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Apstrakti/Abstracts

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Plenarno predavanje
Opening lecture

PERSONALIZED REFERENCE INTERVALS: THE ROAD TO PERSONALIZED LABORATORY MEDICINE

Abdurrahman Coskun

Acibadem Mehmet Ali Aydinlar University, School of Medicine, Department of Medical Biochemistry, Istanbul, Turkey

Interpreting laboratory data is a comparative procedure that requires reliable reference intervals for accurately interpreting patients' test results. Currently, reference intervals are obtained from populations and used for interpreting individual's laboratory data. In other words, individuals are considered as members of a population rather than as individual with unique characteristics. Reference value that are normal for a population may be abnormal for some individuals. Therefore, using population-based reference interval may not be appropriate for individuals. Individual-specific reference interval, derived from the individual's own data, should be used to interpret individual laboratory data. Recently, we developed an algorithm based on prediction intervals to estimate personalized reference intervals (prRI). The general equation for the prRI is as follows: Here, f(St) represents the time-dependent set point derived from the arithmetic mean of the repeated measurements, and f(R) represents the random component derived from the Gaussian combination of within-person/subject biological variation. Five measurement results obtained from repeated samples taken at the same time of day are sufficient to estimate the pRRI. The concentration of the analytes is influenced by three main physiological rhythms: (i) infradian rhythms or within-day variations, (ii) circadian rhythms, which are 24-hour cyclic variations, and (iii) ultradian rhythms, which have periods longer than 24 hours, such as the monthly variation observed in menstrual cycle hormones and seasonal variation observed in vitamin D, lipids, etc. To maintain the set point at a constant level, samples should be taken at the same time of day. Otherwise, due to within-day variation (infradian variation), the pRRI will change depending on the time samples are taken. In contrast to the set point, there isn't sufficient evidence to suggest that the random component of the prRI, which determines the upper and lower limits, is time dependent. The random component of the prRI can be estimated from an individual's own data or from the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Biological Variation database.

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Plenarne sekcije
Plenary sessions

ARTIFICIAL INTELLIGENCE: IS IT THE RIGHT TIME FOR CLINICAL LABORATORIES?

Mario Plebani

University of Padova, Padova, Italy

Laboratory medicine is a constantly progressing field with novel tests and techniques being developed and incorporated into the repertoire of clinical laboratories at an astonishing rate. As a central part of the healthcare system, clinical laboratories have been coping with incremental improvements in informatics for decades and have been pioneers in digitization and computer-assisted tools as software. This fact, in addition to the central role of clinical laboratories in patient healthcare, highlights the importance of improving timely and accurate diagnosis and patient care through AI. As a result, the laboratory medical profession may now be facing a big transformation due to disruptive technologies, namely digitalization, Big Data, AI and machine learning (ML). In particular, the adoption of AI tools seems to receive increasing interest in improving the pre-analytical phase -namely appropriateness in test request- and the post-analytical phase (laboratory report). In addition, AI tools have been found to potentially reduce errors in the total testing process, thus improving quality and patient safety. The potential application of AI and ML models to laboratory data could be relevant, but to manage the change and uncover additional benefits to patient care, there is an urgent need to adapt expertise within laboratories and to improve the cooperation between laboratories and AI experts. In addition, clinical laboratories must ensure that laboratory data are accurate and reliable to avoid the risk of sophisticated systems such as ML and AI using inaccurate results which in turn can lead to inaccurate and potentially harmful information. This concept has been well summarized in the mantra »garbage in, garbage out«.

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Plenarne sekcije
Plenary sessions

FLOWING THROUGH LABORATORY CLINICAL DATA: THE ROLE OF ARTIFICIAL INTELLIGENCE AND BIG DATA

Andrea Padoan

Department of Medicine (DIMED), University of Padova, Italy and Laboratory Medicine Unit, University-Hospital of Padova, Italy

In recent decades, the landscape of clinical laboratories has undergone a profound transformation with the advent of advanced technological tools and instrumentation. Among these innovations, Laboratory Information Systems (LIS), initially introduced in the 1970s, has evolved remarkably from rudimentary software to sophisticated platforms. Today, LIS plays a pivotal role in creating an archive of laboratory results and report generation and communicating with healthcare facilities and regional databases. Nowadays, the evolution of LIS, called Laboratory Information Management Systems (LIMS), has been facilitated by the confluence of several factors. These include significant advancements in various information technologies, integrating cost-effective sensors into analytical instruments, and enhanced interoperability with complementary digital tools. Consequently, this technological progress has catalyzed a surge in the volume of data generated within clinical laboratories (1). A comprehensive understanding of the Total Testing Process (TTP) evaluating the volume of data generated within clinical laboratories might underline that the analytical phase merely represents a fraction of the extensive data continuum inherent in the testing cycle. In this context, beyond the patients' test results and demographic values, LIS can serve as a repository for many supplementary information. Within the analytical phase, LIS captures test results and records ancillary information crucial for quality assurance and interpretation. These may encompass indices such as hemolysis, calibration curves, sample dilutions, assay repetitions, and adherence to technical validation protocols. Further, for »-omics« analyses, LIS plays a pivotal role in managing voluminous datasets, including mass spectra, proteomics, metabolomics, lipidomics, and sequencing files (2). LIMS are further equipped to integrate data from laboratory quality control systems encompassing both internal and external quality controls, as well as validation protocols for analytical methods. Notably, LIS tailored for genetic testing possesses the capability to interface with diverse instrumental software and sophisticated pipelines to distill vast sequences into clinically actionable insights. Data generated throughout the pre-preanalytical and preanalytical phases are relevant since both phases constitute a substantial portion of the TTP dataset (3). For the pre-analytical phase, these include sample collection and transportation information, centrifugation, sample dilution, etc.. During the post-analytical phase, LIS serves as a repository for interpretative comments and documentation regarding reviewing urgent results, issuing provisional reports, and subsequent communication with requesting clinicians. Emerging of the value of these data are of utmost importance for AI tools. These always require huge amount of information; despite the clinical laboratories are a producers of quality data, this fact is not always recognized. An evolution of technology and LIS might facilitate the operational efficiency of clinical laboratories but also favor the development of algorithms based on AI for high-quality, personalized patient care through comprehensive data management and interpretation.

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Plenarne sekcije
Plenary sessions

AI IN THE PREANALYTICAL PHASE

Salomón Martín Pérez

Macarena University Hospital, Seville, Spain

The preanalytical phase, crucial in the clinical analysis process, covers from the request to the preparation of the sample for analysis and is recognized for its high susceptibility to errors, with studies estimating that between 40% and 70% of analysis errors laboratory occur at this stage. These errors can range from incorrect patient or physician identifications to issues in patient preparation and extraction techniques, significantly impacting the quality of results and patient safety. The importance of this phase lies in its direct influence on diagnostic accuracy, highlighting the need for effective strategies to minimize errors, including continuous training and the incorporation of advanced technologies. Artificial intelligence (AI) offers us the potential to radically transform the pre-analytical phase in clinical laboratories, offering innovative solutions to address errors and optimize processes. Through data analysis and automation, AI can improve patient identification and correct association with their samples, significantly reducing human errors. In addition, AI-based systems can predict and alert about possible incompatibilities or errors in analytical requests and optimize sample logistics to ensure their correct preparation and storage. Implementing AI in the preanalytical phase not only increases efficiency and accuracy, but also frees laboratory staff from repetitive tasks, allowing them to focus on more critical aspects of clinical analysis. Ultimately, AI can be a key ally in the continuous improvement of quality and patient safety in the clinical laboratory.

HOW IS LABORATORY DATA USED AND CHARACTERIZED BY MACHINE LEARNING MODELS

Anna Carobene

IRCCS San Raffaele Hospital, Milan, Italy

The integration of artificial intelligence (AI) and machine learning (ML) into clinical practice represents a frontier in laboratory medicine that promises to enhance diagnostic accuracy and patient care. Despite the proliferation of studies in this area, as highlighted by a recent report in Nature Medicine (1), the translation of these technologies into routine clinical applications remains limited. This challenge is echoed in a review in Clinical Chemistry, which notes the modest advancements within the field of Laboratory Medicine (2). The question of reproducibility of ML applications in laboratory medicine, underscores a critical barrier to the wider adoption of these technologies (3). This concern is further validated by the findings of Carobene et al. (4) and Agnello et al. (5), which demonstrate the often-inappropriate use of clinical data by information technologies in contexts such as COVID-19 and sepsis. These studies reveal a significant challenge: the variability introduced by analytical instruments and methods, which is frequently overlooked, can severely impact the performance and consistency of ML models, compromising their utility in clinical settings. The importance of addressing this variability is paramount. It necessitates a comprehensive evaluation approach that not only considers the technical aspects of model development but also rigorously examines the sources of variability that can affect model outcomes. This approach underscores the necessity of effective collaboration between data scientists, clinicians, and laboratory medicine professionals. Such interdisciplinary efforts are crucial for developing models that are not only technically robust but also clinically relevant and interpretable (4, 5). The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) has highlighted the need for laboratory medicine professionals to engage with new technologies (6). By understanding the fundamentals of big data and ML algorithms, professionals can better interpret model results and contribute to the development of more reliable prediction models. This engagement is further emphasized

through the recommendation of external validation as a mean to ensure model replicability and robustness (6, 7). A further important step in this direction is represented by recent research that attempts to integrate this discussion by focusing on the critical factors of harmonization, standardization, and biological variation in the clinical laboratory context (8). In this study the authors showed how intra- and inter-subject biological variation significantly influences model robustness, highlighting the necessity of incorporating these factors into model validation processes. This inclusion is vital for ensuring the clinical utility of prediction models across diverse patient populations and conditions, thereby enhancing their applicability in real-world settings. In conclusion, bridging the gap between research innovations and their application in clinical practice requires a multifaceted approach. Beyond the essential steps of internal and external validation, it is imperative to thoroughly characterize and address sources of variability that impact model performance. The collaborative efforts between laboratory professionals, clinicians, and data scientists are key to achieving this goal, ensuring the development of clinically relevant, interpretable, and reliable prediction models. Such a comprehensive approach not only fosters the translation of AI and ML technologies into effective clinical tools but also advances the field of laboratory medicine towards a future where precision diagnostics play a central role in patient care.

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DUŽINA TELOMERA KAO PREDIKTOR BIOLOŠKE STAROSTI I BOLESTI

Jelena Kotur-Stevuljević

Katedra za medicinsku biohemiju, Farmaceutski fakultet, Univerzitet u Beogradu, Srbija

Starenje je prirodan, progresivan i štetan proces koji se odvija tokom vremena i karakteriše se nagomilavanjem ireverzibilnih promena u ćelijama. Ćelijsko starenje podrazumeva morfološke i funkcionalne promene ćelijskih kontrolnih sistema koje rezultiraju smanjenjem proliferativnog kapaciteta ćelije. Osnovna obeležja starenja su genomska nestabilnost, skraćivanje telomera, epigenetske promene, gubitak homeostaze proteina, disfunkcija mitohondrija, ćelijsko starenje (senescencija), iscrpljivanje stem ćelija, promenjena interćelijska komunikacija, oštećena autofagija, hronična inflamacija, disbioza. Telomere su zaštitni krajevi hromozoma koje se sastoje od ponavljajućih segmenata nukleotida. Sa svakom ćelijskom deobom one se skraćuju, najduže su nakon rođenja, a tokom fiziološkog starenja se skraćuju određenom dinamikom. Veliki broj bolesti ubrzava skraćivanje telomera pa su telomere biomarkeri starenja ali i bolesti. Kada telomere u ćeliji dostignu kritično kratku dužinu, ćelija se nalazi na putu apoptoze ili dolazi do mutacija koje će dovesti do razvoja kancera. Telomere tumorskih ćelija su kratke, ali nikad ne dostignu kritično kratku dužinu koja bi dovela do ćelijske smrti, već naprotiv, ćelije nastavljaju da se dele i proliferišu i pored kratkih telomera. Osim enzima telomeraze koji katalizuje produžavanje telomere, postoje i drugi mehanizmi kojima se odigrava taj proces. Životne navike, kao što su navike u ishrani, fizičkoj aktivnosti, pušenje, konzumiranje alkohola, dužina sna mogu ubrzati skraćivanje telomera ili ih produžiti, u zavisnosti od toga da li u životu pojednica dominiraju pozitivni ili negativni životni stilovi. Dokazano je da pušenje, nedostatak fizičke aktivnosti, gojaznost, stres i izlaganje zagađenjima su faktori koji ubrzavaju skraćivanje telomera, što ubrzava starenje i predstavlja podlogu za kancer, dijabetes, kardiovaskularne i druge hronične bolesti koje se češće pojavljuju kod starijih osoba.

TELOMERE LENGTH AS PREDICTOR OF BIOLOGICAL AGEING AND DISEASES

Jelena Kotur-Stevuljević

Department for Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Serbia

Aging is a natural, progressive and harmful process that takes place over time and is characterized by the accumulation of irreversible changes in cells. Cellular aging involves morphological and functional changes in cellular control systems that result in a decrease in the proliferative capacity of the cell. The main features of aging are genomic instability, telomere shortening, epigenetic changes, loss of protein homeostasis, mitochondrial dysfunction, cellular aging (senescence), depletion of stem cells, altered intercellular communication, impaired autophagy, chronic inflammation, dysbiosis. Telomeres are the protective ends of chromosomes consisting of repeating segments of nucleotides. With each cell division, they shorten, so telomeres are the longest after birth and during physiological aging they shorten with a certain dynamic. A large number of diseases accelerate the shortening of telomeres, which is why telomeres are biomarkers of aging and disease. When the telomeres in a cell reach a critically short length, the cell is on the path to apoptosis or a mutation may occur in the cell that will lead to the development of cancer. Tumour cells' telomeres are short, but they never reach a critically short length that would lead to cell death. On the contrary, cells continue to divide and proliferate despite short telomeres. Apart from the enzyme telomerase, which catalyses the lengthening of telomeres, there are other mechanisms by which this process takes place. Lifestyle habits, such as diet, physical activity, smoking, alcohol consumption, sleep length can accelerate telomere shortening or lengthen them, depending on whether positive or negative lifestyles dominate an individual's life. It has been proven that smoking, lack of physical activity, obesity, stress and exposure to pollution are factors that accelerate the shortening of telomeres, which accelerates aging and is the basis for cancer, diabetes, cardiovascular and other chronic diseases that appear more often in the elderly.

ŠTA JE PROCES STARENJA?

