

# **9. Hrvatski kongres studenata Doktorskog studija „Molekularne bioznanosti“**

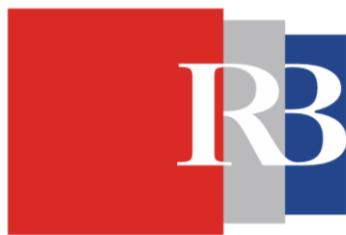
## **Knjiga sažetaka**



*Simpozij studenata poslijediplomskog  
interdisciplinarnog studija  
„Molekularne bioznanosti“*

*Sveučilište Josipa Jurja Strossmayera u  
Osijeku, Odjel za biologiju u suradnji s  
Institutom Ruđer Bošković  
i Sveučilištem u Dubrovniku*

*16.07.2022.*



Poštovane kolegice i kolege,

Veliko nam je zadovoljstvo da Vam poželimo dobrodošlicu na simpozij IX.generacije studenata doktorskog studija „Molekularne bioznanosti” koji se održava 16. srpnja 2022. godine s početkom u 08:30 sati u dvorani br.201, Odjel za biologiju, Ulica cara Hadrijana 8a, Osijek.

Program kongresa čine usmena izlaganja i poster sekcije koje pokrivaju tematiku molekularne biologije i medicine.

Želimo Vam srdačnu dobrodošlicu!

Studenti IX. generacije sveučilišnog poslijediplomskog interdisciplinarnog doktorskog studija „Molekularne bioznanosti”



## **Zahvale**

Studenti IX. Generacije sveučilišnog poslijediplomskog interdisciplinarnog doktorskog studija „Molekularne bioznanosti” zahvaljuju se djelatnicima Sveučilišta Josipa Jurja Strossmayera u Osijeku, Odjela za biologiju na pomoći pri organizaciji i osiguravanja prostora za održavanje kongresa.

Studenti IX. Generacije sveučilišnog poslijediplomskog interdisciplinarnog doktorskog studija „Molekularne bioznanosti” zahvaljuju se i sponzoru:

**Vindija**

## **Članovi organizacijskog odbora**

Marina Bakula, Dino Belić, Matea Bogović, Stjepan Bulat, Ivana Butorac Prpić, Iva Bušić, Dora Dragičević, Tihana Duić, Vanja Đuričić, Elizabeta Jakovac, Valentin Kordić, Sabina Lanča, Nikolina Lazić, Valentino Lisek, Barbara Marić, Ana Mitka, Antonija Mišković, Nataša Moser, Dinko Paulić, Alen Rončević, Karla Rožac, Ivan Vidić, Petar Vranjić, Lovro Vukadinović, Andrea Vukoja, Mladen Vuković.

## **Članovi znanstvenog odbora**

Darija Bogdanić, Mateja Čosić, Luka Fotak, Stjepan Ivandić, Vid Jakovljević, Maja Jirouš, Marin Kutnjak, Matko Leović, Petar Krešimir Lozo, Katarina Mandić, Iva Marković, Ana Mataić, Ana Miletić, Monika Mlinarić, Margareta Mršo, Jure Pavešić, Ana Petelinec, Ivan Pokrovac, Anja Privara, Karlo Prižmić, Mirna Rozić, Tea Škrobo, Silvija Šoprek, Katarina Šunić, Marija Tomić, Filip Uljanić, Damira Veseljak, Marin Volarić, Lana Vujica.

## Program kongresa

08:30-09:30 **Registracija sudionika**

09:30-09:35 **Otvorenje kongresa**

09:35-10:05 **Plenarno predavanje**

Doc.dr.sc. Damir Štimac, dr.med.

*Invazivna dijagnostika bolesti dojki*

10:05-11:15 **Sekcija I. Stanična i klinička onkologija**

Moderatori sekciјe: Jure Pavešić, Matko Leović

10:05-10:15 Marina Bakula: *Dijagnostika i liječenje raka pluća*

10:15-10:25 Luka Fotak: *Lung adenocarcinoma in a COVID-19 patient*

10:25-10:35 Matea Bogović: *Radiološka obrada karcinoma dojke*

10:35-10:45 Antonija Mišković: *Sinkroni planocelularni karcinom grkljana i intrahepatalni kolangiokarcinom*

10:45-10:55 Ivan Vidić: *The Prevalence of Prostate, Urinary Bladder and Kidney Cancer among the Homeland War Veterans*

10:55-11:05 Petar Krešimir Lozo: *Epidemiologija raka vrata maternice u Zadarskoj županiji*

11:05-11:15 RASPRAVA

11:15-11:45 **POSTER SEKCIJA I. Mikrobiologija**

11:45-12:35 **Sekcija II. Biomedicina**

Moderatori sekciјe: Karlo Prižmić, Ana Mataić

11:45-11:55 Darija Bogdanić: *Red blood cell alloantibody evanescence and pretransfusion testing*

11:55-12:05 Margareta Mršo: *Models of drug-induced liver injury (DILI) – current issues and future perspectives*

12:05-12:15 Barbara Marić: *HbA1c iz perspektive laboratorijske medicine*

12:15-12:25 Sabina Lanča: *Zaštitni učinak mediteranske prehrane*

12:25-12:35 RASPRAVA

12:35-13:20 **POSTER SEKCIJA II. Klinička medicina I.**

13:20-14:30 PAUZA - RUČAK

### 14:30-15:20 **Sekcija III. Molekularna biologija stanice**

Moderatori sekcije: Damira Veseljak, Katarina Mandić

14:30-14:40 Marija Tomić: *Role of talins and kanks in integrin αVβ5 focal adhesions and response to paclitaxel treatment in MDA-MB-435S cells*

14:40-14:50 Maja Jirouš: *Deregulated miR-20a/miR-92b circuit underlies changes in circulating γδT cell proportions in psoriasis vulgaris*

14:50-15:00 Marin Kutnjak: *Priming modifies heat stress response in BPM1 -overexpressing Arabidopsis thaliana (L.) Heynh.*

15:00-15:10 Filip Uljanić: *Lipid binding by the N-terminal motif mediates plasma membrane localization of Bordetella effector protein BteA*

15:10-15:20 Lovro Vukadinović: *Stimulacija razvoja biofilma sušom*

15:20-15:30 RASPRAVA

### 15:30-16:25 **POSTER SEKCIJA III. Molekularna biologija stanice**

### 16:25-17:25 **Sekcija IV. Klinička medicina**

Moderatori sekcije: Luka Fotak, Darija Bogdanić

16:25-16:35 Nataša Moser: *Tjelesna masa novooboljelih od šećerne bolesti tipa 2*

16:35-16:45 Karlo Prižmić: *Warfarin: Is it a real enemy or a fictional one?*

16:45-16:55 Nikolina Lazić: *Fizioterapijski pristup u liječenju spuštenih stopala (pes planus) u djece*

16:55-17:05 Vid Jakovljević: *Iskustvo primjene anti-VEGF terapije kod makularnog edema*

17:05-17:15 Alen Rončević: *SARS-CoV-2 Dissemination Through Peripheral Nerves Explains Multiple Organ Injury*

17:15-17:25 RASPRAVA

### 17:25-18:10 **POSTER SEKCIJA IV. Klinička medicina II.**

18:10-19:00 **ZAVRŠNA RIJEČ I ZATVARANJE KONGRESA, UPIS OCJENA**

# POSTER SEKCIJA I. Mikrobiologija

Ivan  
Pokrovac

*Antimikrobni učinak koloidnih  
nanočestica na MSSA i MRSA*

Dinko  
Paulić

*Risk factors and molecular predispositions for cervical  
dysplasia among women from east Croatia*

Dino  
Belić

*Dirty Croatian Money: How Big is the Threat?*

Tea  
Škrobo

*An outbreak of nosocomial bloodstream infection  
due to multidrug-resistant *Acinetobacter  
baumannii* in COVID-19 intensive care unit*

Silvija  
Šoprek

*The first detection of CMY-16-type of  
AmpC  $\beta$ -lactamases in multidrug-resistant  
*Proteus mirabilis* in Croatia: implications for therapy*

Elizabeta  
Jakovac

*Uspješnost detekcije uzročnika  
dijareje djece različitim metodama*

## POSTER SEKCIJA II. Klinička medicina I.

Ana Mitka	Primjena srednjih doza niskomolekularnih heparina za prevenciju tromboembolija u COVID-19 bolesnika
Iva Bušić	<i>Cystoid macular lesions are resistant to topical dorzolamide treatment in enhanced S-cone syndrome child</i>
Petar Vranjić	<i>Anemia in patients with inflammatory bowel diseases in Clinical Hospital Center Osijek</i>
Mladen Vuković	<i>Comparation of inflammatory bowel disease therapy with an emphasis on immune therapy; results of research study of inflammatory bowel disease in the Osijek-Baranja County 2000-2014.</i>
Karla Rožac	<i>Physical activity and evaluation of lower back pain in students of physiotherapy and nursing</i>
Tihana Duić	<i>Using US and MRI in prenatal diagnosis of rare multiple fetal malformations – OEIS Complex</i>
Valentin Kordić	<i>Hipovitaminoza D u depresivnih bolesnika</i>
Adreja Vukoja	<i>Radioterapijske tehnike u KBCO</i>
Vanja Đuričić	<i>Prognostički pokazatelji preživljenja pacijenata u terminalnom stadiju kroničnog bubrežnog zatajenja</i>

# POSTER SEKCIJA III. Molekularna biologija stanice

Mateja Ćosić	Kinetochore- and chromosome-driven transition of microtubules into bundles promotes spindle assembly
Ana Mataić	Peritumoral Clefting and Expression of MMP-2 and MMP-9 in Basal Cell Carcinoma of the Skin
Ana Petelinec	Changes of tunneling nanotubes and tumor microtubes in cellular stress response
Anja Privara	<i>Dictyostelium lggD</i> is a Rho-regulated IQGAP involved in large-scale endocytosis
Lana Vujica	Interaction of environmental contaminants with zebrafish ( <i>Danio rerio</i> ) multidrug and toxin extrusion protein 3 ( <i>Mate3/Slc47a3</i> )
Marin Volarić	Novel method for <i>Tribolium</i> sequencing
Katarina Mandić	Multi-omics analysis of HNSCC reveal non-overlapping epigenomic regulation in gene expression
Damira Veseljak	Satellitome characterization of the black flour beetle <i>Tribolium madens</i>
Katarina Šunić	Occurrence of deoxynivalenol and culmorin and their derivatives at two different locations in naturally and FHB inoculated plants and their possible role in disease severity
Monika Mlinarić	Effect of lipid raft disruption and EGFR signaling pathway activation on NRF2 and AQP3 in breast cancer cell lines of different malignancies
Iva Marković	Salicylic acid contributes to basal defense of <i>Solanum tuberosum</i> against potato spindle tuber viroid (PSTVd)

# POSTER SEKCIJA IV. Klinička medicina II.

Ana Miletić	Periferni živčani blok u kombinaciji sa spinalnom anestezijom za autotransplantaciju kože – prikaz slučaja
Stjepan Ivandić	Transplantacija osteohondralnog allografta lateralnog kondila femura, prikaz slučaja i radne stanice
Stjepan Bulat	Role of Chiari osteotomy in treating degenerative hip arthritis: A review
Valentino Lisek	Laparoscopic splenectomy in KB Dubrava - our experience
Jure Pavešić	Metastatske bolesti kralješnice - iskustvo Klinike za traumatologiju od 11/2019-11/2021
Mirna Rozić	Satisfaction with Perioperative Care in Patients Undergoing General or Regional Anaesthesia
Matko Leović	Hedgehog-GLI signaling controls proliferation and invasiveness of head and neck squamous cell carcinoma
Dora Dragičević	Awareness Regarding Oral Health among Orthodontic Patients and Non-Orthodontic Patients
Ivana Butorac-Prpić	Autologni dentinski graft u prezervaciji alveole

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4. Antonija Mišković: Sinkroni planocelularni karcinom grkljana i intrahepatalni kolangiokarcinom
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6. Petar Krešimir Lozo: Epidemiologija raka vrata maternice u Zadarskoj županiji
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21. Ivan Pokrovac: Antimikrobní učinak koloidnih nanočestica na MSSA i MRSA
22. Dinko Paulić: Risk factors and molecular predispositions for cervical dysplasia among women from east Croatia
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25. Silvija Šoprek: The first detection of CMY-16-type of AmpC  $\beta$ -lactamases in multidrug-resistant *Proteus mirabilis* in Croatia: implications for therapy
26. Elizabeta Jakovac: Uspješnost detekcije uzročnika dijareje djece različitim metodama
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55. Ivana Butorac-Prpić: Autologni dentinski graft u prezervaciji alveole

## **DIJAGNOSTIKA I LIJEČENJE RAKA PLUĆA**

**Bakula M<sup>1</sup>**

*<sup>1</sup>Klinički zavod za patologiju i sudske medicinu, KBC Osijek, Hrvatska*

Rak pluća ima tendenciju metastaziranja vrlo rano nakon formiranja. Osnovna podjela raka pluća je na rak malih stanica SCLC, i rak nemalih stanica NSCLC. Stope preživljjenja raka pluća su niže od preživljjenja raka po drugim primarnim sijelima. Razvoj molekularne medicine vodi k razvoju novih terapijskih postupaka koji su usmjereni prema točno određenim biomarkerima. Svaki tumor pluća ima drugačiju genetsku građu i biološko ponašanje, s toga se u liječenju raka pluća pristupa personalizirano. Inhibicija mogućih onkogenih pokretača raka pluća kao što su EGFR, ALK, PDL1, ROS, NTRK FUZIJE, daje sistemski dobar odgovor na terapiju. Tumori nisu monolitna velika skupina, svaki ima drugačiju genetsku građu kao i biološko ponašanje, ne mogu se liječiti na isti način. Problemi kod personaliziranog pristupa terapiji raka pluća koja se temelji na molekularnoj dijagnostici i genetici su mutacija tumora koja nije rijetka, kao i pojave rezistencije na terapiju. U Hrvatskoj su dosta loši rezultati liječenja raka pluća, vjerojatno zbog iznalaženja sredstava, dok onkologija postaje sve više ekspanzivna.

Ključne riječi: rak pluća, biomarkeri, mutacije, personalizirani pristup liječenju

**LUNG ADENOCARCINOMA IN A COVID-19 PATIENT**

Fotak L<sup>1</sup>

<sup>1</sup>*General hospital Varaždin, Department for Lung diseases and TB*

In this report I present a case of a 64-year-old female patient who was admitted to the emergency department (ED) complaining of dyspnea, dry cough and constant chest pain propagating into her back. Her medical history includes long-term smoking, implanted mechanical aortic valve, past admission to the hospital 2 weeks prior due to cardiac decompensation and right-sided pleural effusion. Upon current admission a progression of pleural effusion is noted on chest X-ray, the laboratory results show an elevated level of white blood cells, elevated CRP and elevated D-dimers due to which a CT-pulmonary angiography was performed - no signs of thromboembolism were found, of note were a progression of pleural effusion and a diffuse bilateral thickening of the lung interstitium - pneumonitis. The initial SARS-CoV-2 PCR result was negative. Due to ongoing COVID-19 hospital guidelines at the time, the patient was admitted to the observation ward and an IV antibiotic therapy was started, along with diuretics and other supportive therapy. A control PCR swab was obtained and the patient tested positive for SARS-CoV-2 and was thus admitted to the COVID-ward where no further diagnostics could be performed due to staff shortage. Upon completing the mandatory isolation period, the patient was admitted to the Department of Pulmonology where further diagnostics was to be performed. Pleurocentesis was performed, the cytological evaluation showed an unspecified malignant pleural effusion. A diagnostic bronchoscopy was performed with samples taken, the finding being an adenocarcinoma with insufficient material for further PD-L1, EGFR, ALK and ROS-1 status determination. By that time the patient's clinical status showed a progressive worsening with marked dyspnea and worsening of arterial blood gasses, a control D-dimer value showed a marked increase due to which a repeat CT-pulmonary angiography was performed, again with no signs of thromboembolism. Additional testing was performed, including bone scintigraphy which showed multiple pathological accumulation of the radiopharmac in the spine, ribs, sternum, pelvis and acetabulum, suggesting a widespread malignancy. The patient and the family requested a hospital discharge and no follow-up was performed.

