Jr, MD, VA Medical Center San Francisco, CA, USA
Pravin J. Gupta, MD, D/9, Laxminagar, Nagpur – 440022 India
Winfried Neuhuber, Medical Faculty, University of Erlangen, Nuremberg, Germany

Editorial Staff
Ivan Jovanović, Gordana Radosavljević, Nemanja Zdravković
Vladislav Volarević

Management Team
Snezana Ivezic, Milan Milojevic, Bojana Radojevic, Ana Miloradovic, Ivan Miloradovic

Corrected by
Scientific Editing Service "American Journal Experts"

Design
PrstJeziklostaliPsi - Miljan Nedeljkovic

Print
Faculty of Medical Sciences

Indexed in
EMBASE/Excerpta Medica, Index Copernicus, BioMedWorld, KoBSON, SCIndeks

Address:
Serbian Journal of Experimental and Clinical Research, Faculty of Medical Sciences, University of Kragujevac
Svetozara Markovića 69, 34000 Kragujevac, PO Box 124
Serbia
izdavacka@medf.kg.ac.rs
www.medf.kg.ac.rs/sjecr

SJECR is a member of WAME and COPE. SJECR is published at least twice yearly, circulation 300 issues The Journal is financially supported by Ministry of Science and Technological Development, Republic of Serbia
ISSN 1820 – 8665
Table Of Contents

Original Article / Orginalni naučni rad
DNA REPAIR MECHANISMS AND CELLULAR REPROGRAMMING: CRITERIA FOR THE SUCCESSFUL GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS AND IN VITRO DISEASE MODELLING ...............................................................125

Original Article / Orginalni naučni rad
FACTORS ASSOCIATED WITH DEATH IN INTENSIVE CARE UNIT PATIENTS WITH VENTILATOR-ASSOCIATED PNEUMONIA FAKTORI RIZIKA ZA SMRTNI ISHOD KOD PACIJENATA U INTENZIVNOJ NEZI NA VEŠTAČKOJ VENTILACIJI KOJI SU DOBILI PNEUMONIJU .............................................................................................................131

Case Report / Prikaz slučaja
SYNCHRONOUS PRIMARY OVARIAN ENDOMETRIOID CARCINOMA AND POORLY DIFFERENTIATED ENDOMETRIAL STROMAL SARCOMA SINHRONI PRIMARNI SLABO DIFERENTOVANI SARKOM STROME ENDOMETRIJUMA I ENDOMETRIOIDNI KARCINOM JAJNIKA ................................................................................................................................................139

Case Report / Prikaz slučaja
MELKERSSON-ROSENTHAL SYNDROME: A CASE REPORT SINDROM MELKERSSON ROSENTHAL: PRIKAZ SLUČAJA ........................................................................................................................................145

Case Report / Prikaz slučaja
PALMOPLANTAR PUSTULOSIS AND ERUPTIVE PSORIASIS: A CASE REPORT PALMOPLANTarna PUSTULOZA I ERUPTIVNA PSORIJAZA: PRIKAZ SLUČAJA ........................................................................................................................................147

Case Report / Prikaz slučaja
RECIDIVANT NEUROBORRELIOSIS: CASE REPORT RECIDIVANTNA NEUROBORELIOZA: PRIKAZ SLUČAJA ........................................................................................................................................151

INSTRUCTION TO AUTHORS FOR MANUSCRIPT PREPARATION ..............................................................................................157
ABSTRACT

Maintenance of genomic integrity is an important aspect of stem cell function

All stem cells, whether they are pluripotent or multipotent tissue-specific cells, need to be able to eliminate genomic mutations if they threaten the ability of the cell to complete its function in vivo. This is of great importance for adult stem cells, the purpose of which is the lifelong repair and regeneration of a specific organ system. It is easy to imagine the potential harm that could accumulate in the haematopoietic system if only a small fraction of the haematopoietic stem cells (HSCs) resident in the endosteal niche of the bone marrow (BM) were allowed to accumulate mutations that altered their ability to generate lymphoid or myeloid lineages. Because the daily requirement for new blood cells is of the order of one billion, damage of this type would soon become apparent, and the patient’s health would decline. Important though this is, the impact of mutations occurring in adult life is minor compared to the possible disruptions that could arise if the genome of the early embryo was damaged to the point that embryonic development was restricted. The inner cell mass (ICM) of blastocyst-stage embryos gives rise to every tissue found in the adult but consists of a very small number of cells (which can be as few as 12 cells). If a mutation is allowed to persist in any of the ICM cells, it will be transferred to the tissues and organs that arise from that cell and could have a profound effect on organ function. Therefore, the accumulation of mutations in the ICM must be prevented at all costs.

Keywords: pluripotent stem cells, DNA damage, DNA repair

PLURIPOTENT STEM CELLS SHARE THE NECESSITY OF MAINTAINING GENOME INTEGRITY

Human pluripotent stem cells is the common term used to describe two types of pluripotent stem cells characterised by an indefinite self-renewal ability and the capacity to give rise to any cell type in the adult: human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs). hESCs are derived from spare in vitro fertilized embryos (1) after parental consent and have been widely used in the last decade as a generic tool to understand the maintenance of pluripotency, human embryonic development, and congenital disease. However, the utilisation of human embryos for research purposes is surrounded by a number of ethical issues, prohibiting hESC derivation and application in several countries. In contrast, human iPSCs bypass both of these issues because they share all the characteristics of hESCs, including the ability to proliferate indefinitely and differentiate into many cell types, but also represent a source of autologous stem cells for therapies, provided that they are derived from the patient in which they are used. Such patient-derived cells represent a unique opportunity to create in vitro disease models that can be exploited to understand disease pathology and drug discovery (see Figure 1).

The enhanced genome defence abilities of pluripotent stem cells decrease markedly upon differentiation (5-7). A number of studies have demonstrated that such terminally differentiated cell types as neurons differ greatly in their DNA repair capacity and ability to remove potentially harmful reactive oxygen species, such as superoxides and peroxides (8, 9). Because the other publications in this field have dealt adequately with...
the mechanisms of such defence systems, these mechanisms will not be discussed in detail here; however, it is apparent that the decrease in genome defence activity that accompanies the differentiation of pluripotent stem cells must be reversed when somatic cells re-acquire pluripotency during hiPSC generation. This reprogramming of genome defence has been demonstrated in a few recent studies showing that the expression levels of DNA repair-related genes were equivalent in hiPSCs and hESCs and were higher in comparison with their differentiated counterparts (10). At a first glance, these data could be considered to simply underline the point that pluripotent cells require efficient DNA repair; however, other studies suggest a more fundamental relationship between pluripotency and genome defence. The pluripotent state depends upon a framework of transcription factors that are largely controlled by the "core" factors OCT4, SOX2 and NANOG (11). These factors, in turn, are subjected to regulation by several "activator" protein complexes; among these, the XPC-nucleotide excision repair complex (12) has been shown to interact with both the OCT4 and SOX2 gene products and is recruited to the NANOG and OCT4 promoters. Importantly, the selective depletion of the components of the XPC complex using siRNA directed against XPC, RAD23B and CETN2 caused hESCs to differentiate, suggesting that, although pluripotent cells have a fundamental need for enhanced DNA repair, the expression of the genes involved in DNA repair at an appropriate level is also central to the maintenance of pluripotency (12).

If pluripotency relies, at least in part, on effective DNA repair, it is then possible that DNA repair processes contribute to the reprogramming of somatic cells into hiPSCs. The reprogramming process is very inefficient; typically less than 1% of the transduced somatic cells give rise to truly pluripotent colonies. However, p53-null mouse embryonic fibroblasts reprogram much more efficiently than do their wildtype counterparts. p53 is an important protein in cell cycle control and the initiation of DNA repair in cells with damaged genomes (13); more importantly, it prevents the propagation of such DNA-damaged cells (14). In view of this fact, it has been suggested that p53 may prevent the reprogramming of cells with DNA damage or chromosomal abnormalities from progressing to pluripotency. This is, of course, highly desirable because we want to eliminate any defective cells from the derived hiPSC lines; however, if p53 arrests the cell cycle in response to the detection of DNA damage, it is quite possible that the cell also

Figure 1. Schematic presentations of iPSC technology and its applications for basic biology, drug discovery and cell replacement therapies.
tries to repair the damage. Furthermore, we know that genome-wide active DNA demethylation is required to reprogramme the genome for totipotency in primordial germ cells (15) and that this process is dependent upon base-excision DNA repair. Some of the original studies of the impact of a p53 knockout were performed using Terc−/− mice with critically short telomeres, a condition that would be expected to trigger a DNA damage response; however, this is a rather exceptional case because we would not expect to find a significant number of somatic cells with such short telomeres in human samples collected for iPSC generation. Studying the impact of DNA repair mechanisms on the reprogramming process would be better achieved by generating hiPSCs from individuals suffering from diseases that result from DNA repair deficiencies.

**GENERATION OF HIPSCS FROM DNA REPAIR-DEFICIENT SOMATIC CELLS**

**Overview of DNA repair pathways and genes that lead to DNA repair diseases**

Several repair systems can be activated in response to a range of lesions that affect DNA. DNA double-strand breaks (DSBs) are one of the most severe forms of DNA damage, and can lead to cell death, genomic instability or oncogenic transformations if un repaired or mis-repaired (see Figure 2). Two key mechanisms exist to repair this type of lesion, namely, the homologous recombination (HR) or non-homologous end-joining (NHEJ) pathways. The former is considered to be less error prone because it relies upon the presence of a DNA template (usually the other allele of a mutated gene) to regenerate the sequence of DNA bases around the site of the DSB. NHEJ ligates the broken DNA strands directly using short regions of homology to guide the repair. If these regions of “microhomology” are not accurate, NHEJ will still function but is imprecise, and the improperly matched nucleotides are eliminated, which can lead to mutation and/or chromosome damage.

The protein complexes that mediate HR or NHEJ comprise several proteins. For example, NHEJ-mediated DSB repair is initiated by the binding of the Ku protein to dsDNA ends, followed by recruitment of the catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs), end processing and recruitment of the ligation complex comprised of DNA Ligase IV, XRCC4 and XLF, which performs the final ligation step. Mutations occurring in the genes that encode these proteins or the polypeptides from which they are constructed affect the functionality of the NHEJ and HR pathways and are known to result in a number of diseases. Not surprisingly, most of these conditions are associated with an increased predisposition to cancer, but there are a number of other developmental and cellular deficits specific to each disease. Several studies have generated hiPSCs that may be used to model the individual diseases in vitro. Single-strand breaks occur more frequently (16), but the repair systems associated with these lesions are very effective. Although other DNA repair systems include mismatch repair and nucleotide excision repair, few data are available concerning the impact of these repair mechanisms on hiPSC generation and survival. Therefore, we will focus on the mutations affecting DSB repair in this review.

**HIPSC MODELS OF DNA REPAIR DEFICIENCIES**

**Fanconi anaemia**

Fanconi anaemia is a BM failure syndrome characterised by developmental abnormalities, an enhanced pre-disposition to cancer and the progressive loss of haematopoietic cells; the disease is usually fatal before the age of 40. Fifteen genes (also known as complementation groups) contribute to this disease, including FANCA, FANCB, FANCC, FANCD1 (BRCA2), FANCD2, FANCE, FANCF, FANCG, FANCI, FANCI, FANCM, FANCN and FANCP (17, 18), but the exact mechanisms by which mutations in these genes can lead to BM failure have not been confirmed. Recent studies in the field have established the existence of an FA pathway in which eight of the FA proteins, namely, FANCA, -B, -C, -E, -F, -G, -L and -M, form a nuclear complex that is thought to function as a ubiquitin ligase in response to DNA damage or DNA replication stress. In response to stress, this multimeric FA core complex monoubiquinates FANCD2 and FANCI, which then recruit other downstream FA proteins, FANCD1/BRCA2, FANCJ, and FANCN, to sites of DNA damage. Once the monoubiquitinated FANCD2 and its associated partners (FANCI, FANC1, FANCJ, and FANCN) are correctly localised on the sites of DNA damage, they associate with other DNA repair proteins, such as NBS1, RAD51 and BRAC1, to initiate the DNA repair process (19).