Danijela Drašković Radojković

Specijalistička internistička ordinacija Naza medic, Beograd, Srbija

Broj osoba starijih od 60 ili više godina drastično će se povećati u naredne tri decenije. Kao najbrže rastuća starosna strategija širom sveta, globalno stanovništvo preko 60 godina premašiće dve milijarde do 2050 godine: 12 puta više u odnosu na 1950 godinu (United Nations Department of Economic and Social Affairs Population Division 2013). U 20. veku, smanjena smrtnost i produžavanje prosečnog ljudskog životnog veka pomerili su svetsku demografsku strukturu ka starenju. Ovo pomeranje je u početku proizašla iz lečenja zaraznih bolesti i kardiovaskularnih poremećaja. SZO definiše zdravo starenje kao »proces razvoja i održavanja funkcionalne sposobnosti koja omogućava blagostanje u starijoj dobi«. Funkcionalna sposobnost se s tim da imate mogućnosti koje omogućavaju svim ljudima da budu i rade ono što smatraju kao vrednost. Međutim, povećanje invaliditeta u poslednje vreme pratilo je dobitke u zdravim godinama života (zdravstveni raspon) i dugovečnosti. Starost predstavlja primarni faktor rizika za hronične bolesti, uključujući kardiovaskularna, maligna i neurodegenerativna stanja. Biološko starenje je povezano sa smanjenjem reparativnog i regenerativnog potencijala u tkivima i organima. Ovo smanjenje se manifestuje kao smanjena fiziološka rezerva kao odgovor na stres (koji se kaže homeostenoza) i vremenski zavisni neuspeh složenih molekularnih mehanizama koji kumulativno stvaraju poremećaj. Starenje se neizbežno javlja sa vremenom u svim organizmima i pojavljuje se na molekularnom, ćelijskom, organskom i organizacionom nivou sa genetskim, epigenetskim i ekološkim modulatorima. Pojedinci sa istim hronološkim dobom i njihovim organima ispoljavaju diferencijalne putanje opadanja starosti, i iz toga sledi da treba da procenimo biološku starost nezavisno od hronološkog doba. Među istraživačima se mnogo raspravlja o mehanizmima koji doprinose procesu starenja. Međutim, široko je prihvaćeno da je oštećenje genetskog materijala, ćelija i tkiva koje se akumulira sa godinama i koje telo ne može da popravi uzrok gubitka funkcije povezane sa starenjem. Ono što je manje jasno je šta uzrokuje ovu štetu na molekularnom nivou i zašto se može popraviti kod mladih organizama ali ne i kod starih. Da bi bolje okarakterisali proces starenja, istraživači su počeli da identifikuju i kategorišu ćelijska i molekularna obeležja starenja. Opšte je prihvaćeno da različita obeležja doprinose procesu starenja i zajedno određuju uočene karakteristike starenja. Relevantan proces smatra se obeležjem starenja ako njegovo pogoršanje uzrokuje prerano starenje, dok njegovo poboljšanje promoviše zdravlje tokom starenja i

WHAT IS THE AGING PROCESS?

Danijela Drašković Radojković

Specialist internal medicine practice Naza medic, Belgrade, Serbia

The number of individuals aged 60 or older will increase dramatically in the next three decades. As the fastest growing age-strata worldwide, the global population over 60 will surpass two billion by 2050: a 12-fold increase from 1950 (United Nations Department of Economic and Social Affairs Population Division 2013). In the 20th century, decreased mortality and lengthening of average human lifespan shifted the worldwide demographic structure toward the aged. This shift stemmed initially from treatment of infectious diseases and subsequently cardiovascular disorders. WHO defines healthy ageing as »the process of developing and maintaining the functional ability that enables wellbeing in older age.« Functional ability is about having the capabilities that enable all people to be and do what they have reason to value. However, an increase in late-life disability has accompanied gains in healthy years lived (health span) and longevity. Age represents the primary risk factor for chronic diseases, including cardiovascular, malignant, and neurodegenerative conditions. Biological aging is associated with a reduction in the reparative and regenerative potential in tissues and organs. This reduction manifests as decreased physiological reserve in response to stress (termed homeostenosis) and a time-dependent failure of complex molecular mechanisms that cumulatively create disorder. Aging inevitably occurs with time in all organisms and emerges on a molecular, cellular, organ, and organismal level with genetic, epigenetic, and environmental modulators. Individuals with the same chronological age and their organs exhibit differential trajectories of age-related decline, and it follows that we should assess biological age distinctly from chronological age. There is much debate among researchers about the mechanisms that contribute to the ageing process. However, it is widely accepted that damage to genetic material, cells and tissues that accumulates with age and cannot be repaired by the body is the cause of the loss of function associated with ageing. What is less clear is what causes this damage at the molecular level and why it can be repaired in young organisms but not in old ones. To better characterize the ageing process, researchers have begun to identify and categorize the cellular and molecular hallmarks of ageing. It is generally accepted that different hallmarks contribute to the ageing process and together determine the observable features of ageing. A relevant process is considered a hallmark of ageing if its deterioration causes premature ageing, whereas its improvement promotes health during ageing and extends lifespan. The hallmarks of ageing are genome instability,

produžava životni vek. Obeležja starenja su; nestabilnost genoma, degradacija telomera, epigenetske promene, gubitak proteostaze, oštećena svarljivost hranljivih materija,mitohondrijska disfunkcija, ćelijska senescencija, iscrpljenost matičnih ćelija, izmenjena intracelularna komunikacija, pogoršana autofagija, hronična upala, disbalans creva. Razumevanje molekularnih i fizioloških fenomena koji pokreću složene i multifaktoralne procese koji se odnose na biološko starenje kod ljudi informisaće kako istraživači procenjuju i istražuju zdravlje i bolesti tokom životnog toka. Svi mogu da iskuse zdravo starenje. Zdravo starenje vrednuje stvaranje sredine i mogućnosti koje omogućavaju ljudima da budu i rade ono što žele tokom celog života. Biti oslobođen bolesti ili nemoći nije uslov za zdravo starenje, jer mnogi stariji odrasli imaju jedno ili više zdravstvenih stanja koja, kada se dobro kontrolišu, nemaju veliki uticaj na njihovo blagostanje.

telomere degradation, epigenetic changes, loss of proteostasis, Impaired perception of nutrients, mitochondrial dysfunction, cellular senescence, exhaustion of stem cells, altered intracellular communication, deteriorated autophagy, chronic inflammation, imbalance of the intestinal flora (dysbiosis). Understanding the molecular and physiological phenomena that drive the complex and multifactorial processes underlying biological aging in humans will inform how researchers assess and investigate health and disease over the life course. Everybody can experience healthy ageing. Healthy ageing is about creating the environments and opportunities that enable people to be and do what they value throughout their lives. Being free of disease or infirmity is not a requirement for healthy ageing, as many older adults have one or more health conditions that, when well controlled, have little influence on their wellbeing.

OKSIDATIVNI STRES U KARDIOVASKULARNIM BOLESTIMA

Milica Miljkovic Trailovic, Aleksandra Stefanovic, Jelena Kotur-Stevuljevic

Katedra za medicinsku biohemiju, Farmaceutski fakultet, Univerzitet u Beogradu, Beograd, Srbija

Kardiovaskularne bolesti predstavljaju jedan od glavnih uzroka smrtnosti širom sveta i značajno doprinose gubitku zdravlja i prekomernim troškovima zdravstvenog sistema. Oksidativni stres, koji karakteriše neravnoteža između stvaranja reaktivnih vrsta kiseonika (ROS) i antioksidativnog odbrambenog sistema, igra ključnu ulogu u patogenezi i napredovanju kardiovaskularnih bolesti. Generisanje niskog nivoa ROS je od suštinskog značaja za brojne ćelijske funkcije, kao što su transdukcija signala, odbrana od mikroorganizama i ekspresija gena, ali disregulacija signalizacije oksidansa može izazvati ili ubrzati različita patološka stanja, uključujući kardiovaskularne bolesti. Naime, povećana proizvodnja ROS može oštetiti lipide, proteine i DNK. Shodno tome, ovo oštećenje može dovesti do endotelne disfunkcije, upale, formiranja plaka i stanja kao što su ateroskleroza, hipertenzija i koronarna arterijska bolest. Potencijalni izvori ROS uključuju mitohondrijalnu disfunkciju, NADPH oksidazu, ksantin oksidazu, azot oksid sintazu, inflamaciju i ishemijsko-reperfuzionu povredu. Ovi izvori oksidativnog stresa interaguju jedni sa drugima i sa drugim patološkim procesima, formirajući složenu mrežu koja intenzivira kardiovaskularne bolesti. Takođe, oksidativni stres je jedan od uobičajenih patoloških mehanizama preko kojih različiti faktori rizika doprinose oštećenju kardiovaskularnog sistema. Tako dislipidemija, dijabetes, hipertenzija, gojaznost i

OXIDATIVE STRESS IN CARDIOVASCULAR DISEASE

Milica Miljkovic Trailovic, Aleksandra Stefanovic, Jelena Kotur-Stevuljevic

Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

Cardiovascular diseases are leading cause of death globally and substantially contribute to loss of health and excess health system costs. Oxidative stress, characterized by an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense mechanism, plays a pivotal role in the pathogenesis and progression of cardiovascular disease. The generation of low level of ROS is essential for numerous cellular functions, such as signal transduction pathways, defense against microorganisms and gene expression, but dysregulation of oxidant signaling may cause or accelerate different pathological conditions, including cardiovascular disease. Namely, increased production of ROS can damage lipids, proteins, and DNA. Consequently, this damage can lead to endothelial dysfunction, inflammation, plaque formation and conditions like atherosclerosis, hypertension, and coronary artery disease. Potential sources of ROS in the heart include mitochondrial dysfunction, NADPH oxidase, xanthine oxidase, uncoupled nitric oxide synthase, inflammation, and ischemia-reperfusion injury. These sources of oxidative stress interact with each other and with other pathological processes, forming a complex network that intensifies cardiovascular disease. Also, oxidative stress is one of the common pathological mechanisms through which different risk factors contribute to the development of vascular disease. Thus, dyslipidemia, diabetes, hyper-

pušenje kroz oksidativni stres dodatno oštećuju srce, doprinoseći progresiji kardiovaskularnih bolesti. Sva naša dosadašnja istraživanja oksidativnog stresa su pokazala značajno poremećenu ravnotežu između proizvodnje ROS i antioksidativne zaštite, ne samo kod kardiovaskularnih bolesti, već i kod različitih srodnih bolesti, kao što su bubrežna insuficijencija, sindrom policističnih jajnika, preeklampsija, prekomerna težina, dijabetes, kao i kod kolorektalnog karcinoma. Iz svega navedenog sledi da bi dalje razumevanje uloge oksidativnog stresa u kardiovaskularnoj patofiziologiji moglo biti od suštinskog značaja za razvoj efikasnih tretmana za prevenciju kardiovaskularne bolesti.

ULOGA SASP U PATOGENEZI PREEKLAMPSIJE

Vesna D. Garovic[1], Tamara Gojkovic[2], Vladimir Djokic[1], Daniela Ardalic[3], Zeljko Mikovic[3,4], Sonja Suvakov[1]

[1]Odeljenje za nefrologiju i hipertenziju, Klinika Mejo, Ročester, Minesota, SAD
[2]Katedra za medicinsku biohemiju, Farmaceutski fakultet Univerziteta u Beogradu, Srbija
[3]Ginekološko akušerska klinika Narodni front, Beograd, Srbija
[4]Katedra za ginekologiju i akušerstvo, Medicinski fakultet Univerziteta u Beogradu, Srbija

Incidenca hipertenzivnih poremećaja u trudnoći je u stalnom porastu, što može biti posledica nekoliko faktora, uključujući kasniju starosnu dob trudnica, te veći broj komorbiditeta koji prate zdravstveno stanje tokom kasnog reproduktivnog perioda. Preeklampsija (PE), najteži hipertenzivni poremećaj u trudnoći, ostaje jedan od vodećih uzroka morbiditeta i mortaliteta majki i fetusa, a žene koje tokom trudnoće razviju PE su pod većim rizikom za nastanak i razvoj kardiovaskularnih i bubrežne bolesti kasnije tokom života majki. Njegova etiologija ostaje nepoznata, te trenutno nisu dostupni specifični terapijski pristupi zasnovani na mehanizmima. Ćelijsko starenje, odnosno senescencija, predstavlja proces zaustavljanja ćelijskog ciklusa kao odgovor na mnoge etiološke faktore, ali i fiziološke stimuluse, igra važnu ulogu u patogenezi PE, te se u novijoj literature pridaje pažnja mehaničkoj vezu senescencije sa budućom bolešću. Naša hipoteza je potkrepljena eksperimentalnim podacima, te rezultatima dobijenim u našoj laboratoriji, kao i podržana u objavljenim radovima. Prvo smo ispitali ulogu mezenhimalnih matičnih ćelija (MSC) u procesu narušavanja normalne angiogeneze, koja predstavlja jednu od ključnih karakteristika PE. Pokazali smo da je smanjeni pro-angiogeni potencijal MSC kod žena koje su razvile PE u poređenju sa zdravim, normotenzivnim, trudnicama obnovljen tretmanom dasatinibom, senolitičkim

tension, obesity and smoking through oxidative stress additionally damage the heart, contributing to injury of cardiovascular system. All our previous research of oxidative stress has shown significantly disrupted balance between ROS production and antioxidative defense, not only in cardiovascular disease, but also in different related disease, such as renal injury, polycystic ovary syndrome, preeclampsia, overweight, diabetes, as well as in colorectal carcinoma. Taking all together, further understanding the role of oxidative stress in cardiovascular pathophysiology could be essential for developing effective treatments for cardiovascular disease.

THE ROLE OF SASP IN PATHOGENESIS OF PRE-ECLAMPSIA

Vesna D. Garovic[1], Tamara Gojkovic[2], Vladimir Djokic[1], Daniela Ardalic[3], Zeljko Mikovic[3,4], Sonja Suvakov[1]

[1]Division of Nephrology and Hypertension, Mayo Clinic, Rochester, Minnesota, USA
[2]Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Serbia
[3]Gynecology and Obstetrics Clinic Narodni Front, Belgrade, Serbia
[4]Department of Gynecology and Obstetrics, Faculty of Medicine, University of Belgrade, Serbia

The incidence of hypertensive disorders of pregnancy is increasing, which may be due to several factors, including an increased age at pregnancy and more comorbid health conditions during reproductive years. Preeclampsia (PE), the most severe hypertensive disorder of pregnancy, remains one of the leading causes of maternal and fetal morbidity and mortality, and affected women are at increased risk for future cardiovascular and renal disease. Its etiology remains unknown, thus no specific, mechanism-based treatments, are currently available. Cellular senescence, the process of cell cycle arrest in response to many physiologic and maladaptive stimuli, may play an important role in the pathogenesis of PE and provide a mechanistic link to future disease. Our hypothesis is supported both by published studies and data from our laboratory. First, we examined the contribution of mesenchymal stem cells (MSC) to impaired angiogenesis, one of the key features of PE. We showed that decreased pro-angiogenic potential of MSC from PE compared to normotensive pregnancies was restored with treatment with Dasatinib, a senolytic agent that specifically targets MSC. Second, we adopted an animal model of PE, IL-10 knock-out mice intraperitoneally injected with PE sera, which recapitulates key features associated with PE. This model demonstrates increase in senescent cell bur-

agensom koji specifično cilja MSC. Drugo, uspostavili smo životinjski model PE, intraperitonealnim ubrizgavanjem seruma žena koje su razvile PE u IL-10 knockout miševe, koji imitira ključne patološke karakteristike povezane sa PE. Ovaj model pokazuje povećanje broja senescentnih ćelija, što je poslužilo kao osnova hipoteze našim daljim istraživanjima, podržanim preliminarnim podacima, da će terapisjko ciljanje na sprečavanje procesa senescencije sprečiti pojavu PE fenotipa. Treće, pokazali smo da žene sa PE u odnosu na normotenzivne zdrave trudnice: i) prolaze kroz ubrzano epigenetsko starenje tokom trudnoće; ii) pokazuju više nivoe i/ili ekspresije sekretornog fenotipa povezanog sa starenjem (SASP) u krvi i masnom tkivu; iii) pokazao povećanu ekspresiju markera senescencije $p16^{Ink4A}$ u bubrezima; iv) smanjeni nivoi α-Klotho u urinu (protein koji suprimira senescenciju) tokom porođaja. Četvrto, naša populaciona studija je pokazala da PE predstavlja rizik za nastanak i razvoj budućih multimorbiditet, kao dokaz ubrzanog starenja. Buduća istraživanja bi trebalo da istraže uloge senescencije u PE u cilju razumevanje patogeneze ove bolesti, te pronalaženju adekvatnih terapijskih pristupa. Konkretno, senolitici (agensi koji selektivno indukuju apoptozu u starim ćelijama) ili senomorfni agensi (lekovi koji blokiraju proizvodnju SASP) mogu poslužiti kao novi terapeutici u pristupu lečenja PE, te prevenciji njenih dugoročnih komplikacija.