Keywords: adenocarcinoma, COVID-19, D-dimers

## **RADIOLOŠKA OBRADA KARCINOMA DOJKE**

Bogović M<sup>1</sup>

<sup>1</sup> Odjel za kliničku radiologiju, Nacionalna memorijalna bolnica Vukovar

Rak dojke je heterogena bolest koju čini više bioloških podtipova s određenim kliničkim tijekom i ponašanjem. Također je karcinom dojke najčešći stalište sijelo karcinoma u žena kako u Republici Hrvatskoj tako i u svijetu. Kako bismo mogli i znali pravovremeno i pravilno upućivati žene na dijagnostičke pretrage i kliničke pregledne potrebno je imati dobre smjernice za dijagnosticiranje i praćenje kako suspektnih pacijentica tako i pacijentica oboljelih od karcinoma dojke. Nove smjernice za pravilno dijagnosticiranje i praćenje pacijentica oboljelih od karcinoma dojke u Republici Hrvatskoj objavljene su u veljači 2022.godine. Radiološka dijagnostika, uz laboratorijske pretrage, patohistološke analize i pregledne kliničara, je osnova dijagnostike i praćenja oboljelih od karcinoma dojke. Mamografija, mamografija s tomosintezom, ultrazvuk, elastografija, kontrastni ultrazvuk (ceus), color doppler, magnetska rezonancija i invazivna dijagnostika su radiološki modaliteti čije prednosti, nedostatke, ograničenja, specifičnosti i osjetljivosti moramo dobro poznavati da bismo ih mogli pravilno koristiti u svrhu brže, točnije i kvalitetnije dijagnoze.

Ključne riječi: karcinom dojke, dijagnostika, radiologija, smjernice

**SINKRONI PLANOCELULARNI KARCINOM GRKLJANA I  
INTRAHEPATALNI KOLANGIOKARCINOM – PRIKAZ SLUČAJA**

Mišković A<sup>1</sup>

<sup>1</sup>*Odjel za otorinolaringologiju, Opća bolnica „dr. Josip Benčević“ Slavonski Brod, Hrvatska*

Postaviti dijagnozu zločudne bolesti tijekom prvog pregleda bolesnika u hitnoj ambulanti nije jednostavno. Ukoliko se tijekom obrade proširenosti bolesti posumnja na inoperabilnost karcinoma, metastaziranje ili drugu zločudnu novotvorinu, zadatak postaje još teži. U ovom prikazu slučaja predstavljamo jedinstveni slučaj 64-godišnje bolesnice sa dva sinkrona karcinoma – planocelularnim karcinomom grkljana i intrahepatalnim kolangiom. Anamnestički se radi o prethodno zdravoj bolesnici koja se javila u hitnu ambulantu radi pogoršanja promuklosti i razvoja obostrane otekline na vratu što je primjetila unazad 3 mjeseca. Na temelju kliničkog pregleda postavljena je radna dijagnoza karcinoma epiglotisa s obostranim metastazama u vrat. Bolesnica se zaprimi na Odjel otorinolaringologije, učini se laringomikroskopija s biopsijom tumorske tvorbe i patohistološki se postavi dijagnoza planocelularnog karcinoma grkljana. U sklopu obrade proširenosti bolesti učini se CT vrata i prsnog koša koji ukaže na neočekivanu hipovaskularnu leziju jetre i brojne suspektne sekundarizme u limfne čvorove abdomena. Core biopsija jetre s patohistološkom analizom uzorka govori u prilog adenokarcinoma, no ne može se sa sigurnošću izjasniti o točnom tipu te se učini mamografija i gastoskopija kako bi se isključio karcinom dojke i karcinom želuca. S obzirom na intrahepatalnu lokalizaciju i karakteristike bioptata postavi se dijagnoza kolangiomu jetre. Bolesnica se upućuje onkologu koji indicira kemoiradioterapiju sa djelomično uspješnim odgovorom. U tercijarnoj ustanovi se učini perkutana mikrovalna ablacija lezije jetre. Tijekom jednogodišnjeg liječenja prati se regresija planocelularnog karcinoma grkljana i stacionaran nalaz kolangiomu jetre. Motivirana za nastavak liječenja, bolesnica je u tijeku drugog ciklusa kemoterapije po protokolu za kolangiomu jetre.

Ključne riječi: planocelularni karcinom grkljana, intrahepatalni kolangiom, prikaz slučaja

**THE PREVALENCE OF PROSTATE, URINARY BLADDER AND KIDNEY  
CANCER AMONG THE HOMELAND WAR VETERANS**

Sorić T<sup>1,2</sup>, Gusar I<sup>1</sup>, Zekanović A<sup>2</sup>, Vidić I<sup>2</sup>, Dželalija B<sup>1</sup>

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Relevant literature mentions exposure to war events as a significant factor in the emergence of not only mental but also physical illnesses. The Homeland War in the Republic of Croatia was fought between 1990 and 1995, and it is estimated that over 500.000 soldiers fought in the war. Croatian defenders can unquestionably be singled out as a particularly vulnerable group due to the long-term and serious consequences of war-related sufferings. The aim of this study was to investigate the prevalence of prostate, urinary bladder, and kidney cancer among the Homeland War veterans 50–70 years of age in relation to the general male population of the same age in the Republic of Croatia. The cross-sectional study was conducted in Zadar General Hospital, Croatia, in the period from March 2017 to June 2018 within the Preventive Physical Examination Program for Croatian Homeland War Veterans. Of the 2101 veterans that were processed, 1425 men were included in the research. They fulfilled the following criteria: participants in the Homeland War; participated in the war for more than 1500 days; aged 50–70; and had not previously received treatment for prostate, urinary bladder, and kidney cancer. Results showed that total proportion of urogenital cancer patients was 2.4% (35); 24 (1.6%) patients had prostate cancer, 7 (0.4%) patients had urinary bladder cancer and 4 (0.2%) kidney cancer. There was a statistically significant difference in the prevalence of prostate and urinary bladder cancer, but not for a kidney cancer. The exposure of Homeland War veterans to war events and long-term war-related stress is a strong risk factor for the development of prostate and bladder cancer.

Keywords: war veterans, prostate cancer, urinary bladder cancer, kidney cancer

## EPIDEMIOLOGIJA RAKA VRATA MATERNICE U ZADARSKOJ ŽUPANIJI

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Cilj rada je bio procijeniti učestalost i specifične epidemiološke značajke raka vrata maternice stanovnica Zadarske županije od 2007. do 2016. godine te moguće razlike u odnosu na druge županije Hrvatske.

Izvor podataka za oboljele su bili agregirani podaci Zdravstveno-statističkih ljetopisa Zadarske i Istarske županije, Hrvatskog zavoda za javno zdravstvo i Registra za rak. Državni zavod za statistiku bio je izvor podataka o stanovništvu. Standardizirane stope su izračunate na Europsko standardno stanovništvo 2013. godine (ESP 2013).

U Zadarskoj županiji (ZŽ) u razdoblju 2007.-2016. od raka vrata maternice (RVM) ukupno je bilo 537 oboljelih. Prosječna godišnja stopa pobola, koja predstavlja približnu vrijednost stope prevalencije RVM za ZŽ istog razdoblja je 62,17/100.000. Novooboljelih od RVM u ZŽ u razdoblju 2007.-2014. bilo je ukupno 99, prosječno godišnje 12 novooboljelih, prosječna godišnja dobno standardizirana stopa novooboljelih 14,48/100.000. Počevši od 25-29 godina u svim petogodišnjim dobним razredima do dobi 85 i više godina života registrirane su novooboljele od RVM. Dobno najmlađe novooboljele bile su u dobnom razredu od 25 do 29 godina, najstarija novooboljela je bila dobi 85 i više godina. Najveći broj i najveće dobno-specifične stope incidencije su bili u kasnijoj fertilnoj dobi 40-49 godina, s 33 novooboljele, što čini 1/3 svih novooboljelih. Ukupno je od RVM 31 umrla u ZŽ u razdoblju 2007.-2016., prosječno godišnje 3 umrle. Prosječna godišnja gruba stopa smrtnosti od RVM za ZŽ iznosila je 3,60/100.000.

Rezultati ovog rada su pokazali da su u ZŽ u razdoblju 2007.-2016. najveći broj i najveće dobno-specifične stope incidencije RVM bili u kasnijoj fertilnoj dobi 40-49 godina. U istom razdoblju ukupno je umrla 31 stanovnica ZŽ od RVM, koje po standardima suvremene zdravstvene zaštite razvijenih zemalja nisu smjele umrijeti.

Ključne riječi: epidemiologija, rak vrata maternice, Zadarska županija

## RED BLOOD CELL ALLOANTIBODY EVANESCENCE AND PRETRANSFUSION TESTING

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Clinically significant non-ABO blood group alloantibodies may develop after exposure to foreign red blood cell (RBC) antigens during transfusion or pregnancy. Alloimmunization risk depends on several factors that can be divided into those pertaining to the donor, recipient, RBC unit, and intrinsic RBC factors. The particular problem is the alloantibody evanescence. Undetected alloantibodies may cause acute and delayed hemolytic transfusion reactions. This study was conducted by analysing the persistence and evanescence of the clinically significant RBC antibodies in alloimmunized patients.

Retrospective data analysis of patients alloimmunized between 2011 and 2019 was performed. Patients who did not have additional antibody screening in the indirect antiglobulin test (IAT) after the first positive IAT, were excluded from the study. If subsequent IAT was found negative, it was regarded that alloantibody is no longer present in the bloodstream. In the case of every subsequent positive IAT during pretransfusion testing ( $\geq 72$  h), antibody identification was performed. All together, 544 patients were evaluated. The mean age of the patients was 64 years (range, 0-93 years). The great majority of analysed patients were older than 50 years (76.5 %). Additionally, the majority of patients were females (65.3%), had a positive IAT at the first admission to the hospital (57.2%), were transfused (77.2%), and had a longer than 6 months follow-up (54.3%). A total of 656 antibodies were detected, yielding a rate of 1.2 antibodies per patient. Patients with single alloantibodies (N=455, 83.6%) outnumbered those with multiple alloantibodies (N=89, 16.4%). Antibody specificity with the most frequent evanescence was anti-Jka, while anti-D alloantibody persisted in the majority of cases. The evanescence of alloantibodies represents a significant problem in routine pretransfusion testing.

Keywords: alloantibody, transfusion, indirect antiglobulin test, red blood cell

## MODELS OF DRUG INDUCED LIVER INJURY (DILI) – CURRENT ISSUES AND FUTURE PERSPECTIVES

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Drug-induced Liver Injury (DILI), caused by conventional drugs, herbal medications, dietary supplements, as well as by other xenobiotics, includes liver injury that can sometimes be associated with severe outcomes such as acute liver failure. Therefore, it represents an important cause of morbidity and mortality and remains a common cause of withdrawal of drugs in both preclinical and clinical phases. The etiopathogenesis of DILI is complex and involves genetic, metabolic and immune factors. Despite classification difficulties, researchers agree that DILI generally can be divided into two categories. The first category is predictable and dose dependent. The second is idiosyncratic, mostly dose independent. Idiosyncratic DILI (iDILI) is further subdivided into two sub-categories: allergic, immune-mediated, and non-allergic, nonimmune-mediated. We performed a structured search of bibliographic databases - Web of Science Core Collection, Scopus and Medline for peer-reviewed articles on models of DILI. The reference lists of relevant studies was prepared and a citation search for the included studies was carried out. In addition, the characteristics of screened studies were described. One hundred and six articles about the existing knowledge of appropriate models to study DILI in vitro and in vivo with special focus on hepatic cell models, variations of 3D co-cultures, animal models, databases and predictive modeling and translational biomarkers developed to understand the mechanisms and pathophysiology of DILI are described. Besides descriptions of current applications of existing modeling systems, associated advantages and limitations of each modeling system and future directions for research development are discussed as well.

Keywords: drug evaluation studies, drug-induced, evidence-based toxicology, liver injury, preclinical, side effects

## HbA1c IZ PERSPEKTIVE LABORATORIJSKE MEDICINE

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Laboratorijska medicina koristi HbA1c u postavljanju dijagnoze, praćenju uspješnosti terapije i procjeni rizika kasnih komplikacija oboljelih od šećerne bolesti ,kao indirektni biokemijski biljeg prosječne glikemije u prethodna dva do tri mjeseca. HbA1c nastaje posttranslacijskom modifikacijom HbA u spontanoj dvostupanjskoj ne-enzimskoj reakciji kovalentnog vezanja glukoze na N terminalnu AK Valin u β-lancu globina pri čemu najprije nastaje nestabilni aldimin (Schiff-ova baza) koji amadorijevom pregradnjom prelazi u stabilan ketoamin. Analit je u uzorku krvi stabilan nekoliko tjedana na 2-8C°, mjesecima na -20C°, a čak godinama na -70C°. Laboratorijske metode određivanja HbA1c temelje se na dva principa; prva je metoda razdvajanja na osnovi razlika u naboju molekule ,a druga na osnovu razlike u strukturi molekule. Poznate analitičke interferencije različite su kod različitih metoda ,a najpoznatije su talasemije, prisutstvo HbS, C, E ili F, izrazita lipemija uzorka te neke kemijske posttranslacijske promjene same molekule. Sva klinička stanja koja mijenjaju vrijeme poluživota eritrocita utjecati će na rezultat i kliničko tumačenje nalaza poput poremećaja eritrocitopoeze, poremećaji sinteze hemoglobina, kronična bolest jetre, splenomegalija, splenektomija, sideropenična anemija, akutna i kronična krvarenja, terapija transfuzijom, hemolitička anemija, trudnoća, kronična bubrežna bolest. IFCC i NGSP pokrenuli su program standardizacije s ciljem uspostavljanja referentne metode i definiranje referentnog kalibracijskog materijala te harmonizacije u kliničkoj praksi. Uspostavljena su dva referentna sustava standardizacije; rezultat u postotku i u jedinicama SI sustava (mmol/mol), međusobno potpuno usporedive master jednadžbom. Harmonizaciju u kliničkoj primjeni omogućio je Globalni koncenzus 2010.godine koji je definirao kalibraciju metode prema IFCC referentnim sustavom kao jedinu valjanu. HDMBLM pokrenulo je vanjsku kontrolu kvalitete za određivanje HbA1c u RH 2005.godine, pri čemu je sudjelovalo 28 laboratorija sa CV 9,6%. Danas sudjeluje 132 laboratorija sa 12 metoda i CV neovisan o metodi 4% što ukazuje na zadovoljavanje svih strogih kriterija analitičke kvalitete određivanja HbA1c u RH na svim razinama zdravstvene zaštite.