Fanconi anaemia can be treated by BM transplants from HLA-identical siblings; if such individuals are not available, non-related donors can be used, but the outcomes are less favourable. The ultimate goal of many groups working on Fanconi anaemia is to develop protocols to transplant genetically corrected haematopoietic stem cells. These cells should have a selective growth advantage over those present in the patient’s bone marrow and should progressively replace the affected stem cells over time. The generation of genetically corrected hiPSCs would be a useful first step in this development; however, initial studies have suggested that reprogramming the dermal fibroblasts directly from Fanconi anaemia patients into hiPSCs might not be possible (20). Human iPSCs were only generated from fibroblasts that had been previously corrected by stable transfection of FANCA or FANCD2, suggesting that the DNA repair / chromosome stability function mediated by the Fanconi core complex is important for reprogramming. Subsequent studies have shown that hiPSCs can be generated from FANCA and FANCC fibroblasts, though the efficiency of this process is much lower (21) and is feasible only under hypoxic conditions. These data highlight the probable involvement of the Fanconi anaemia repair pathway in hiPSC generation. The pathway activation that results from cellular stress during reprogramming increases the expression levels
of p53, p21 and p19 in both wildtype and Fanconi-patient fibroblasts. Interestingly, the number of DSBs (indicated by γ-H2A.X foci) increases during reprogramming, even in wildtype cells, suggesting a possible means of activating the Fanconi pathway. However, this result requires cautious interpretation because pluripotent cells show higher levels of γ-H2A.X foci than their differentiated counterparts, which may be related to a γ-H2A.X function unrelated to DNA repair (22). Even before reprogramming, Fanca-/- fibroblasts have a higher level of γ-H2A.X foci, DSBs and chromosomal abnormalities; thus, it is possible that the low reprogramming efficiency may simply arise because a large number of cells are eliminated (by apoptosis) during the reprogramming process because they exceed a given threshold level of DNA damage. Work performed in our labs shows that hiPSCs can also be generated from FANCC-/- fibroblasts (23) under normoxic conditions. These hiPSCs express the typical pluripotency marker genes and demonstrate robust multi-lineage differentiation in vitro (embryoid bodies); however, they produce abnormal teratomas consisting almost primarily of immune cells, indicating deficiencies in their in vivo differentiation potential. The levels of karyotypic abnormalities present in the FANCC-/- hiPSCs were significantly higher than in the parent fibroblasts. As a comparison, the stable knockdown of the FANCC gene by shRNA in hESCs showed a relatively stable karyotype, indicating that the additional chromosomal abnormalities may be a consequence of the hiPSC reprogramming process. In terms of enhancing our ability to model Fanconi anaemia in vitro, the FANCC-/- hiPSCs demonstrated a greatly reduced ability to form haematopoietic colonies in methylcellulose plus haematopoietic growth factors. Although committed haematopoietic progenitors did form and proliferated at similar rates to those derived from wildtype hiPSCs, the numbers of such cells destroyed by apoptosis was much greater in the FANCC-/- hiPSCs. These combined data indicate that the activation of the Fanconi pathway is indispensable for cellular reprogramming, the maintenance of genomic stability and the survival of embryonic haematopoietic progenitors.

**MUTATIONS IN THE NHEJ PATHWAY**

- **XLF AND LIGASE IV**

Although the derivation of hiPSCs from cells with mutations in Fanconi pathway genes has indicated some aspects of the impact of DNA repair mechanisms on reprogramming, there are other diseases affecting different pathways that may help to shed more light on the subject. Two genes in this pathway, Cernunnos (also known as XLF) and DNA ligase IV, are important for the final ligation step to re-join the two sides of the double-strand break. Mutations in these genes are known to cause diseases. Cernunnos-deficiency syndrome is a rare recessive autosomal disorder caused by mutations in the XLF gene, a key factor involved in the end-joining step of the DNA non-homologous end-
joining (NHEJ) process. In humans, this disease manifests as severe immunodeficiency coupled to developmental defects (microcephaly and growth delays) (24). At the cellular level, the inhibition of NHEJ produces higher levels of unrepaired DSBs, but the consequence of this for haematopoietic differentiation appears to be similar to that observed for the Fanconi pathway in that haematopoietic progenitors do arise but are subjected to apoptotic attrition. Human iPSCs carrying the C169T (R57X) mutation in the XLF gene were also morphologically similar to unaffected control iPSCs but were unable to form teratomas following injection into immune-compromised mice (25). Defects have been observed in other differentiated cell types derived from XLF-deficient iPSCs; for example, neuronal-like cells showed differences in the expression patterns of the microtubule protein beta-III-tubulin, suggesting an altered cytoskeletal structure. The reduced NHEJ activity in XLF-deficient hiPSCs was demonstrated using fluorescent reporter constructs of NHEJ and HR activity (26). However, this analysis also indicated that the cells attempt to compensate for the reduction in NHEJ activity by increasing HR, suggesting some degree of crosstalk between the two methods for repairing double-strand breaks. Despite this, XLF-deficient hiPSCs were unable to repair DSBs efficiently after exposure to ionising radiation and showed a much higher percentage of cells with unrepaired double-strand breaks under normal conditions. This result suggests that NHEJ is required to repair the damage induced by the normal culture of hiPSCs.

Patients with LIG4 deficiency (OMIM # 606593) show a range of phenotypic abnormalities (27, 28). Most of the mutations identified to date impact the ability of DNA Ligase IV to interact with its partner, XRCC4, or disrupt the ligase catalytic domain. However, the precise nature of the mutations determine the level of Ligase IV activity present in the cell, thus the disease is subject to wide variation in humans. We have been able to generate hiPSCs from Ligase IV-deficient patients, which, in parallel with the other DNA repair defects mentioned thus far, are morphologically indistinguishable from control hiPSCs (29). As expected, the efficiency of derivation is low, though the predicted karyotypic instability of Ligase IV iPSCs manifests in an unusual manner. Tilgner et al reported that hiPSCs with an apparently normal karyotype could be derived from patient fibroblasts with pronounced karyotypic abnormalities, whereas the hiPSCs derived from patient fibroblasts with a normal karyotype were prone to the accumulation of chromosomal abnormalities. The low reprogramming efficiency and karyotypic abnormalities observed for Ligase IV hiPSCs suggest that NHEJ-mediated DSB repair is important for both cellular reprogramming and the maintenance of genomic stability in hiPSCs. Similar to the hiPSCs derived from FA and XLF patients, Ligase IV patient-specific hiPSCs show in vitro and in vivo deficiency in differentiation capacity, the inability to repair DSBs and enhanced apoptosis (29). Although all the above-mentioned patient-specific hiPSC lines are able to undergo differentiation to blood progenitor cells, the proliferating subset of those undergo apoptosis, resulting in a reduced number of committed blood progenitor cells, which is most likely the underlying cause of the pancytopenia observed frequently in these patients early in childhood. Together, these data suggests that the proper functioning of NHEJ-mediated DSB repair is important for the cellular reprogramming of differentiated cells to hiPSCs, for the maintenance of their genomic stability and for the survival of proliferating blood progenitors.

CONCLUDING REMARKS

The generation of hiPSCs from somatic cells with DNA repair defects is still in its infancy, but the fact that cellular reprogramming works, albeit at a much lower efficiency, for cells with the possible accumulation of unrepaired DNA damage encourages the belief that in vitro models of DNA repair diseases can be created. This activity should be of great value in furthering our understanding of the consequences of DNA repair defects for embryonic development and the long-term survival of adult, tissue-specific stem cells and their genomic stability. A note of caution needs to be exercised if the hiPSCs are to be used for future cell-replacement therapies. Our work and that of others has shown that both the fibroblasts and hiPSCs derived from patients with mutations in DNA damage repair genes show a great degree of chromosomal abnormalities. It is, therefore, logical to screen primary human fibroblasts as early as possible for the presence of chromosomal abnormalities. Given that DNA damage repair proteins are needed during the cellular reprogramming process, it is imperative to perform gene correction in fibroblasts prior to reprogramming. The differentiation of genetically corrected hiPSC lines from such patients should then provide the functional and necessary target cells needed for the treatment of their conditions.

References


FACTORS ASSOCIATED WITH DEATH IN INTENSIVE CARE UNIT
PATIENTS WITH VENTILATOR-ASSOCIATED PNEUMONIA

Slobodan M. Jankovic1,2, Zorana Djordjevic2
1 Medical Faculty, University of Kragujevac, Kragujevac, Serbia
2 Kragujevac Clinical Center, Serbia

FAKTORI RIZIKA ZA SMRTNI ISHOD KOD PACIJENATA U
INTENZIVNOJ NEZI NA VEŠTAČKOJ VENTILACIJI
KOJI SU DOBILI PNEUMONIJU

Slobodan M. Jankovic1,2, Zorana Djordjevic2
1 Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Srbija
2 Klinički centar Kragujevac, Srbija

Short Title:
DEATH AFTER VENTILATOR-ASSOCIATED PNEUMONIA

ABSTRACT

Background: The incidence of ventilator-associated pneumonia (VAP) among patients on mechanical ventilation ranges from 15% to 25%, and mortality ranges from 33% to 38%.

Aim: The aim of our study was to analyse the importance of previously uninvestigated potential risk factors for death in intensive care unit (ICU) patients with VAP.

Methods: A case-control design was chosen for this study. The study population consisted of all patients who developed ventilator-associated pneumonia in the central ICU of a tertiary care hospital (n = 65) during a period of 6 months. Cases (n=45) included patients who died during their treatments in the ICU, if their primary cause of death was ventilator-VAP. Controls (n=20) included patients with VAP who survived their treatments in the ICU and who subsequently were subsequently transferred to other hospital wards.

Results: Significant associations were found between death and age over 65 (OR adjusted = 10.66; CI: 1.22, 93.12; p = 0.032), death and infection upon admission to the ICU (OR adjusted = 434.39; CI: 3.07, 61449.65; p = 0.016), and death and administration of ceftriaxone prior to VAP (OR adjusted = 69.32; CI: 1.74, 2768.92; p = 0.024). A synergistic effect on death was found only for age over 65 and infection upon admission to the ICU.

Conclusions: ICU patients with VAP experience have increased risk of mortality if they receive ceftriaxone prophylactically, if they have an infection upon admission to the ICU and if their age is advanced.

Key Words: Ventilator-associated pneumonia; risk factors; death; ceftriaxone.

SAŽETAK

Uvod: Učestalost pneumonije kod pacijenata na veštačkoj ventilaciji (PVV) se kreće između 15% i 25%, a smrtnost pacijenata sa takvom pneumonijom je između 33% i 38%.

Cilj: Cilj naše studije je bio analiza značajna prethodno nedovoljno ispitanih potencijalnih faktora rizika za smrtni ishod pacijenata u intenzivnoj nezi sa PVV-om.

Metoda: Studija je dizajnirana kao studija tipa slučaj-kontrola. Ispitivanu populaciju činili su svi pacijenti koji su dobili PVV u centralnoj intenzivnoj nezi Kliničkog centra (n = 65) tokom perioda od 6 meseci. Slučajevi (n=45) su pacijenti koji su umrli tokom lečenja u intenzivnoj nezi, ukoliko je njihov primarni uzrok smrti pneumonija vezana za veštačku ventilaciju. Kontrole (n=20) su pacijenti sa PVV-om koji su preživeli lečenje u intenzivnoj nezi, a zatim prebačeni na druga odeljenja.

Rezultati: Pronađena je značajna veza između smrtnog ishoda i starosti preko 65 godina (OR adjusted = 10.66; CI 1.22, 93.12; p = 0.032), smrtnog ishoda i infekcije na prijemu u intenzivnu negu (OR adjusted = 434.39; CI 3.07, 61449.65; p = 0.016), i smrtnog ishoda i primene ceftriaksona pre nastanka PVV (OR adjusted = 69.32; CI 1.74, 2768.92; p = 0.024). Sinergistički efekat na smrtni ishod je bio pronađen samo za starost preko 65 godina i infekciju na prijemu u jedinicu intenzivne nege.

Zaključak: Pacijenti iz intenzivne nege sa pneumonijom udruženom sa veštačkom ventilacijom češće umiru ako profilaktički primaju ceftriakson, ako imaju infekciju na prijemu u intenzivnu negu i ako su stariji od 65 godina.

Ključne reči: pneumonija kod pacijenata na veštačkoj ventilaciji; faktori rizika; smrtni ishod; ceftriakson

Conflict of interest:
The authors do not have any conflicts of interest with respect to the contents of this manuscript.

DOI: 10.5937/SJECR13/2669

Correspondence to: Professor Slobodan M. Jankovic; Medical Faculty, University of Kragujevac; Ul. Svetozara Markovica 69; 34000, Kragujevac, Serbia
Tel./Fax. +381 34 306800 ext 117; e-mail: slobodan.jankovic@medf.bg.ac.rs
INTRODUCTION

Ventilator-associated pneumonia (VAP) is a frequent complication of mechanical ventilation in intensive care unit (ICU) patients. The incidence of VAP among patients on mechanical ventilation ranges from 15% to 25%, and mortality ranges from 33% to 38%. The serious clinical and economic consequences of ventilator-associated pneumonia (e.g., patients who develop VAP are twice as likely to die, stay 6.1 days longer in intensive care units on average, and generate more than $10,019 of additional hospital costs as compared with similar patients without VAP) make efficacious treatment of VAP an extremely important health issue.

A number of risk factors for death in patients with VAP have been identified in previous studies, the following factors showing high strong associations: inadequate initial treatment with antibiotics, concomitant bacteraemia, advanced age, female sex, disease severity at VAP onset, nonfermenting Gram-negative bacilli or methicillin-resistant S. aureus as VAP-causative pathogens, prolonged mechanical ventilation dependency, persistent fever, severity of lung injury, septic shock, severe sepsis, previous carbapenem usage within 72 hours, presence of neurologic disease at admission, and failure of the Pao2/Fio2 ratio to improve by day three. However, there are numerous factors that play significant roles in the treatment of patients with VAP whose relationships to death have not been investigated, or were were investigated in inadequately powered studies, such as individual antibiotic agents, prophylactic use of antibiotics, duration of antibiotic use, dosage of antibiotics, and concomitant medication. The aim of our study was to analyse the importance of previously uninvestigated potential risk factors for death in ICU patients with VAP.