SENESCENCIJA – MEHANIZMI I IMPLIKACIJE U FIZIOLOGIJI I PATOLOGIJI

Tamara Gojkovic[1], Sonja Suvakov[2], Sandra Vladimirov[1], Vesna D. Garovic[2]

[1]Katedra za medicinsku biohemiju, Farmaceutski fakultet Univerziteta u Beogradu, Srbija
[2]Odelenje za nefrologiju i hipertenziju, Klinika Mejo, Ročester, Minesota, SAD

Senescencija predstavlja stanje stabilnog zaustavljanja ćelijskog rasta koje karakterišu restrukturiranje hromatina, metaboličke promene, pojačana upala i aktivacija puteva autofagije. Različiti stimulusi, različitim metaboličkim putevima mogu da pokrenu proces ulaska ćelije u senescenciju, najčešće aktivacijom p53. Nadalje, ovi putevi konvergiraju ka inhibiciji ciklin zavisnih kinaza (CDK) pomoću inhibitora kao što su p16, p15, p21 i p27. Ova inhibicija ima za cilj da zaustavi proliferaciju ćelija, pri čemu hipofosforilisani oblik proteina retinoblastoma (RB) igra ključnu ulogu u izvršenju senescencije. Specifični mehanizmi koji dovode do senescencije mogu varirati u zavisnosti od tipa ćelije, te stimulusa i uslova koji su uključeni. Telomere funkcionišu kao molekularni sat koji beleži replikativnu istoriju ćelije. Svaka ćelijska

den, which served as the basis of the hypothesis, supported by preliminary data, that targeting senescence will prevent the emergence of PE phenotype. Third, we have shown that women with PE vs. normotensive controls: i) undergo accelerate epigenetic aging during pregnancy; ii) exhibit higher levels and/or expressions of senescence-associated secretory phenotype (SASP) in blood and adipose tissue; iii) displayed increased kidney expression of $p16^{Ink4A}$ (marker of senescence); iv) and decreased levels of urinary α-Klotho (an anti-aging protein) at the time of delivery. Fourth, our population-based study showed that PE is a risk for future multimorbidity, a marker of accelerated aging. Future research should explore how better understanding of the role of cellular senescence in PE may lead to therapeutic trials. Specifically, senolytics (agents that induce apoptosis selectively in senescent cells) or senomorphics (drugs that block the production of SASP) may serve as novel therapeutics for PE and its long-term complications.

SENESCENCE – MECHANISMS AND IMPLICATIONS IN PHYSIOLOGY AND PATHOLOGY

Tamara Gojkovic[1], Sonja Suvakov[2], Sandra Vladimirov[1], Vesna D. Garovic[2]

[1]Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Serbia
[2]Division of Nephrology and Hypertension, Mayo Clinic, Rochester, Minnesota, USA

Senescence represents a state of stable cellular growth arrest characterized by chromatin remodeling, metabolic shifts, enhanced inflammation, and the activation of autophagy pathways. Various stimuli can initiate the process of cellular senescence through different metabolic pathways, frequently involving p53 activation. These pathways typically converge on inhibiting cyclin-dependent kinases (CDKs) by inhibitors such as p16, p15, p21, and p27. This inhibition aims to halt cell proliferation, with the hypophosphorylated form of the retinoblastoma protein (RB) playing a critical role in executing senescence. The specific mechanisms leading to senescence can vary depending on cell type, stimuli, and conditions involved. Telomeres function as molecular clocks tracking the replicative history of a cell. Each

deoba dovodi do skraćivanja telomera, a kada one dostignu kritično kratku dužinu, pokreću se mehanizmi replikativnog starenja. Ovaj proces ćelija tumači kao oštećenje DNK, koje pokreće odgovor na oštećenje DNK (DDR) i vodi ćeliju u senescenciju. Takođe, oksidativni stres ima značajan doprinos razvoju senescencije. Visoki nivoi reaktivnih vrsta kiseonika (ROS) aktiviraju puteve koji dovode do aktivacije p38 mitogen-aktiviranih protein kinaza (p38 MAPK), te regulacije aktivnosti p53 i p21, čime doprinose progresiji senescencije. Kada u ćeliji dođe do aktivacije različitih onkogena, ćelija aktivira puteve senescencije. Ovo služi kao zaštitni mehanizam koji sprečava nekontrolisanu deobu ćelija, te potencijalno formiranje tumora. Senescencija izazvana onkogenima, kao zaštitni mehanizam, zaustavlja ćelijski ciklus kao odgovor na onkogene signale, čime se smanjuje rizik od maligne transformacije i održava integritet ćelija i tkiva. Slično tome, gubitak tumorskih supresora može aktivirati puteve koji uzrokuju senescenciju, delujući kao zaštitna barijera tokom rane tumorigeneze. Senescentne ćelije pokazuju složeni proinflamatorni odgovor poznat kao sekretorni fenotip povezan sa starenjem (SASP), koji promovišu transkripcioni faktori – nuklearnim faktor kB (NF-kB) i CCAAT/pojačivač vezujući protein β (CEBPβ). SASP karakteriše lučenje citokina, hemokina, faktora rasta i proteaza. Kako na mikrookruženje tkiva utiču prisutni fenotipovi ćelija i okolni rastvorljivi i nerastvorljivi faktori, time SASP sekretom može negativno da utiče na okolne zdrave ćelije doprinoseći stvaranju mreže senescentnih ćelija unutar tkiva, što dovodi do značajnih metaboličkih promena koje mogu in icirati tumorogenezu. Ova dualnost naglašava ambivalentnu prirodu senescencije: iako u osnovi ima za cilj sprečavanje tumorogeneze, takođe može podstaći progresiju tumora kroz svoj proinflamatorni sekretom. Iako se tradicionalno povezuje sa oštećenjem ćelija, senescenciji podležu i različite embrionalne strukturame. U ovim strukturama otkrivena sa žarišta senescencije zahvaljujući bojenju beta-galaktozidazom (SAbGAL) koje je povezano sa starenjem, zatim nedostatku proliferacije, povećanju markera heterohromatina i povećanju koncentracije inhibitora ćelijskog ciklusa (p15, p21, p27), ali bez prisustva markera koji ukazuju na oštećenje DNK. Ovo razvojno programirano starenje u velikoj meri se oslanja na p21. Pored toga, senescencija je fiziološki programirana u određenim tipovima ćelija odraslih, kao što su normalni megakariociti i placentni sinciciotrofoblasti koji uključuju puteve senescencije kao deo njihovog fiziološkog procesa njihovog sazrevanja. Uloga senescencije u fiziološkim i patološkim procesima naglašava njenu složenu prirodu. Ova ambivalentnost ukazuje na potrebu za daljim istraživanjem kako bi se identifikovali molekuli ili putevi koji vode tkiva ka fiziološkim procesima ili dubljim patološkim promenama. Razumevanje ovih mehanizama moglo bi utrti put terapijskim intervencijama koje koriste povoljne aspekte senescencije dok istovremeno ublažavaju njene štetne efekte.

cell division shortens telomeres, and when they reach a critically short length, they trigger replicative senescence, perceived by the cell as DNA damage. This activates the DNA damage response (DDR), leading the cell into senescence. Oxidative stress also significantly contributes to senescence. High levels of reactive oxygen species (ROS) activate pathways that lead to the activation of p38 mitogen-activated protein kinase (p38 MAPK) and the upregulation of p53 and p21, thus promoting senescence progression. When cells encounter the activation of various oncogenes, they typically enter a state of cellular senescence. This serves as a protective mechanism to prevent uncontrolled cell division and potential tumor formation. Oncogene-induced senescence acts as a biological safeguard, halting the cell cycle in response to oncogenic signals, thereby reducing the risk of malignant transformation and maintaining cellular and tissue integrity. Similarly, the loss of tumor suppressors can trigger pathways leading to senescence, acting as a barrier during early tumorigenesis. Senescent cells exhibit a complex pro-inflammatory response known as the senescence-associated secretory phenotype (SASP), driven by transcription factors like nuclear factor kappa B (NF- B) and CCAAT/enhancer-binding protein β (CEBPβ). SASP involves the secretion of cytokines, chemokines, growth factors, and proteases. The phenotypes of cells and the surrounding soluble and insoluble factors within the tissue microenvironment significantly influence the tissue's state. The SASP secretome can negatively impact surrounding healthy cells, contributing to the formation of a network of senescent cells within the tissue, leading to profound metabolic changes and potentially initiating tumorigenesis. This duality underscores the ambivalent nature of senescence: while it fundamentally aims to prevent tumorigenesis, it can also promote tumor progression through its pro-inflammatory secretome. Although senescence is traditionally associated with cellular damage, it is also observed in various embryonic structures. These structures exhibit senescence markers such as senescence-associated beta-galactosidase (SAβGAL) staining, absence of proliferation, increased heterochromatin markers, and elevated levels of cell cycle inhibitors (p15, p21, p27), but they do not exhibit DNA damage markers. Developmentally programmed senescence relies heavily on p21. Moreover, senescence is physiologically programmed in certain adult cell types, such as normal megakaryocytes and placental syncytiotrophoblasts, which incorporate senescence pathways as part of their natural maturation processes. The role of senescence in both physiological and pathological processes highlights its complex nature. This ambivalence suggests a need for further research to identify molecules or pathways that drive tissues toward recovery or deeper pathological changes. Understanding these mechanisms could pave the way for therapeutic interventions that leverage the beneficial aspects of senescence while mitigating its harmful effects.

EVOLUTIVNI ASPEKTI MENOPAUZE

Ljiljana Marina

Nacionalni centar za infertilitet i endokrinologiju pola, Klinika za endokrinologiju, dijabetes i bolesti metabolizma, Univerzitetski klinički centar Srbije, Medicinski fakultet Univerziteta u Beogradu, Srbija

Menopauza predstavlja biopsihosocijalni fenomen koji podrazumeva prestanak reproduktivne funkcije i folikularno iscrpljivanje kod žena koji se najčešće javlja između 45. i 55. godine života. Sa starenjem žene dolazi do pojave iregularnosti i smanjivanja dužine trajanja menstrualnog ciklusa, smanjene reproduktivne sposobnosti usled slabljenja funkcije jajnika i čestih izostanaka ovulacije, kao i do pojave različitih tegoba. Menstrualni ciklusi se javljaju sve ređe i ovaj period života žene se naziva perimenopauza. Kada menstrualni ciklusi žena u potpunosti prestanu u periodu od 12 meseci, a izlučivanje polnih hormona se značajno smanji, prestaje reproduktivna sposobnost žene i nastupa menopauza. Iako predstavlja normalnu fazu u životnom ciklusu žene, poreklo, razvoj i svrha menopauze još uvek nisu potpuno jasni i privlače veliku pažnju naučnika i istraživača. Brojne bolesti žena nastaju upravo u menopauzi koja osim toga kod preko 80% žena dovodi do nastanka tegoba koje značajno utiču na i remete kvalitet života žene. Nadoknada hormona u ovom periodu dokazano dovodi do otklanjanja ovih tegoba, te prevenciju i makar odlaganje mnogih bolesti, ali i dalje predstavlja razlog za brojne rasprave na temu opravdanosti savetovanja terapije u menopauzi. Sa druge strane, poslednji stavovi kažu da je menopauza prolazna faza ženskog fertiliteta koja može de-evoluirati, odložiti se, ako ne i potpuno nestati.

MENOPAUSE – EVOLUTIONARY ASPECTS

Ljiljana Marina

National Center for Infertility and Gender Endocrinology, Clinic for Endocrinology, Diabetes and Metabolic Diseases, University Clinical Center of Serbia, Faculty of Medicine, University of Belgrade, Serbia

Menopause is a biopsychosocial phenomenon presented by the cessation of reproductive function and follicular exhaustion in women, most often occuring between the age of 45 and 55. With aging menstrual cycle becomes irregular and shorter, reproductive potential weakens and this period of a woman's life is called perimenopause. When a woman's menstrual cycles stop completely within a period of 12 months, and the secretion of sex hormones decreases significantly, the reproductive potential of a woman ends and menopause occurs. Although it represents a normal phase in a woman's life cycle, the origin, development and purpose of menopause are still not completely clear and attract a lot of attention from scientists and researchers. Numerous diseases of women arise precisely during menopause, which, in addition, in over 80% of women, leads to the appearance of complaints that significantly affect and disturb the quality of life of a woman. Hormone replacement during this period has been proven to eliminate these complaints, and to prevent and even postpone many diseases, but it is still the reason for numerous discussions on the justification of advising therapy in menopause. On the other hand, the latest theories say that menopause is a transitory phase of female fertility that can de-evolve, be delayed, if not completely disappear.