Ključne riječi : HbA1c, dijabetes, interferencije, standardizacija, HDMBLM

## PROTECTIVE EFFECTS OF MEDITERRANEAN DIET

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Interest in the Mediterranean diet began in the 1950s when it was noted that heart disease was not as common in Mediterranean countries as other parts of the world. Since then, numerous studies have confirmed that the Mediterranean diet helps prevent heart disease and stroke.

The Mediterranean diet is based on the traditional cuisines of Greece, Italy and other Mediterranean countries. Plant-based foods, such as whole grains, vegetables, legumes, fruits, nuts, seeds, herbs and spices form the basis diet. Olive oil is the main source of additional fats. Fish, seafood, dairy and poultry are included in moderation. Red meat and sweets are consumed only occasionally. Olive oil is the primary source of added fat in the Mediterranean diet. Olive oil provides monounsaturated fats that lowers total cholesterol and level of low – density lipoproteins ( or „bad“ cholesterol). Nuts and seeds also contain monounsaturated fats. Fatty fish, such as mackerel, herring, sardines, albacore tuna and salmon, are rich in omega-3 fatty acids. These polyunsaturated fats help fight inflammation in the body. Omega-3 fatty acids also help decrease triglycerides, reduce blood clotting, and lower the risk of stroke and heart failure. Data from southern Mediterranean countries confirm that 60 percent of deaths were related to diabetes, obesity and cardiovascular disease, of which as many as 52 percent of deaths were caused by heart disease. The conclusion is that the main role in the prevention of cardiovascular diseases is played by reducing body weight with the help of exercise and the Mediterranean diet. Living the Mediterranean way also means being physically active and sharing meals with loved ones.

Keywords: mediteran diet, plant based food, olive oil, omega – 3, stroke, heart failure

**ROLE OF TALINS AND KANKS IN INTEGRIN  $\alpha V\beta 5$  FOCAL ADHESIONS  
AND RESPONSE TO PACLITAXEL TREATMENT  
IN MDA-MB-435S CELLS**

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Multiple signalling pathways that can control cytoskeletal network organisation, as well as cell proliferation and motility are triggered by cell binding to the extracellular matrix (ECM) via integrins. Upon binding to the ECM, these receptors cluster and form multimolecular integrin adhesion complexes (IACs). IAC composition analysis in MDA-MB-435s, previously described that integrin  $\alpha V\beta 5$  is the predominant integrin used in long term culture and showed increased sensitivity to microtubule (MT) poison paclitaxel (PTX), upon integrin  $\alpha V$  knockdown. The analysis also revealed that  $\alpha V\beta 5$  IACs contain talins (TLN) 1 and 2, and KANKs 1 and 2. KANK2 has already been shown to have a key role in connecting the  $\alpha V\beta 5$  focal adhesions (FAs) to MTs, and influencing cell sensitivity to PTX. Since talins and KANKs bind, our goal was to investigate their localisation, distinguish the mutual binding of their isoforms as well as elucidate their role in formation of IACs. We performed immunofluorescence and western blot analyses of TLNs and KANKs localisation/expression, actin and MTs visualisation upon knockdown of each TLNs and KANKs. TLN1 knockdown resulted in decreased expression and changed localisation of KANK2 in cell, eliminated integrin  $\alpha V\beta 5$  FAs, changed cell morphology and proliferation as well actin and MT appearance. On the other hand, knockdown of TLN2 did not affect cell morphology and proliferation, nor KANK2 expression and localisation, but it slightly changed FA size, and altered the MT appearance. Interestingly, KANK1 knockdown does not affect sensitivity to PTX. We are currently performing microtubule dynamics measurements upon each TLN and KANK protein. These data will elucidate the differential role of TLN and KANK isoforms in  $\alpha V\beta 5$  FAs which contribute to actin-MT crosstalk and response to MT poisons.

Keywords: melanoma, talin, KANK, paclitaxel

## Deregulated miR-20a/miR-92b circuit underlies changes in circulating gamma-delta T cell proportions in psoriasis vulgaris

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Psoriasis vulgaris (PV) is an autoinflammatory dermatosis driven by T cells. The role played by unconventional, innate-like  $\gamma\delta$ T cell populations in PV has been less well recognised, but the next generation of multi-messenger biology provides an opportunity to break this impasse. To this end, we investigated the composition of flow-sorted  $\gamma\delta$ T cells (CD3 $\epsilon$ , panTCR $\gamma\delta$ , TCRV $\delta$ 1/ $\delta$ 2, FACS Canto II) in relation to their microRNA expression (hsa-miR-20a-5p/-29a-3p/-92b-5p, qPCR, reference: hsa-miR-423-3p,  $2^{-\Delta\Delta Ct}$ ) by using a deeply phenotyped cohort of 12 affected and 14 control individuals. We also analyzed gene expression in bulk  $\gamma\delta$ T cells (EOMES, RUNX3, TBX21, RORC, CCR6, ZBTB16, SELPLG and IL18R, qPCR, reference: TBP,  $2^{-\Delta\Delta Ct}$ ) and serum levels of IL-17A/F, IL-18, IL-23, CCL20 and CCL27. V $\delta$ 1 $\cdot$  $\delta$ 2 $^-$  (median 35 % vs. 17 %,  $P=0.039$ , Wilcoxon test) and V $\delta$ 1 $\cdot$  $\delta$ 2 $^-$   $\gamma\delta$ TCR $^{int}$  compartments (40 % vs. 22 %,  $P=0.02$ ) were expanded in  $\gamma\delta$ T cells from PV patients. The bulk  $\gamma\delta$ T cells in PV were enriched in miR-92b ( $FC=13.2$ ,  $P=0.013$ ), but depleted of miR-20a ( $FC=0.27$ ,  $P=0.0014$ ). A strong inverse relationship was also noted between miR-20a abundance and the number of V $\delta$ 1 $\cdot$  $\delta$ 2 $^-$   $\gamma\delta$ TCR $^{int}$  cells in the blood ( $\rho=-0.61$ ,  $P=0.0035$ , Spearman test). ZBTB16, SELPLG and IL18R transcripts followed miR-20a dynamics ( $0.44 < \rho < 0.56$ ,  $0.038 > P > 0.0054$ ), suggesting that cell homing and trafficking may underlie numerical evolution of certain  $\gamma\delta$ T subsets in PV. In contrast, miR-92b lacked association with cell numbers. Age, sex, BMI, the burden of inflammation and CMV status had no major effect on miR-20a/92b expression in bulk  $\gamma\delta$ T cells. In conclusion, we provide an updated constraint on miR-20a/29a/92b expression in purified  $\gamma\delta$ T blood cells from PV donors. A further study into the cellular landscape of miRNA expression is warranted.

Keywords: psoriasis vulgaris, gamma-delta T cells, miRNA

**PRIMING MODIFIES HEAT STRESS RESPONSE IN *BPM1*-OVEREXPRESSING *ARABIDOPSIS THALIANA* (L.) HEYNH.**

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Exposure of plants to stressors affects their physiological processes. Increased temperature is an environmental stressor that causes heat stress through a series of changes in plant cells. Resistance to future stress conditions can be improved by applying moderate stress before exposing the plant to severe stress, i.e., priming. The aim of this study was to determine the effects of priming on oxidative stress and antioxidative response (by measuring H<sub>2</sub>O<sub>2</sub> content, lipid peroxidation level, proline content, and antioxidant enzymes activity), HSP70 protein expression (by western blotting), and photosynthetic efficiency (by the OJIP method). Assays were performed on wild-type seedlings of *Arabidopsis thaliana* and a *BPM1*-overexpressing line (*oeBPM1*). One group of plants was exposed to an increased temperature of 42 °C for six hours, another group was exposed to 42 °C after priming at a temperature of 37 °C, while the control group was not exposed to an increased temperature. After a 24-hour recovery period, the seedlings were used for analysis of the aforementioned parameters. Priming led to better long-term survival of plants after heat stress. Primed plants showed a significant increase in protein and proline content and a decrease in lipid peroxidation level compared to plants exposed to 42 °C only. Heat stress resulted in induction of antioxidant enzymes activity in cells, increased HSP70 protein expression, and significant disruption of photosynthetic efficiency. The observed lipid peroxidation level and catalase activity indicate that the *oeBPM1* line has a partially better response to heat stress than wild-type plants, suggesting possible roles of the *BPM1* protein. Finally, the results of this study have shown that overexpression of the *BPM1* gene in *A. thaliana* and priming have an impact on the heat stress response. This raises many questions for future research on the *BPM1* gene and potential applications of priming in plants.

Keywords: *Arabidopsis thaliana*, heat stress, priming, *BPM1* gene

**LIPID BINDING BY N-TERMINAL MOTIF MEDIATES  
PLASMA MEMBRANE LOCALIZATION OF *BORDETELLA*  
EFFECTOR PROTEIN BTEA**

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The respiratory pathogens, *Bordetella pertussis* and *Bordetella bronchiseptica* employ a type III secretion system (T3SS) to inject a 69-kDa BteA effector protein into host cells. This effector is known to contain two functional domains, including an N-terminal lipid raft targeting (LRT) domain and a cytotoxic C-terminal domain that induces non-apoptotic and caspase-1-independent host cell death. However, the exact molecular mechanisms underlying the interaction of BteA with the plasma membrane (PM) as well as its cytotoxic activity in the course of *Bordetella* infections remain poorly understood. Using a protein–lipid overlay assay and surface plasmon resonance, we show here that the recombinant LRT domain binds negatively charged membrane phospholipids. Specifically, we determined that the dissociation constants of the LRT domain–binding liposomes containing phosphatidylinositol 4,5-bisphosphate, phosphatidic acid, and phosphatidylserine were ~450 nM, ~490 nM, and ~1.2 μM, respectively. Both phosphatidylserine and phosphatidylinositol 4,5-bisphosphate were required to target the LRT domain and/or full-length BteA to the PM of yeast cells. The membrane association further involved electrostatic and hydrophobic interactions of LRT and depended on a leucine residue in the L1 loop between the first two helices of the four-helix bundle. Importantly, charge-reversal substitutions within the L1 region disrupted PM localization of the BteA effector without hampering its cytotoxic activity during *B. bronchiseptica* infection of HeLa cells. The LRT-mediated targeting of BteA to the cytosolic leaflet of the PM of host cells is, therefore, dispensable for effector cytotoxicity.

Keywords: lipid–protein interaction, surface plasmon resonance, *Saccharomyces cerevisiae*, BteA effector protein, *Bordetella pertussis*

**STIMULACIJA RAZVOJA BIOFILMA SUŠOM**

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U radu je eksperimentalno ispitana sposobnost bakterijskih kultura za stvaranje biofilma te utjecaj perioda izloženosti nedostatku vode i povećane temperature na tu sposobnost s hipotezom pojačanog stvaranja biofilma kao odgovor na stres sušom. Eksperimentalni dio rada je bio podijeljen u tri eksperimenta u kojima je testirano ukupno 28 bakterijskih kultura izoliranih s područja sjeveroistočne Hrvatske iz različitih tipova staništa. Od 28 bakterijskih kultura odabранo je pet kultura za dodatni eksperiment olakšavanja stresa sušom pri uzgoju pšenice, no njihov utjecaj na olakšavanje stresa sušom nije pokazao značajan rezultat.

Ključne riječi: biofilm, suša, bakterije promotori rasta biljaka

## TJELESNA MASA NOVOOBOLJELIH OD ŠEĆERNE BOLESTI TIPA 2

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Debljina kao kompleksna bolest ima rastuću prevalenciju u cijelom svijetu. Često donosi mnoge komplikacije, a jedna njih je šećerna bolest tipa 2 (ŠBT2). Poznato je da oko 85% bolesnika sa ŠBT2 ima prekomjernu tjelesnu masu ili pretilost. Udio visceralnog masnog tkiva bolji je predskazatelj rizičnog učinka debljine na zdravlje od apsolutne količine masnog tkiva. Ciljevi: Ustanoviti stupanj pretilosti, udio masnog i visceralnog masnog tkiva kod novootkrivenih bolesnika sa ŠBT2. Istraživali smo tjelesnu masu, udio masnog i visceralnog masnog tkiva u novootkrivenih bolesnika sa ŠBT2, (N = 161) tijekom 6 mjeseci. Pratili smo dob i spol, indeks tjelesne mase (ITM), glikirani hemoglobin. Sastav tijela analiziran je bioelektričnom impedancijom, digitalnom vagom OMRON BF 511. Od ukupnog broja ispitanika (N = 161, 78 žena (Ž) i 83 muškarca (M)) 91% Ž i 87% M ispitanika imalo je ITM iznad 25 kg/m<sup>2</sup>. ITM između 30 i 34,9 kg/m<sup>2</sup> imalo je 31% Ž i 26% M, između 35 i 39,9 kg/m<sup>2</sup> je imalo 27% Ž i 18% M, a ITM iznad 40 kg/m<sup>2</sup> 13% Ž i 8% M. Udio masnog tkiva bio je povišen kod 88% Ž i 72% M, a udio visceralnog masnog kod 74% Ž i 83% M. U trenutku dijagnosticiranja ŠBT2 pretilost je bila zastupljena kod 61,5% ispitanika, kod 28% ispitanika pretilost 1. stupnja, kod 22% pretilost 2. stupnja, a kod 10% pretilost 3. stupnja. Naše istraživanje potvrđuje od ranije poznatu činjenicu da je prekomjerna tjelesna masa uz slabu tjelesnu aktivnost i neadekvatnu prehranu jedan od ključnih čimbenika za nastanak ŠBT2. Osobe s povećanim udjelom visceralnog masnog tkiva imaju značajno veći rizik za nastanak šećerne bolesti tipa 2.

Ključne riječi: šećerna bolest tip 2, tjelesna masa, pretilost

**INFLUENCE OF ORAL ANTICOAGULATION WARFARIN THERAPY  
ON TREATMENT OUTCOME OF CHRONIC SUBDURAL HEMATOMAS  
AT THE UNIVERSITY HOSPITAL OF SPLIT: DEPARTMENT OF  
NEUROSURGERY FROM 2010 TO 2018.**

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The goal of this study was to determine if taking chronic oral anticoagulant warfarin therapy affects treatment outcome of chronic subdural hematoma, with special attention to: anamnestic data, epidemiological data, clinical presentation and usage of anticoagulation therapy.

This study included 240 participants in period between January 2010 and December 2018. They were divided into two groups whether they were or they were not taking warfarin anticoagulation therapy. The primary outcome measures were: size of preoperative and postoperative CT finding, Markwalder grade, revision surgeries and duration of time patients were hospitalized at the neurosurgical ward (shown in days).

Patients taking oral anticoagulant warfarin were averagely hospitalized longer ( $22.7 \pm 15.3$  days) than those who did not take oral anticoagulation treatment ( $17.4 \pm 13.5$  days). This correlation has been shown to be statistically significant.

This study has successfully demonstrated only statistically significant correlation between number of days patients spent at the neurosurgical ward and taking warfarin as chronic oral anticoagulation therapy. Other examined correlations showed no statistical significance. Further research and analysis of larger number of subjects are required in order to define the influence of other oral anticoagulation drugs (warfarin excluded) on occurrence and treatment outcome of chronic subdural haematoma.