MATERIALS AND METHODS

Setting

Our study was conducted in a tertiary care university hospital (Clinical Center) in Kragujevac, Republic of Serbia, which covers a population of approximately 1.5 million inhabitants. The study population consisted of all patients who developed ventilator-associated pneumonia in the central ICU of the Kragujevac hospital (n = 65) during the 6-month period lasting from May 1st, 2010, to October 31st, 2010. Demographic, drug prescription and (co)morbidity data were obtained from the medical records. All data were obtained anonymously, with previous written consent of the patients or their relatives, and the study protocol was approved by the Ethics Committee of the Kragujevac Clinical Center. Ventilator-associated pneumonia was diagnosed using any one of the following criteria: rapid cavitation of a pulmonary infiltrate in the absence of cancer or tuberculosis, a positive pleural fluid culture, a species with the same antibiogram isolated from blood and respiratory secretions without another identifiable source of bacteraemia, histopathologic examination of lung tissue at autopsy or non-bronchoscopic bronchoalveolar lavage through a distally wedged catheter with \( \geq 10^6 \) CFU/mL or \( \leq 1\% \) squamous epithelial cells in the retrieved fluid.

Study design

The design of our study was of a case-control type, with an aim to assess the association between various risk factors and the occurrence of death in patients with ventilator-associated pneumonia. Cases and controls (comprising the study population) were selected from the list of patients with VAP in the central ICU of Kragujevac Clinical Center, with “cases” being defined as patients who died in the ICU and “controls” as patients who survived treatment of VAP and were transferred to non-intensive care wards of the hospital. Patients younger than 18 and pregnant females were not included in the study population.

Cases

Cases (n=45) were chosen from the study population if they died during their treatment in the central ICU of Kragujevac Clinical Center and if their primary cause of death was ventilator-associated pneumonia, as judged by their physicians.

Controls

Controls (n=20) were chosen from the study population (patients with VAP) if they survived their treatment in the central ICU of Kragujevac Clinical Center and were transferred to non-intensive care wards of the clinical centre.

Potential risk factors

To identify potential risk factors, the following data were collected for each patient: age, sex, emergency hospitalisation, diagnosis upon admission to the ICU, infection upon admission to the hospital, concomitant chronic diseases, causative agent of VAP, resistance of the causative agent to antibiotics, type of surgery, duration of hospitalisation after operation, sepsis, use of a peripheral intravenous catheter, duration of peripheral intravenous (IV) catheterisation, duration of peripheral IV catheterisation prior to VAP, use of a central intravenous catheter, duration of central IV catheterisation, duration of central IV catheterisation prior to infection, infection of the urinary tract, use of a urinary catheter, duration of urinary tract catheterisation, duration of urinary tract catheterisation prior to infection, use of endotracheal intubation, duration of intubation, duration of intubation prior to infection, use of artificial ventilation, duration of artificial ventilation, time from the beginning of artificial ventilation until infection, duration of hospitalisation in the ICU, previous hospitalisation, duration of hospitalisation prior to admission to the ICU, total duration of hospitalisation, indication for antibiotics, antibiotic administration prior to hospital infection, use of cefuroxime, metronidazole, ciprofloxacin, ceftriaxone, azithromycin, ampicillin-sulbactam, meropenem, imipenem, ertapenem, and ...
vancomycin, ampicillin, gentamicin, cefotaxime or piperacillin-tazobactam prior to VAP, daily dose of cefuroxime, metronidazole, ciprofloxacin, ceftriaxone, azithromycin, ampicillin-sulbactam, meropenem, imipenem, ertapenem, vancomycin, ampicillin, gentamicin, cefotaxime or piperacillin-tazobactam prior to VAP, use of cefuroxime, metronidazole, ciprofloxacin, ceftriaxone, azithromycin, ampicillin-sulbactam, meropenem, imipenem, ertapenem, vancomycin, ampicillin, gentamicin, cefotaxime or piperacillin-tazobactam after the occurrence of VAP, daily dose of cefuroxime, metronidazole, ciprofloxacin, ceftriaxone, azithromycin, ampicillin-sulbactam, meropenem, imipenem, ertapenem, vancomycin, ampicillin, gentamicin, cefotaxime or piperacillin-tazobactam after the occurrence of VAP, use of tigecycline after the occurrence of VAP and its daily dose, and transfusion of blood or its derivatives.

Data analysis
The prevalence of each risk factor was determined for both cases and controls. The differences between cases and controls in the observed characteristics were assessed by a Student t-test for continuous variables and a chi-squared test for frequencies. The differences were considered significant if the probability of falsely rejecting a null hypothesis was less than 0.05. To estimate the association between variables and death from VAP, crude and adjusted odds ratios (OR) with 95% confidence intervals (95% CI) were calculated using logistic regression.14,15

RESULTS

Sixty-five patients were enrolled in the study. The baseline characteristics of cases and controls and the differences between them are shown in Table 1. Cases and controls did not significantly differ in terms of age, sex, emergency hospitalisation, diagnosis upon admission to the ICU, infection upon admission to the hospital, concomitant chronic diseases, causative agent of VAP, resistance of the causative agent to antibiotics, type of surgery, duration of hospitalisation after the operation, sepsis, use of a peripheral intravenous catheter, duration of peripheral IV catheterisation, duration of peripheral IV catheterisation prior to VAP, use of a central intravenous catheter, duration of central IV catheterisation, duration of central IV catheterisation prior to infection, infection of the urinary tract, use of a urinary catheter, duration of urinary tract catheterisation, duration of urinary tract catheterisation prior to infection, use of endotracheal intubation, duration of intubation, duration of intubation prior to infection, use of artificial ventilation, duration of artificial ventilation, time from the beginning of artificial ventilation until infection, duration of hospitalisation in the ICU, previous hospitalisation, duration of hospitalisation prior to admission to the ICU, total duration of hospitalisation, indication for antibiotics, antibiotic administration prior to hospital infection, use of cefuroxime, metronidazole, ciprofloxacin, ceftriaxone, azithromycin, ampicillin-sulbactam, meropenem, imipenem, ertapenem, vancomycin, ampicillin, gentamicin, cefotaxime or piperacillin-tazobactam prior to VAP, daily dose of cefuroxime, metronidazole, ciprofloxacin, ceftriaxone, azithromycin, ampicillin-sulbactam, meropenem, imipenem, ertapenem, vancomycin, ampicillin, gentamicin, cefotaxime or piperacillin-tazobactam prior to VAP, use of cefuroxime, metronidazole, ciprofloxacin, ceftriaxone, azithromycin, ampicillin-sulbactam, meropenem, imipenem, ertapenem, vancomycin, ampicillin, gentamicin, cefotaxime or piperacillin-tazobactam after the occurrence of VAP, daily dose of cefuroxime, metronidazole, ciprofloxacin, ceftriaxone, azithromycin, ampicillin-sulbactam, meropenem, imipenem, ertapenem, vancomycin, ampicillin, gentamicin, cefotaxime or piperacillin-tazobactam after the occurrence of VAP, use of tigecycline after the occurrence of VAP and its daily dose, and transfusion of blood or its derivatives.

The results of the logistic regression analysis (Cox & Snell R-square = 0.386, Nagelkerke R-square = 0.545, Hosmer and Lemeshow chi-square = 2.516, df = 8, p = 0.961), with adjustment for potential confounders, are shown in Table 2. The only significant associations were between the variables death and age over 65 (OR adjusted = 10.66; CI: 1.22, 93.12; p = 0.032), death and infection upon admission to ICU (OR adjusted = 434.39; CI: 3.07, 61449.65; p = 0.016), and death and administration of ceftriaxone prior to VAP (OR adjusted = 69.32; CI: 1.74, 2768.92; p = 0.024). Although the crude odds ratios for administration of tigecycline for treatment of VAP and multi-drug resistance of the causative agent were significantly different from one (see Tables 1 and 2), confidence limits of these odds ratios after adjustment included the value of one.

The interactions between factors likely to introduce greater risk for death after VAP were investigated (Table 3). The analyses showed clear synergistic effects only for the interaction of age over 65 and infection upon admission to the ICU. Although crude and adjusted odds ratios increased when age over 65 interacted with administration of ceftriaxone prior to VAP and when infection upon admission to the ICU interacted with administration of ceftriaxone prior to VAP, synergistic effects could not be confirmed by either crude or adjusted odds ratios because their confidence limits also widened substantially to include the value of one.

DISCUSSION

Although several previous studies16,17,18 found the deaths of patients with VAP to be associated with the severity of the primary disease precipitating ICU admission and surgery, this association was not confirmed in our study. One of the reasons for our finding this difference could be the very high mortality rate suffered by our patients (69%), which
### Table 1. Baseline characteristics of cases and controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n=45)</th>
<th>Controls (n=20)</th>
<th>Test value and significance of the null hypothesis</th>
<th>Crude odds ratios with confidence intervals (1.96*SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>30/15 (67%/33%)</td>
<td>15/5 (75%/25%)</td>
<td>$\chi^2 = 0.451, p = 0.502$</td>
<td>1.44 (0.78, 2.67)</td>
</tr>
<tr>
<td>Age (years, mean ± SD)</td>
<td>62.4 ± 16.2</td>
<td>51.6 ± 19.3</td>
<td>$T = 1.725, p = 0.194$</td>
<td>1.03 (1.01, 1.05)</td>
</tr>
<tr>
<td>Emergency hospitalisation (yes/no)</td>
<td>42/3 (93%/7%)</td>
<td>17/3 (85%/15%)</td>
<td>$\chi^2 = 1.148, p = 0.284$</td>
<td>3.35 (1.51, 7.43)</td>
</tr>
<tr>
<td>Principal diagnosis (internal disease/trauma/surgical disease/infectious disease)</td>
<td>8/4/26/7 (18%/9%/58%/15%)</td>
<td>3/3/14/0 (15%/15%/70%/0%)</td>
<td>$\chi^2 = 3.991, p = 0.262$</td>
<td>0.96 (0.66, 1.41)</td>
</tr>
<tr>
<td>Infection upon admission to ICU (yes/no)</td>
<td>8/37 (18%/82%)</td>
<td>1/19 (5%/95%)</td>
<td>$\chi^2 = 1.895, p = 0.169$</td>
<td>1.68 (0.72, 3.97)</td>
</tr>
<tr>
<td>Having a chronic disease (yes/no)</td>
<td>18/27 (40%/60%)</td>
<td>5/15 (25%/75%)</td>
<td>$\chi^2 = 1.363, p = 0.243$</td>
<td>1.28 (0.69, 2.37)</td>
</tr>
<tr>
<td>Age over 65 (yes/no)</td>
<td>25/20 (56%/44%)</td>
<td>6/14 (30%/70%)</td>
<td>$\chi^2 = 3.625, p = 0.057$</td>
<td>2.41 (1.33, 4.37)</td>
</tr>
<tr>
<td>Causative agent (Stenotrophomonas/Acinetobacter/Proteus/ S.aureus/Pseudomonas/Klebsiella/E.coli/Providencia)</td>
<td>3/14/1/2/11/13/0/1 (7%/31%/2%/4%/24%/29%/0%/3%)</td>
<td>1/7/1/2/3/5/1/0 (5%/35%/5%/10%/15%/25%/5%/0%)</td>
<td>$\chi^2 = 6.077, p = 0.639$</td>
<td>1.36 (1.19, 1.54)</td>
</tr>
<tr>
<td>Multiresistance of the causative agent (yes/no)</td>
<td>41/4 (91%/9%)</td>
<td>19/1 (95%/5%)</td>
<td>$\chi^2 = 0.295, p = 0.587$</td>
<td>5.12 (2.74, 9.65)</td>
</tr>
<tr>
<td>Surgery (yes/no)</td>
<td>35/10 (78%/22%)</td>
<td>19/1 (95%/5%)</td>
<td>$\chi^2 = 2.921, p = 0.087$</td>
<td>0.50 (0.24, 1.05)</td>
</tr>
<tr>
<td>Having a central intravenous catheter (yes/no)</td>
<td>33/12 (73%/27%)</td>
<td>13/7 (65%/35%)</td>
<td>$\chi^2 = 0.465, p = 0.495$</td>
<td>1.67 (0.92, 3.01)</td>
</tr>
<tr>
<td>Administration at another hospital ward prior to admission to the ICU (yes/no)</td>
<td>28/17 (62%/38%)</td>
<td>14/6 (70%/30%)</td>
<td>$\chi^2 = 3.366, p = 0.045$</td>
<td>0.63 (0.34, 1.16)</td>
</tr>
<tr>
<td>Reason for the administration of antibiotics prior to pneumonia (prophylaxis/treatment of a hospital infection/treatment of an infection upon admission/unknown)</td>
<td>31/29/3 (69%/4%/20%/7%)</td>
<td>18/0/1 (90%/0%/5%/5%)</td>
<td>$\chi^2 = 3.795, p = 0.284$</td>
<td>1.38 (1.04, 1.83)</td>
</tr>
<tr>
<td>Administration of ceftriaxone prior to VAP (yes/no)</td>
<td>16/29 (36%/64%)</td>
<td>8/12 (40%/60%)</td>
<td>$\chi^2 = 0.662, p = 0.718$</td>
<td>1.06 (0.61, 1.84)</td>
</tr>
<tr>
<td>Administration of ciprofloxacin prior to VAP (yes/no)</td>
<td>5/40 (11%/89%)</td>
<td>1/19 (5%/95%)</td>
<td>$\chi^2 = 0.616, p = 0.432$</td>
<td>0.66 (0.27, 1.61)</td>
</tr>
<tr>
<td>Administration of ceftriazone prior to VAP (yes/no)</td>
<td>9/36 (20%/80%)</td>
<td>2/18 (10%/90%)</td>
<td>$\chi^2 = 0.985, p = 0.321$</td>
<td>3.55 (1.49, 8.46)</td>
</tr>
<tr>
<td>Administration of ampicillin+ sulbactam prior to VAP (yes/no)</td>
<td>3/42 (7%/93%)</td>
<td>2/18 (10%/90%)</td>
<td>$\chi^2 = 0.217, p = 0.642$</td>
<td>2.04 (0.53, 7.84)</td>
</tr>
<tr>
<td>Administration of meropenem prior to VAP (yes/no)</td>
<td>7/38 (16%/84%)</td>
<td>3/17 (15%/85%)</td>
<td>$\chi^2 = 0.003, p = 0.954$</td>
<td>1.67 (0.69, 4.08)</td>
</tr>
<tr>
<td>Administration of vancomycin prior to VAP (yes/no)</td>
<td>1/44 (2%/98%)</td>
<td>2/18 (10%/90%)</td>
<td>$\chi^2 = 1.903, p = 0.168$</td>
<td>0.94 (0.22, 4.06)</td>
</tr>
<tr>
<td>Administration of meropenem for the treatment of VAP (yes/no)</td>
<td>11/34 (24%/76%)</td>
<td>4/16 (20%/80%)</td>
<td>$\chi^2 = 0.154, p = 0.695$</td>
<td>4.30 (1.45, 12.77)</td>
</tr>
<tr>
<td>Administration of imipenem for the treatment of VAP (yes/no)</td>
<td>8/37 (18%/82%)</td>
<td>2/18 (10%/90%)</td>
<td>$\chi^2 = 0.643, p = 0.422$</td>
<td>5.89 (1.57, 22.20)</td>
</tr>
<tr>
<td>Administration of tigecycline for the treatment of VAP (yes/no)</td>
<td>14/31 (31%/69%)</td>
<td>4/16 (20%/80%)</td>
<td>$\chi^2 = 0.854, p = 0.356$</td>
<td>5.18 (1.78, 15.09)</td>
</tr>
<tr>
<td>Administration of ciprofloxacin for the treatment of VAP (yes/no)</td>
<td>6/39 (13%/87%)</td>
<td>1/19 (5%/95%)</td>
<td>$\chi^2 = 1.001, p = 0.317$</td>
<td>3.40 (0.99, 11.73)</td>
</tr>
<tr>
<td>Administration of ampicillin+ sulbactam for the treatment of VAP (yes/no)</td>
<td>4/41 (9%/91%)</td>
<td>1/19 (5%/95%)</td>
<td>$\chi^2 = 0.295, p = 0.587$</td>
<td>4.14 (0.78, 21.91)</td>
</tr>
<tr>
<td>Administration of piperacillin+tazobactam for the treatment of VAP (yes/no)</td>
<td>8/37 (18%/82%)</td>
<td>5/15 (25%/75%)</td>
<td>$\chi^2 = 0.451, p = 0.502$</td>
<td>1.89 (0.66, 5.46)</td>
</tr>
<tr>
<td>Administration of vancomycin for the treatment of VAP (yes/no)</td>
<td>2/43 (4%/96%)</td>
<td>4/16 (20%/80%)</td>
<td>$\chi^2 = 3.999, p = 0.046**</td>
<td>0.51 (0.10, 2.61)</td>
</tr>
<tr>
<td>Duration of endotracheal intubation prior to VAP (days)</td>
<td>10.6 ± 10.4</td>
<td>9.2 ± 7.1</td>
<td>$T = 0.602, p = 0.549$</td>
<td>1.16 (1.08, 1.24)</td>
</tr>
<tr>
<td>Time elapsed from the beginning of artificial ventilation to VAP (days)</td>
<td>10.6 ± 10.6</td>
<td>8.8 ± 6.1</td>
<td>$T = 0.723, p = 0.472$</td>
<td>1.17 (1.09, 1.25)</td>
</tr>
<tr>
<td>Duration of hospitalisation prior to admission to the ICU (days)</td>
<td>5 ± 6.9</td>
<td>4.5 ± 4.4</td>
<td>$T = 0.298, p = 0.766$</td>
<td>0.99 (0.94, 1.04)</td>
</tr>
</tbody>
</table>