ULOGA ĆELIJSKE SENESCENCIJE U TERAPIJI TUMORA

Maja Milanovic

Charite University Medicine, Berlin, Nemačka

Ćelijska senescencija je terminalni blok ćelijskog ciklusa izazvan genomskim stresom. Ako je uzrokovana terapijom koja oštećena DNK, naziva se terapijski izazvana senescencija (TIS). Zajedno sa apoptozom, TIS predstavlja bitan tumor supresivni mehanizam koji sprečava proliferaciju ćelija raka i ograničava rast tumora. Međutim, senescencija (za razliku od apoptoze) održava ćelije raka u vitalnom i metabolički aktivnom stanju, zbog čega je neophodno razumeti njihovu dugoročnu sudbinu u tkivima i njihov uticaj

THE ROLE OF CELULAR SENESCENCE IN TUMOR THERAPY

Maja Milanovic

Charite University Medicine, Berlin, Germany

Cellular senescence is a terminal cell-cycle arrest induced by genomic stress. If triggered by DNA-damaging therapy, it is referred to as treatment-induced senescence (TIS). Together with apoptosis, TIS represents a major tumour-suppressive mechanism which prevents proliferation of cancer cells and limits tumor growth. However, senescence (unlike apoptosis) keeps cancer cells in a viable and metabolically active condition, making it imperative to understand their long-term fate in tissues and their impact on disease

na ishod bolesti. Nedavno otkrića koja dovode u pitanje dugogodišnju dogmu o ireverzibilnosti proliferativne blokade u senescenciji je otvorila brojna mehanistička i pragmatična pitanja o TIS-indukujućim terapijama u lečenju raka. Obzirom na to da se TIS razvija kao nusproizvod svake hemoterapije ili zračenja, kritička cena reverzibilnosti senescencije i njenog tumorogenog kapaciteta je ključna za razvoj bezbednih terapijskih režima u lečenju raka. Naš nedavni rad pokazuje da određene genomske mutacije ili funkcionalni defekti u senescentnim ćelijama raka mogu dovesti do reaktivacije ćelijskog ciklusa. Štaviše, demonstrirali smo da takve post-senescentne ćelije dobijaju dodatne biološke karakteristike koje potiču iz epigenetskih preuređivanja u TIS stanju. Pokazali smo da TIS korelira sa otvaranjem regiona hromatina koji su bili kondenzovani u procesu ćelijske diferencijacije. Ovo otključava primitivnije karakteristike nalik stem ćelijama, koje u kontekstu raka dovode do povećanne agresivnosti bolesti. Naš rad je baziran na mišijem Eμ-Mic modelu, dobro poznatom modelu agresivnih limfoma B-ćelija. Dodatno smo modifikovali ćelije limfoma da uslovno eksprimiraju esencijalni medijator senescencije Suv39h1. Ako je Suv39h1 aktiviran, ćelije limfoma masovno aktiviraju TIS u odgovoru na hemoterapiju. Deaktivacija Suv39h1 u punom TIS-stanju dovodi do postepenog deaktiviranja senescentne molekularne kaskade i pokretanja proliferacije. Transkripciono profilisanje ćelija limfoma u TIS-stanju otkrilo je iznenađujuću aktivaciju stem ćelijskih markera i funkcija, kao latentni transkripcioni program dok je ćelijski ciklus pod kontrolom. Reaktivacija ćelijskog ciklusa (mutacijom Suv39h1) u potpunosti aktivira latentni stem-ćelijski program i rezultuje rastom ćelija limfoma, koje sad poseduju povećani tumorogeni kapacitet. Dalja funkcionalna analiza TIS-preraslih ćelija otkrila je aktivaciju Vnt signalizacije kao glavnog pokretača agresivnih post-senescentnih karakteristika. Intrigantno, kod nativnih limfoma koji nisu genetski manipulisani, identifikovali smo podgrupu limfoma koji su bili skloni recidivu nakon terapije i ćelije recidiva su bile okarakterisane povećanjem TIS i stem programa, posebno Wnt signalizacije. Ovo potvrđuje da nalazi iz našeg genetskog modela verno reflektuju biologiju nativnih limfoma i sugerišu TIS-a i senescentni stem-ćelijski kapacitet kao novi mehanizam relapsa. Naši rezultati, zajedno sa sličnim nalazima u primarnim uzorcima pacijenata sa B-ćelijskim limfomom, otkrivaju senescentni stem-ćelijski kapacitet kao novi rizik hemoterapije i novi mehanizam relapsa, koji nije povezan sa apoptotičkom rezistencijom. Naši dalji napori su usmereni na razumevanje molekularnih mehanizama TIS i njene veze sa neuspehom lečenja. Naš krajnji cilj je da sprečimo recidiv hemoterapije novim strategijama lečenja, koje posebno ciljaju TIS-reprogramirane ćelije i eliminišu njihov senescentičnu stem-ćelijski kapacitet, kao kritičnu osobinu koja izaziva relaps.

outcome. Recent findings that challenge the long-standing dogma about the irreversibility of senescent cell cycle arrest opened numerous mechanistic and pragmatic questions about senescence-inducing therapies in cancer treatment. Considering that TIS develops as a by-product of any chemo- or irradiation therapy, assessment of a senescence reversibility and of tumorigenic capacity of senescent cells appears to be crucial for the development of safe cancer treatment regimens. Our recent work demonstrates that particular genomic mutations or functional defects in fully senescent cancer cells can lead to re-initiation of the cell cycle. Moreover, we show that such post-senescent cells acquire additional biological features that stem from epigenetic rearrangements in the TIS state. TIS associates with opening of chromatin regions that were compacted in the process of cellular differentiation. This unlocks more primitive, stem cell-like features, which in cancer context lead to increased aggressivity of the disease. We used Eμ-myc mice as a well-established model of aggressive B-cell lymphomas and engineered further lymphoma cells to conditionally express an essential senescence mediator Suv39h1. If Suv39h1 is activated, lymphoma cells massively undergo TIS upon chemotherapy. Deactivation of Suv39h1 in fully senescent cells leads to gradual deactivation of senescence molecular machinery and restarted proliferation. Transcriptional profiling lymphoma cells in TIS state revealed a surprising upregulation of stem cell markers and functionalities, but remained latent as long as the cell cycle was kept in check. Breaching the cell cycle block by the mutation of Suv39h1 fully engaged the latent stemness program and resulted in regrowth of lymphoma cells with highly increased tumorigenic capacity. Further functional analysis of TIS-outgrown cells revealed activation of canonical Wnt signalling as a major driver of aggressive post-senescent biology. Intriguingly, in native, non-genetically manipulated lymphomas, we identified a subset of samples which were prone to relapse after chemotherapy and relapsed cells were characterized with upregulated TIS and stemness signatures, in particular Wnt singaling. This confirms that the findings from our genetic model apply to real life lymphoma biology and suggest TIS outgrowth and senescence-associated stemness as a novel mechanism of relapse. Our results, along with consistent findings in primary samples from B-cell lymphoma patients, uncover senescence-associated stemness as an unrecognized chemotherapy peril and novel relapse mechanism, not related to apoptotic resistance. We seek to further mechanistically dissect TIS and its link to treatment failure. Our ultimate goal is to prevent chemotherapy relapse by inventing treatment strategies, which specifically target TIS-reprogrammed cells or eliminate senescence-associated stemness, as their detrimental, relapse-driving feature.

STATUS RETKIH BOLESTI U REPUBLICI SRBIJI – GDE SMO DANAS?

Bojana Mirosavljević

ICON pc, Novi Sad, Srbija

Retka bolest – podrazumeva svaku bolest koja se javlja kod najviše jedne od 2000 osoba. Prema procenama, postoji između 6000 i 7000 retkih bolesti. Prema proceni Evropske komisije kojom se služimo i u Srbiji, 6% do 8% populacije ima neku retku bolest. U Srbiji se procenjuje da oko pola miliona građana živi sa nekom retkom bolešću.

Najčešće karakteristike su:
– 80% retkih bolesti su genetskog porekla, ostale su posledica infekcija, alergija, uticaja faktora životne sredine ili su degenerativne i proliferativne;
– kod 50% osoba sa retkim bolestima, prvi simptomi bolesti se javljaju već na rođenju ili u ranom detinjstvu;
– 30% dece sa retkom bolešću žive kraće od pet (5) godina;
– za više od 95% retkih bolesti ne postoji nikakva registrovana terapija.

Najčešća posledica retkih bolesti je trajni invaliditet.
Uprkos međusobnoj različitosti, osobe sa retkim bolestima i njihove porodice suočavaju se sa istim brojnim teškoćama koje proističu iz retkosti:
– Nedostupnost dijagnoze i/ili višegodišnje traganje za dijagnozom;
– nedostatak informacija o bolesti, pomoći, nedostatak stručnjaka…
– nepostojanje naučnih istraživanja, nepostojanje lekova i odgovarajućih medicinskih pomagala;
– visoka cena postojećih lekova i terapija, dovodi do smanjivanja životnog standarda porodice i smanjenja dostupnosti lečenja;
– socijalne posledice: stigmatizacija, izolacija, diskriminacija, smanjenje profesionalnih mogućnosti;
– nedostatak kvalitetne zdravstvene zaštite: isključenost iz zdravstvene zaštite, čak i kada je postavljena ispravna dijagnoza.
– nejednakost: nailazak na administrativne prepreke u pokušajima da se leče ili ostvare prava iz domena socijalne zaštite.

Udruženje »Život« je osnovano 2010. godine sa misijom da pruži podršku i informacije pacijentima sa retkim bolestima i njihovim porodicama. Cilj udruženja je da ujedini zajednicu pacijenata, grupe pacijenata i lekare u cilju poboljšanja života i statusa pacijenata sa retkim bolestima i njihovih porodica. Aktivni radi na podizanju svesti o problemima retkih bolesti, obezbeđivanju terapije i medicinske opreme, kao i

THE STATUS OF RARE DISEASES IN THE REPUBLIC OF SERBIA – WHERE WE ARE TODAY?

Bojana Mirosavljević

ICON pc, Novi Sad, Serbia

Rare disease – any disease that occurs in no more than one in 2000 people. According to estimates, there are between 6000 and 7000 rare diseases. According to the estimate of the European Commission, which we also use in Serbia, 6% to 8% of the population has a rare disease. In Serbia, it is estimated that around half a million citizens live with a rare disease.

The most common features are:
– 80% of rare diseases are of genetic origin, the rest are the result of infections, allergies, the influence of environmental factors or are degenerative and proliferative;
– in 50% of people with rare diseases, the first symptoms of the disease appear already at birth or in early childhood;
– 30% of children with a rare disease live less than five (5) years;
– for more than 95% of rare diseases there is no registered therapy.

The most common consequence of rare diseases is permanent disability.
Despite their diversity, people with rare diseases and their families face the same many difficulties that arise from rarity:
– Unavailability of diagnosis and/or years of searching for a diagnosis;
– lack of information about the disease, help, lack of experts…
– lack of scientific research, lack of medicines and appropriate medical aids;
– the high price of existing drugs and therapies leads to a decrease in the standard of living of the family and a decrease in the availability of treatment;
– social consequences: stigmatization, isolation, discrimination, reduction of professional opportunities;
– lack of quality health care: exclusion from health care, even when the correct diagnosis has been made;
– inequality: encountering administrative obstacles in attempts to heal or realize rights from the domain of social protection.

Association »Life« was founded in 2010 with the mission to provide support and information to patients with rare diseases and their families. The aim of the association is to unite the patient community, patient groups and doctors in order to improve the life and status of patients with rare diseases and their families. Active works to raise awareness about the problems of rare diseases, provide therapy and medical

unapređenju položaja u društvu kako obolelih od retkih bolesti tako i njihovih porodica, kao i skraćivanju vremena do dijagnoze i okupljanju na jednom mestu svih zainteresovanih za temu retkih bolesti, kreirajući zajednicu.

Aktivnosti udruženja
Najveće dostignuće je inicijativa za donošenje Zojinog zakona – Zakon o prevenciji i dijagnostici genetičkih bolesti, genetički uslovljenih anomalija i retkih bolesti, koji je jednoglasno usvojen 2015. godine. Od 2020. godine je aktivna internet platforma Baza retkih bolesti koja je relevantan izvor informacija za lekare, pacijente i članove njihovih porodica. Pretraga retkih bolesti je omogućena na srpskom, makedonskom, hrvatskom i engleskom jeziku. Baza podataka zahteva svakodnevno ažuriranje jer ima oko 7.000 retkih bolesti. Godišnja regionalna konferencija o retkim bolestima, kao i organizovanje edukativnih vebinara za lekare i edukativnih vebinara za pacijente. Okupljanje svih relevantnih aktera iz Srbije ali i regiona na temu retkih bolesti u okviru konferencije kao i veća vidljivost obolelih od retkih bolesti od kojih je preko 85% osoba sa invaliditetom. Osnaživanje zajednice obolelih od retkih bolesti kao i povećanje dostupnosti informacija. Takođe, od aktivnosti posebno izdvajamo kreiranje onlajn izdanja prvog i jedinog časopisa o retkim bolestima na Balkanu »Reč za život«, koji postoji od 2015. godine. »Reč za život« je dobio veliku evropsku nagradu Evropske organizacije za retke bolesti Black Pearl Award 2018.

equipment, as well as improve the position in society of both those suffering from rare diseases and their families, as well as shortening the time to diagnosis and gathering in one place all those interested in the topic of rare diseases, creating a community.

Association activities
The most important achievement is the initiative to adopting Zoya's Law – the Law on Prevention and Diagnosis of Genetic Diseases, Genetically Conditioned Anomalies and Rare Diseases, which was unanimously adopted in 2015. Since 2020, the Internet platform Rare Disease Database has been active and is a relevant source of information for doctors, patients and their family members. Search for rare diseases is possible in Serbian, Macedonian, Croatian and English. The database requires daily updates as there are around 7,000 rare diseases. Annual regional conference on rare diseases, as well as organizing educational webinars for doctors and educational webinars for patients. Gathering of all relevant actors from Serbia and the region on the topic of rare diseases within the conference, as well as greater visibility of people suffering from rare diseases, of which over 85% are disabled. Empowering the community of people suffering from rare diseases as well as increasing the availability of information. Also, among the activities we highlight the creation of the online edition of the first and only magazine on rare diseases in the Balkans, »Word for Life«, which has been in existence since 2015. »Word for Life« received the European Organization for Rare Diseases Black Pearl Award 2018.

GENSKE TERAPIJE ZA RETKE BOLESTI

Maja Stojiljković

Institut za molekularnu genetiku i genetičko inženjerstvo, Univerzitet u Beogradu, Srbija

Svaka bolest čija je učestalost manja od 1 u 2000 ljudi definiše se kao retka bolest (RB). Do sada je opisano preko 6000 različitih RB i taj broj se iz godine u godinu povećava. Smatra se da preko 80% RB ima monogensku genetičku osnovu. Uprkos neverovatnom napretku u razvoju terapija za RB, i dalje za >95% njih ne postoji specifičan efikasan tretman. Ovo su razlozi zašto su znanja iz molekularne genetike od neprocenjivog značaja za istraživanje molekularne osnove RB. Sekvenciranje nove generacije ima veliku ulogu u postavljanju tačne dijagnoze i identifikaciji novih meta koje će poslužiti kao osnov za razvoj inovativnih terapeutika. Takođe, bazična istraživanja, poput funkcionalne karakterizacije genetičkih varijanti i istraživanje molekularnih mehanizama nastanka bolesti su neophodni preduslovi za razvoj različitih molekularnih terapeutika, pa tako i

GENE THERAPIES FOR RARE DISEASES

Maja Stojiljković

Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Serbia

Every disease with a prevalence of less than 1 in 2000 people is defined as a rare disease (RD). So far, over 6000 different RDs have been described, and this number increases year by year. It is considered that over 80% of RDs have a monogenic genetic basis. Despite incredible progress in developing therapies for RDs, still, for more than 95% of them, there is no specific effective treatment. Next-generation sequencing plays a significant role in making accurate diagnoses and identifying new targets that will serve as a foundation for developing innovative therapeutics. Also, basic research, such as the functional characterization of genetic variants and exploring the molecular mechanisms of disease onset, are necessary prerequisites for developing various molecular therapeutics, including gene therapy. Gene therapy involves introducing genetic material into cells to

genske terapije. Genska terapija podrazumeva unošenje genetičkog materijala u ćelije kako bi se nadomestio urođeni genetički nedostatak i kako bi se omogućila kontinuirana sinteza funkcionalnog proteina i izlečila bolest. Genetički materijal koji se unosi može biti funkcionalna kopija gena (DNK molekul), oligonukleotid (kratak nekodirajući RNK molekul) koji se po principu komplementarnosti vezuju za iRNK ili pre-iRNK i dovodi do željene modulacije ekspresije proteina (npr. modulacija iskrajanja, blokiranje translacije ili aktivacija degradacije iRNK) ili kratke RNK koje precizno koriguju gen unutar same ćelije (CRISPR/Cas9). Neke od bolesti za koje je do sada registrovana genska terapija su deficijencija adenozin deaminaze, spinalna mišićna atrofija, retinalna distrofija, a mnoga klinička ispitivanja su u toku. Nesumnjiva je važnost bazičnih istraživanja, ali i njihova brza translacija u medicinsku praksu.