Keywords: warfarin, anticoagulation, chronic subdural hematomas, Markwalder grade

**FIZIOTERAPIJSKI PRISTUP U LIJEČENJU SPUŠTENIH STOPALA  
(PES PLANUS) U DJECE**

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Stopalo je složena struktura ljudskog tijela čija je važnost u kretanju podcijenjena sve dok jedna od njezinih sastavnica ne prestane primjereno funkcionirati. Uobičajena abnormalnost kompleksa stopala i gležnja kod djece je spušteno stopalo (lat.*pes planus*). Spušteno stopalo ima dva oblika: fleksibilno i kruto spušteno stopalo. Kod većine djece deformacija fleksibilnih spuštenih stopala ne uzrokuje nikakve kliničke simptome, stoga ne zahtijeva liječenje, ali ako dijete ima simptomatsko ili kruto spušteno stopalo, potrebno ga je pažljivo ispitati i primjereno liječiti. S obzirom da se vrste spuštenog stopala razlikuju prema načinu njihova deformiranja i razvoja te postojanju ili nepostojanju simptomima, vrlo je važno znati pravilno i adekvatno liječiti svaku vrstu spuštenog stopala. Cilj ovog rada je pružiti pregled dokaza o načinu liječenja spuštenih stopala u djece te pokušati odgovoriti na pitanje koji su to trenutni dokazi o načinu liječenja spuštenih stopala u djece. Pregledom dostupne literature zaključujemo da unatoč tome što postoje brojne studije o različitim načinima liječenja spuštenih stopala, dokazi su još uvijek nepotpuni i ne dopuštaju donošenje čvrstih zaključaka o nizu uobičajenih pristupa.

**Ključne riječi:** djeca; deformacije stopala; liječenje; pes planovalgus; pes planus; spušteno stopalo

**ISKUSTVO PRIMJENE ANTI-VEGF TERAPIJE  
KOD MAKULARNOG EDEMA**

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U prvoj skupini pacijenata pratili smo grupu koja je primila anti-VEGF (bevacizumab, ranibizumab i aflibercept) terapiju u razmaku od 28 do 350 dana, te je napravljena analiza prosjeka debljine makule za pozitivni i negativni učinak terapije. U drugoj skupini pacijenata pratili smo u točnim vremenskim intervalima djelovanje pojedine anti-VEGF terapije. Prvu skupinu pacijenata podjelili smo na grupu A (bevacizumab 1.25 mg/0.05mL), grupu B (ranibizumab 0.5 mg/0.05 mL) i grupu C (aflibercepta 2 mg/0.05 mL). Od navedenih grupa napravljene su 2 podgrupe gdje je (-) grupa imala smanjenje edema, a (+) grupa je imala povećanje edema. U svakoj (-) i (+) grupi uzeo se srednji prosjek debljine makule i vremenskog perioda aplikacije. Druga skupina je podijeljena u grupu I (bevacizumab 1.25 mg/0.05mL), grupu II (ranibizumab 0.5 mg/0.05 mL), grupu III (aflibercept 2 mg/0.05 mL). Liječeni su u pravilnim vremenskim intrevalima od 0, 30, 60 i 90 dana. U grupi A za (-) grupu trebalo je 55,14 dana da se smanji edem za -70,95  $\mu\text{m}$ . U (+) grupi dobili smo svakih 141,22 dana povećanje edema za 79,61  $\mu\text{m}$ . U grupi B za (-) grupu uočili smo smanjenje edema za -93,77  $\mu\text{m}$  unutar 80,5 dana. U (+) grupi dobili smo svakih 85,3 dana porast debljine od 51,83  $\mu\text{m}$ . U grupi C dobili smo za (-) grupu smanjenje edema za -170,75  $\mu\text{m}$  unutar 42,5 dana. U (+) grupi uočili smo svakih 171,33 dana pogoršenje edema za 172,66  $\mu\text{m}$ . Prosjek debljine makule u grupi I, II i III odgovara dosadašnjim spoznajama. Napravili smo preliminarno ispitivanje na malom broju subjekata koje će pomoći da postavimo što jasnije kriterije buduće analize. Ovo istraživanje ukazuje na potrebu racionalnog odabira lijekova za suzbijanje edema makule.

Ključne riječi: bevacizumab, ranibizumab, aflibercept

## SARS-COV-2 DISSEMINATION THROUGH PERIPHERAL NERVES EXPLAINS MULTIPLE ORGAN INJURY

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Coronavirus disease (COVID-19), caused by recently identified severe acute respiratory distress syndrome coronavirus 2 (SARS-CoV-2), is characterized by inconsistent clinical presentations. While many infected individuals remain asymptomatic or show mild respiratory symptoms, others develop severe pneumonia or even respiratory distress syndrome. SARS-CoV-2 is reported to be able to infect various organs and tissues. More than a third of cases displayed neurological involvement, and many severely ill patients developed multiple organ infection and injury. However, less than 1% of patients had a detectable level of SARS-CoV-2 in the blood, raising a question of how the virus spreads throughout the body. We propose that nerve terminals act as entry points for the brain invasion, allowing SARS-CoV-2 to infect the brainstem. By exploiting the subcellular membrane compartments of infected cells, SARS-CoV-2 is capable to disseminate from the brain to periphery via vesicular axonal transport and passive diffusion through axonal endoplasmic reticula, causing multiple organ injury independently of an underlying respiratory infection. The proposed model clarifies a wide range of clinically observed phenomena in COVID-19 patients, such as neurological symptoms unassociated with lung pathology, protracted presence of the virus in samples obtained from recovered patients, exaggerated immune response, and multiple organ failure in severe cases with variable course and dynamics of the disease. We believe that this model can provide novel insights into COVID-19, its long-term sequelae, and establish a framework for further research.

Keywords: SARS-CoV-2, neurotropic infection, axonal transport, peripheral nerves, neurological symptoms, multiple organ failure

**ANTIMIKROBNI UČINAK KOLOIDNIH NANOČESTICA  
NA MSSA I MRSA**

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Sojevi bakterija koje pokazuju otpornost na antibiotike u uporabi svakim danom postaju sve veći javnozdravstveni problem te se pojavljuje potreba za znanstvenim pronalaskom alternativnih izvora biološko-aktivnih tvari. Nanočestice, čestica promjera između 1 i 100 nanometara, pokazuju širok spektar antimikrobnog učinka, čak i na višestruko rezistentnim sojevima bakterija, te su obećavajući spojevi u dalnjem razvoju antimikrobne terapije.

Cilj ovog rada bio je utvrditi učinak nekoliko koloidnih otopina nanočestica u nekoliko različitih sistema u uvjetima *in vitro* utvrđivanjem najmanjih baktericidnih (MBC) i najmanjih inhibitornih (MIC) koncentracija metodom modificirane serijalne mikrodilucije te utvrditi utjecaj medija (albumin, goveđi serum) na ishod istoimene metode i utvrđivanjem antimikrobnog učinka tkanine impregnirane slojem smjese nanočestica primjenom tzv. „time-kill“ metode. Kao mikrobi model korištena je vrsta *Staphylococcus aureus* (sojevi MSSA i MRSA). Nanočestice korištene su elementarno srebro (10 i 40 nm) i platina, titanov dioksid, te cinkov i itrijev oksid.

Reultati pokazuju da se MBC i MIC vrijednosti koloidnih otopina fiziološkoj otopini nalaze u rasponima od 0.1 do 80 ppm-a. Od ispitivanih nanočestica najmanje MBC i MIC pokazuje cinkov oksid, a najveće titanov dioksid. Nisu uočene baktericidne vrijednosti kod kompleksnijih i koncentriranijih medija što indicira da ti kompleksniji mediji značajno snižavaju antibakterijski učinak nanočestica.

Zaključno, tkanina, dimenzija 1cm<sup>2</sup>, impregnirana slojem smjese nanočestica pokazuje stopostotnu redukciju vijabilnih sojeva MSSA i MRSA nakon 18-satne inkubacije.

Ključne riječi: nanočestice, antibakterijski učinak, *MRSA*, *MSSA*

**RISK FACTORS AND MOLECULAR PREDISPOSITIONS FOR CERVICAL DYSPLASIA AMONG WOMEN FROM EAST CROATIA**

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The aim of this study was to investigate possible association between high-risk Human papillomavirus (HR HPV) – induced cervical infection, HR HPV-related cervical dysplasia, HR HPV genotypes with two Toll-like receptor (TLR) 9 gene polymorphisms and other risk factors. During a three-year period, 100 women positive for cervical HR HPV infection (97 with cervical dysplasia and 3 positive women without dysplasia) were genotyped using the Linear Array HPV Genotyping assay (Roche Diagnostics). Furthermore, two polymorphisms of TLR9 (-1486T/C, rs187084 and 2848C/T rs352140) were determined using real-time Polymerase Chain Reaction; 50 HR HPV negative women of similar ethnicity were included as controls. This study showed that infections with HPV 16 in women with cervical dysplasia were more frequently found compared with HPV 18 infections ( $p=0.0539$ ). Comparison between HR HPV positive and negative women showed significant association between age  $>35$  years ( $p=0.0058$ ), being unmarried women ( $p=0.0001$ ), no condom usage ( $p=0.0304$ ) and active tobacco smoking ( $p=0.0376$ ) with HR HPV cervical infection. No significant associations between two TLR9 gene polymorphisms, HR HPV infection and cervical dysplasia were found. Our results indicated following findings: women with cervical dysplasia showed significant higher rate of HR HPV 16 infection compared to HR HPV 18, HR HPV – infection was strongly correlated with social risk factors and lastly, TLR9 gene polymorphisms (rs187084; rs352140) did not correlate with HR HPV infection and cervical dysplasia. Further genome-wide association studies could open a new frontier in understanding the relationship between polymorphisms at TLR9 and immunological mechanisms in HPV-induced carcinogenesis.

**Keywords:** carcinogenesis, Human papillomavirus 16, Human papillomavirus 18, Toll-like receptor 9, uterine cervical dysplasia

**DIRTY CROATIAN MONEY: HOW BIG IS THE THREAT?**

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The objective of this study was to determine the extent of bacterial contamination, expressed as colony forming units (CFU), on individual banknotes and coins of Croatian Kuna (HRK). The purpose of the study was to define if the fear of money-transmitted diseases is founded.

One-hundred twenty pieces of banknotes and coins were collected for the experiment, 10 bills of 10, 20, 50, 100, 200 and 500 HRK and 10 coins of 10, 20 and 50 Croatian Lipa and 1, 2 and 5 HRK. At the Department of Microbiology and Parasitology, Faculty of Medicine, University of Osijek, swabs were taken from money, moistened in saline, planted on blood agar and incubated for 24 hours under ambient conditions at 37 °C. After growing the bacteria, CFU were counted and replanted for further identification, which was performed in accordance with the microbiological professional standards.

In total, 739 bacterial CFU were grown and six bacterial species have been identified: *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus viridans*, *Bacillus sp.*, *Klebsiella sp.*, *Neisseria sp.* Almost 30% of the money was bacteriologically clean. There were no statistical differences between the prevalence of bacterial contamination of banknotes and coins. The most common bacteria isolated was *S. epidermidis* (86.33%) with statistical significance both on banknotes and coins ( $p<0.0001$ ).

The identified bacterial species are mostly part of the normal human flora. Pathogenic, and potentially pathogenic bacterial species were not found on Croatian banknotes and coins in a respect for one colony of *Klebsiella sp.*

Keywords: bacterial contamination, normal bacterial flora, banknotes, coins

**AN OUTBREAK OF NOSOCOMIAL BLOODSTREAM INFECTION DUE  
TO MULTIDRUG-RESISTANT *ACINETOBACTER BAUMANNII*  
IN COVID-19 INTENSIVE CARE UNIT**

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Nosocomial bloodstream infections (NBSIs) are an emerging cause of morbidity and mortality in COVID-19 intensive care units (ICUs) worldwide. The aim of this study was to assess the incidence rate and microbiological characteristics of NBSI among critically ill COVID-19 patients. A single-center retrospective study was conducted at a tertiary care hospital in Zagreb, Croatia between October 1, 2020, and March 1, 2021. The incidence of BSIs/1000 ICU days was calculated, etiology of BSI and antimicrobial resistance pattern of the isolates were analyzed. Pulsed-field gel electrophoresis (PFGE) and multilocus-sequence typing (MLST) were performed in order to assess the molecular epidemiology. A total of 193 episodes of BSI were registered in 176 patients, with an incidence rate of 25 episodes per 1000 patient-days at risk. A significant proportion of BSIs were polymicrobial (73/193, 37%). The majority of isolates were gram negative bacteria, prevalently *A.baumannii* (112/278, 38.9%). PFGE and MLST showed 2 clone types (ST2 and ST636) dominated by clone type ST2. The blaOXA-23 gene was detected in ST2 isolates while blaOXA-24 gene was ST636 specific. During COVID-19 second wave, number of ICU beds increased from 35 to 75 and microbiological laboratory was closed. Due to work overload in ICU, health care workers used the same personal protective equipment (PPE) while attending to patients, hand hygiene was inadequate (wearing 2-3 pairs of gloves), and environment was insufficiently cleaned and disinfected. Education regarding hand hygiene, proper use of PPE as well as decrease in number of COVID-19 cases stopped the outbreak. Adherence to infection control measures regarding PPE alongside with antimicrobial stewardship and availability of microbiology are essential in preventing NBSI in COVID-19 ICUs.

Keywords: nosocomial bloodstream infections, *Acinetobacter baumannii*, COVID-19, infection control measures

**THE FIRST DETECTION OF CMV-16-TYPE OF AmpC  $\beta$ -LACTAMASES IN  
MULTIDRUG-RESISTANT *PROTEUS MIRABILIS* IN CROATIA:  
IMPLICATIONS FOR THERAPY**

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Coexistence of different resistance mechanisms often leaves very few therapeutic options. In this study we characterized the types of  $\beta$ -lactamases that coexist in multidrug-resistant (MDR) *P. mirabilis* isolates from University Hospital Centre Split, and determined in vitro antimicrobial susceptibilities for this type of strain.

Consecutive non-repeated *P. mirabilis* isolates were collected from different clinical samples during a 1-year period, based on the resistance to third-generation cephalosporins and cefoxitin. Double-disk synergy test was performed for detection of extended-spectrum  $\beta$ -lactamase (ESBL), and cefoxitin Hodge test for detection of AmpC  $\beta$ -lactamase. Genes encoding beta-lactamases were detected by polymerase chain reaction (PCR) and identified by DNA sequencing. Clonal relatedness of isolates was analyzed by Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR fingerprinting. Antimicrobial susceptibility was done by disk diffusion method and minimal inhibitory concentrations (MICs) by gradient strip method. A total of 132 isolates were analysed. DNA sequencing revealed coexistence of TEM-116 and CMY-16  $\beta$ -lactamases. ERIC-PCR demonstrated clonal relatedness of the isolates. All isolates were multidrug-resistant, with 100% resistance to amoxicillin/clavulanate, cephalosporins except the fourth generation, quinolones and trimethoprim-sulfamethoxazole. Seventeen isolates were susceptible to amikacin, and only 6 of them to all aminoglycosides. Piperacillin/tazobactam and ceftazidime showed variable degrees of resistance: 52.3% isolates were susceptible to piperacillin/tazobactam in disk diffusion test, and 55.1% were susceptible in gradient strip test. All isolates were 100% susceptible to carbapenems.

This study shows that carbapenems should be the drug of choice for serious infections caused by MDR *P. mirabilis* coproducing TEM-116 and CMY-16  $\beta$ -lactamases. Piperacillin/tazobactam and ceftazidime are less reliable agents for therapy of serious infections. Acquisition of carbapenemase genes, along with an intrinsic resistance to tigecycline and colistin, indicates the possibility of development MDR *P. mirabilis* into a pan-resistant strain.