*For the sake of clarity, variables occurring with less than 2% frequency were omitted from the table, as were several less important variables having insignificant differences between cases and controls.  
**Significant difference
tration of antibiotics as a part of selective decontamination of the digestive tract (SDD) reduces VAP incidence by over 50% and mortality by approximately 25%, but this effect no longer holds true if antibiotics are administered for a prolonged period of time and in empiric dose regimens, as was the practice at our study site. Therefore, the high mortality observed in our study could be the consequence of an inappropriate use of antibiotics for the prophylaxis of VAP.

To further explore this presumption, we analysed the associations of individual antibiotics administered both for prophylaxis and for treatment of VAP with death. Out of a set of more than 10 different antibiotics, prophylactic administration of only one antibiotic, the third-generation cephalosporin ceftriaxone, was strongly linked to death in the patients with VAP (see Tables 2 and 3). The patients who prophylactically received ceftriaxone had more than
3-times greater risk of death than those who did not receive such prophylaxis; the risk increased to more than 6 times in patients older than 65 years. This adverse effect of third-generation cephalosporins on mortality was previously shown among other types of patients in ICUs and has been explained by the selection of multidrug-resistant bacteria producing extended-spectrum beta-lactamases.

Although in our study, multi-drug resistance of isolated causative agents was not statistically associated with death, more than 90% of our patients in both groups were suffering from VAP caused by multi-drug resistant bacteria. In such a situation, other disabling factors that compromise defence against infection, such as advanced age and additional infection upon admission to the ICU, can add to the negative effects of ceftriaxone and increase the likelihood of death, as was observed in our study.

Assessment of the individual risk of death for each patient who may develop VAP must take into account prophylactic ceftriaxone administration, the presence of infection upon admission to the ICU, and the advanced age of the patient. If one of these factors applies to a given patient’s case, he or she deserves special attention and careful selection of antibiotics, both for prophylaxis and for treatment of VAP.

ACKNOWLEDGEMENTS

This study was partially financed by grant No. 175007, given by Serbian Ministry of Science and Ecology, and by a grant provided by the Ministry of Science, Montenegro.

REFERENCES


SYNONOUS PRIMARY OVARIAN ENDOMETRIOID CARCINOMA AND POORLY DIFFERENTIATED ENDOMETRIAL STROMAL SARCOMA

Petar Arsenijević1,2, Janko Đurić1,2, Slodoban Arsenijević1,2, Miloš Z. Milosavljević1 and Slobodanka Mitrović1,3
1 Faculty of Medical Science, University of Kragujevac, Kragujevac, Serbia
2 Department of Gynaecology and Obstetrics, Clinical Center Kragujevac, Kragujevac, Serbia
3 Department of Pathology, Clinical Center Kragujevac, Kragujevac, Serbia

ABSTRACT

Introduction: The presence of two concomitant genital malignant tumours is a rare phenomenon with an incidence of 0.7%. They are typically localised in the uterus and ovary, and approximately 50% are identical endometrioid-endometrioid histological type. In the presence of two different histological types of tumours, diagnosis is easier, but because of the small number of reported cases, there are no precise data on their clinical course. Generally, women because of the small number of reported cases, there are no precise data on their clinical course. Generally, women multiparous and in menopause compared with women who have metastatic ovarian cancer or endometrial cancer.

Case Report: In this work, we present a 53-year-old woman who contacted a gynaecologist because of irregular haemorrhaging. The gynaecological exam revealed a tumour protruding through the cervical canal. The biopsy with initial histopathological diagnosis and additional ECHO and CT evaluations indicated the presence of a cervical tumour spreading over the uterus and both uterine adnexa. After surgical intervention, macroscopic and microscopic analyses of the postoperative material showed the simultaneous presence of two different malignancies, ovarian endometrioid carcinoma and poorly differentiated endometrial stromal sarcoma, which infiltrated all layers of the uterus, cervix and both fallopian tubes.

Conclusion: The prognosis of synchronous tumours of the ovary and the uterus is primarily dependent on the stage and histological type of each tumour. Because of the rather small number of reported cases, a large part of this phenomenon remains unknown. Our report is the first description of the synchronous occurrence of endometrial ovarian carcinoma and uterine stromal sarcoma.

Keywords: Ovarian Neoplasms; Endometrial Neoplasms; Neoplasms, Multiple Primary; Sarcoma; Endometrial Stromal; Carcinoma; Endometrioid.

Clír rada: Prisustvo dva genitalna maligna tumora u istom vremenu je redak fenomen sa zabeleženom učestalosti od 0,7%. Njihova lokalizacija često je u uterusu i jajniku, ali zbog okolnih faktora, dijagnoza se lakše postavlja, ali zbog malog broja prikazanih slučajevima nema preciznijih podataka o njihovom kliničkom toku. Generalno, žene sa sinchronim tumoralnim proporcijama i endometrioidnom carcinomom i endometrioidnom sarcomom, češće su mlade, gojazne, premenopauzalne i neradijasne, u odnosu na obolele od carcinoma jajnika ili metastatskog carcinoma endometrijuma.

Prikaz slučaja: U ovom radu prezentujemo ženu staru 53 godine kod koje se na ginekološkom pregledu javljaju kontaktno krvavljenje, otkriva tumor koji prolazi kroz endocervikalni kanal. Inicijalna biopsija sa histološkom orijentacionom dijagnozom, dodatne CT i EHO pretrage ukazuju na prisustvo tumora u uterusu koji zahvata grlic i obe adnekse. Makroskopska i mikroskopska analiza operativnog materijala pokazuje da se radi o istovremenom prisustvu dva različita maligniteta: endometrioidnog carcinoma jajnika i slabo diferentovanoj sarcomi stromalne endometrijuma koji je infiltrisao sve slojeve zida uterusa, grlic i oba tuba.

Zaključak: Prognoza sinchronih tumora ovarijuma i uterusa premostrano zavisi od stadijuma i histološkog tipa svakog pojedinačnog tumora. Naš prikaz predstavlja prvi opis sinchronog tumora ovarijuma i jajnika u kombinaciji sarkoma stromalne endometrijuma i endometrioidnog carcinoma jajnika. Zbog malog broja opisanih slučajeva kombinacije različitih histoloških tipova ovaj fenomen je još uvek nedovoljno poznat.

Ključne reči: sinchroni tumori ovarijuma i endometrijuma, sarkom strome endometrijuma, endometrioidni karcinom jajnika.

DOI: 10.5937/SJECR13/3193

Correspondence to: Slodobanka Mitrović; Department of Pathology, Faculty of Medical Science, University of Kragujevac, Serbia; Svetozara Markovica 69, Kragujevac 34000, Serbia
Home address: Lepenički bulevar, 23/9, 34000 Kragujevac, Serbia; Tel: +381 65 80 80 877; +381 34 370 073; E-mail: smitrovic@medf.kg.ac.rs
INTRODUCTION

Synchronous malignant tumours of the female genital tract are a rare but known phenomenon, with an incidence of only 0.7%.1 Approximately 50% of synchronous gynaecological malignancies are synchronous tumours of the uterus and ovary (STOUs).2 The most common variant of STOUs is the combination of endometrioid carcinomas of the ovary and endometrium, while the occurrence of uterine stromal sarcoma and adenocarcinoma of the ovary has not been yet described in the literature. The diagnosis and treatment of STOUs is no different from the diagnosis and treatment of solitary cancers of the female genital tract. The treatment of choice is surgery, and additional therapeutic procedures, such as chemotherapy and radiotherapy, are used depending on the stage of the disease and histological type of the tumour.

CASE REPORT

A 53-year-old female patient who was multiparous, in menopause and had a medium body composition reported to the ambulatory gynaecological examination service after irregular uterine haemorrhaging. The haemorrhaging had occurred for the first time since the patient had entered menopause, and she reported no other symptoms. Her medical history indicated that menarche had started at 14 years, and her last regular menstrual cycle was at 51 years of age. The patient did not use oral contraceptive pills or hormone replacement therapy. A bimanual gynaecologic examination was conducted and showed an enlarged uterus. The cervical examination identified a whitish lesion protruding from the endocervical canal. A cervical biopsy was performed, and a spotted lesion of approximately 6 mm was excised. Haematoxylin-eosin (HE) staining indicated the existence of a tumour with a high-grade malignancy. The differential diagnosis included poorly differentiated adenocarcinoma, stromal sarcoma and mixed Mullerian tumour. It was suggested that a definitive histological diagnosis with the simultaneous staging of the disease could be made after analysing the postoperative materials. Before the operation, the patient underwent ultrasonography (ECHO) and computed tomography. Transabdominal ECHO showed an enlarged uterus, 210x130 mm in diameter, with a wall thickness of up to 70 mm and a right adnexal solid mass, 125x58 mm in diameter. In addition to visualizing the enlarged uterus and right adnexal mass, computed tomography (CT) of the abdomen and pelvis indicated the existence of focal intra-

Figure 1: Macroscopic appearance of the resected operative material (a) and the tissue cross section (b).