SMA – OD DIJAGNOSTIKE DO GENETIČKE TERAPIJE

Miloš Brkušanin

Univerzitet u Beogradu – Biološki fakultet, Beograd, Srbija

Bez primene terapije, spinalna mišićna atrofija (SMA) predstavlja progresivno neuromišićno oboljenje karakteristično po prevremenom, intenzivnom i nepovratnom odumiranju motornih neurona u prednjim rogovima kičmene moždine. Ovaj najčešći genetički uzrok smrtnosti u dečijem uzrastu uzrokovan je potpunim odsustvom funkcionalnog gena SMN1 i postojanjem različitog broja kopija gena SMN2, tzv. rezervnog gena. Decenijama unazad, nakon postavljanja dijagnoze SMA, lečenje se zasnivalo isključivo na primeni simptomatske terapije i standarda nege, čiji su efekti bili izuzetno ograničeni. Jedina prevencija koja je vršena u porodicama pogođenim bolešću bila je testiranje nosilaca (ispitivanje srodnika sa ciljem identifikovanja greške koja uzrokuje SMA), praćena prenatalnom analizom u svakoj trudnoći. Međutim, 2016. godine odobrena je prva, a do danas ukupno tri, inovativne genetički dizajnirane terapije za lečenje SMA. Sva tri terapijska pristupa pokazala su odlične rezultate u prekliničkim i kliničkim studijama, kao i u realnoj upotrebi terapija. Budući da direktno deluju na uzrok bolesti, njihovi efekti u lečenju su revolucionarni. Sva dosadašnja istraživanja pokazala su da se najveći efekat sva tri primenjena terapijska pristupa postiže isključivo ukoliko se primene pre pojave bilo kakvih simptoma. To je potpuno promenilo našu strategiju u lečenje osoba sa SMA i usmerilo napore na rano otkrivanje bolesti putem neonatalnog skrininga i ranu primene terapije.

compensate for an inborn genetic defect and to enable the continuous synthesis of a functional protein and cure the disease. The genetic material introduced can be a functional copy of a gene (DNA molecule), an oligonucleotide (a short non-coding RNA molecule) that binds by complementarity to mRNA or pre-mRNA and leads to the desired modulation of protein expression (e.g., modulation of splicing, blocking of translation, or activation of mRNA degradation), or short RNAs that precisely correct a gene within the cell itself (CRISPR/Cas9). Some of the diseases for which gene therapy has been registered so far include adenosine deaminase deficiency, spinal muscular atrophy, retinal dystrophy, and many clinical trials are ongoing. The importance of basic research is undeniable, as is its rapid translation into medical practice.

SMA – FROM DIAGNOSTICS TO GENETIC THERAPY

Miloš Brkušanin

University of Belgrade – Faculty of Biology, Belgrade, Serbia

In the absence of therapy, spinal muscular atrophy (SMA) represents a progressive neuromuscular disorder characterized by the premature, intense, and irreversible degeneration of motor neurons in the anterior horns of the spinal cord. This most common genetic cause of mortality in childhood is caused by the complete absence of the functional SMN1 gene and the presence of variable number of SMN2 gene copies, serving as a backup gene. For decades, following the establishing of the diagnosis of SMA, treatment has been exclusively based on symptomatic therapy and standard care, with extremely limited effects. Within families afflicted by the disease, preventative measures have predominantly involved carrier testing, aimed at identifying the causative error leading to SMA, followed by prenatal analysis during subsequent pregnancies. However, in 2016, the first, and to date a total of three, innovative genetically designed therapies for treating SMA were approved. All three therapeutic approaches have shown outstanding results in preclinical and clinical studies, as well as in real-world therapy use. Since they directly target the disease cause, their effects in treatment are revolutionary. All previous research has shown that the greatest effect of all three applied therapeutic approaches is achieved only if they are administered before any symptoms appear. This paradigm shift has fundamentally reshaped our approach to managing individuals with SMA, pivoting toward early disease detection via neonatal screening initiatives and the prompt application of therapeutic interventions.

NOVOROĐENAČKI PROBIR U REPUBLICI HRVATSKOJ

Ksenija Fumić[1], Ana Škaričić[2], Iva Bilandžija Kuš[2], Korana Lipovac[2], Ivana Križić[2]

[1]Klinički zavod za laboratorijsku dijagnostiku Kliničkoga bolničkog centra Zagreb i Medicinskog fakulteta Sveučilišta u Zagrebu; Farmaceutsko-biokemijski fakultet Sveučilišta u Zagrebu
[2]Klinički zavod za laboratorijsku dijagnostiku Kliničkoga bolničkog centra Zagreb i Medicinskog fakulteta Sveučilišta u Zagrebu

Početak novorođenačkog probira (NBS) u Republici Hrvatskoj (RH) seže u 1978. godinu kada je uveden Guthriejev test za fenilketonuriju/hiperfenilalaninemiju (PKU/HPA), te 1985. godinu kada je uveden probir za konatalnu hipotireozu (CH). Tijekom godina pojavile su se nove tehnologije koje su se pokazale robusnima i prikladnima za istodobno određivanje velikog broja analita iz jednog uzorka suhe kapi krvi (DBS). Tandemska spektrometrija masa udružena s tekućinskom kromatografijom visoke djelotvornosti, LC-MS/MS, kao jedna od tih tehnologija, našla je svoje mjesto u kliničkim laboratorijima i omogućila proširenje probira. Od listopada 2017. započeo je pilot projekt s dodanih šest novih bolesti u postojeći program novorođenačkog probira u RH. Nove bolesti uključene u nacionalni probir bile su: dvije organske acidurije, izovalerijanska acidurija (IVA) i glutarna acidurija tipa 1 (GA1), te četiri poremećaja razgradnje masnih kiselina, CUD (manjak karnitinskog nosača), MCADD (manjak srednjelančane acil-CoA dehidrogenaze), VLCADD (manjak dugolančane acil-CoA dehidrogenaze) i LCHADD/TFP (manjak 3-OH-dugolančane acil-CoA dehidrogenaze, izoliran ili kao dio manjka trifunkcionalnog proteina). U ožujku 2023. započeo je još jedan pilot projekt, ovaj put za probir na spinalnu mišićnu atrofiju (SMA). Kako bi se NBS mogao odgovarajuće provoditi, bilo je potrebno prevladati brojne izazove. Na službene stranice KBC-a Zagreb smo postavili osnovne informacije o novorođenačkom probiru, kako bi bile dostupne široj javnosti. Kreirali smo i službenu adresu elektroničke pošte putem koje komuniciramo s trideset i dva rodilišta zadužena za praćenje novorođenčadi otkrivene probirom. Trajna edukacija jedna je od najvažnijih značajki cjelokupnog NBS programa, zbog čega smo pripremili edukativne materijale za osoblje rodilišta te održali brojne usmene prezentacije o pravilnom uzorkovanju krvi i kvaliteti uzoraka DBS. Također imamo blisku suradnju s pedijatrima specijalistima za metaboličke poremećaje i neuropedijatrima. Održavamo redovne tjedne sastanke gdje raspravljamo o aktualnim problemima u NBS-u. U analitičkom dijelu probira, morali smo uspostaviti vlastite granične vrijednosti novorođenačke populacije za nasljedne

NEWBORN SCREENING – EXPERIENCES FROM THE REPUBLIC OF CROATIA

Ksenija Fumić[1], Ana Škaričić[2], Iva Bilandžija Kuš[2], Korana Lipovac[2], Ivana Križić[2]

[1]Clinical Institute for Laboratory Diagnostics, University Hospital Centre Zagreb, Croatia; Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia
[2]Clinical Institute for Laboratory Diagnostics, University Hospital Centre Zagreb, Croatia

The beginnings of newborn screening (NBS) in the Republic of Croatia date back to 1978 when the Guthrie test for phenylketonuria/hyperphenylalaninemia (PKU/HPA) was introduced, and to 1985 when congenital hypothyroidism (CH) screening was added to the national screening program. Over the years, new technologies emerged which proved to be robust and suitable for simultaneous determination of a large number of analytes from single dried blood spot (DBS) sample. Tandem mass spectrometry coupled with high performance liquid chromatography, LC-MS/MS, as one of those technologies, found its place in clinical laboratories and allowed expansion of screening programs. As of October 2017, a pilot project with addition of six new diseases to the existing NBS program in Croatia has started. The new diseases included in the national screening were: two organic acidurias, isovaleric aciduria (IVA) and glutaric aciduria type 1 (GA1), and four fatty acid oxidation disorders, CUD (carnitine uptake deficiency), MCADD (medium-chain acyl-CoA dehydrogenase deficiency), VLCADD (long-chain acyl-CoA dehydrogenase deficiency) and LCHADD/TFP (3-OH-long-chain acyl-CoA dehydrogenase deficiency, isolated or as a part of TFP deficiency). In March 2023, another pilot project started, but this time for spinal muscular atrophy (SMA) screening. We came across a lot of challenges in order to enable the NBS program to function properly. Essential information about newborn screening was made available on the official University Hospital Center Zagreb website for public access. We have also created an official e-mail address through which we communicate with 32 maternity wards responsible for follow-up of positive children detected through NBS. Continous education is one of the most important aspects of the whole NBS program, which is why we have prepared educational materials for maternity hospitals nursery staff and also held numerous oral presentations about correct blood spot sampling and good quality specimens for NBS. We also have a close collaboration with metabolic pediatricians and neuropediatricians through weekly meetings where we discuss current NBS issues. In the analytical part of the screening process, we needed to

metaboličke bolesti (NMB) uključene u probir. U tu smo svrhu analizirali približno 2000 DBS kartica zdrave novorođenčadi i odabrali granične vrijednosti za sve karakteristične biljege bolesti. Nakon što smo otkrili veći broj stvarno pozitivne novorođenčadi novorođenačkim probirom, prilagodili smo početne granične vrijednosti za neke primarne i sekundarne biljege. Ovo iskustvo omogućilo nam je da razvijemo odgovarajuće upute za postupanje nakon pozitivnog rezultata probira. Jedan od najvećih izazova bila je izrada laboratorijskoga informacijskog sustava (LIS) posebno osmišljenog za novorođenački probir. Upis uzorka i generiranje jedinstvenoga crtičnog koda provodi se u laboratoriju, a ne u rodilištima. Projekt E-novorođenče predviđa dodjeljivanje crtičnog koda već u rodilištu. Nažalost, još se nisu stekli svi uvjeti da ovaj projekt zaživi. Od početka probira na SMA susreli smo se s nekoliko odbijanja probira, vjerojatno zbog nedostatka informacija i posljedičnog straha od manipulacije genskim materijalom. U posljednjih šest godina analizirali smo približno 220 000 uzoraka novorođenčadi i otkrili 56 PKU/HPA, 95 CH, 17 MCADD, 12 VLCADD, 2 GA1, 2 IVA, 2 CUD i 1 LCHADD/TFP. U prvoj godini novorođenačkog probira na SMA otkrili smo pet pacijenata. Također smo otkrili četiri asimptomatske majke s NMB preko probira njihove djece i četiri asimptomatska brata i sestre dojenčadi pozitivne na poremećaje razgradnje masnih kiselina. Sve sumnje na NMB potvrđene su specifičnijim metaboličkim testovima i analizama odgovarajućih gena. Za potvrdu pozitivnih rezultata probira, razvili smo genski panel koji sadrži sve gene za bolesti uključene u probir. Nakon završetka novorođenačkog probira na sve bolesti, DBS kartice čuvaju se pet godina, što nije pravno obvezujući postupak. Do kraja ove godine planiramo proširiti probir i uvesti homocistinuriju kao novu bolest u nacionalni program probira. U budućnosti je plan NBS programu dodati još nekoliko bolesti, uključujući metilmalonsku aciduriju, propionsku aciduriju i poremećaje metabolizma kobalamina. Trenutno razvijamo drugostupanjski test koji će nam pomoći u diferencijalnoj dijagnostici tih poremećaja.

establish our own population cut-off values for screened inborn errors of metabolism (IEM). For this purpose, we have analyzed approximately 2000 DBS cards from healthy newborns and chosen the cut-off values for each of the screened disease markers. During time, after detecting more true positive infants through NBS, we have adjusted initial cut-off values for some primary and secondary markers. This experience allowed us to develop appropriate algorithms for follow up procedures after positive screening result. One of the biggest challenges was the creation of laboratory information system (LIS) specifically designed for NBS. Sample registration and creation of a unique barcode is carried out in NBS laboratory, not in the maternity wards. E-newborn project suggests the assignment of a barcode in the maternity hospital. Unfortunately, not all conditions have been met for this project to take off yet. Since the beginning of the SMA screening, we have encountered several screening refusals, probably beacuse of misinformation and consequent fear of genetic material manipulation. In the last six years we have analyzed approximately 220 000 newborn samples and have detected 56 PKU/HPA, 95 CH, 17 MCADD, 12 VLCADD, 2 GA1, 2 IVA, 2 CUD and 1 LCHADD/TFP infants. In the first year of SMA screening, we have detected 5 patients. We have also discovered four asymptomatic mothers with IEM through their children's screening, and four asymptomatic siblings of infants positive for fatty acid oxidation disorders. All suspected disorders have been confirmed with more specific metabolic tests and by genetic analyses of corresponding genes. For confirmation of positive screening results, we have developed a gene panel that includes all the genes for the diseases we screen for. After NBS for all diseases has been concluded, DBS cards are stored for five years, which is not a legally binding procedure. By the end of this year, we are planning to expand the screening panel and introduce homocystinuria as another disease in the national screening program. In the future, the plan is to add several more diseases to the NBS program, including methylmalonic aciduria, propionic aciduria and cobalamin metabolism disorders. Currently we are developing a second-tier test which would help us re-evaluate and differentiate positive NBS results for those IEMs.