Keywords: *Proteus mirabilis*, multidrug-resistant, antimicrobial susceptibility

**USPJEŠNOST DETEKCIJE UZROČNIKA DIJAREJE DJECE  
RAZLIČITIM METODAMA**

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Klasične kultivacijske metode usmjerene na izolaciju patogena sve više postaju postupak koji se rabi u drugom aktu, samo kod obrade pozitivnih uzoraka nakon molekularnog testiranja. Cilj istraživanja bio je usporediti razlike u detekciji enteropatogena kod upotrebe molekularnih i konvencionalnih metoda u djece s gastroenteritisom na području Zagreba. Uzastupno zaprimljeni uzorci stolice tijekom dva mjeseca prikupljeni od 34 djece od jednog mjeseca do osam godina starosti s dijagnozom gastroenteritisa i dijarejom testirani su pomoću FilmArray GI panela (BioFire Diagnostics, Salt Lake City, UT, USA) i konvencionalnim metodama. Metode klasične kultivacije za bakterijske uzročnike, mikroskopska identifikacija parazita i brzi imunokromatografski testovi za antigene virusa i parazita korišteni su pri testiranju konvencionalnim metodama. Od 34 uzorka, njih pet je bilo negativno, u 13 je detektiran samo jedan mikroorganizam, a u 16 više mikroorganizama. U 15 pacijenata enteropatogeni su detektirani samo pomoću FilmArray-a. Rotavirusi, EPEC i sapovirusi bili su najčešće detektirani patogeni. Usporedbom molekularnih i brzih testova koji detektiraju antigene virusa u uzorku tvrđeno je šest lažno negativnih i čak 16 lažno pozitivnih rezultata brzih imunokromatografskih testova za virusne enteropatogene. Višestruki PCR testovi mogu značajno poboljšati rutinsku dijagnostiku detekcije uzročnika dijareje u pacijenata s gastroenteritisom, posebice u djece. FilmArray GI panel je jednako uspješan u detekciji bakterijskih patogena kao metode klasične kultivacije, dok je pokazao zabrinjavajuće visoku učestalost lažnih rezultata kada se brzi imunokromatografski testovi upotrebljavaju za detekciju virusnih enteropatogena u djece s gastroenteritisom.

Ključne riječi: dijareja, dijagnostički testovi, virusni gastroenteritis, PCR, bakterijski gastroenteritis

**PRIMJENA SREDNJIH DOZA NISKOMOLEKULARNIH HEPARINA ZA  
PREVENCIJU TROMBOEMBOLIJA U COVID-19 BOLESNIKA**

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Od početka pandemije uočena je visoka učestalost tromboembolija u pacijenata hospitaliziranih radi COVID-19. Stručna javnost je odmah prihvatile potrebu primjene tromboprofilakse u svih hospitaliziranih bolesnika. Cilj je bio ispitati učinkovitost i sigurnost tromboprofilakse srednjim dozama NMH u COVID-19 bolesnika sa srednje teškom i teškom bolesti. Retrospektivnom analizom obuhvaćeno je 98 pacijenata koji su liječeni prema internom algoritmu tromboprofilakse za COVID-19 bolesnike. Za bolesnike sa srednje teškim i teškim oblikom bolesti predviđeno je korištenje srednjih doza NMH. Terapijska doza je 1 mg/kg/2x dnevno. Doze između ovih predstavljaju srednje profilaktičke doze. Primarna mjera ishoda je pojava tromboembolijskog događaja u arterijskoj ili venskoj cirkulaciji te dismeinirana intravaskularna koagulacija. Sekundarni ishodi su pojava krvarenja i smrt bilo kojeg uzroka. Istraživanje je provedeno na 98 ispitanika, od kojih je 55 muškaraca i 43 žena, bez značajne razlike u raspodjeli prema težini bolesti. Od ukupno 88 ispitanika starijih od 50 godina, njih 59 ima teški oblik COVID-19 (Fisherov egzaktni test, P = 0,04). Od 98 ispitanika njih devet je primalo profilaktičku dozu, 60 je primalo srednju dozu, a na terapijskoj dozi NMH bilo je 29 ispitanika. Ukupno se bilježi pet trombotičkih nuspojava, po 1 ispitanik s dubokom venskom trombozom i moždanim udarom, dok ih je tri imalo DIK, bez značajne razlike u raspodjeli u odnosu na razinu tromboprofilakse. S obzirom na prisutna krvarenja, blaga krvarenja su značajnije prisutna kod ispitanika na terapijskoj dozi, kod četiri ispitanika (Fisherov egzaktni test, P = 0,01). Ukupno je 26 pacijenata umrlo. Mali broj ispitanika ne dopušta nam zaključivati o utjecaju intenziviranja tromboprofilakse na pojavnost tromboembolija i smrtnost, te je istraživanje potrebno napraviti na više ispitanika.

**Ključne riječi:** COVID-19, tromboembolija, krvarenje, tromboprofilaksa, niskomolekularni heparin

**CYSTOID MACULAR LESIONS ARE RESISTANT TO  
TOPICAL DORZOLAMIDE TREATMENT IN ENHANCED  
S-CONE SYNDROME CHILD**

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The purpose of this paper was to evaluate whether cystoid macular lesions respond to treatment with dorzolamide 2% drops in the enhanced S-cone syndrome (ESCS) child, as several case reports document favorable efficacy in adults.

Seven-year-old boy with ESCS and cystoid macular lesions was treated with dorzolamide 2% in both eyes three times a day for a period of 7 months. The efficacy of treatment was analyzed by visual acuity assessment (ETDRS), multifocal electroretinography and SD-OCT central foveal thickness (CFT) measurement.

Baseline RE CFT was 540 and 453 µm in the LE, with amplitude of P1-wave density 39.8 and 50.4 nV/deg(2), respectively. Best corrected visual acuity (BCVA) was 0.3 logMAR RE and 0.3 logMAR LE at distance. At 7-month follow-up examination, CFT showed no reduction in thickness (RE 599 µm, LE 521 µm). P1-wave density increased (RE 49.1 nV/deg(2), LE 84.9 nV/deg(2)), with BCVA 0.3 logMAR RE and 0.2 logMAR LE.

To the best of our knowledge, this is the youngest ESCS patient treated with dorzolamide drops and the first report recording that cystoid macular lesions are resistant to topical dorzolamide treatment. Furthermore, these data are in favor of the hypothesis that microcystoid changes in ESCS appear due to defects in cell-to-cell adhesion rather than the disintegration of the retinal barrier. The marked differences in treatment response to carbonic anhydrase inhibitors between the adults and the child here presented suggest that the breakdown of the blood-retinal barrier may play a more important role later in life.

**Keywords:** amblyopia, carbonic anhydrase inhibitors, enhanced S-cone syndrome

**ANEMIA IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE  
IN CLINICAL HOSPITAL CENTER OSIJEK**

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Crohn's disease and ulcerative colitis are autoimmune idiopathic diseases whose incidence is constantly increasing, consequently representing a significant health problem of a developed Western society. They are characterized by chronic inflammation of the gastrointestinal wall and the high occurrence of relapse. Drug groups used in the treatment of IBDs are aminosalicylates, immunomodulators, corticosteroids and biological agents. Beside the basic pathology, patients with IBDs often have comorbidities and anemia is the most significant one. Different disorders occur, in iron metabolism, erythropoiesis or shortening the life span of erythrocytes. The most common is mild anemia with concentration of haemoglobin between 70 and 110 g/L, MCV and MCHC are within physiological limits or mildly decreased. The aim of this study was to determine the correlation between hemoglobin concentration and duration of disease. The research was based on medical documentation of patients affected by inflammatory bowel diseases, treated at the Department of gastroenterology and hepatology at Clinical Hospital Center Osijek. In total, 86 patients were involved, of which 44 of them were diagnosed with ulcerative colitis and 42 with Crohn's disease. Anemia was found in anamnesis of 63 (72%) patients with inflammatory bowel disease. Hemoglobin values ranged from 49 to 155 g/L, with a median (interquartile range) of 119 g/L (109,5 to 132 g/L). No statistically significant correlation was found between hemoglobin values and duration of disease in patients with IBDs at the time of research ( $p = 0,052$ ,  $P = 0,635$ ). Anemia is present in most patients with inflammatory bowel disease. The average hemoglobin concentration does not depend on the duration of the inflammatory bowel disease.

**Keywords:** anemia, hemoglobin, inflammatory bowel disease, Crohn's disease, ulcerative colitis

**COMPARATION OF INFLAMMATORY BOWEL DISEASE THERAPY  
WITH AN EMPHASIS ON IMMUNE THERAPY;  
RESULTS OF RESEARCH STUDY OF INFLAMMATORY BOWEL DISEASE  
IN THE OSIJEK-BARANJA COUNTY 2000-2014**

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One of the significant health issues in modern era are ulcerative colitis (UC) and Crohn's disease (CD). Beneath clinical manifestation of those inflammatory bowel diseases (IBDs) lays chronic inflammation of gastrointestinal wall, mediated by the release of interleukins, cytokines and TNF. Drugs used in treatment are antibiotics, corticosteroids, immunomodulators and biological agents. The aim of this study was to compare the usage use of immunomodulating drugs vs. antibiotics and corticosteroids in treatment of IBDs during the time. The results of this study are part of the results of the doctoral dissertation by M. Žulj, MD. This study is based on the medical documentation of IBD patients treated at KBC Osijek and OB Našice in the period of 2000-2014. In total of 78 patients, 51 were diagnosed with UC and 27 with CD. This study is a part of the university project "Povezanost polimorfizama jednog nukleotida u NOD 2 i MDR 1 genima s nastankom upalnih bolesti crijeva" VIF 2016-MEFOS 16, conducted at the University J.J.Strossmayer led by prof.dr.sc. A.Včev. At the beginning of treatment there was a statistically significant difference in usage the use of antibiotics between the groups with UC and CD (32 UC, 10 CD, p=0, 03 χ<sup>2</sup>test), immunosuppressants (11 UC, 15 CD, p=0, 001 χ<sup>2</sup>test) and biological agents (7 UC, 9 CD, p=0, 04 χ<sup>2</sup>test), while there was no statistically significant difference in usage the use of corticosteroids (36 UC, 21 CD, p=0, 59, χ<sup>2</sup>test). During this study, drugs used in the treatment of those patients were modified. Statistically significant differences were found in usage the use of biological agents (7 UC, 17 CD, p<0, 001, χ<sup>2</sup>test), while there was no statistically significant difference in usage the use of antibiotics (3 UC, 1 CD, p>0, 99, χ<sup>2</sup>test), corticosteroids (13 UC, 3 CD, p=0, 13, χ<sup>2</sup>test) and immunosuppressants (6 UC, 4 CD, p=0, 72, χ<sup>2</sup>test). Although the drug groups used in the treatment of UC and CD are similar, biological agents are mostly used in CD while corticosteroids are used in UC and CD equally, regardless of time of the treatment. Immunosuppressants are mostly used in patients with CD, while antibiotics in patients with UC at the beginning of treatment.

**Keywords :** inflammatory bowel disease, antibiotics, corticosteroids, immunosuppressants, biological agents

**PHYSICAL ACTIVITY AND EVALUATION OF LOWER BACK PAIN IN  
STUDENTS OF PHYSIOTHERAPY AND NURSING**

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The younger population today often has problems with pain affecting the lower back caused by certain lifestyle habits. Since sedentary lifestyle is present on a daily basis, it was investigated how low back pain is related to physical activity. The study involved 113 participants who were interviewed through a questionnaire that included respondents' opinions and assessments of the relationship between physical activity and low back pain. General and specific quality of life were assessed using the VAS Pain Assessment Scale and the Oswestry Questionnaire. The results of the study show that certain lifestyle habits, such as sitting for hours, have a statistically significant difference in 58.41% of cases, as well as the influence of pain on concentration in learning. The results show that the index of the Oswestry questionnaire reaches 26.611% (27%), confirming the percentage of disability, which in this case is moderate disability according to the published instructions (21-40%). A statistically significant difference was found in the Oswestry questionnaire total according to the type of biomedical and health students, with the highest median (16 (11-21)) recorded in the graduate nursing student respondents and the lowest (9.5 (9-13)) in the non-graduate nursing student respondents. In addition, a positive correlation was found between mean force and pain level in the Oswestry questionnaire results ( $\tau = 0.448$ ).

**Keywords:** physical activity, life habits, sedentary lifestyle, quality of life, lower back pain, moderate disability

**USING US AND MRI IN PRENATAL DIAGNOSIS OF RARE MULTIPLE FETAL MALFORMATIONS – OEIS COMPLEX**

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OEIS complex is extremely rare group of congenital malformations consisting of an omphalocele, exstrophy of cloaca, imperforate anus and spinal defects. OEIS complex represents the most severe manifestation of a spectrum of birth defects with incidence of 1 in 200 000-400 000 pregnancies. It is a midline defect occurring in early blastogenesis or in mesodermal migration. The ethiology is heterogeneous. Several reports show occurrence in siblings, concurrent occurrence in monozygotic twins and frequent occurrence in IVF pregnancies. We report a case of OEIS complex in an IVF pregnancy, diagnosed prenatally at 35 weeks gestation by ultrasound (US) and confirmed by magnetic resonance imaging (MRI). We report a case of a 30-year-old prim gravida, pregnancy after IVF fertilization, was referred to our clinic at 35 weeks gestation with a neural tube defect. US showed a lumbosacral meningomyelocele with no visualisation of fetal bladder and vessels. A solid tubular anterior abdominal-wall mass was detected with a close proximity to a single umbilical artery (SUA). Fetal US suggested prenatal diagnosis of OEIS complex. MRI (MR „Simens, Esbree 1,5 T“) confirmed a lumbosacral neural tube defect 3x5cm in size, omphalocele 6cm in length (with liver and small bowel herniating into the sac) and the absence of fetal bladder. Brain ventricular system was mildly dilated (up to 12mm). Internal organs and limbs were normal, but genital organs could not be clearly identified. Defect in pelvic bones could not have been excluded. A vigorous baby with ambiguous genitalia was born by a scheduled C-section at 38 weeks, birth weight and length 3149(50th percentile) and 49cm (50th percentile) respectively. Immediately after birth the child was admitted to the NICU of a tertiary care centre. As a conclusion, MRI imaging is an excellent method for exact diagnosis and useful adjunct to US for complex fetal anomalies. MRI helps to visualise fetal anatomy and structural defects more exactly. In our case where suspicion was made with US, we were able to confirm the diagnosis using MRI, provide better prenatal counselling, alert a tertiary centre and plan reconstructive surgery in advance.

**Keywords:** OEIS complex, MRI, prenatal diagnosis, multiple congenital abnormalities, ultrasonography

## **HIPOVITAMINOZA D U DEPRESIVNIH BOLESNIKA**

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Vitamin D ili kalciferol odnosno vitamin sunca je esencijalan mikronutrijent važan za naše cjelokupno zdravlje. Njegova specifičnost je široki spektar djelovanja na organizam u usporedi sa ostalim vitaminima radi čega vitamin D nalikuje na hormone. Očekuje se njegova sve veća primjena u svrhu liječenja i prevencije brojnih bolesti. Danas je u literaturnim podacima općeprihvaćeno da je nedostatak vitamina D to jest hipovitaminoza D javnozdravstveni problem koji utječe na zdravlje te mnoge akutne i kronične bolesti. Smatra se kako je hipovitaminoza D povezana sa različitim psihičkim entitetima.