Figure 2: Macroscopic appearance of the right tumour-containing ovary (a) with a solid and partly cystic appearance at the cross section (b).
peritoneal metastases. CT of the thorax and a bone scan did not register pathomorphological changes. The gynecological council decision was to carry out radical surgical treatment due to the possibility of metastases in the right adnexa. The patient underwent exploratory laparotomy, total hysterectomy, lymphadenectomy, total omentectomy, peritoneal biopsy, and peritoneal lavage.

A histopathologic analysis was performed. The uterus, left adnexa and right fallopian tube were initially submitted together, while the right ovarian tumour, samples of the omentum and peritoneum, 30 lymph nodes and 12 ml of ascites fluid were submitted separately. The uterus weight was 700 g, and it was 184x136x84 mm in diameter, while the cervix was 38 mm long with a protruding tumour mass (Figure 1a). The tissue cross section demonstrated that the walls of the uterus and cervix were infiltrated by a grey tumour mass, with yellowish areas of necrosis and red areas of haemorrhage. Tumours with similar macroscopic characteristics filled the lumen and infiltrated the walls of both fallopian tubes. The largest diameter of the left ovary was 24 mm, with no visible macroscopic changes (Figure 1b). The right ovarian tumour was enlarged to 72x44 mm in diameter, with a solid and partly cystic structure at the cross section (Figure 2a, b). The adipose tissue in the omental and peritoneal samples was of regular morphology, and the lymph node structure was homogeneous, with diameters ranging from 7 to 15 mm.

Microscopically, the existence of two morphologically distinct tumours was confirmed. HE staining showed characteristics similar to the tissues of the uterus, cervix and fallopian tubes. The tumour was present in all layers of the uterus wall, with infiltrations into the cervix and fallopian tubes. The tumour contained extensive areas of necrosis and areas of angioinvasion and was composed of small and moderately sized cells, with scant cytoplasm and blurred cell boundaries (HE staining technique, x200). Immunohistochemically, the tumour cells were diffusely positive for only vimentin, (x400) and remained negative for CKAE1/AE3 (x200) and CD10 (x400).

Figure 3: Histological features of a high-grade endometrial stromal sarcoma. a) The tumour contained small and moderately sized cells, with scant cytoplasm and blurred cell boundaries (HE staining technique, x200). Immuno- histochemically, the tumour cells were diffusely positive for only b) vimentin, (x400) and remained negative for c) CKAE1/AE3 (x200) and d) CD10 (x400).

A microscopic analysis of the HE-stained samples that matched the cyst found in the right ovary revealed a tumour composed of pseudo-glandular, cribriform and solid areas of atypical, cylindrical and cuboidal cells with eosinophilic cytoplasm (Figure 4a). The nuclei were hyperchromatic or vesicular, with a moderate number of mitotic figures. The stroma was relatively sparse and contained an inflammatory mononuclear cell infiltrate (Figure 4b). Immunohistochemically, the tumour cells were positive for CK7, CA125, ER and PR and remained negative for vimentin and CEA. The proliferation index was intermediate, with approximately 25% of the nuclei expressing Ki-67 (Figure 4c-f). Based on the immunophenotype, the diagnosis of a moderately differentiated endometrioid carcinoma of the ovary with no tumour extension (FIGO Ia stage) was made. A microscopic analysis of the HE-stained samples that matched the cyst found in the right ovary revealed a tumour composed of pseudo-glandular, cribriform and solid areas of atypical, cylindrical and cuboidal cells with eosinophilic cytoplasm (Figure 4a). The nuclei were hyperchromatic or vesicular, with a moderate number of mitotic figures. The stroma was relatively sparse and contained an inflammatory mononuclear cell infiltrate (Figure 4b). Immunohistochemically, the tumour cells were positive for CK7, CA125, ER and PR and remained negative for vimentin and CEA. The proliferation index was intermediate, with approximately 25% of the nuclei expressing Ki-67 (Figure 4c-f). Based on the immunophenotype, the diagnosis of a moderately differentiated endometrioid carcinoma of the ovary with no tumour extension (FIGO Ia stage) was made. The left ovary, lymph nodes, and tissue samples of the omentum and peritoneum were all free of tumour deposits. Finally, a cytological analysis of the peritoneal lavage fluid showed no presence of malignant cells.
The postoperative course passed without complication, and the patient was released from the hospital on the 10th postoperative day. The oncology council decided to continue the course of treatment with chemotherapy and radiotherapy. However, despite the therapeutic measures implemented, the disease progressed, and secondary deposits were identified in the liver; the patient died 12 months after the diagnosis.

DISCUSSION

Synchronous malignant tumours of the female genital tract are rare, with an incidence ranging from 0.7% to 6%. They are typically localised in the uterus and ovaries and are rarely found in the cervix and ovaries or cervix and uterus. The phenomenon of more than two simultaneous tumours is extremely rare, although Atasever et al previously described up to 5 simultaneous ovarian cancers in one patient.

Studies have shown that approximately 50% of synchronous gynaecological malignancies are tumours of the uterus and ovaries. Their incidence and prevalence is still a subject of debate because of the large differences in the observed results, which are primarily due to different research approaches. For instance, population studies including women with ovarian cancer have reported an incidence of less than 3%, while analyses of first-stage endometrial carcinoma have reported an incidence of STOUs of 0.31%. Zaino et al have claimed that the incidence of STOUs in women with ovarian cancer is 10% and no more than 5% in women with endometrial cancer, while Irving has reported an incidence of 15-20%. This inconsistency is associated with the different characteristics of the analysed populations, sample size, more organised prevention methods and more precise diagnostics. This is particularly important in the case of cancers with identical endometrioid histological types, with many previously well-defined pathomorphological criteria for the detection of STOUs. The histopathological diagnosis of different histological types of STOUs is not a problem due to the clearly defined microscopic images and different tumour immunophenotypes.

The most common histological type of STOUs is endometrioid carcinoma in both locations. Serous and clear-cell carcinomas are less frequent, and few cases, if any, are
related to the combination of granulosa cell tumour of the ovary and endometrial carcinoma. In a series of 30 cases of STOUs, Rodolakis et al described only one case of uterine endometrioid carcinoma and carcinosarcoma of the ovary. The relatively small number of individual cases of non-endometrioid types of STOUs is the most likely reason for the lack of their precise analysis. Ours is the first report of STOUs with endometrial stromal sarcoma (SSE) and endometrioid carcinoma of the ovary.

SSE is the second most common mesenchymal tumour of the uterus, with a prevalence of 10% among all malignancies with a mesenchymal component. According to the WHO classification, uterine sarcomas are classified into two categories: low-grade SSE and undifferentiated SSE. Low-grade malignant SSE typically consists of tumour cells similar to stromal cells in the proliferative phase of the menstrual cycle, which infiltrate the myometrium, parametrium and surrounding lymph vessels. In contrast, undifferentiated SSE is characterised by the absence of stromal differentiation and is referred to as poorly differentiated or high-grade undifferentiated. Endometrioid carcinomas are present in approximately 10-30% of malignant ovarian tumours, bilateral in 28% of cases and most frequent in the fifth and sixth decades of life. They derive from epithelial metaplasia or the focus of ovarian endometriosis. A potential reason for the identification of STOUs comprising SSE and endometrioid carcinoma may be the existence of adenomyosis and ovarian endometriosis.

Various genetic, embryonic and hormonal factors are possible reasons for the phenomenon of STOUs. STOUs are thought to be associated with hyperoestrogenism during chronic anovulation, polycystic ovary syndrome, obesity, oestrogen-producing ovarian tumours and hormone therapy. The multiple genetic alterations observed suggest that the tumours develop independently, and Lynch syndrome is present in 7-9% of patients with STOUs. Most likely, STOUs are caused by different and still unknown aetiological factors. The average age of patients with STOUs is 49-52 years. In contrast to STOUs, ovarian and endometrial cancers occur in older, multiparous, postmenopausal, obese women who often suffer from diabetes or hypertension. The patient we presented was 52 years old and multiparous with no other features characteristic of patients with STOUs.

The GOG study showed that patients with STOUs have a much better prognosis compared with patients with metastatic ovarian and endometrial cancers. The ten-year survival rate is 80%, and it is mainly related to the most common endometrioid/endometrioid type of STOUs. The better prognosis of STOUs is most likely due to the early detection of ovarian cancer because the illness typically presents as abnormal bleeding from the uterus. This does not apply to our patient, in whom the disease was discovered in an advanced stage, which is why her overall survival was only thirteen months. The best course of treatment is surgery with adjuvant chemotherapy and radiotherapy. Despite all the available treatment measures, most patients do not live for more than two years after the diagnosis due to the occurrence of metastasis or relapse within the first six months of this pernicious illness.

CONCLUSION

The prognosis of synchronous tumours of the ovary and the uterus is primarily dependent on the stage and histological type of each tumour. Because of the rather small number of reported cases, a large part of this phenomenon remains unknown. Our report is the first description of the synchronous occurrence of endometrial ovarian carcinoma and uterine stromal sarcoma.

ABBREVIATIONS

STOUs - synchronous tumours of the ovary and uterus;
HE - haematoxylin-eosin;
ECHO - ultrasonography;
CT - computed tomography;
LCA - leucocyte common antigen;
EMA - epithelial membrane antigen;
CK - cytokeratin;
ER - oestrogen receptor;
PR - progesterone receptor;
CEA - carcinoembryonic antigen;
FIGO - International Federation of Gynecology and Obstetrics;
SSE - endometrioid stromal sarcoma.

REFERENCES

ABSTRACT

This paper presents a case of a 60-year-old patient with an oligosymptomatic form of Melkersson-Rosenthal syndrome. This syndrome is characterized by three symptoms: swelling of the lips and face, unilateral facial palsy, and lingua plicata. The literature often includes information on monosymptomatic forms of the syndrome and granulomatous cheilitis or the existence of only two symptoms of the classic triad. The anamnesis, clinical, and histopathological findings in this case confirmed the existence of chronic granulomatous inflammation confined to the lower lip, indicating an oligosymptomatic form of Melkerson-Rosenthal syndrome. The treatment included prednisone application at a dose of 25 mg daily, histamine H1 and H2 antagonists, and the antibiotic ofloxacin for urinary tract infection.

Keywords: Melkersson-Rosenthal syndrome, granulomatous cheilitis, prednisone.

INTRODUCTION

In 1928, Melkersson first described a case of recurrent facial paresis with mouth edema. A few years later (in 1931), Rosenthal added a third symptom, lingua plicata, to the description, and Melkersson-Rosenthal syndrome was established (1). It usually occurs in adulthood and middle age in both sexes. The etiology of Melkersson-Rosenthal syndrome is unknown, and many believe that there is a genetic predisposition.

This syndrome is characterized by three symptoms: swelling of the lips and face, unilateral facial nerve palsy, and fissured tongue. There are numerous case reports on monosymptomatic variants of the syndrome with granulomatous cheilitis or the existence of only two symptoms. In addition to the swelling of the lips and facial region, patients also lose taste and smell, secretion from the salivary and lacrimal glands is reduced, and mobility of the lips is decreased. Sometimes, the patients have difficulties speaking (1,2,3).

Treatment of the syndrome is based on the local administration of triamcinolone every 4 to 6 months. Using clofazimine 100 mg twice daily for 10 days followed by twice a week for 4 months is also recommended. Surgical treatment along with the previously mentioned therapy is also possible (4,5).

THE CASE REPORT

The patient was a 60-year-old male who was admitted to the hospital for the evaluation of chronic swelling of his lower lip. From the patient history, the first incidence of swelling and redness of the lower lip occurred in 1989. He was treated then with both local and systemic antibiotics, but there was no improvement. After a tooth extraction, the swelling spontaneously subsided but returned in 2007 and persisted until the current presentation, regardless of treatment. He had attempted to treat himself with local antibiotics and antihistamines. He had no hives, rashes, or swelling of other soft tissues. He denied the existence of other diseases and allergies to food, medication, insect bites, and surgery. His family history was unremarkable.

The patient was conscious during the hospital admission, properly oriented, eupneic, and without fever. His body shape and weight were normal, and there were no signs of peripheral lymphadenopathy or hemorrhagic syndrome. The excessive swelling and redness in the area of the lower lip were the most prominent findings (Figures 1 and 2); the tongue was coated with whitish skim, dry and lightly scarred. The heart rhythm was regular, with clear tones; the arterial blood pressure was
180/120 mmHg. His anterior abdominal wall was above chest level when the patient was lying on his back, insensitive to palpation and without swelling. There were no deformities or venous varices on the lower extremities. The laboratory findings were as follows: erythrocyte sedimentation rate 6 mm in the first hour, fibrinogen 3.1 g/l, leukocyte count 5.89 x 10^9/l (lymphocytes 1.38 x 10^9/l, monocytes 0.54 x 10^9/l, eosinophils 0.29 x 10^9/l, and basophils 0.04 x 10^9/l), erythrocyte count 4.39 x 10^12/l, hemoglobin 147 g/l, hematocrit 0.439, MCV 97 nL, platelet count 255 x 10^9/l, glucose 4.1 mmol/l, creatinine 74 μmol/l, total bilirubin 29 μmol/L, direct bilirubin 16.4 μmol/L, total blood proteins 73 g/L, albumin 43 g/L, cholesterol 5.23 mmol/L, triglycerides 1.25 mmol/L, potassium 3.7 mmol/L, sodium 138 mmol/L, calcium 2.39 mmol/L, AST 36 IU/L, ALT 41 IU/L, ALP 70 IU/L, gamma-GT 12 IU/L, and LDH 517 IU/L. The immunological results were as follows: antinuclear antibodies 0, anti-neutrophil cytoplasmic antibodies 0, anti-mitochondrial antibodies 0, anti-smooth muscle antibodies 0, anti-liver kidney microsomal antibodies-1 0, IgG 13.9 g/L, IgA 2.72 g/L, IgM 0.76 g/L, C4 0.115 g/L, C3c 1.32 g/L, CRP 0.44 nm/L, IgE 0.204 mg/L, CH50 12.78 IU/ml, alternative pathway 2.29 g/L, C3 1.41 g/L, C4 0.3 g/L, Cl kv 0.33 g/L, Cl C46.2%, and ClL 0.081 g/L.