HITOTRIOZIDAZA – ZNAČAJ U DIJAGNOSTICI POJEDINIH RETKIH BOLESTI

Zorica Šumarac[1,2], Aleksandar Šumarac[3]

[1]Farmaceutski fakultet Novi Sad, Univerzitet Privredna akademija u Novom Sadu, Srbija
[2]Centar za medicinsku biohemiju, Univerzitetski klinički centar Srbije, Beograd, Srbija
[3]Medicinski fakultet, Univerzitet u Beogradu, Srbija

Hitotriozidaza je glikozil hidrolaza koja pripada grupi humanih hitinaza a koje imaju zaštitnu ulogu od patogena koji sadrže hitin, kao što su gljivice, nematode i insekti. Luči se iz aktiviranih makrofaga. Hitotriozidaza se smatra učesnikom signalnih puteva uključenih u inflamatornim procesima i potencijalnim markerom imunoaktivacije, zbog čega se humane hitinaze i generalno smatraju delovima urođenog imunog sistema. Upadljivo povećanje aktivnosti hitotriozidaze se uočava u serumu obolelih od Gošeove bolesti (potiče od aktiviranih makrofaga–Gošeovih ćelija) i sarkoidoze, a može se naći i kod galaktosijalidoze, sarkoidoze, amiotrofične lateralne skleroze, multiple skleroze, tuberkuloze, ateroskleroze, akutne malarije, parazitnih i drugih bolesti. Hitotriozidaza se koristi kao koristan biomarker težine bolesti, za razlikovanje aktivnosti bolesti i predviđanje pogoršanja. Problem u korišćenju hitotriozidaze kao biomarkera je recesivno nasleđeni deficit, uočen kod 5–6% opšte populacije. I pored ove osobine, hitotriozidaza je jedan od najspecifičnijih biomarkera u postavljanju dijagnoze Gošeove bolesti i praćenju enzim-supstitucione i supstrat-redukcione terapije. Uz ulogu koju ima u dijagnostici sarkoidoze, poslednjih godina pokazala je veliki značaj u praćenju efekata terapije, gde je smanjenje enzimske aktivnosti u korelaciji sa kliničkim simptomima i efikasnošću ostalih dijagnostičkih metoda (napr. 18F-FDG PET). Danas se aktivnost hitotriozidaze koristi i u praćenju dijabetes melitusa tip 2 (pojava ateroskleroze), postavljanju dijagnoze sindroma policističnih jajnika, nealkoholne masne bolesti jetre, b-talasemije, HBV i HCV hepatitisa, raznih karcinoma i velikog broja autoimunih bolesti (SLE, Hronova bolest, psorijaza, juvenilni idiopatski artritis i druge).

CHITOTRIOSIDASE – SIGNIFICANCE IN THE DIAGNOSIS OF CERTAIN RARE DISEASES

Zorica Šumarac[1,2], Aleksandar Šumarac[3]

[1]Faculty of Pharmacy Novi Sad, University Business Academy Novi Sad, Serbia
[2]Center for Medical Biochemistry, University Clinical Center of Serbia, Belgrade, Serbia
[3]Medical School, University of Belgrade, Serbia

Human chitotriosidase is a chitin-degrading glycosyl hydrolase secreted by activated macrophages and various monocyte-derived cell lines. Chitotriosidase belongs to a group of human chitinases that have a protective defense against chitin-containing pathogens such as fungi, nematodes and insects. Chitotriosidase is considered as a participant of signaling pathways involved in inflammation and potential marker of immunoactivation processes. Because of this fact, human chitinases are generally considered as a parts of the innate immune system. A striking increase of plasma chitotriosidase activity is observed in serum from Gaucher's disease (originating from activated macrophages – Gaucher cells), galactosialidosis, sarcoidosis, amyotrophic lateral sclerosis, multiple sclerosis, tuberculosis, atherosclerosis, acute malaria and other infectious and parasitic diseases. Chitotriosidase is used as a useful biomarker of disease severity, for differentiating disease activity and predicting of deterioration. A recessively inherited deficiency in chitotriosidase was previously revealed (in 5–6% of global population), which sometimes represents a problem in the using of this biomarker. Despite this feature of the enzyme, chitotriosidase is one of the most specific biomarkers in diagnosis of Gaucher's disease and monitoring of enzyme replacement and substrate reduction therapy. In addition to its importance in the diagnosis of sarcoidosis, chitotriosidase has shown a particularly great importance in the monitoring of sarcoidosis therapy, where a decreasing of chitotriosidase activity shows an excellent correlation with clinical symptoms and the efficiency of other diagnostic methods (e.g. 18F-FDG PET). Nowadays, chitotriosidase is determined also in the diagnosis of diabetes mellitus type 2, polycystic ovary syndrome, non-alcoholic fatty liver disease, b-thalassemia, HBV and HCV hepatitis, various cancers and a large number of autoimmune diseases (SLE, Chron's disease, psoriasis, juvenile idiopathic arthritis and others).

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TIME FOR A SUSTAINABLE TRANSITION WITHIN THE MEDICAL LABORATORIES

Tomris Ozben

International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), President
European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), Past-President
EFLM Task Force-Green and Sustainable Labs, Chair

Laboratory medicine should contribute to a sustainable healthcare system ensuring that resources are used efficiently from ecological, social, and economical perspectives, while providing high-quality services to patients and physicians. It will be a challenge for clinical laboratories to achieve sustainable operations. Clinical laboratories use more energy and water than offices and generate huge amounts of hazardous and non-hazardous wastes every year. Clinical laboratories can limit their environmental impact and provide sustainable laboratory services making reductions in four key areas—energy consumption, water consumption, waste production, and use of hazardous chemicals. Establishing sustainable development goals and applying multiple means for reductions in these key areas, clinical laboratories can reduce their environmental impact. By being mindful of the environmental impact of everyday actions in a lab, and by taking steps to minimize energy, water, and hazardous chemical use, as well as waste generation, a clinical lab can be transformed into a safe, sustainable space. Sustainability measures should be a key feature in the rapidly changing healthcare environment to reduce their negative impacts on the environment and economy. Laboratory medicine community should lead the shift to carbon neutrality by decreasing their deleterious environmental impact and implementing efficient approaches to address the effects of climate change and pollution without compromising the quality of healthcare. In order to provide high-quality, effective, and safe healthcare services, sustainable healthcare systems need to overcome major economic and social challenges. Though there will be initial capital costs, there is a long-term cost-saving potential of a more efficient use of energy and other resources in healthcare systems. Despite this, there is a long way to go for environment-friendly hospitals, healthcare structures, and clinical laboratories to become the norm. Good collaboration among the healthcare systems and a common vision for future actions would help to achieve such goals.

PRIMENA SEKVENCIRANJA NAREDNE GENERACIJE U PRENATALNOJ DIJAGNOSTICI

Ivana Joksić

Ginekološko-akušerska klinika »Narodni front«, Beograd, Srbija

Kongenitalne anomalije se detektuju u 2–4% trudnoća i mogu dovesti do značajnih strukturnih ili funkionalinih oštećenja zahvaćenih organa i sistema, sa teškim posledicama koje uključuju intrauterinu ili neonatalnu smrt, hronična oboljenja i dugotrajne invaliditete, što predstavlja veliko opterećenje za pogođene porodice i zdravstveni sistem u celini. Različite vrste genetičkih promena mogu uzrokovati razvoja anomalija, a najznačajnije su hromozomske aberacije (strukturne i numeričke), submikroskopski rearanžmani (copy number variations-CNV; mikrodelecije-mikroduplikacije) i monogenske bolesti. Svim trudnicama kod kojih se ultrazvučnim pregledom detektuju anomalije fetusa, preporučuje se genetičko ispitivanje ploda. Tradicionalno, primenom metoda citogenetike i molekularne citogenetike uzrok anomalija se mogao ustanoviti u oko 35% slučajeva, te je njihova etiologija u velikom broju slučajeva ostajala nerazjašnja. Iz navedenih razloga postojala je potreba za implementacjiom novih metoda, posebno za dijagnostiku monogenskih bolesti. Razvoj cenovno prisupačnih i brzih tehnologija sekvenciranja naredne generacije (next generation sequencing-NGS) kao i njihova implementacija u prenatalnoj dijagnostici u poslednjih nekoliko godina, dovela do revolucionarnih pomaka u oblasti fetalne medicine. Najveća prednost metode u odnosu na prethodno korišćene (sekvenciranje po Sangeru) su istovremena analiza velikog broja gena, tj nije potrebno prethodno odabrati jedan gen od interesa što je zbog poteškoća u određivanju tačnog fenotipa fetusa često i bilo nemoguće. U kliničkoj dijagnostici su u upotrebi različiti genski paneli ili sekvenciranje celog egzoma (WES, whole exome sequencing), dok se sekvenciranje genoma (whole genome sequencing-WGS) za sada primenje u naučne svrhe. Dijagnostički prinos ES u prenatalanoj dijagnostici zavisi od vrste i broja kongentalnh anomalija i kreće se od 6–80%. Razvoj novih metoda koje omogućavaju multiomički pristup doveo je do značajnog poboljšanja dijagnostičkog prinosa u prenatalnoj medicini, što omogućava da se pacijentima predoči preciznija prognoza bolesti, potencijani modaliteti lečenja, rizici za rekurenciju u narednim trudnoćama i donošen je informisanih odluka o daljem toku trudnoće.

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APPLICATION OF NEXT GENERATION SEQUENCING IN PRENATAL DIAGNOSTICS

Ivana Joksić

Gynecology and Obstetrics Clinic »Narodni front«, Belgrade, Serbia

Congenital anomalies affect 2–4% of pregnancies and can lead to significant structural or functional damage to the affected organs and systems, with severe consequences that include intrauterine or neonatal demise, chronic diseases and long-term disabilities, which represent a great burden for the affected families and the health system. Various types of genetic changes can cause development of anomalies; the most significant are chromosomal aberrations (structural and numerical), copy number variations (CNV; microdeletions-microduplications) and single gene disorders. Genetic testing of the fetus is recommended to all pregnant women when fetal anomalies are detected by ultrasound examination. Traditionally, with the application of cytogenetics and molecular cytogenetics techniques, the cause of anomalies could be established in about 35% of cases, so etiology remained unclear in significant portion of patients. This has led to a need for the implementation of new methods, especially for the diagnosis of single gene disorders. The development of affordable and fast next generation sequencing technologies (NGS) as well as their implementation in prenatal diagnostics in the last few years has led to revolutionary developments in the field of fetal medicine. The biggest advantage of the method compared to the previously used ones (Sanger sequencing) is the simultaneous analysis of a large number of genes; now it is not necessary to select one gene of interest beforehand, which was often impossible due to the difficulties in fetal phenotyping. Various gene panels or whole exome sequencing (WES) are used in routine diagnostics, while whole genome sequencing (WGS) is currently used for scientific purposes only. The diagnostic yield of ES in prenatal diagnosis depends on the type and number of congenital anomalies and ranges from 6–80%. The development of new methods that enable a multiomic diagnostic approach has led to a significant improvement in prenatal medicine, allowing for individualized and personalized genetic counseling regarding potential treatment modalities, risks for recurrence in subsequent pregnancies, and informed decisions about the further course of pregnancy.

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ANALIZA LIPIDOMA I PROTEOMA HDL ČESTICA U TRUDNOĆI

Jelena Vekić[1], Aleksandra Zeljković[1], Danijela Ardalić[2], Marija Sarić-Matutinović[1], Tamara Gojković[1], Jasmina Ivanišević[1], Milica Miljković-Trailović[1], Jelena Munjas[1], Snežana Jovičić[1], Vesna Spasojević-Kalimanovska[1], Željko Miković[2], Aleksandra Stefanović[1]

[1]Katedra za medicinsku biohemiju, Univerzitet u Beogradu-Farmaceutski fakultet, Beograd, Srbija
[2]Ginekološko-akušerska klinika »Narodni front«, Beograd, Srbija

Razvoj komplikacija u trudnoći, poput gestacijskog dijabetesa, gestacijske hipertenzije, preeklampsije, prevremenog porođaja i zastoja u rastu fetusa, posredovan je različitim uzrocima, koji su usko povezani sa poremećajima u metabolizmu majke. Poznato je da tokom trudnoće u organizmu majke dolazi do metaboličkih promena koje su neophodne za adekvatan rast fetusa. Iako su promene u lipidnom profilu tokom trudnoće uobičajene, njihovi različiti aspekti, poput promena u strukturi i funkciji lipoproteina visoke gustine (HDL), još uvek nisu dobro shvaćeni. Dosadašnje naučno i kliničko iskustvo podržava hipotezu o protektivnoj ulozi HDL čestica tokom metaboličke adaptacije na trudnoću. Neadekvatno povećanje koncentracije HDL-holesterola tokom drugog tromestra udruženo je sa razvojem komplikacija u trudnoći. Danas je dobro potvrđeno da protektivna svojstva HDL čestica daleko prevazilaze ulogu u procesu reverznog transporta holesterola. Njihov proteom i lipidom obuhvata više od 100 različitih komponenti, što ukazuju na brojne specifične fiziološke funkcije. Međutim, strukturne i funkcionalne promene HDL čestica tokom trudnoće su retko proučavane, dok je veza između HDL proteoma i lipidoma sa ishodom trudnoće i kardiometaboličkog zdravlja u kasnijem životnom dobu majke i deteta skoro potpuno neispitana. Istraživanje lipidoma i proteoma HDL čestica tokom nekomplikovane i visokorizične trudnoće omogućava otkrivanje komponenti HDL čestica čije su promene najizraženije, te stoga mogu pomoći u odabiru novih biomarkera za predviđanje i praćenje komplikacija trudnoće, kao iskorak ka personalizovanoj prevenciji.

ANALYSIS OF HDL LIPIDOME AND PROTEOME IN PREGNANCY

Jelena Vekić[1], Aleksandra Zeljković[1], Danijela Ardalić[2], Marija Sarić-Matutinović[1], Tamara Gojković[1], Jasmina Ivanišević[1], Milica Miljković-Trailović[1], Jelena Munjas[1], Snežana Jovičić[1], Vesna Spasojević-Kalimanovska[1], Željko Miković[2], Aleksandra Stefanović[1]

[1]Department of Medical Biochemistry, University of Beograde-Faculty of Pharmacy, Belgrade, Serbia
[2]The Obstetrics and Gynecology Clinic »Narodni front«, Belgrade, Serbia

The development of pregnancy complications, such as gestational diabetes, gestational hypertension, pre-eclampsia, premature birth and fetal growth restriction, has various causes, all of which are closely linked to disturbances in maternal metabolism. Metabolic changes in the mother's body during pregnancy are essential for adequate fetal growth. Although the changes in the lipid profile during pregnancy are common, their various aspects, such as changes in the structure and function of high-density lipoproteins (HDL), are not yet well understood. Current scientific and clinical experience supports the hypothesis of a protective effect of HDL in the context of metabolic adaptation to pregnancy. An insufficient increase in HDL-cholesterol concentration during the second trimester is positively associated with development of pregnancy complications. It is now firmly established that the protective function of HDL particles goes far beyond the role in reverse cholesterol transport. Their proteome and lipidome comprises more than 100 different compounds that indicate many specific physiological functions. However, structural and functional changes in HDL during pregnancy are rarely studied, while the link of HDL proteome and lipidome with pregnancy outcome and subsequent cardiometabolic health of the mother and child are almost completely unexplored. Investigating lipidomic and proteomic aspects of HDL particles during uncomplicated and high-risk pregnancy allows the detection of HDL-related parameters that undergo the most striking changes and could therefore help in the selection of novel biomarkers for the prediction and monitoring of pregnancy complications, as a step toward personalised prevention.