U radu će se prikazati rezultati istraživanja koje je obuhvatilo hospitalizirane bolesnike u periodu od veljače 2021. g. do svibnja 2022. g. na Zavodu za integrativnu psihijatriju, Klinike za psihijatriju, Kliničkog bolničkog centra u Osijeku. Većina ispitanih bolesnika na Zavodu su bili sa dijagnosticiranim depresivnim poremećajem, njih 37% od čega su njih velika većina, čak 84%, imali verificiranu hipovitaminozu D. Autori će u istraživanju analizirati varijable koje se odnose na sociodemografske podatke o bolesnicima, dijagnostičke kategorije sa laboratorijskim vrijednostima 25-OH vitamin D3. Ovim istraživanjem htjeli smo naglasiti utjecaj vitamina D na mentalno zdravlje pojedinca i skrenuti pozornost na važnost tog aspekta u liječenju i praćenju bolesnika te istaknuti potrebu za dalnjim randomiziranim kontroliranim ispitivanjima vitamina D sa svrhom prevencije i liječenja psihičkih poremećaja.

Ključne riječi: vitamin D, hipovitaminoza D, psihijatrijski poremećaji, depresivni poremećaj

## **RADIOTERAPIJSKE TEHNIKE U KBCO**

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U KBC-u Osijek pacijenti se zrače dvjema radioterapijskim tehnikama: 3D CRT (3D konformalna terapija) i VMAT (volumetrijski modulirana lučna terapija). Obje tehnike imaju isti cilj, isporučiti terapijsku dozu zračenja na ciljni volumen uz maksimalnu poštedu okolnih organa. U periodu od 01.01.2015. - 01.01.2018. ozračeno je 2 544 pacijenta 3D CRT tehnikom, dok je u periodu od 01.03.2019. - 01.03.2022. ozračeno 3 226 VMAT tehnikom, što je porast broja pacijenata od 21,11%. Za usporedbu radioterapijskih tehnika promatran je period od 3 godine. Regije od interesa bile su pluća, želudac i prostata. U zadanim periodu ukupno je 756 pacijenata zračenih 3D konformalnom terapijom te 1 003 zračenih VMAT-om. Porast broja pacijenata se bilježi za regije pluća i prostate (15,87% i 33,98%) dok je jedini pad za regiju želuca. Izradom plana zračenja za obje tehnike na istom pacijentu, osigurani su isti uvjeti za usporedbu tehnika. Na DVH-u (dozno-volumetrijskom histogramu) prikazana je usporedba doza zračenja za klinički ciljni volumen (CTV), planirani ciljni volumen (PTV) i organe od rizika (OARs). Zračenje VMAT tehnikom ima strože kriterije od zračenja 3D konformalnom tehnikom. Pridodata je veća važnost organima od rizika te njihovoj zaštiti. Najbolji prikaz prednosti zračenja VMAT tehnikom u odnosu na 3D konformalnu možemo vidjeti iz DVH, gdje je vidljiva bolja pokrivenost CTV-a i PTV-a u VMAT tehnici te su organi od rizika primili manju dozu zračenja.

VMAT radioterapijska tehnika pruža bolju pokrivenost ciljnog volumena od interesa i poštedu organa od rizika u idealnoj situaciji. Samo u pojedinim situacijama se nije pokazala boljom, kada se radi o nesuradljivom pacijentu s puno komorbiditeta te nemogućnošću mirnog postavljanja i zadržavanja tog položaja od strane pacijenta.

Ključne riječi: VMAT, 3D CRT, pluća, želudac, prostata

**PROGNOSTIC INDICATORS OF PATIENT SURVIVAL IN THE TERMINAL STADIUM OF CHRONIC RENAL FAILURE**

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The objective of this study was to determine whether the values of some biochemical parameters from the blood may be associated with mortality in patients who are in the terminal stadium of chronic renal failure and are treated with chronic hemodialysis.

Therefore, I wanted to determine the importance of biochemical parameters from the blood as prognostic indicators, comparing the values of these parameters in a group of patients who died during the treatment with a group of patients who survived.

The study was conducted as a retrospective cohort study with 160 included patients who were treated by hemodialysis at the Department of dialysis at Clinical Hospital Center Osijek.

Patients have been followed for the past few years measuring laboratory values from the blood every 2 months. The data were taken from the archive of Clinical Hospital Center Osijek and processed using the SPSS 16.0 program (Statistical Package for Social Science Inc..., Chicago, IL, USA ).

Analysis of the results has shown that average number of erythrocytes had had an effect on the outcome of patients ( $p = 0.028$ ), as well as concentration of hemoglobin ( $p=0.015$ ), MCHC ( $p=0.020$ ), RDW ( $p = 0.044$ ), the value of total cholesterol ( $p = 0.009$ ), triglycerides ( $p = 0.014$ ), LDL ( $p=0.037$ ) and albumin ( $p <0.001$ ). For other variables, there are changes to treatment outcome, but they are not statistically significant.

As a conclusion, variables associated with the outcome of the treatment have been gradually excluded by applying multivariate logistic regression and the final result has shown that the outcome of treatment is associated mostly with values of albumin. Albumin values as an indicator of the general condition of the whole organism could be a significant prognostic indicator of survival.

Keywords: chronic renal failure, hemodialysis, albumin, survival

**KINETOCHEORE- AND CHROMOSOME-DRIVEN TRANSITION OF  
MICROTUBULES INTO BUNDLES PROMOTES SPINDLE ASSEMBLY**

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Mitotic spindle assembly is crucial for chromosome segregation and relies on bundles of microtubules that extend from the poles and overlap in the middle. However, how these structures form remains poorly understood. Here we show that overlap bundles arise through a network-to-bundles transition driven by kinetochores and chromosomes. STED super-resolution microscopy revealed that PRC1-crosslinked microtubules initially form loose arrays, which become rearranged into bundles. Kinetochores promote microtubule bundling by lateral binding via CENP-E/kinesin-7 in an Aurora B-regulated manner. Bundle separation is driven by steric interactions of the bundle-associated chromosomes at the spindle midplane since spindles with uncondensed or unengaged chromosomes are narrower. In agreement with experiments, theoretical modeling suggests that bundles arise through competing attractive and repulsive mechanisms. Finally, perturbation of overlap bundles led to inefficient correction of erroneous kinetochore-microtubule attachments. Thus, kinetochores and chromosomes drive coarsening of a uniform microtubule array into overlap bundles, which promote not only spindle formation but also chromosome segregation fidelity.

**Keywords:** overlap bundles, kinetochores, CENP-E, Aurora B, spindle assembly, segregation fidelity

**PERITUMORAL CLEFTING AND EXPRESSION OF MMP-2 AND MMP-9  
IN BASAL CELL CARCINOMA OF THE SKIN**

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Peritumoral clefting is one of the main histologic features of basal cell carcinoma of the skin (BCC). The aim of the study was to analyze the expression of MMP-2 and MMP-9 both in cells of basal cell carcinoma and in the adjacent stroma and to correlate the findings of immunohistochemical analysis with the presence of peritumoral clefting.

The study was made on archival material comprising 48 cases of BCC. These were scanned for the presence of peritumoral clefts. The results of immunohistochemical staining for MMP-2 and MMP-9 were determined semiquantitatively using immunohistochemical staining index (ISI).

Peritumoral retractions were found in 40 BCC cases. Positive immunohistochemical reaction for MMP-2 in tumor cells was found in 47 cases and in all cases in the adjacent stroma. Positive immunostaining for MMP-9 in BCC tumor cells was observed in 37 cases and in all cases in the adjacent stroma. There was no statistically significant association between peritumoral retractions and expression of MMPs. A statistically significant correlation was found in the expression of both MMP-2 and MMP-9 between the tumor and the stroma.

Tumor cells elaborate MMP-2 and -9, but they also produce some other factors that may induce production of MMPs in adjacent stromal cells. The role of MMPs in the development of peritumoral clefts could not be confirmed.

Keywords: basal cell carcinoma, MMP-2; MMP-9, histopathology, peritumoral clefting

**CHANGES OF TUNNELING NANOTUBES AND TUMOR MICROTUBES IN  
CELLULAR STRESS RESPONSE**

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The cell environment and intercellular connections have a major impact in cellular stress response. One type of cell to cell connections are tunneling structures that interconnect the cells. These are tunneling nanotubes (TNTs) and tumor microtubes, (TMs). These are membranous structures made of F-aktin. In some cell types they also have microtubules. Their main role is transport of organelles, small vesicles and molecules between adjacent and remote cells. In this work, tunneling structures in U2OS cells are described. To visualize tunneling structures, fluorescent staining methods and confocal microscopy were used. Cells were treated with cold shock and with sHSP27 inhibitor J2 to see how environmental and oxidative stress affect the number of tunneling structures. As a result, cold shock treatment was found to increase the number of TNTs and TMs, while the treatment with sHSP27 inhibitor J2 reduced the number of structures. Additionally, localization of protein sHSP27, mitochondria and membranous structures that refer to the transport is noted.

Keywords: tunneling nanotubes, tumor microtubes, sHSP27

**DICTYOSTELIUM IQGD IS A RHO-REGULATED IQGAP INVOLVED  
IN LARGE-SCALE ENDOCYTOSIS**

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IqgD is an IQGAP-related protein from the amoeba *Dictyostelium discoideum*. IQGAPs are evolutionarily conserved, multidomain proteins that serve as scaffolds to integrate diverse signaling pathways. They regulate various cellular processes such as migration, adhesion, and vesicle trafficking. IQGAP proteins directly bind actin filaments via their calponin homology domain (CHD). They can further cross-link them into bundles, which is dependent on the dimerization and oligomerization of IQGAP molecules. Oligomerization is facilitated by binding of active Cdc42 and Rac1, members of the Rho family GTPases, to the GAP-related domain (GRD). IQGAPs also regulate actin dynamics via interaction with the nucleation-promoting factor N-WASP and the actin-assembly factors Arp2/3 complex and formin Dia1, thus promoting the formation of protrusive structures at the cell leading edge. *Dictyostelium* IqgD is a fimbrin-related RasGAP that contains a CHD duplex, a coiled-coil region, a GRD/RasGAP domain, and a RasGAP\_C-terminal (RG Ct) extension. We show by confocal microscopy that fluorescently labeled IqgD in live *Dictyostelium* cells localizes to the entire cell cortex. However, it is enriched at the membrane patches that are primed for macropinocytosis. Similarly, IqgD is also strongly enriched at the base of the phagocytic cup during large particle engulfment. Next, we examined its presumed interactions with actin via CHD, and with Ras and Rho GTPases involved in large-scale endocytosis, via GRD. Using a yeast two-hybrid assay, we demonstrated a direct interaction between IqgD and Rac1A and Rac1C GTPases. Interestingly, while IqgD showed a higher affinity for constitutively active Rac1A, it prefers binding to dominant-negative Rac1C. Interaction with endogenous actin was demonstrated by Co-IP. The presented data strongly suggest that the *Dictyostelium* protein IqgD regulates actin cytoskeleton in large protrusions such as macropinocytic and phagocytic cups and that Rho GTPases Rac1A and Rac1C regulate its activity.

Keywords: IqgD, IQGAP, Rho, actin, *Dictyostelium*

**INTERACTION OF ENVIRONMENTAL CONTAMINANTS WITH  
ZEBRAFISH (*Danio rerio*) MULTIDRUG AND TOXIN EXTRUSION  
PROTEIN 3 (Mate3/Slc47a3)**

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Multidrug and toxin extrusion proteins belong to the superfamily of solute carriers. They function as bidirectional transporters (efflux of substrates is linked to proton-coupled electroneutral exchange) and they primarily mediate the elimination of cationic compounds. Phylogenetic analysis revealed six Mate transporters in teleost fishes annotated as Mate3-8. Because knowledge of non-mammalian Mates is still scarce, for molecular characterization of Mate transporters we used zebrafish (*Danio rerio*) as an established model organism. The zebrafish *mate3* gene, encoding the transmembrane protein Mate3, is constitutively expressed during embryonic development, followed by marked tissue-specific expression in adulthood. Mate3 is highly expressed in the kidney, intestine, testis, and brain of males, while expression is very low to moderate in females. It has been shown to interact with xenobiotic compounds, suggesting a role in efflux of toxic compounds. The aim of this study was to analyze the interaction of environmental pollutants with zebrafish Mate3 using a high-throughput screening assay. For this purpose, we have developed a stable zebrafish Mate3 cell line (Flp-In-293 / drM3 cell line) and standardized a cellular uptake assay using DAPI and ASP<sup>+</sup> as fluorescent model substrates. The developed assay was used to identify interactors of the zebrafish Mate3 transporter and to discriminate the nature of the interaction with a broad spectrum of 67 different environmental contaminants (industrial chemicals, pesticides, and pharmaceuticals). As a result, highly potent Mate3 interactors were identified in all of the above groups, with the pharmaceuticals pyrimethamine ( $IC_{50} = 1 \mu\text{M}$ ) and mitoxantrone dihydrochloride ( $IC_{50} = 1 \mu\text{M}$ ) being the most potent interactors. Some of the identified interactors could be of environmental concern and their interaction with Mate3 could lead to impairment of its normal efflux function, making the fish more sensitive to environmental pollutants. Furthermore, knowledge on pharmaceuticals that act as Mate3 substrates and inhibitors may be relevant for biomedical applications.

Keywords: Mate3, Zebrafish, High throughput screening assay, HEK293 Flp-In cells, Molecular docking

**NANOPORE SEQUENCING OF HARD-CUTICLES *TRIBOLIUM* BEETLES**

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Recent advances in the field of genome sequencing, especially Nanopore technology, have enabled the assembly of complex genomes and the analysis of repeat-rich regions. One of the most important factors for successful sequencing and assembly is the isolation of high molecular weight (HMW) DNA of adequate purity in sufficient quantity. Isolation of DNA with these qualities proves to be a difficult task for tissues, but becomes even more complicated when hard exoskeletons are involved. In addition, commercially available kits are often not tested and optimized for insects. For that purpose, we have developed a reproducible method for the isolation of HMW DNA from *Tribolium* beetles and its use in Nanopore sequencing. In short, the isolation protocol consists of isolating the cell nuclei using a specific buffer and then purifying the DNA on an anion-exchange chromatography column. This procedure results in HMW DNA of adequate properties for library preparation, which showed to be insufficient amount for multiple runs on a single Nanopore MinION sequencing flow cell, yielding up to 13 Gb of output. We have also found that DNA shearing increased average N50 read values up to 26 kb, together with better overall pore health during sequencing experiments. Even though this protocol was tested on *T. castaneum* and two closely related species, described optimization steps could be beneficial when applied to any organism that poses a challenge using conventional DNA isolation methods.

Keywords: Nanopore sequencing, *Tribolium castaneum*, high molecular weight DNA

**MULTI-OMICS ANALYSIS OF HNSCC REVEAL NON-OVERLAPPING  
EPIGENOMIC REGULATION IN GENE EXPRESSION**

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Head and neck squamous cell carcinoma (HNSCC) is one of the most common malignancies demonstrating large heterogeneity, partly due to the different anatomical sites of origin of the tumour, even though it arises from a single cell type. Moreover, depending on the HPV status, we distinguish two different groups of patients, which differ significantly in terms of lifestyle, age and 5-year survival rates. The additional role of (rather common) HPV infection on the aetiology, genetics, and epigenetics of cancer cells is not fully understood. In this study, we aim to identify different epigenetic patterns in HNSCC, namely regulation by DNA methylation and microRNAs, as epigenetic regulation is thought to be the major driving force in HNSCC development. The study cohort consisted of tumour samples from 20 individuals (10 HPV-positive), 10 of which were collected from the oropharynx (6 HPV-positive) and another 10 from the oral cavity (4 HPV-positive), with 6 tonsil samples from healthy participants as the control group. We performed a multi-omics analysis that included transcriptome, microRNA transcriptome and DNA methylation profiles to investigate the association between the two mechanisms to the gene expression levels, as well as the changes upon HPV infection. Expectedly, the tumour samples showed an overall decrease in DNA methylation levels. Finally, by using predictions of target genes under transcriptional regulation by microRNA or DNA methylation, we observed that the differentially expressed genes in tumour samples were mostly regulated by only one of the two epigenetic mechanisms, with distinct biological pathways for each regulatory mechanism.