Microbiological culturing of the patient’s urine revealed the presence of Klebsiella and Enterobacter spp. Culturing of the throat and nose swabs revealed only normal microbial flora. Skin prick tests with standard respiratory and nutritive allergens were negative. Neurological examination showed only cheilitis, and there were no other abnormalities. After consulting with an ear-nose-larynx specialist, the lower lip was biopsied. Histologic examination of the biopsied specimen revealed fragments of mucosa with signs of chronic inflammation and a moderate number of rare, poorly formed epithelioid granulomas without caseous necrosis.

Based on the abovementioned findings, the existence of chronic granulomatous inflammation of the lower lip was confirmed, and the diagnosis of the oligosymptomatic form of Melkersson-Rosenthal syndrome was made.

The patient was treated with oral prednisolone 25 mg daily, H1 and H2 blockers, and the antibiotic ofloxacin for a urinary tract infection. The patient was advised to go to a dentist and a gastroenterologist for further treatment.

**DISCUSSION**

Melkerson-Rosenthal syndrome is characterized by a triad of symptoms: swollen lips, facial nerve paresis, and an enlarged and grooved tongue. The majority of the reports in literature describe monosymptomatic forms of this syndrome (3,5). Our patient is another example of monosymptomatic syndrome characterized by granulomatous cheilitis. The data from the patient’s history and clinical and histological analysis indicated the existence of chronic granulomatous inflammation of the lower lip, without the other two characteristic elements of Melkersson-Rosenthal syndrome. After unspecific anti-inflammatory therapy (prednisolone 25 mg daily, H1 and H2 blockers), the inflammation of the lips subsided, with satisfactory aesthetic results.

Because Melkerson-Rosenthal syndrome can be associated with Crohn’s disease, physicians should be aware of this association (6) and refer their patients with the syndrome for further diagnostic evaluation. Moreover, sarcoidosis may have similar clinical features as Melkerson-Rosenthal syndrome (6); thus, further evaluation by a pulmonologist is also warranted.

**REFERENCES**

Palmoplantar Pustulosis and Eruptive Psoriasis: A Case Report

Tanja Jevtić Milić
Health Centre Kosovska Mitrovica

Abstract

This paper presents the case of a 50-year-old female patient with palmoplantar pustular changes within the course of eruptive (guttate) psoriasis. The diagnosis was based on the patient's history and clinical and histology findings. The patient was treated with an overnight local administration of betamethasone dipropionate and 3% salicylic acid applied twice daily to the palms and soles with occlusive dressings. The therapy was continued with 25 mg of acitretin daily (in the morning, after breakfast), betamethasone ointment applied twice daily to the palms and soles, and mometasone furoate cream applied in the morning and evening. This treatment led to a significant improvement in the patient's condition.

Keywords: psoriasis; palmoplantar pustulosis, betamethasone, salicylic acid.

Introduction

Palmoplantar pustulosis (pustular bacterid, bacterid Andrews) is a chronic inflammatory disorder that affects the palms and soles. The peak occurrence of this disorder is during the third, fourth and fifth decades of life. It remains unclear whether this disorder is a form of psoriasis or a separate disease entity, and only ~20% of patients with psoriasis also suffer from palmoplantar pustulosis (1). The aetiology of palmoplantar pustulosis is unclear, although associations with focal infections elsewhere in the body have been reported. The sudden eruption of pustules on the palms and soles is followed by their coalescence, drying out and desquamation. The patients feel itching and burning on the affected skin, and in some patients, the nails become thickened and brittle (2). The disorder is typically treated with corticosteroids and phototherapy without great success.

Guttate psoriasis begins as tiny scaly papules, typically oval or circular in shape, that spread centrally. This form of psoriasis is more frequently associated with palmoplantar pustulosis and is designated as psoriasis pustulosa palmoplantaris. It manifests as the appearance of scaly plaques sprinkled with sterile pustules that are localised on the palms and soles. This form is most common in children and typically occurs after streptococcal infections, upper respiratory infections or influenza (3,4). This paper presents the case of a 50-year-old female patient with palmoplantar pustular changes within the course of eruptive (guttate) psoriasis.

The Case

A 50-year-old female patient was admitted to the ward due to redness of the palms and soles and guttate scaly plaques covering the body surface. She had a 5-year history of festering blisters and redness on the soles of her feet...
Figure 1. The pustules and the tarnished erythaema of the patient’s soles.

Figure 2. The pustules and residual lesions on the palms.

Figure 3. Scaly plaques on the dorsal side of the hands.

Figure 4. Scaly plaques on the thighs.

Figure 5. Exfoliative desquamation of the soles.

Figure 6. Diffuse erythaema and desquamation of the palms.
(Figure 1). The plaques appeared during the summer and disappeared during the winter, during which the patient was not treated. One month before her admission, the normal changes occurred on her palms (Figure 2), and less intensely coloured plaques appeared on her trunk and extremities (Figures 3 and 4). She had smoked for 28 years and denied any other symptoms, allergy, and other diseases. The skin of her palms and soles was covered with numerous pustules and dark remnants of erythaema followed by desquamation. There were scaly individual plaques, up to 1 cm in diameter, on the skin of her trunk and extremities.

The laboratory findings of the patient were as follows: erythrocyte sedimentation rate, 14 mm during the first hour; total cholesterol, 6.48 mmol/L; LDL cholesterol, 4.51 mmol/L; and normal blood glucose, urea, creatinine, total bilirubin, triglycerides, HDL cholesterol, AST, ALT, LDH, TSH, triiodothyronine, and thyroxine. The patch tests for standard allergens were negative. A skin biopsy was conclusive for psoriasis.

The patient was treated with an overnight local administration of betamethasone dipropionate and 3% salicylic acid applied twice daily to her palms and soles with occlusive dressings.

The therapy was continued with 25 mg of acitretin daily (in the morning, after breakfast), betamethasone ointment applied twice daily to the palms and soles, and mometasone furoate cream applied in the morning and evening. This treatment led to a significant improvement in the patient's condition and desquamation of the soles and palms (Figures 5 and 6).

**DISCUSSION**

Palmoplantar pustular psoriasis and pustular bacterid Andrews are separately defined in the literature according to their diagnostic, etiologic and therapeutic characteristics, although they appear to have many similarities. One important difference between these two conditions is their occurrence at different ages. Our patient experienced both guttate psoriasis and palmoplantar pustulosis concomitantly. In the medical literature, the association of these two rare diseases has been described in only 1-2% of patients with guttate psoriasis (3,4). In 2007, the International Psoriasis Council designated palmoplantar pustulosis as a condition separate from psoriasis. However, the existing literature on the aetiology, epidemiology and clinical and histological characteristics does not provide a clear distinction between these two conditions, which are most likely two presentations of the same underlying pathological mechanism (5).

Palmoplantar pustulosis is resistant to therapy. Effective treatment requires the combination of drugs and therapeutic procedures, which we performed for our patient with satisfactory results. Similar experiences have been reported by the other authors who followed small cohorts of such patients (6). Our case report provides a small contribution to better the understanding of this enigmatic condition and outline a more efficient therapy. This disease may adversely influence quality of life, which is the universal measure used to compare diseases and the effectiveness of therapies (7).

**REFERENCES**

ABSTRACT

We present a case of a young woman, age 34, who presented with recurrent Lyme neuroborreliosis (LNB). Her clinical profile consisted of a rare combination of two third-stage manifestations, namely, progressive encephalomyelitis and peripheral neuritis, in both bouts of the disease. The epidemiological data were controversial, as she reported a tick bite only two months prior to the onset of symptoms. Negative magnetic resonance imaging (MRI) results excluded multiple sclerosis, vascular causes and tumours. Serological tests confirmed the Bb infection in the recommended two-step serological approach consisting of an enzyme-linked immunosorbent assay (ELISA) test for Borrelia burgdorferi (Bb) immunoglobulin G (IgG) and immunoglobulin M (IgM) and a Western blot (WB) as confirmatory analysis in the blood. Another controversial issue is the lack of pleocytosis. Atypical findings in our patient can be explained by a possible rare genotype of Bb. After treatment with oral doxycycline, she made an apparent remission, but after three months, she had another episode with signs of central and peripheral nervous system involvement, increased Bb antibodies and white matter changes on the MRI. This time, she was treated with intravenous ceftriaxone, 2 grams daily for four weeks. She showed no signs of LNB, both clinically and serologically, during a follow up lasting about a year. This case emphasises the importance of the clinical and serological findings and the use of ceftriaxone as the first line of treatment in LNB.

Key words: Lyme neuroborreliosis, progressive encephalomyelitis, neuritis, ceftriaxone

SAŽETAK

Mi predstavljamo slučaj mlade žene starosti 34 godine sa rekurentnom Lajmskom neuroboreliozom (LNB). Njena klinička slika se sastojala od retke kombinacije dve manifestacije treće faze, naime, progresivnog encefalomijelitisa i perifernog neuritisa u obe epizode bolesti. Epidemiološki podaci su kontroverzni jer se ubod krpelja desio samo dva meseca ranije. Magnetna rezonanca (MR) mozga je isključila multiplu sklerozu, vaskularne uzroke i tumor. Serološki testovi su potvrdili Bb infekcije u preporučenoj serologiji u dva koraka, sa enzyme-linked immunosorbent assay (ELISA) testom za Borrelia burgdorferi (Bb) specifične immunoglobuline G (IgG) i immunoglobulin M (IgM) i Western Blot (WB) testom za konfirmaciju. Drugo kontroverzno pitanje je nedostatak pleocitoze u likvoru. Atipični nalazi u naše bolesnice mogu se objasniti retkim genotipom Bb. Nakon tretmana sa oralnim doksiciklinom, bolesnica je postigla remisiju, ali je posle tri meseca imala još jednu epizodu sa znacima zahvaćenosti centralnog i perifernog nervnog sistema, povećanim titrom specifičnih Bb antitela kao i promenama bele mase na MR mozga. Ovaj put, ona je trećirana ceftiraksonom intravenski, dva grama dnevno tokom četiri nedelje. Bolesnica je ponovo ušla u remisiju. LNB koja se održavala i klinički i serološki tokom praćenja u trajanju od oko godinu dana. Ovaj slučaj naglašava značaj kliničkih i seroloških nalaza u dijagnosticu kao i opravdanost ceftiraksona, kao prve linije lečenja u LNB.

Ključne reči: Lajmska neuroborelioza, progresivni encefalomijelitis, neuritis, ceftirakson

DOI: 10.5937/SJECR13/3134
Correspondence: Merdin S. Markisic, General Hospital Berane, Montenegro
e-mail: docomm@t-com.me
INTRODUCTION

Lyme neuroborreliosis (LNB) is a disease of the central nervous system (CNS) and/or peripheral nervous system (PNS) caused by the spirochete Borrelia burgdorferi (Bb) as part of a general infection referred to as Lyme borreliosis (LB) or Lyme disease (1, 2). In Europe, LNB is caused by Bb sensu stricto—mainly Borrelia garinii and Borrelia afzelii—and rarely Bb sensu stricto, whereas in North America, the only bacterium is Bb sensu stricto (3, 4).

LB clinically manifests in the following three stages: first or early localised disease, second or early disseminated disease and the third or late disseminated disease (1, 5). Lyme neuroborreliosis upcomprises 10-15% of all cases of LB.

The first stage

In the first stage of LB, the typical skin manifestation is Erythema migrans (EM), a red circle at least 5 cm in diameter. As this circle enlarges, its centre becomes pale. Neurological disorders are less common in the early stages and may manifest by headache, muscle pain and fever, with or without EM (6). The titre of antibodies in the blood at this stage is negative, and the diagnosis is based solely on the identification of the clinical and epidemiological data regarding the tick bite.

The second stage

In the second stage of LB, there are neurological, rheumatological, cardiac and skin manifestations (4). The most typical manifestations of the second stage of LNB are as follows: painful meningoradiculitis or Garin-Bujadoux-Bannwarth syndrome (GBBS), meningitis, encephalitis, cranial mono- and polyneuritis, and myelitis. Less common symptoms include myalgia, myositis, pseu-dotumour of the brain, vasculitis, ataxia and other clinical manifestations (7).

Painful meningoradiculitis occurs within the first four months after the tick bite (4). The position of pain in the body changes changes and arm and leg weakness can occur, as well as neck stiffness. Cerebrospinal fluid (CSF) pleocytosis is characteristic with hyperproteinorachia and sometimes a positive oligoclonal response.