MIKRORNK U NEALKOHOLNOJ MASNOJ BOLESTI JETRE – NOVI DIJAGNOSTIČKI I PROGNOSTIČKI BIOMARKERI

Ana Ninić[1], Sanja Erceg[1], Jelena Munjas[1], Miron Sopić[1], Jelena Kotur-Stevuljević[1], Aleksandra Klisić[2], Milica Mamić[3], Miloš Mitrović[4], Nataša Miljković[5], Ratko Tomašević[6]

[1]Katedra za medicinsku biohemiju, Univerzitet u Beogradu-Farmaceutski fakultet, Beograd, Srbija
[2]Univerzitet Crne Gore-Medicinski fakultet, Podgorica, Crna Gora
[3]Služba za laboratorijsku dijagnostiku, KBC Zemun, Beograd, Srbija
[4]Kliničko odeljenje za gastroenterologiju i hepatologiju, KBC Zvezdara, Beograd, Srbija
[5]Služba za laboratorijsku dijagnostiku, KBC Zvezdara, Beograd, Srbija
[6]Klinika za internu medicinu, KBC Zemun, Beograd, Srbija

Nealkoholna masna bolest jetre (NAFLD), jedna od najčešćih hroničnih bolesti jetre, obuhvata širok spektar histopatoloških promena u jetri. Razvija se od intrahepatične akumulacije lipida (steatoze) preko nealkoholnog steatohepatitisa (NASH), na kraju do ciroze i hepatocelularnog karcinoma. Primenom rutinskih biohemijskih markera ne može da se predvidi napredovanje i ishod NAFLD-a, dok epigenetički markeri, kao što su mikro ribonukleinske kiseline (miRNA), koje utiču na ekspresiju gena i fenotip, dobijaju sve veći značaj u patogenezi ove bolesti. MiRNA su kratke endogene nekodirajuće jednolančane ribonukleinske kiseline koje dovode ili do degradacije informacione ribonukleinske kiseline (mRNA) ili do sprečavanja translacije zrelih mRNA. Povećanje ekspresije miRNA-122 stimuliše sinteze masnih kiselina u jetri i holesterola. MiRNA-34a je visoko eksprimirana u steatozi i NASH-u, a stimulacija njegove ekspresije povećava oksidativni stres i anabolizam lipida uzrokujući progresiju steatoze i inflamaciju jetre. Visoka ekspresija miRNA-21 povećava akumulaciju holesterola, oksidativni stres i inflamaciju, što dovodi do razvoja steatoze, inhibicije metabolizma lipoproteina i insulinske rezistencije u jetri. Sve ispitivane miRNA imaju veći potencijal da se primene kao neinvazivni biomarkeri u praćenju progresije NAFLD-a i kao indikatori stadijuma NAFLD-a u odnosu na klasične biohemijske markere funkcije jetre.

MICRORNA IN NONALCOHOLIC FATTY LIVER DISEASE – NOVEL DIAGNOSTIC AND PROGNOSTIC BIOMARKERS

Ana Ninić[1], Sanja Erceg[1], Jelena Munjas[1], Miron Sopić[1], Jelena Kotur-Stevuljević[1], Aleksandra Klisić[2], Milica Mamić[3], Miloš Mitrović[4], Nataša Miljković[5], Ratko Tomašević[6]

[1]Department for Medical Biochemistry, University of Belgrade-Faculty of Pharmacy, Belgrade, Serbia
[2]University of Montenegro-Faculty of Medicine, Podgorica, Montenegro
[3]Department of Laboratory Diagnostics, Clinical Hospital Center Zemun, Belgrade, Serbia
[4]Clinical Department of Gastroenterology and Hepatology, Clinical Hospital Center Zvezdara, Belgrade, Serbia
[5]Department of Laboratory Diagnostics, Clinical Hospital Center Zvezdara, Belgrade, Serbia
[6]Clinic for Internal Medicine, Clinical Hospital Center Zemun, Belgrade, Serbia

Nonalcoholic fatty liver disease (NAFLD), one of the most common chronic liver diseases, comprises of a wide range of histopathological changes in liver. It develops from the intrahepatic lipid accumulation (steatosis) through nonalcoholic steatohepatitis (NASH) ultimately to cirrhosis and hepatocellular carcinoma. Routinely used laboratory markers are not able to predict NAFLD progression and outcome, whereas epigenetic markers, such as micro ribonucleic acids (miRNA), which affect gene expression and phenotype gain great significance in the disease pathogenesis. MiRNAs are short endogenous noncoding single-stranded RNAs that cause either messenger RNA (mRNA) degradation or prevention of mature mRNAs translation. Upregulation of miRNA-122 increases hepatic fatty acids and cholesterol synthesis. MiRNA-34a is highly expressed in liver steatosis and NASH and its upregulation enhances oxidative stress and lipid anabolism, aggravating hepatic steatosis and inflammation. High expression of miRNA-21 increases cholesterol accumulation, oxidative stress, and inflammation, which lead to steatosis development, inhibition of lipoprotein metabolism and hepatic insulin resistance. All the examined miRNAs have more potential to be used as noninvasive tools in monitoring NAFLD progression and NAFLD severity indicators than classic biochemical liver function markers.

PRIMENA MULTIOMIČKOG PRISTUPA U DIJAGNOZI AKUTNOG KORONARNOG SINDROMA

Jelena Munjas[1], Sandra Vladimirov[1], Tamara Gojković[1], Milica Miljković-Trailović[1], Ana Ninić[1], Aleksandar Davidovic[2,3], Luka Vukmirovic[1], Miron Sopić[1]

[1]Katedra za medicinsku biohemiju, Univerzitet u Beogradu-Farmaceutski fakultet, Beograd, Srbija
[2]Kliničko odeljenje za kardiovaskularne bolesti, Kliničko-bolnički centar Zvezdara, Beograd, Srbija
[3]Katedra za Internu Medicinu, Stomatološki fakultet, Univerzitet u Beogradu, Beograd, Srbija

Ishemijska bolest srca (IBS) je posledica stvaranja aterosklerotskih plakova koji ometaju normalan protok krvi kroz srčani mišić. Bolest je hronična, ali najčešće progresivna, čak i u naizgled klinički asimptomatskim periodima. IBS može imati duge, klinički stabilne periode, ali takođe može postati klinički nestabilna u bilo kom trenutku, najčešće kao posledica akutnog aterotrombotičkog događaja uzrokovanog rupturom ili erozijom plaka. Klinička slika IBS varira od stabilne angine pektoris (SAP) do akutnog koronarnog sindroma (AKS); u zavisnosti od sastava plaka, stabilnosti plaka i njegove interakcije sa vaskularnim mikrookruženjem. Tradicionalna dijagnostička klasifikacija i menadžment IBS ne odražavaju na pravi način heterogenost patofizioloških mehanizama koji dovode do destabilizacije plaka i akutnih koronarnih događaja. Zbog toga su neophodni novi dijagnostički pristupi koji bi omogućili bolju stratifikaciju visokorizičnih pacijenata, predikciju razvoja velikih neželjenih kardiovaskularnih događaja i adekvatniji menadžment bolesti. Projekat MSCA SE CardioSCOPE, zasnovan na dobro definisanim prospektivnim kohortama, koristi analizu transkriptoma, proteoma i metaboloma, uz korišćenje strategije mašinskog učenja/veštačke inteligencije (ML/AI), za otkrivanje novih patoloških činilaca u AKS-u. Ovakvim pristupom omogućava se konstruisanje personalizovanih multi-marker modela korišćenjem ML algoritama, koji se mogu primeniti za otkrivanje visoko-rizičnih pacijenata sa IBS i bolju dijagnostiku AKS.

APPLICATION OF MULTIOMIC APPROACH IN DIAGNOSIS OF ACUTE CORONARY SYNDROME

Jelena Munjas[1], Sandra Vladimirov[1], Tamara Gojković[1], Milica Miljković-Trailović[1], Ana Ninić[1], Aleksandar Davidovic[2,3], Luka Vukmirovic[1], Miron Sopić[1]

[1]Department for Medical Biochemistry, University of Belgrade-Faculty of Pharmacy, Belgrade, Serbia
[2]Intern Clinic, Clinical Ward for Cardiovascular Diseases, Clinical-Hospital Centre Zvezdara, Belgrade, Serbia
[3]Department for Internal Medicine, Faculty of Dentistry, University of Belgrade, Belgrade, Serbia

Coronary artery disease (CAD) is a consequence of the narrowing or hardening of arteries, restricting blood flow to the heart, driven by atherosclerosis. The disease is chronic, but most often progressive, even in apparently clinically silent periods. CAD can have long, stable periods but can also become unstable at any time, typically due to an acute atherothrombotic event caused by plaque rupture or erosion. Clinical presentation of CAD varies from stable angina pectoris (SAP) to acute coronary syndrome (ACS); depending on the plaque composition, plaque stability, and its interaction with the vascular microenvironment. The traditional diagnostic classification of CAD and its therapeutic management do not properly address the heterogeneity of pathophysiological mechanisms of plaque destabilization, leading to acute coronary events, which urges novel therapeutic and diagnostic approaches. The MSCA SE CardioScope project, relaying on well-defined prospective cohorts, employs transcriptomic, proteomic, and metabolomics signatures with the use of machine learning/artificial intelligence (ML/AI) strategy to discover novel pathological players of ACS. This approach will enable the construction of personalized multi-marker models using ML algorithms for identification of high-risk patients and for improved ACS diagnosis and prediction of the development of major adverse cardiovascular outcomes.

ZNAČAJ PERSONALIZOVANE ISHRANE U POSTIZANJU OPTIMALNOG NUTRITIVNOG STATUSA

Ana Petrović

Nutricionistički studio Ana Petrović, Beograd, Srbija

Dijetarne preporuke za postizanje optimalnog zdravlja i održavanje normalne telesne mase se uglavnom zasnivaju na opštim principima za ishranu i kao takvi su namenjeni opštoj, zdravoj populaciji. Zaista, randomizovana kontrolisana ispitivanja su pokazala da samo 40% ljudi ima koristi od ovih opštih dijetarnih intervencija, a koje su uspostavljene sa ciljem smanjenja rizika od hroničnih nezaraznih bolesti u čitavoj populaciji. Ograničena efikasnost može se pripisati inter-individualnoj varijabilnosti na koju utiče niz faktora kao što su genetika, epigenetika, bihejvioralne i psihološke karakteristike, ali i uticaj životne sredine. Sve je veći broj dokaza koji ukazuju da je personalizovana ishrana efikasnija strategija za postizanje optimalnog zdravlja i prevenciji hroničnih oboljenja. Dva glavna razloga koji podržavaju primenu personalizovane ishrane u postizanju dugoročnih zdravstvenih ishoda su pre svega: 1) biološki razlozi, odnosno interindividualne varijacije koje su uslovljene već nabrojanim faktorima i 2) individualizovani pristup pokazuje pozitivan uticaj na motivaciju i pridržavanje datim preporuka o načinu ishrani. Naime, individualno dizajnirana ishrana uzima u obzir lične preferencije pojedinca u vezi izbora hrane, kao i prisustvo alergija i intolerancija na određene namirnice što značajno povećava motivaciju pojedinca da se pridržava propisanog dijetarnog režima i usvoji ga kao deo zdravog stila života.

THE SIGNIFICANCE OF PERSONALIZED DIET IN ACHIEVING OPTIMAL NUTRITIVE STATUS

Ana Petrović

Nutritionist studio Ana Petrović, Belgrade, Serbia

Dietary recommendations for achieving optimal health and maintaining normal body weight are generally based on general nutritional principles and as such are intended for the general, healthy population. Indeed, randomized controlled trials have shown that only 40% of people benefit from these general dietary interventions, which have been established to reduce the risk of chronic noncommutable diseases in the entire population. The limited effectiveness can be attributed to inter-individual variability influenced by a number of factors such as genetics, epigenetics, behavioral and psychological characteristics, but also the influence of the environment. A growing body of evidence indicates that personalized nutrition is a more effective strategy for achieving optimal health and preventing chronic disease. The two main reasons that support the application of personalized nutrition in achieving long-term health outcomes are first of all: 1) biological reasons, i.e. inter-individual variations that are conditioned by the factors already listed and 2) an individualized approach shows a positive impact on motivation and adherence to given recommendations on the way of eating. Namely, an individually designed diet takes into account the individual's personal preferences regarding the choice of food, as well as the presence of allergies and intolerances to certain foods, which significantly increases the individual's motivation to adhere to the prescribed dietary regimen and adopt it as part of a healthy lifestyle.

UTICAJ PROMENE ŽIVOTNOG STILA NA NIVO ADIPOCITOKINA KOD GOJAZNIH ISPITANIKA SA POREMEĆENOM GLIKOREGULACIJOM

Margarita Dodevska

Institut za javno zdravlje Srbije »Dr Milan Jovanović Batut«, Beograd, Srbija

Savremeni način življenja karakterističan je po smanjenom nivou fizičke aktivnosti i promenjenim dijetarnim navikama, što za posledicu ima poremećaj telesne mase. Povećana izloženost hrani sa većom energetskom gustinom i manjom količinom vlakana i

THE INFLUENCE OF LIFESTYLE CHANGES TO THE LEVEL OF ADIPOCYTOKINES IN OBESE PERSONS WITH DISTURBED GLYCOREGULATION

Margarita Dodevska

Institute for Public Health of Serbia »Dr Milan Jovanovic Batut«, Belgrade, Serbia

The modern way of life is characterized by a reduced level of physical activity and changed dietary habits, which results in a disorder of body mass. Increased exposure to food with higher energy density and less fiber and with a sedentary lifestyle leads to a positive

uz sedentarni način života, dovodi do pozitivnog energetskog bilansa i povećanje telesne mase i gojaznosti. Povišen indeks telesne mase (ITM) ili prekomerna adipoznost u zrelom dobu značajan je faktor rizika za brojne hronične bolesti kao što su dijabetes, kardiovaskularne bolesti, nealkoholna masna bolest jetre, hronična bolest bubrega i niz karcinoma povezanih sa gojaznošću. Jedna od strategija u lečenju gojaznosti je dijetarna intervencija uz povećanu fizičku aktivnost. U dijetarnim intervencijama za redukciju telesne mase akcenat je stavljen i na značaj konzumiranja biljne hrane koja je bogata dijetnim vlaknima (cela zrna žitarica, voće, povrće, mahunarke i jezgrasto voće). Vlakna su polimeri ugljenih hidrata sa tri ili više monomernih jedinica, koji se ne apsorbuju u tankom crevu. Vlakna predstavljaju veliki broj jedinjenja različitih molekulskih masa, fizičkih osobina i fizioloških efekata, pa zato postoje više klasifikacija. Na osnovu fizičkih karakteristika dele se na: viskozna i neviskozna, fermentabilna i nefermentabilna, kao i na rastvorljiva i nerastvorljiva u vodi. Istraživanja ukazuju na direktnu povezanost između unosa vlakana i regulaciju telesne mase, homeostazu glukoze, lipidnog profila i smanjenju sistemskih inflamatornih markera. Takođe, literaturni podaci ukazuju i da su rastvorljiva vlakna efikasna u smanjenju i regulaciju određenih inflamatornih adipocitokina.