**Keywords:** Head and neck squamous cell carcinoma, epigenetic regulation, transcriptomics

**SATELLITOME CHARACTERIZATION OF THE  
BLACK FLOUR BEETLE *TRIBOLIUM MADENS***

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Heterochromatic regions of eukaryotic genomes consist of large portions of repetitive DNA elements. Repetitive DNA elements are often dominated by satellite DNAs (satDNAs), tandemly repeated non-coding sequences located primarily in the (peri)centromeric and (sub)telomeric chromosomal regions. Although detecting these sequences in a genome used to be difficult, high-throughput sequencing technologies now allow the disclosure of entire satellitomes, i.e., comprehensive collections of satDNAs in a single genome. The black flour beetle *Tribolium madens* belongs to the genus *Tribolium*, whose species are known for a large share of satDNAs in the genome content. So far, the two satDNAs have been detected in *T. madens*, comprising together 34% of the genomic DNA. However, there is yet no evidence of other *T. madens* satDNAs. In this work, we addressed the *T. madens* satellitome by applying a high-throughput sequencing approach. First, we sequenced the whole *T. madens* genome by two different technologies - Illumina and HiFi PacBio. Based on the unassembled short Illumina reads, the computational pipeline TAREAN defined the putative satDNA consensus repeat units. By mapping the consensus sequences to the long and highly accurate PacBio HiFi reads, we explored their tandem organization. In this way we discovered ten new satDNAs. As each of newly detected satDNA comprises less than 0.05% of the genome, these sequences represent low copy satDNAs. Fluorescence *in situ* hybridization revealed that the low-copy satDNAs are scattered on different chromosomes of the complement (2n=20 + supernumerary chromosomes). To gain insight into the evolution of *T. madens* satDNAs, we further investigated whether there were similar sequences in the genomes of closely related species *Tribolium castaneum* and *Tribolium freemani*. The satDNAs, which have the orthologous sequences in the related genomes, have been shown to evolve according to the principles of concerted evolution.

Keywords: *Tribolium madens*, satellitome, Illumina, PacBio

**OCCURRENCE OF DEOXYNIVALENOL AND CULMORIN AND  
THEIR DERIVATIVES AT TWO DIFFERENT LOCATIONS IN NATURALLY  
AND FHB-INOCULATED PLANTS AND THEIR  
POSSIBLE ROLE IN DISEASE SEVERITY**

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Fusarium head blight (FHB) is a serious disease of wheat that can cause yield and quality losses. This is accompanied by mycotoxin production and toxic effects in humans and animals. Culmorin (CUL) can coexist with trichothecene mycotoxins and potentially affect their toxicity. The aim of this study was to analyse the occurrence of deoxynivalenol (DON) and CUL and their derivatives with LC/MS-MS in six winter wheat varieties at Osijek and Tovarnik in naturally infected and FHB-inoculated plants. We aimed to determine whether the inhibitory effects of CUL could lead to synergistic toxicity of DON and CUL. At location Osijek, the highest accumulation of CUL occurred in the FHB-susceptible variety Golubica ( $29087 \mu\text{g kg}^{-1}$ ), while 15-hydroxyculmorin, 15-hydroxyculmoron and 5-hydroxyculmorin showed the same tendency of increase in wheat varieties as for DON and its derivatives. In addition, FHB inoculations resulted in increase in the concentration of all mycotoxins studied. DON, its derivatives and 5-hydroxyculmorin were increased at Tovarnik due to higher FHB disease pressure for all wheat varieties, compared to the Osijek. FHB severity was only moderately correlated with CUL/DON ratio, with a negative correlation at Tovarnik and a positive correlation at Osijek. A negative correlation at Tovarnik indicated that varieties with the higher area under the disease progress curve (AUDPC) values for FHB severity had more DON than CUL contamination. The sum of CUL and DON was closely correlated with disease infestation, suggesting that the production of these two metabolites contributes to FHB severity. However, further studies are needed to verify whether CUL may play a possible role as a *Fusarium* virulence factor involved in increased phytotoxicity.

Keywords: culmorin, deoxynivalenol, *Fusarium*, wheat

**EFFECT OF LIPID RAFT DISRUPTION AND EGFR SIGNALING  
PATHWAY ACTIVATION ON NRF2 AND AQP3 IN BREAST CANCER CELL  
LINES OF DIFFERENT MALIGNANCIES**

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Aquaporins are transmembrane channels that facilitate the transport of water across the plasma membrane and are crucial for cellular homeostasis and volume regulation. In addition to water, aquaporins can transport other small polar molecules, such as glycerol, ions, CO<sub>2</sub>, urea, and hydrogen peroxide, important in cell proliferation, migration, and adhesion. Because of these functions, tumor cells often have a higher expression of aquaporins, which supports their growth. Of the 13 mammalian aquaporins, six can facilitate hydrogen peroxide and are therefore called peroxiporins. The mere existence of peroxiporins implies their involvement in the regulation of oxidative stress and antioxidative response and may affect NRF2 expression/activity. In addition to redox signaling pathways, peroxiporins can regulate other signaling pathways such as EGFR. Our aim was to study whether lipid raft disruption or EGFR signaling pathway activation affects aquaporin activity and thus affects NRF2 expression and PI3K/Akt signaling pathway activity. In this study, lipid rafts were disrupted by cholesterol depletion using methyl-β-cyclodextrin. The effect of depletion was measured by MTT assay, expression of target proteins by Western blot, and aquaporin activity was assessed by H<sub>2</sub>O<sub>2</sub> permeability test. Relative gene expression was evaluated with quantitative real-time PCR. Depending on the breast cancer cell line, lipid raft disruption and EGFR signaling pathway activation had different effects on AQP3 and NRF2 expression, as well as PI3K/Akt signaling pathway activation.

Keywords: peroxiporins, EGFR signaling pathway, NRF2 expression, lipid rafts

**SALICYLIC ACID CONTRIBUTES TO BASAL DEFENSE OF *SOLANUM TUBEROSUM* AGAINST POTATO SPINDLE TUBER VIROID (PSTVd)**

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Viroids are small, circular, noncoding RNAs currently known to infect only plants. Potato spindle tuber viroid (PSTVd), the type species of Pospiviroidae, is the most important subviral pathogen of potato that can severely limit potato crop yields. Unlike plant resistance to most other pathogens, no naturally occurring resistance to PSTVd has been detected in potato cultivars. The ability of viroids to cause disease is determined by molecular interactions between the host plant and the viroid RNA. These interactions directly affect the development of symptoms and host plant defense responses, in which the signaling compound salicylic acid (SA) plays a key role. To evaluate the role of SA in the potato-PSTVd compatible interaction, we used transgenic NahG potato plants that are unable to accumulate SA. After infection with PSTVd, NahG transgenic lines showed pronounced symptom development such as stunted growth, necrosis of old leaves, and reduction in the size of young leaves and tubers. The dynamics of viroid replication and the expression of host genes, which are markers of defense responses, were followed in a time-course experiment during symptom development using real-time PCR. At 4 and 5 wpi, viroid RNA levels were significantly higher in infected leaves of NahG plants compared with wild-type plants, indicating delayed viroid accumulation in the wild-type plants. At 6 wpi, this difference in viroid replication between genotypes disappeared, suggesting that SA plays a role in the early phase of PSTVd infection of potato. The increased susceptibility of NahG plants correlated with the accumulation of reactive oxygen species (ROS), including hydrogen peroxide, impaired callose formation, and decreased expression of genes involved in SA -regulated basal defense responses, such as the pathogenesis-related genes PR -1 and PR -2. The obtained results suggest that SA is an important compound required for the basal defense of potato against PSTVd.

Keywords: salicylic acid, viroid, PSTVd

**PERIFERNI ŽIVČANI BLOK U KOMBINACIJI SA  
SPINALNOM ANESTEZIJOM ZA AUTOTRANSPLANTACIJU KOŽE  
– PRIKAZ SLUČAJA**

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Bolesnik u dobi od 71 godine, dijabetičar, sa loše reguliranom hipertenzijom, četiri preboljela cerebrovaskularna inzulta i tumorom mokraćnog mjehura primljen je radi kirurškog liječenja, devet dana nakon što je zadobio opekline obje potkoljenice i stopala, ukupne površine oko 11%, IIA i IIB stupnja.

Kod bolesnika je u dva akta planirano odizanje kožnog autotransplantata s gornjih ekstremiteta, a potom nekrektomija kože potkoljenica i stopala s aplikacijom istog.

Odizanje transplantata učinjeno je u supraklavikularnom bloku uz dodatnu primjenu lokalnog anestetika u interskalenski prostor zbog uzimanja transplantata s ramena. Nekrektomija u području donjih ekstremiteta učinjena je u spinalnom bloku. Pod kontrolom ultrazvuka prikaže se brahijalni pleksus u supraklavikularnom prostoru i spinalnom iglom 25G aplicira 10 ml 0,5% levobupivakaina, a zatim se u inteskalenski prostor aplicira još 10 ml istog lokalnog anestetika. U intervertebralni prostor na razini L3-L4, spinalnom iglom 25G aplicira se 2,8 ml 0,5% levobupivakaina.

Prije operacije je bolesnik premediciran peroralno midazolatom, bez potrebe za dodatnom sedacijom tijekom zahvata.

Kombinacija regionalne anestezije i neuroaksijalnog bloka omogućila je primjerenu anesteziju za planirani operacijski zahvat uz produljenu postoperacijsku analgeziju.

Bolesnik je za vrijeme zahvata bio u kontaktu, bez potrebe za dodatnom intravenskom sedacijom, uz održanu hemodinamsku stabilnost.

Opća anestezija u bolesnika opterećenog komorbiditeom povezana je sa višim rizikom nastanka perioperacijskih kardiovaskularnih i respiratornih komplikacija. Regionalnom anestezijom osigurali smo dobru perioperacijsku kontrolu boli uz izostanak značajnih oscilacija vitalnih parametara u odnosu na prijeoperacijski period.

Ključne riječi: periferni živčani blok, spinalna anestezija, autotransplantacija kože, opekлина

**TRANSPLATACIJA OSTEOHONDRALNOG ALOGRAFTA LATERALNOG  
KONDILA FEMURA, PRIKAZ SLUČAJA I RADNE STANICE**

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Transplantacija osteohondralnog alografta tehnika je kojom se može tretirati širok spektar patologije koja uključuje hrskavicu i podležeu kost. Tehnika je posebno pogodna za mlađu populaciju pacijenata sa značajnim ( $> 2.5 \text{ cm}^2$ ) osteohondralnim defektom, kao primarni ili kao revizijski zahvat. Predstavljamo slučaj 28-godišnjeg pacijenta koji se prezentirao dugogodišnjom boli u području lateralnog kompartimenta desnog koljena, najizraženijom prilikom fleksije. Učinjenom radiološkom obradom nađena je osteohondralna lezija dimenzija 3x2cm u području lateralnog kondila femura koja je shvaćena kao nestabilni OCD. Učinjen je pokušaj fiksacije s dva chondral darta. Nakon inicijalnog poboljšanja dolazi do pogoršanja stanja te je pacijent 8 mjeseci kasnije podvrgnut operativnom zahvatu u vidu artroskopske toalete te mikrofrakturne. Nakon drugog zahvata izostaje adekvatan klinički odgovor. Naposljetku se odlučujemo za kirurški zahvat transplantacije osteohondralnog alografta. U suradnji s Bankom tkiva Klinike za traumatologiju te Bankom tkiva i kosti Barcelona Španjolska pribavlja se smrznuti alograft lateralnog kondila femura. Korišten je Arthrex BioUni® OATS® instrumentarij. Zbog tehničkih uvjeta nije bilo moguće osigurati originalnu Arthrex radnu stanicu te dizajniramo vlastitu radnu stanicu za fiksaciju alografta tokom obrade koja se temelji na modifikaciji standarnog Ilizarov okvira. Operativni zahvat je učinjen u spinalnoj anesteziji. Izmjerena je veličina defekta te je prema mjeri obrađen alograft fiksiran na radnoj stanicici. Zatim je učinjena transplantacija grafta. Operativni zahvat i postoperativni tijek protekao je uredno. Šest tjedana nakon zahvata radiološki su vidljivi znakovi koštanog preraštavanja, bez znakova odbacivanja implantata. Pacijent je subjektivno bez tegoba, urednog opsega pokreta. Zaključak prikaza slučaja je da je transplantacija osteohondralnog alografta operacija s izvrsnim ranim rezultatima za velike osteohondralne defekte u mlađoj populaciji. Također, naša radna stanica može biti izrađena uz pomoć materijala koji se nalaze u inventaru svakog traumatološkog odjela bolnice te je prikladna alternativa komercijalno dostupnom setu.

Ključne riječi: osteohondralni alograft, koljeno, transplantacija

**ROLE OF CHIARI OSTEOTOMY IN TREATING  
DEGENERATIVE HIP ARTHRITIS: A REVIEW**

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Chiari pelvic osteotomy (CPO) or medial displacement pelvic osteotomy is a surgical procedure of making a congruent shelf above intact hip joint by using the cancellous bone of ilium along with capsular interpositioning to contain the femoral head and bear weight. CPO is usually considered a salvage procedure indicated in patients with dysplastic acetabular sockets as a part of developmental hip dysplasia. It has been widely performed for several decades but since 1990s number of CPO procedures has decreased because of use of other pelvic osteotomies, total hip arthroplasty (THA), ultrasound screening and narrowing indications for this procedure. However, CPO is not a historical procedure and it has viable indications in modern orthopaedic surgery, especially in patients who prefer joint-conserving procedure or in situations when THA is not feasible. Moreover, keeping the native hip as long as possible should be preferred modern orthopaedic approach. The Chiari osteotomy can produce excellent results in patients who have right indications and when other procedures are not suitable. On the other hand, there are several disadvantages of this procedure such as narrowing of the pelvis, shortening of the limb and risk of sciatic nerve palsy. With in-depth analysis of literature, we discuss indications for CPO, surgical technique of CPO, THA conversion rate after CPO, the interval from the CPO to THA and CPO survival rate.