The third stage

The third stage of LB consists of neurological, rheumatological and dermatological manifestations. The third stage may occur without any apparent manifestations of the first two stages, and in many cases, patients do not recall a tick bite. In the third stage, the most prominent clinical entities are progressive encephalomyelitis, encephalopathy, neuropathy (with or without acrodematitis chronica atrophicans), dementia, myositis, granulomatous CNS tumour and vasculitis (4).

Progressive encephalomyelitis is characterised by spastic paresis, ataxia, cranial nerve damage (ofnamely, cranial nerves VII and VIII), micturition disturbances and other signs (8). The CSF signs are pleocytosis, hyperproteinorachia, oligoclonal response and intrathecal synthesis of the IgG and IgM immunoglobulins es(9). In the case of CNS involvement, it is beneficial to perform magnetic resonance imaging (MRI) of the brain and possibly the spinal cord (10). It is possible for cranial neuropathy and meningitis to appear on the MRI due to the enhancement after contrast application.

Encephalopathy of LNB is expressed byas fatigue, memory disturbances, headache, confused state, depression, daytime sleepiness, irritability and dysnomia (4). Additionally, there are various neuropsychological deficits (11). An MRI can reveal small white matter lesions of the brain. Specific antibodies to Bb in the CNS are found in 50% of these cases.

In neuropathy, muscle stretch reflexes are reduced or absent and there is weakness and numbness of the limbs, pain and muscle cramps.

Diagnosis of Lyme neuroborreliosis

Microbiological methods of diagnosis, which can prove LB, can be direct and indirect (12, 13). Direct diagnostic methods include culturing pathogens in appropriate substrates and detection of genetic material (14). Indirect methods can confirm infection by the detection of specific antibodies (15, 16).

Borrelia cultivation is the best and the only accurate evidence of an infection with Bb, especially when Bb is in atypical clinical forms and for patients who do not develop a proper specific immune response. However, the cultivation of Bb sensu lato is a very complex and time-consuming method (9 weeks and even longer) and can be performed only in well-equipped laboratories (12, 17).

It is necessary in the case of suspected LB, based on symptoms, to start a serology examination with an enzyme-linked immunosorbent assay (ELISA) test for Bb immunoglobulin G (IgG) and immunoglobulin M (IgM) (4). Determination of rheumatoid factor (RF) and Venereal Disease Research Laboratory (VDRL) in the blood is mandatory to exclude the most common false-positive results: rheumatoid arthritis and syphilis. It is also necessary that the ELISA be positive in at least two different samples. If the ELISA is positive, a confirmation test is required (because of the possibility of false positive results) with Western blot (WB) analysis, which demonstrates the specificity of previous findings (9). False-positive and/or misinterpreted laboratory findings are often the cause of patients wandering from one doctor to another doctor and not having adequate support. It is suggested that all serological tests are performed using both the serum and the CSF (9).

The new European criteria for LNB includes two levels of LNB diagnosis: definite and possible LNB (18). A definite LNB must include at least two of the following three criteria: 1. neurological symptoms; 2. CSF pleocytosis; and/or 3. intrathecal synthesis of antibodies to Bb. Antibodies may be negative in the first 6 weeks. It is always necessary to exclude other causes of current symptoms,
as diagnostic errors are common. IgM has no diagnostic value in late LNB, and if it is an isolated finding, it is usually indicative of a false positive and is thus likely caused by some other disease (19). IgM class antibodies appear by the third week and IgG antibodies from the sixth week after the infected tick bite.

IgG antibodies to Bb usually remain positive throughout the patient's life, even after successful treatment of Lyme disease (20). IgG and IgM antibodies can persist in the CSF long after receiving adequate therapy and showing no further evidence of active neurologic disease.

**Treatment of Lyme disease and Lyme neuroborreliosis**

Antibiotic therapy is causal treatment for LB and LNB (21). It is necessary to perform the correct diagnostic procedure before treatment to prevent the application of a long-term therapy that is inadequate for a disease other than LNB. Such a misstep can lead to unnecessary complications.

In the earlier stages of LB, the efficacy of oral doxycycline 100 milligrams every 12 hours daily for 14 days was shown to be effective. The drug of choice for the third stage of the LNB is ceftriaxone, which is given intravenously 2 grams a day for three to four weeks (21). Improvement in acute cases occurs in a few days and in chronic cases after several months or even longer (8 months or more). An improvement or cure is found in 90% of cases. Alternatively, intravenous cefotaxime or penicillin or amoxicillin orally is given. If there is possible LNB, one course of antibiotic therapy is sufficient, and if there is no improvement, one should look for other causes. If the criteria have been met for definite LNB and the therapy was ineffective after more than six months, the condition is called post Lyme syndrome (22). In this syndrome, antibiotic therapy is not effective. In children younger than 9 years of age, doxycycline is contraindicated.

**A CASE REPORT**

Our patient was a 34-year-old woman who was admitted to the neurology department of the General Hospital Berane on two separate occasions, in April and July of 2008, suffering from fatigue, headaches, weakness in the arms and legs, difficulty walking, ataxia, numbness of the face and neck stiffness. Anamnestic data stated a tick bite two months before her first admission.

During her first hospitalisation in April, the patient complained of malaise, headaches, weakness in the arms and legs (difficulty in walking, ataxia), numbness of the face and neck stiffness. Neurological examination on admission showed she had hypesthesia of the left half of the face. Her neck was slightly stiff, but she tested negative for meningeal signs. She had weakness in both arms, as well as in both legs. Her muscle stretch reflexes were increased in all four extremities, and her extended reflex zone was found with patellar and feet clonus on both sides. Her plantar reflex was absent.

On the basis of the clinical presentation disseminated, the CNS disease was supposed and the necessary diagnostic procedures were performed. Magnetic resonance imaging of the brain and cervical spine were performed in April, and the findings were normal. The ELISA results for IgG and IgM antibodies to Bb in serum were positive and confirmed by WB. The serodiagnosis was confirmed positive on several occasions. Control serologic tests in May were positive, whereas in June, the findings were normal. The cytological findings in the CSF were within normal limits. Isoelectric focusing of CSF showed normal results. Electromyoneurography (EMNG) of the upper and lower extremities indicated predominantly axonal neuropathy, which is aetiologically associated with the underlying disease, i.e., LNB. Routine laboratory tests showed normal results.

During her first hospitalisation in April, after receiving positive serological findings for Bb, the patient received doxycycline tablets at a dose of 100 mg per 12 hours for one month, after which there was an improvement and a reduction of symptoms in May.

A recurrence of symptoms occurred in July of the same year when she was readmitted to the hospital. A neurological examination revealed the following findings: her cranial nerve findings were normal, her meningeal signs were negative, she had a slightly stiff neck, both her arms and legs were weak predominantly on the left side; her muscle stretch reflexes were increased bilaterally and had enhanced reflexogenic zone and she had patellar and feet clonus present on both sides with a normal plantar reflex.

All routine laboratory tests were within normal range. Her brain MRI results revealed the presence of changes in the deep paraventricular white matter which corresponded to changes observed in LNB. An Her ELISA test showed a rise in Bb antibody titre of IgG and IgM in serum. Additionally, a confirmatory WB test in July showed the she had increased titres of specific antibodies. New CSF cytological and biochemical findings and isoelectric focusing of CSF again showed normal findings. The EMNG finding was unchanged compared to the previous test.

Given that LNB reoccurred despite previous treatment with doxycycline, therapy with ceftriaxone at a dose of one gram per 12 hours as an intravenous infusion during four weeks was administered in July. An improvement in symptoms occurred after a few days, and the neurological status upon discharge from the second hospitalisation showed only increased muscle stretch reflex on the left leg.

In August, after one month, follow up controls were performed, and then in September, October and November of the same year with normal neurological findings. In August and November of 2008 and March of 2009, the serological results were negative. Based on clinical and serological criteria, there was a complete remission of the disease.
DISCUSSION

We presented a case of a female patient with LNB, which fulfilled the criteria for the third stage of LNB, with progressive encephalomyelitis and neuropathy (4, 21). The short time interval between the occurrence of the tick bite and the presentation of the symptoms and the dynamics of serology are arguments against a causal relationship, so these occurrence of these issues was most likely a coincidence. Therefore, the infection was most likely acquired during an earlier infected tick bite that had not been registered.

The visibility of ticks on the skin depends on the tick’s size and the duration of its stay on the host’s skin (4). Young forms, such as larvae, often go unnoticed because they are less than 1 mm in diameter and can release themselves or be accidentally removed before they are noticed. However, the larvae are also less contaminated. Nymphs, the intermediate developmental stage of ticks, are the most typical carriers of infection. Nymphs have a higher percentage of infection with Bb, and even though they are larger than larvae, are small enough to be less detectable and can stay on the skin of the host long enough (more than 24 hours) to transfer the infection. Adult ticks are larger (a few millimetres in diameter) after the blood meal and can be observed easily and removed quickly; therefore, they usually do not have enough time to transfer Bb to the host. After infection with Bb, EM may be lacking in half of patients with LB (23), as was the case for our patient.

Pertaining to LNB, in almost half of the cases, the data on the tick bite and/or EM are missing (9). This gap in the available data makes the diagnosis of LNB difficult, but even the information about the tick bite, as was the case in our patient, can be misleading. It is considered that for the development of the third stage of LNB to occur, at least 6 months have to pass, which in our case was not fulfilled, thus indicating an earlier infection. Also, the tick bite, which occurred two months before the onset of symptoms, was not accompanied by the appearance of EM or other manifestations of the first stage of LB. There were no data on the manifestation of the second stage in our patient. Many patients with third stage LNB not only lack epidemiological data about the tick bite, but also lack information about the first/second stage clinical manifestations (4). In such cases, there is a particular need for a good deferential diagnosis that encompasses multiple sclerosis, small vessels disease of the brain, neuropathy, and various other neurological, rheumatological, and even psychiatric diseases (20).

An interesting point of our case is the simultaneous existence of two manifestations of late LNB—namely, progressive encephalomyelitis and neuropathy—and no signs of skin manifestations of acrodermatitis chronica atrophicans. An immediate response of neuropathy to ceftriaxone is also intriguing, as usually one has to wait several months for a response to occur (21). The emergence of resistance to therapeutic antibiotics or recurrence of clinically expressed infection may be explained by multiple mechanisms. Doxycycline is usually ineffective during the later stages of the disease, but dissemination leads to improvement, or there is a “decapitation” of infection, but it is not eradicated and consequently reappears. Less frequently, there might be a new infection, but then the time period between the two episodes of the disease necessarily has to be longer.

The value of ELISA IgG and IgM tests in the first steps of serological diagnosis has been confirmed. Positive findings are followed up by WB analysis, which serves as a confirmatory test because of its higher specificity (22). Typically, there is an increase in specific IgM antibodies in the beginning of the infection, and then the subsequent appearance of IgG antibodies, followed by the disappearance of IgM and the persistence of IgG antibodies as a type of “immunological scar” (4). The persistence of IgM antibodies is rare but possible. Increased persistence of only IgM positivity is a false positive finding (19). The diagnosis of LB is primarily based on clinical findings, and serological results should be used only to confirm the diagnosis (24).

One of the controversies surrounding LNB involves CSF inflammatory syndrome. Inflammatory syndrome in CSF is commonly present in patients with meningitis and/or encephalitis in LNB. Although some authors consider inflammatory syndrome in CSF mandatory for the diagnosis of LNB, the experience of other authors shows that it is not always present (25). A positive inflammatory response of pleocytosis and hyperproteinorachia supports the diagnosis of neuroinfection along with other data—in particular, the presence of specific Bb antibodies, data on the tick bite and the corresponding clinical manifestations. The disappearance of inflammatory syndrome follows therapy. European criteria include pleocytosis while the U.S. criteria does not include pleocytosis (25). While inpleocytosis is usually present in European LNB, the absence of pleocytosis can be explained by early stage disease, immunosuppression, infection with Bb sensu stricto, or the presence of atypical genotypes. In addition, some of our previous patients who fulfilled all of the other criteria for LNB and underwent successful antibiotic therapy did not have pleocytosis, so we propose the presentation of different microbiological characteristics of Bb genotypes in our region (26, 27).

Polyneuropathy in LNB is rarely not accompanied with acrodermatitis chronica atrophicans (ACA) and usually has normal CSF findings. Polyneuropathy without ACA has been reported previously, but it occurs much more frequently in North America than in Europe. (28). This feature was also a distinctive symptom of our patient and is another argument in favour of the involvement of a less frequent Bb genotype.

In some patients with involvement of the brain, non-specific white matter changes on MRI are found (10). In the diagnosis of LNB, MRI plays more of a role in excluding other causes than providing direct proof of the disease (10, 29), as was the case for our patient.
Ceftriaxone is the drug of choice for later stages of LB, especially the third stage of LNB (18). Although the published literature recommends courses of two to four weeks of intravenous ceftriaxone, in our experience, it is more efficient and safer to give 2 grams per day for longer courses of three to four weeks, so that the infection may be eradicated. However, there are different opinions on this possibility, as there may be asymptomatic chronic persistence of Bb even after therapy (19, 21).