**Keywords:** Chiari osteotomy, total hip arthroplasty, degenerative hip arthritis

## **LAPAROSKOPSKA SPLENEKTOMIJA- NAŠA ISKUSTVA**

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Prva laparoskopska splenektomija učinjena je 1991.godine (Delaitre). Slezena je organ koji je pogodan za laparoskopsku tehniku operiranja jer ima relativno standardnu anatomijsku strukturu, a nakon odstranjenja ne zahtijeva rekonstrukciju. S obzirom na dobro poznate prednosti laparoskopske u odnosu na otvorenu kiruršku tehniku, laparoskopska splenektomija kroz godine počela se nametati kao metoda izbora za gotovo sve bolesti slezene. U Kliničkoj bolnici Dubrava prva laparoskopska splenektomija učinjena je 23.5.2003. godine. Ovim radom želimo prikazati naša iskustva u vidu indikacija za laparoskopsku splenektomiju kao i komplikacije postupka. Od 2003. do 2022. u našoj ustanovi učinjeno je 78 laparoskopskih splenektomija. 50 pacijenata imalo je slezenu normalne veličine, dok ih je 28 zadovoljavalo kriterije za splenomegaliju. 60 pacijenata imalo je benignu bolest, a 18 bolesnika malignu bolest. Operirano je 26 muških bolesnika i 52 žene. Raspon godina operiranih bolesnika bio je od 18 do 81 (prosjek 49.6). Od intraoperativnih komplikacija za spomenuti je lezija kolona transverzuma u jednog pacijenta, koja je prepoznata odmah i zbrinuta laparoskopskim šivanjem. Od postoperativnih komplikacija imali smo 2 slučaja krvarenja iz aa.gastrica breves, od kojih je jedno zbrinuto laparoskopskom tehnikom. 5 bolesnika postoperativno je razvilo trombozu vena porte, od kojih je 4 imalo asimptomatsku kliničku sliku. Prosječna veličina uvećanih slezena bila je 22.8 cm, a vrijeme trajanja operacije uvećanih slezena bilo je dulje za 24 minute. Prosječno trajanje hospitalizacije bilo je 5 dana. Nije bilo perioperacijske smrtnosti. U zaključku možemo reći da je laparoskopska splenektomija sigurna i učinkovita metoda liječenja malignih i benignih bolesti slezene, ali mora biti izvedena od strane iskusnog laparoskopskog kirurga, posebno u slučaju splenomegalije.

Ključne riječi: laparoskopija, splenektomija, splenomegalija, komplikacije

**METASTATIC SPINE DISEASE –  
GUIDELINES OF TREATMENT AND INSTITUTIONAL EXPERIENCE  
FROM 11/2019 TILL 11/2021**

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Metastatic spine disease is common in patients with 3<sup>rd</sup> or 4<sup>th</sup> stage of lung and breast cancer. Only 25% of spine tumors are primary, 75% are the metastases from tumors of other known origin. In 25% of cases, the metastases are the first symptoms of the disease, and further diagnostics are necessary for primary tumor detection. Axial pain or neurologic deficits are usually the first signs of the disease. The routine X-ray and CT (computed tomography) scans are performed as initial diagnostic procedures with obligatory magnetic resonance for soft tissue invasion assessment. In most patients only palliative surgical treatment is planned: tumor reduction and spine stabilization.

Over a two year period, from 11/2019 to 11/2021, we performed 59 spinal stabilization procedures in patients with spinal tumors. Tumor biopsy confirmed metastatic spine disease in 44 patients, with lung and breast cancer being the most common primary tumors. After recovery, patients were transferred to the oncology department for adjuvant chemotherapy or radiotherapy.

Keywords: spine, metastatic disease, palliative surgery, oncologic surgery

**ZADOVOLJSTVO PERIOPERACIJSKOM SKRBI U PACIJENATA  
PODVRGNUTIH OPĆOJ ILI REGIONALNOJ ANESTEZIJI**

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Opća i regionalna anestezija dvije su različite, ali komplementarne tehnike usporedive po stupnju sigurnosti odnosno rizika, sa svojim realnim prednostima i nedostacima te među pacijentima doživljavane na različite načine. Cilj istraživanja je bio utvrditi stav ispitanika prema općoj (OA) i regionalnoj anesteziji (RA), zadovoljstvo nakon iskustva OA ili RA, razliku u stupnju zadovoljstva nakon OA u odnosu RA te čimbenike koji utječu na ocjenu ukupne anestezijske skrbi. U ovom presječnom istraživanju ispitanici su pacijenti liječeni u KBC Osijek u razdoblju od 1. 2. do 30. 4. 2018. Kriterij uključivanja je planirana operacija na gornjim i donjim udovima te genito- urinarnom području izvodiva u OA ili RA. Nisu uključeni hitni pacijenti, roditelje i osobe s kognitivnim smetnjama. Relevantni podatci prikupljeni su putem anonimnog upitnika. Podijeljeno je 288, a vraćeno 240 ispunjenih upitnika. Većina (62,5 %) sudionika izjasnila se za OA s razlikom među pripadnicima različitih odjela. Ispitanici s ortopedije (63,9 %) odabrali bi RA, 51,1 % sudionika s traumatologije OA, a 48,9 % RA. Ispitanici s vaskularne kirurgije, urologije i ginekologije odabrali bi OA (69 %, 80,5 %, 85,7 %). Zadovoljstvo anestezijskom skrbi sudionici su iskazali ocjenom 4 (24,2 %) i 5 (69,2 %). Nakon iskustva s anestezijom 61,7 % ispitanika ponovo bi odabralo OA. Više od 60 % ispitanika podržava OA. Izkustvo i okruženje utječu na njihov stav prema OA i RA. Ispitanici s ortopedije i ranijim iskustvom RA, odabrali bi RA. Sudionici s traumatologije bi, neovisno o iskustvu prihvatali OA i RA, a ispitanici s odjela na kojima je manje zastupljena RA, odabrali bi OA. Više od 90% ispitanika pokazalo je visok stupanj zadovoljstva anestezijskom skrbi. Obaviještenost, mogućnost sudjelovanja u izboru anestezije, psihološka potpora i pojavnost neželjenih događaja povezanih s anestezijom utjecali su na ocjenu ukupne anestezijske skrbi.

Ključne riječi: opća anestezija; perioperacijska skrb; regionalna anestezija; zadovoljstvo pacijenata

## **HEDGEHOG-GLI SIGNALING CONTROLS PROLIFERATION AND INVASIVENESS OF HEAD AND NECK SQUAMOUS CELL CARCINOMA**

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Head and neck squamous cell carcinoma (HNSCC) is the 6th most malignant tumor type. They usually develop in men over 50 years of age, and are most often associated with smoking, alcohol consumption, bad oral hygiene and infection with human papillomaviruses. HNSCC is mostly treated surgically, and only higher stages (III or IV) receive radio- or chemotherapy after surgery. Chemotherapy is very general, cisplatin is most commonly used. The prognosis for high stages is poor, and there is a requirement for better and more effective therapeutics. One of the signaling pathways involved in the development of HNSCC is the Hedgehog-GLI (HH- GLI) signaling pathway. HH-GLI is a proliferative pathway mostly active during embryonic development, and deactivated in differentiated adult tissues, except in the stem cell compartment where it is involved in stem cell maintenance and response to injury. Its aberrant activation is associated with many tumors, and this occurs by different mechanisms in different tumor types. Pathway activation results in accumulation of transcription factors GLI (GLI1, GLI2 or GLI3), which are responsible for transcription of genes involved in proliferation, cell cycle, self-renewal, angiogenesis and epithelial- mesenchymal transition. In this work we wanted to examine the effect of several HH-GLI pathway inhibitors on HNSCC cell proliferation, migration and colony formation capabilities, and to determine which of the three GLI proteins is the main effector of the pathway. We tested the effect of cyclopamine, GANT-61 and lithium chloride on properties of 5 HNSCC cell lines, and analyzed expression of GLI proteins by Western blot. All the cell lines show better responsiveness to downstream inhibitors such as GANT-61 and lithium chloride in comparison to the upstream inhibitor cyclopamine, suggesting that the pathway is at least partly activated non- canonically. GLI3 was found to be the most expressed on both RNA and protein levels, and it was the most responsive of all three GLI proteins to pathway inhibition. Until now most publications have focused on GLI1 protein, and no data is available on the role of GLI3 in HNSCC. In this work we have demonstrated that GLI3 plays a major role in HNSCC cell lines.

Keywords : HNSCC; GLI ; GANT-61; LiCl

**AWARENESS REGARDING ORAL HEALTH AMONG  
ORTHODONTIC PATIENTS AND NON-ORTHODONTIC PATIENTS**

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The aim of the study is to examine oral health awareness among orthodontically treated and orthodontically untreated patients, to examine their attitude about the importance of oral hygiene, their familiarity with toothbrushing techniques and familiarity and source of information on the use of toothbrushes and other toothpastes. Also, the aim was to examine their opinion on the importance of brushing teeth and their interest in expanding their own knowledge about oral hygiene. The study is organized as a cross-sectional study. Study included 98 patients of dental and orthodontic surgeries of the Health Center in Osijek. The examination was conducted during April 2020. An anonymous survey questionnaire was used to conduct the research. 98 patients participated in the study. Orthodontically treated patients use a toothbrush three times a day, far more than orthodontically untreated patients. They use a soft toothbrush and interproximal (interdental) toothbrushes significantly more than orthodontically untreated patients. They no longer use mouthwash, compared to orthodontically untreated patients, but they go to regular check-ups much more than orthodontically untreated patients. Also, the orthodontist / dentist far more explains to them the importance of brushing teeth, which is not the case with orthodontically untreated patients. This study has shown that orthodontically treated patients have significantly more awareness regarding oral health and the importance of oral hygiene protocols, than orthodontically untreated patients.

**Keywords:** oral hygiene, teeth, orthodontically patients, knowledge, attitudes

## **AUTOLOGNI DENTINSKI GRAFT U PREZERVACIJI ALVEOLE**

Butorac Prpić I<sup>1,2</sup>, Marković L<sup>3</sup>

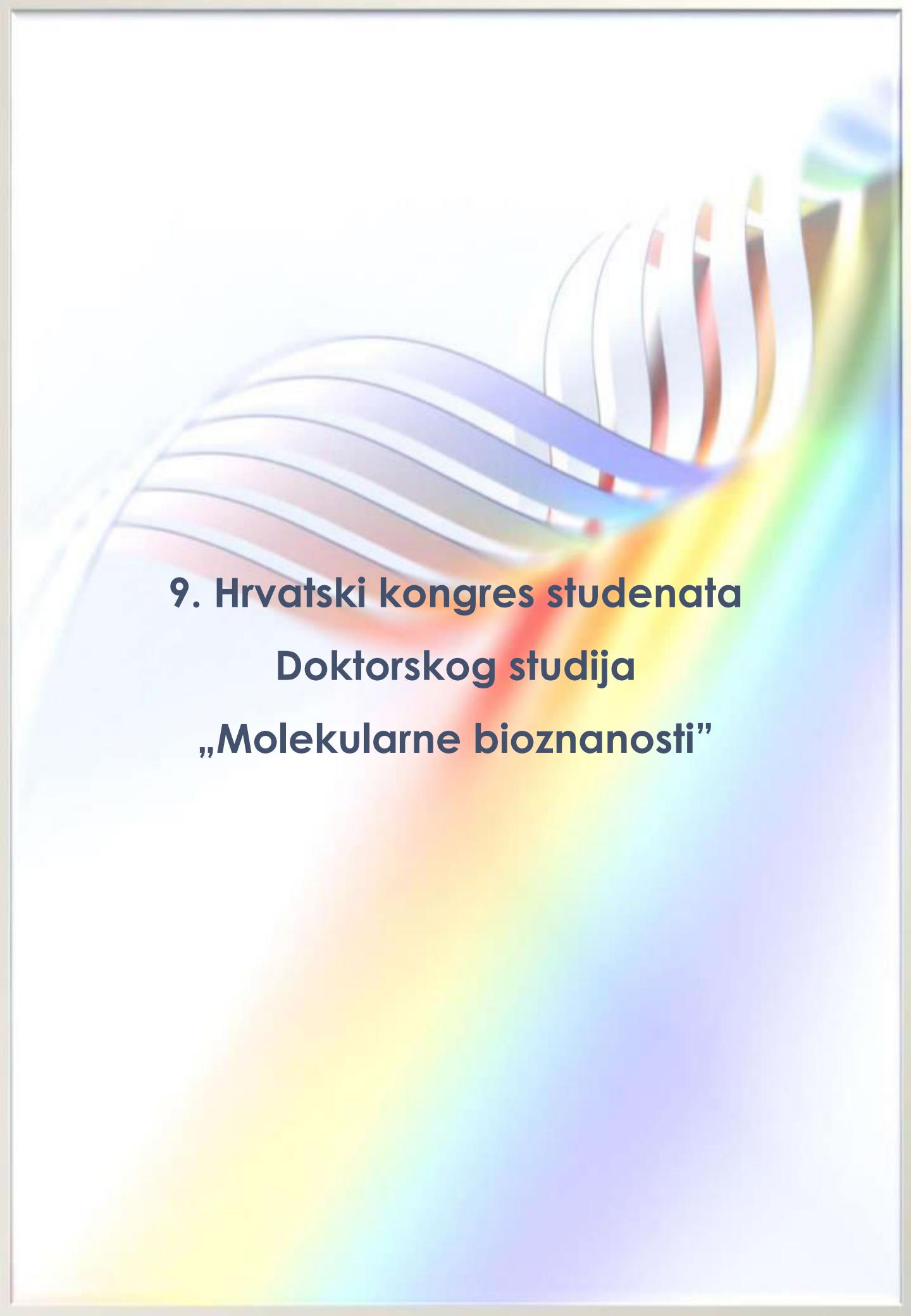
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Nakon ekstrakcije zuba dolazi do promjena u horizontalnoj i vertikalnoj dimenziji alveolarnog grebena koje ugrožavaju buduću implantološku terapiju. U današnje vrijeme postoje mnogi augmentacijski materijali kojima se dimenzionalne promjene pokušavaju reducirati i na taj način poboljšati rezultati implantološke terapije. Premda postoje mnogi augmentacijski materijali koji se koriste, do danas se zlatnim standardnom smatra autogeni koštani transplantat zbog svojih osteoinduktivnih i osteokonduktivnih svojstava. Postoje neki problemi s autogenim koštanim graftom, kao što su infekcije na donatorskom mjestu, ograničena količina dostupne koštane mase i brza resorpcija autogene kosti. Zbog svega navedenog, zadnjih godina radi se na istraživanju dentina kao alternativne zamjene autogenoj kosti. Mineralni i organski sastav zuba gotovo je identičan sastavu kosti. Stvoreni su novi uređaji, pomoću kojih se usitnjavaju zubi i pripremaju dentinski augmentativi u vremenskom razdoblju od 15 do 20 minuta od ekstrakcije zuba. Uvidom u ortopantomogram pacijentice uočena je periapikalna lezija zuba 46 te je predložena ekstrakcija zuba, uz prezervaciju alveole primjenom autolognog dentinskog grafta. Na dan operacije ekstrahiran je Zub 16, ekstrahirani Zub je bio očišćen od mekog tkiva i karijesnih lezija te je zdrobljen u mlincu. Dobiveni graft je pripremljen po specificiranom protokolu za upotrebu te pomiješan s autolognim faktorima rasta dobivenim tehnikom PRF-a. Nakon ekstrakcije zuba 46 alveola je augmentirana pripremljenim dentinskim graftom. Napravljen je ortopantomogram odmah nakon operacije i kontrolni 2 godine nakon. Dentin predstavlja korisnu zamjenu drugim graftovima: posjeduje biokompatibilnost, ne izaziva imunološke reakcije kod pacijenta, sličnog je sastava kao kost koja se i smatra idealnim graftom. Zbog svega navedenog, njegova primjena u prezervaciji alveole postala je sve više primjenjiva.

Ključne riječi: ekstrakcija zuba, augmentacija, koštani graft, dentin, prezervacija alveol



**9. Hrvatski kongres studenata  
Doktorskog studija  
„Molekularne bioznanosti“**