Long courses of oral antibiotics, either alone or after parenteral therapy, are controversial, and there is not enough evidence to support its increased efficacy (18). Giving ceftriaxone, an antibiotic proven to be effective for the treatment of Bb, parenterally at a dose of 2 grams a day for three to four weeks, fulfils the old clinical maxim: give the appropriate drug, in the appropriate dose, long enough. After such treatment, infection recurrences are significantly lower, as was the case for our patient. Symptoms of successfully treated LNB can persist for up to 8 months and, in the case of neuropathy, even for as long as two years (4). It is interesting that in our patient, a longer course of ceftriaxone led to the prompt withdrawal of both forms of late LNB, which co-existed. LNB can be successfully treated with adequate antibiotics (22).

It should be emphasised that there is a logistical problem of diagnosing LNB in smaller towns in which the availability of additional diagnostic methods is significantly lower than in the large centres. Clinical diagnosis of neurological diseases is critical and requires vigilance on several levels: a high level of suspicion for LNB and a detailed disease history, as well as a detailed and accurate neurological examination. Medical histories should particularly address the epidemiological information about the tick bite, the presence of EM or other more specific manifestations of LB or LNB in earlier stages, as in the case of patients such as ours, suffering from the third stage of LNB. The absence of data on the tick bite does not exclude diagnosis of LNB, so in such cases, one should insist on learning if there were possible trips to the countryside with greenery and travel to endemic areas where tick habitats exist (30). Serodiagnostics of LB takes two steps (9). The first step is an ELISA test for Bb specific IgG and IgM antibodies in serum (preferably also in the CSL), and in the case of positive findings, a WB to detect IgG and IgM in serum (preferably in the CSL) can be performed to confirm the diagnosis. In the early stages, serological findings are negative and antibodies appear after about a month or two of repeated testing (4). Neurologists in smaller towns should establish cooperation in advance with one of the reference laboratories dealing with such diseases. Proving pleocytosis in CSF is controversial, as already discussed, and the presence of an increased number of lymphocytes supports LNB along with other positive findings, but the absence of pleocytosis does not make a case against LNB. Further diagnostic processing in the case of CNS involvement should consist of brain and/or spinal cord MRI and, in the case of PNS, the involvement of EMNG performed on all four limbs. Other specific diagnostic procedures are not standard, even in the major centres of LNB, because of their lack of sensitivity and specificity (polymerase chain reaction - PCR, Bb isolation, etc.). PCR for clinical samples has demonstrated a low sensitivity for the diagnosis of LB using blood and CSF (29).

In our patient, this order was respected and LNB was proven, as well as its recurrence. LNB therapy is often a complex process and requires an assessment of the whole set of circumstances (possible neurological and/or other comorbidities, allergies to antibiotics, immune status, etc.) (23).

Given the relative resistance of pathogen, we suggest as a reliable solution, therapy with ceftriaxone, two grams per day of intravenous infusion, preferably divided in two daily doses, for three to four weeks, less in second, more in the third stage. This therapy proved to be successful in our patient (Pavlović, 2012; Bhate, Schwartz, 2011) (21, 22). We emphasise a rare coincidence of two events of the third stage of LNB, namely, progressive encephalomyelitis and peripheral neuritis in our patient, which complicates treatment.

CONCLUSION

Lyme disease manifests itself through a whole range of different clinical manifestations, especially in the skin, nervous system, heart and joints, and is divided into three stages. Our patient was a young woman in her thirties who presented with the third stage of LNB. She presented with a clinical picture of progressive encephalomyelitis and peripheral neuritis, which is a rarity. Serological tests are important in the diagnosis of LB but only along the clinical findings, and these tests are not, by themselves, the determining factor in establishing the aetiological diagnosis. An effort should be made in the case of suspected LNB cases to simultaneously examine CSL and serum for specific Bb antibodies. A positive ELISA test in the first step requires confirmation with a WB test. Additional testing includes MRI and/or EMNG. LNB treatment with intravenous ceftriaxone or oral doxycycline depends on the stage of the disease. Due to the potential for less sensitivity of Bb, it is advisable to use ceftriaxone as antibiotic of first choice in all cases of proven LNB, as was confirmed in our patient.

REFERENCES

INSTRUCTION TO AUTHORS FOR MANUSCRIPT PREPARATION

Serbian Journal of Experimental and Clinical Research is a peer-reviewed, general biomedical journal. It publishes original basic and clinical research, clinical practice articles, critical reviews, case reports, evaluations of scientific methods, works dealing with ethical and social aspects of biomedicine as well as letters to the editor, reports of association activities, book reviews, news in biomedicine, and any other article and information concerned with practice and research in biomedicine, written in the English.

Original manuscripts will be accepted with the understanding that they are solely contributed to the Journal. The papers will be not accepted if they contain the material that has already been published or has been submitted or accepted for publication elsewhere, except of preliminary reports, such as an abstract, poster or press report presented at a professional or scientific meetings and not exceeding 400 words. Any previous publication in such form must be disclosed in a footnote. In rare exceptions a secondary publication will acceptable, but authors are required to contact Editor-in-chief before submission of such manuscript. the Journal is devoted to the Guidelines on Good Publication Practice as established by Committee on Publication Ethics-COPE (posted at www.publicationethics.org.uk).

Manuscripts are prepared in accordance with „Uniform Requirements for Manuscripts submitted to Biomedical Journals“ developed by the International Committee of Medical Journal Editors. Consult a current version of the instructions, which has been published in several journals (for example: Ann Intern Med.1997;126:36-47) and posted at www.icmje.org, and a recent issue of the Journal in preparing your manuscript. For articles of randomized controlled trials authors should refer to the „Consort statement“ (www.consort-statement.org). Manuscripts must be accompanied by a cover letter, signed by all authors, with a statement that the manuscript has been read and approved by them, and not published, submitted or accepted elsewhere. Manuscripts, which are accepted for publication in the Journal, become the property of the Journal, and may not be published anywhere else without written permission from the publisher.

Serbian Journal of Experimental and Clinical Research is owned and published by Medical Faculty University of Kragujevac. However, Editors have full academic freedom and authority for determining the content of the journal, according to their scientific, professional and ethical judgment. Editorial policy and decision making follow procedures which are endeavoring to ensure scientific credibility of published content, confidentiality and integrity of authors, reviewers, and review process, protection of patients’ rights to privacy and disclosing of conflict of interests. For difficulties which might appear in the Journal content such as errors in published articles or scientific concerns about research findings, appropriate handling is provided. The requirements for the content, which appears on the Journal internet site or Supplements, are, in general, the same as for the master version. Advertising which appears in the Journal or its internet site is not allowed to influence editorial decisions.

Manuscripts can be submitted by using the following link: http://scindeks-eur.ceon.rs/index.php/sjecr

MANUSCRIPT

Original and two anonymous copies of a manuscript, typed double-spaced throughout (including references, tables, figure legends and footnotes) on A4 (21 cm x 29,7 cm) paper with wide margins, should be submitted for consideration for publication in Serbian Journal of Experimental and Clinical Research. Use Times New Roman font, 12 pt. Manuscript should be sent also on an IBM compatible floppy disc (3.5”), written as Word file (version 2.0 or later), or via E-mail to the editor (see above for address) as file attachment. For papers that are accepted, Serbian Journal of Experimental and Clinical Research obligatory requires authors to provide an identical, electronic copy in appropriate textual and graphic format.

The manuscript of original, scinetific articles should be arranged as following: Title page, Abstract, Introduction, Patients and methods/Material and methods, Re-
sults, Discussion, Acknowledgements, References, Tables, Figure legends and Figures. The sections of other papers should be arranged according to the type of the article.

Each manuscript component (The Title page, etc.) should begins on a separate page. All pages should be numbered consecutively beginning with the title page.

All measurements, except blood pressure, should be reported in the System International (SI) units and, if necessary, in conventional units, too (in parentheses). Generic names should be used for drugs. Brand names may be inserted in parentheses.

Authors are advised to retain extra copies of the manuscript. Serbian Journal of Experimental and Clinical Research is not responsible for the loss of manuscripts in the mail.

TITLE PAGE

The Title page contains the title, full names of all the authors, names and full location of the department and institution where work was performed, abbreviations used, and the name of corresponding author.

The title of the article should be concise but informative, and include animal species if appropriate. A subtitle could be added if necessary.

A list of abbreviations used in the paper, if any, should be included. The abbreviations should be listed alphabetically, and followed by an explanation of what they stand for. In general, the use of abbreviations is discouraged unless they are essential for improving the readability of the text.

The name, telephone number, fax number, and exact postal address of the author to whom communications and reprints should be sent are typed at the end of the title page.

ABSTRACT

An abstract of less than 250 words should concisely state the objective, findings, and conclusions of the studies described in the manuscript. The abstract does not contain abbreviations, footnotes or references.

Below the abstract, 3 to 8 keywords or short phrases are provided for indexing purposes. The use of words from Medline thesaurus is recommended.

INTRODUCTION

The introduction is concise, and states the reason and specific purpose of the study.

PATIENTS AND METHODS/MATERIAL AND METHODS

The selection of patients or experimental animals, including controls, should be described. Patients’ names and hospital numbers are not used.

Methods should be described in sufficient detail to permit evaluation and duplication of the work by other investigators.

When reporting experiments on human subjects, it should be indicated whether the procedures followed were in accordance with ethical standards of the Committee on human experimentation (or Ethics Committee) of the institution in which they were done and in accordance with the Helsinki Declaration. Hazardous procedures or chemicals, if used, should be described in details, including the safety precautions observed. When appropriate, a statement should be included verifying that the care of laboratory animals followed accepted standards.

Statistical methods used should be outlined.

RESULTS

Results should be clear and concise, and include a minimum number of tables and figures necessary for proper presentation.

DISCUSSION

An exhaustive review of literature is not necessary. The major findings should be discussed in relation to other published work. Attempts should be made to explain differences between the results of the present study and those of the others. The hypothesis and speculative statements should be clearly identified. The Discussion section should not be a restatement of results, and new results should not be introduced in the discussion.

ACKNOWLEDGMENTS

This section gives possibility to list all persons who contributed to the work or prepared the manuscript, but did not meet the criteria for authorship. Financial and material support, if existed, could be also emphasized in this section.

REFERENCES

References should be identified in the text by Arabic numerals in parentheses. They should be numbered consecutively, as they appeared in the text. Personal communications and unpublished observations should not be cited in the reference list, but may be mentioned in the text in parentheses. Abbreviations of journals should conform to those in Index Serbian Journal of Experimental and Clinical Research. The style and punctuation should conform to the Serbian Journal of Experimental and Clinical Research style requirements. The following are examples:

Article: (all authors are listed if there are six or fewer; otherwise only the first three are listed followed by “et al.”)


The authors are responsible for the exactness of reference data.

For other types of references, style and interpunction, the authors should refer to a recent issue of Serbian Journal of Experimental and Clinical Research or contact the editorial staff.

Non-English citation should be preferably translated to English language adding at the end in the brackets native language source, e.g. (in Serbian). Citation in old language recognised in medicine (e.g. Latin, Greek) should be left in their own. For internet sources add at the end in small brackets URL address and date of access, e.g. (Accessed in Sep 2007 at www.medf.kg.ac.yu). If available, instead of URL cite DOI code e.g. (doi: 10.1111/j.1442-2042.2007.01834.x)

TABLES

Tables should be typed on separate sheets with table numbers (Arabic) and title above the table and explanatory notes, if any, below the table.

FIGURES AND FIGURE LEGENDS

All illustrations (photographs, graphs, diagrams) will be considered as figures, and numbered consecutively in Arabic numerals. The number of figures included should be the least required to convey the message of the paper, and no figure should duplicate the data presented in the tables or text. Figures should not have titles. Letters, numerals and symbols must be clear, in proportion to each other, and large enough to be readable when reduced for publication. Figures should be submitted as near to their printed size as possible. Figures are reproduced in one of the following width sizes: 8 cm, 12 cm or 17 cm, and with a maximal length of 20 cm. Legends for figures should be given on separate pages.

If magnification is significant (photomicrographs) it should be indicated by a calibration bar on the print, not by a magnification factor in the figure legend. The length of the bar should be indicated on the figure or in the figure legend.

Two complete sets of high quality unmounted glossy prints should be submitted in two separate envelopes, and shielded by an appropriate cardboard. The backs of single or grouped illustrations (plates) should bear the first authors last name, figure number, and an arrow indicating the top. This information should be penciled in lightly or placed on a typed self-adhesive label in order to prevent marking the front surface of the illustration.

Photographs of identifiable patients must be accompanied by written permission from the patient.

For figures published previously the original source should be acknowledged, and written permission from the copyright holder to reproduce it submitted.

Color prints are available by request at the authors expense.

LETTERS TO THE EDITOR

Both letters concerning and those not concerning the articles that have been published in Serbian Journal of Experimental and Clinical Research will be considered for publication. They may contain one table or figure and up to five references.

PROOFS

All manuscripts will be carefully revised by the publisher desk editor. Only in case of extensive corrections will the manuscript be returned to the authors for final approval. In order to speed up publication no proof will be sent to the authors, but will be read by the editor and the desk editor.
SERBIAN Journal of Experimental and Clinical Research

Je nastavak: Medicus (Kragujevac) = ISSN 1450 – 7994
ISSN 1820 – 8665 = Serbian Journal of Experimental and Clinical Research
COBISS.SR-ID 149695244