VISOKO-PRERAĐENA HRANA I NUTRITIVNI STATUS

Nevena Ivanović

Katedra za bromatologiju, Univerzitet u Beogradu-Farmaceutski fakultet, Beograd, Srbija

Pod visoko-prerađenom hranom (engl. Ultra-processed food, UPF) se najčešće podrazumevaju namirnice koje se dobijaju procesima višestruke prerade u industrijskim uslovima od formulacije sastojaka specijalno pripremljenih za industrijsku primenu. Ova hrana se najčešće karakteriše visokim sadržajem soli, šećera i zasićenih masti, odnosno nutrijentima čiji prekomerni unos ima negativne efekte na nutritivni status, dok sa druge strane ne sadrži dovoljne količine esencijalnih nutrijenata (vitamina i minerala) i dijetnih vlakana. što takođe nosi rizik od razvoja različitih nutritivnih deficita. Poslednjih decenija ova hrana čini značajan deo dijetarnog obrasca prosečnog potrošača u Evropi i Severnoj Americi, čineći i do 50–60% od ukupnog energetskog unosa. Istraživanja ukazuju da povećanje zastupljenosti visoko prerađenih proizvoda u globalnoj ponudi hrane direktno korelira sa sve većom incidencom hroničnih nezaraznih oboljenja na globalnom nivou. Štaviše, rezultati urađenih meta-analiza koje su analizirale uticaj ove hrane na zdravlje pokazuju da je visok unos visoko-prerađene hrane značajno povezan sa značajnim povećanjem rizika za razvoj gojaznosti,

energy balance and an increase in body weight and obesity. An elevated body mass index (BMI) or excess adiposity in adulthood is a significant risk factor for a number of chronic diseases such as diabetes, cardiovascular disease, non-alcoholic fatty liver disease, chronic kidney disease and a number of obesity-related cancers. One of the strategies in the treatment of obesity is dietary intervention with increased physical activity. Dietary interventions for weight reduction emphasize the importance of consuming plant-based foods rich in dietary fiber (whole grains, fruits, vegetables, legumes and nuts). Fibers are polymers of carbohydrates with three or more monomer units, which are not absorbed in the small intestine. Fibers represent a large number of compounds of different molecular weights, physical properties and physiological effects, which is why there are several classifications. Based on their physical characteristics, they are divided into: viscous and non-viscous, fermentable and non-fermentable, as well as soluble and insoluble in water. Research indicates a direct connection between fiber intake and regulation of body mass, glucose homeostasis, lipid profile and reduction of systemic inflammatory markers. Also, literature data indicate that soluble fibers are effective in reducing and regulating certain inflammatory adipocytokines.

HIGHLY PROCESSED FOOD AND NUTRITIONAL STATUS

Nevena Ivanović

Department of Bromatology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

Ultra-processed food (Ultra-processed food, UPF) usually means foods that are obtained through multiple processing processes in industrial conditions from the formulation of ingredients specially prepared for industrial use. This food is most often characterized by a high content of salt, sugar and saturated fat, i.e. nutrients whose excessive intake has negative effects on nutritional status, while on the other hand it does not contain sufficient amounts of essential nutrients (vitamins and minerals) and dietary fiber. which also carries the risk of developing various nutritional deficits. In recent decades, this food has formed a significant part of the dietary pattern of the average consumer in Europe and North America, accounting for up to 50–60% of the total energy intake. Research indicates that the increase in the representation of highly processed products in the global food supply directly correlates with the increasing incidence of chronic non-communicable diseases at the global level. Moreover, the results of the meta-analyses that analyzed the impact of this food on health show that a high intake of highly processed food is significantly associated with a signifi-

visokim obimom struka, niskim nivoima »dobrog« odnosno HDL-holesterola i razvojem metaboličkog sindroma. Takođe, visoka zastupljenost ove hrane u obrascu ishrane povezuje se sa povećanim rizikom od mortaliteta bez obzira na uzrok, kao i rizikom od razvoja kardivaskularnih i cerebrovaskularnih oboljenja, pa čak i razvoja depresije i kancera.

IMUNOMETABOLIČKA DIVERGENCIJA KOD PRIPADNIKA BILJNE I OMNIVORNE ISHRANE

Marta Despotović

Departman za ishranu i metabolizam, Institut za medicinska istraživanja, Univerzitet u Beogradu, Beograd, Srbija

Savremena istraživanja naglašavaju značaj ishrane bogate voćem, povrćem, integralnim žitaricama, zdravim mastima i proteinima za održavanje optimalnog zdravlja. Ova studija istražuje imunometaboličke razlike između biljne (veganske) i omnivorne (svaštojedske, tradicionalne) ishrane, fokusirajući se na to kakav je status imunskog sistema, lipidni profil, hematološki markeri, sastav crevne mikrobiote i metaboličko zdravlje. Analizom ključnih fizioloških parametara, ovo istraživanje teži da doprinese sveobuhvatnom shvatanju kako ishrana utiče na fiziološku homeostazu, ali i rizik od nastanka bolesti. Istraživanje imunometaboličkih diferencijacija između biljne i omnivorne ishrane nosi značajne implikacije za preventivnu medicinu i strategije javnog zdravlja. Cilj studije jeste pružanje uvida u potencijalne načine za optimizaciju zdravlja putem dijetalnih intervencija, otvarajući mogućnosti za personalizovane pristupe ishrani i prevenciji bolesti. Urađen je detaljan pregled naučne literature u periodu od januara 2000. do maja 2024. godine o biljnoj i omnivornoj ishrani gde su ispitivani: status imunskog sistema, lipidni profil, hematološki markeri, sastav crevne mikrobiote i metabolički markeri. Korišćeni su PubMed, Scopus i Web of Science baze podataka, sa sistematskim i detaljnim pregledom relevatnih studija. Selekcija podataka i rezultata obuhvatila je dizajn studije, karakteristike učesnika i ishrane, analizu biomarkera, metode i statističke analize. Kvalitet uključenih studija ocenjen je primenom utvrđenih kriterijuma i jačinom studije. Sinteza rezultata omogućila je razlikovanje i precizno određivanje diferencijalnih efekata dva tipa ishrane. Istraživanja su pokazala da individue na biljnoj ishrani imaju manju koncentraciju pro-inflamatornih markera u poređenju sa individuama na omnivornoj ishrani. Najistaknutiji inflamatorni markeri u većini studija koji su nađeni u nižoj koncentraciji su C-reaktivni protein (CRP), interleukin-6 (IL-6) i faktor nekroze tumora-alfa (TNF-alfa), ali ih ima

cant increase in the risk of developing obesity, a high waist circumference, low levels of »good« or HDL-cholesterol and the development of metabolic syndrome. Also, a high prevalence of this food in the diet is associated with an increased risk of mortality regardless of the cause, as well as the risk of developing cardiovascular and cerebrovascular diseases, and even the development of depression and cancer.

IMMUNOMETABOLIC DIVERGENCE IN PLANT-BASED AND OMNIVOROUS DIET ADHERENTS

Marta Despotović

Department for Nutrition and Metabolism, Institute for Medical Research, National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia

This study aims to explore the intricate immunometabolic disparities between adherents of plant-based and omnivorous diets. By meticulously investigating immune system status, lipid status, hematological markers, gut microbiota composition and metabolic markers, it seeks to uncover nuanced distinctions between these dietary cohorts. Through this investigation, we endeavor to deepen our understanding of the multifaceted physiological responses to dietary choices, thereby offering novel insights into the optimization of health outcomes and the prevention of disease. By analyzing these key physiological parameters, this research aims to contribute to a comprehensive understanding of how dietary choices impact health outcomes and disease susceptibility. The exploration of immunometabolic distinctions between plant-based and omnivorous diets holds significant implications for preventive medicine and public health strategies, offering insights into potential avenues for optimizing health and wellness through dietary interventions. A systematic review of scientific literature spanning from January 2000 to May 2024 was conducted to investigate the effects of plant-based diets on immune system response and status, lipid profiles, hematological markers, gut microbiota composition, and metabolic markers compared to omnivorous diets. PubMed, Scopus, and Web of Science databases were systematically searched for relevant studies. Studies comprising dietary interventions, observational cohorts, and cross-sectional analyses were considered eligible for inclusion. Data extraction encompassed study design, participant characteristics, dietary assessments, biomarker measurements, and statistical analyses. The quality of included studies was assessed using established criteria. Synthesis of results enabled the delineation of the differential effects of plant-based and omnivorous diets on various health outcomes. Evidence are demonstrating that individuals on plant-based diets exhibit lower levels of systemic inflammation and enhanced

mnogo više. Ovakav status imunskog sistema se najviše objašnjava visokim nivoom unosa fitonutrijenata, antioksidanasa i vlakana koji imaju anti-inflamatorni efekat. Kada je lipidni status u pitanju, kod individua na biljnoj ishrani pronađen je niži nivo ukupnog holesterola, LDL holesterola i triglicerida u poređenju sa omnivornom ishranom. Ovakav lipidni status ima kardioprotektivnu prirodu i povezan je sa smanjenim rizikom od kardiovaskularnih bolesti, ateroskleroze i metaboličkog sindroma. Hematološki status ima različite rezultate u odnosnu na studiju koja je rađena. Najveći broj studija pokazuje niže nivoe leukocita i eozinofila kod pojedinaca na biljnoj ishrani, dok neke studije pronalaze slične nivoe hematoloških markera između ova dva tipa ishrane, što ukazuje na potrebu za daljim istraživanjem kako bi se u potpunosti razumeo uticaj ishrane na ove markere. Na biljnoj ishrani je pronađena veća raznovrsnost i obilje bakterijskih vrsta, kao što su Bifidobacterium i Lactobacillus, koje utiču na zdravlje digestivnog i imunskog sistema, ali i niži nivo određenih patogenih bakterija i upalnih markera u crevima. Probiotske bakterije koje se umnožavaju pri konzumaciji namirnica biljnog porekla, fermentišu vlakna da bi proizvele kratkolančane masne kiseline (engl. short-chain fatty acids (SCFA)). Ove masne kiseline služe kao izvor energije za kolonocite, održavaju integritet crevne barijere i imaju imunomodulatorni efekat, dok smanjuju koncentraciju patogenih bakterija uključenih u disbiozu i inflamaciju creva. Biljna ishrana je povezana sa nižim rizikom od metaboličkog sindroma, dijabetesa tipa 2 i gojaznosti. Detektovana je povećana osetljivost na insulin i bolja kontrola glikemije. Pronađen je metabolički povoljan uticaj na adiponektin i leptin koji dovodi do poboljšanja energetske homeostaze i modulacije ekspresije gena uključenih u metabolizam lipida i funkciju mitohondrija, što doprinosi ukupnom metaboličkom zdravlju. Zaključak: Stanje imunskog sistema i inflamacija su direktno pod uticajem načina ishrane. Ovo istraživanje naglašava potencijal biljne ishrane i namirnica kako bi se poboljšala imunska funkcija, lipidni i hematološki status, stanje mikrobiote kao i metaboličko zdravlje. Razumevanje ovih imunometaboličkih razlika ključno je pri izboru optimalne ishrane, čiji glavni cilj jeste imunološko zdravlje i prevencija hroničnih bolesti. Kako su informacije u vezi dijetarnih izbora često kontradiktorne ili nedovoljne, potrebno je detaljno ispitati koja ishrana je adekvatna za ljudsku fiziologiju i održavanje zdravlja, što naglašava potrebu za dodatnim istraživanjima. Stoga, potrebno je donositi informisane izbore o ishrani gde se daje prioritet hranljivim namirnicama koje doprinose fiziološkom zdravlju. Usvajanje takvog pristupa ne samo da pomaže u prevenciji hroničnih bolesti, već i poboljšava kvalitet života kroz bolje mentalno i fizičko zdravlje.

immune function compared to those on omnivorous diets. Specifically, plant-based diet adherents have reduced levels of pro-inflammatory markers. Some of them are C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha), but there are many more. These improvements are largely attributed to the high intake of phytonutrients and antioxidants which bolster immune surveillance and regulation. In terms of lipid status, plant-based diets are associated with favorable profiles, including lower levels of total cholesterol, LDL cholesterol and triglycerides. These lipid improvements are linked to a reduced risk of cardiovascular diseases, atherosclerosis and metabolic syndrome, underscoring the cardioprotective nature of this diet patterns. The influence of plant-based diets on hematological markers is more nuanced. While some studies report lower levels of leukocytes and eosinophils in individuals consuming plant-based diets, other studies find comparable levels between plant-based and omnivorous groups, indicating the need for further research to fully understand these effects. Plant-based diets also promote a more diverse and beneficial gut microbiota compared to omnivorous diets. They enhance the growth of beneficial bacteria such as Bifidobacterium and Lactobacillus, which ferment dietary fiber to produce short-chain fatty acids (SCFAs). These SCFAs serve as an energy source for colonocytes, maintain gut barrier integrity and modulate immune responses, while reducing the abundance of pathogenic bacteria implicated in gut dysbiosis and inflammation. Finally, plant-based diets improve metabolic markers such as insulin sensitivity, glycemic control and adiposity. These diets are associated with lower risk factors for metabolic syndrome, type 2 diabetes and obesity. Moreover, plant-based diets influence biomarkers like adiponectin and leptin, enhancing energy homeostasis and insulin sensitivity, and modulate gene expression involved in lipid metabolism and mitochondrial function, contributing to overall metabolic health. In conclusion, the choice of dietary pattern profoundly influences immune responses and systemic inflammation. This research underscores the potential of plant-based dietary patterns to improve immune function, lipid profiles, gut microbiota composition and metabolic health, supporting their role in disease prevention and health promotion. Understanding these immunometabolic disparities is crucial for informing dietary recommendations aimed at optimizing immune function and preventing chronic diseases. This dichotomy in immunometabolic outcomes underscores the profound impact of dietary patterns on immune homeostasis and systemic health. As we navigate the complexities of modern nutrition, understanding the immunological repercussions of dietary choices becomes paramount. Therefore, by making informed dietary choices that prioritize nutrition that makes us healthy, individuals can effectively reduce inflammation and enhance their immune strength, leading to overall well-being and better health outcomes.

FORMIRANJE I INTERPRETACIJA MREŽE GREŠAKA ZA KVANTITATIVNE DIJAGNOSTIČKE TESTOVE

Nataša Bogavac Stanojević

Univerzitet u Beogradu – Farmaceutski fakultet, Srbija, Beograd, Srbija

Svojstva kvantitativnih dijagnostičkih testova (KDT) mogu se opisati analitičkom ili kliničkom tačnošću. Analitička tačnost se procenjuje upoređivanjem dva merenja u istim uzorcima, od kojih se jedno izvodi pomoću KDT, a drugo referentnom metodom. Tipičan statistički metod za procenu analitičke tačnosti je linearna regresija, a tipične metričke veličine analitičke tačnosti su prosečna razlika između izmerenih vrednosti referentnom metodom i KDT (MARD engl. – mean absolute relative difference) i stopa slaganja. Međutim, analitička tačnost ne ukazuje na kliničke posledice koje nastaju zbog neslaganje u rezultatima između dve metode. Preporučeni postupak za procenu kliničke tačnosti je analiza mreže grešaka. Ova analiza ukazuje na potencijalne terapijske greške uzrokovane pogrešnim rezultatima KDT. Nemaju sve greške merenja isti klinički uticaj. Male greške će verovatno prouzrokovati manju štetu po pacijenta u odnosu na velike greške, a šteta uzrokovana malim greškama je i manje ozbiljna. Analiza grešaka pomuću ovog postupka može se koristiti za svrstavanje grešaka merenja u zone niskog, srednjeg i visokog kliničkog rizika. Pomoću ove analize procenjuje se procenat grešaka u svakoj zoni rizika. Glavni korisnici mreže grešaka su proizvođači KDT i regulatorna tela. Zone mreže grešaka su prvenstveno definisane za «point of care« (PoC) za samokontrolu glukoze u krvi. Za regulatornu upotrebu preporučena su tri tipa mreže grešaka: Clarke Error Grid, Parkes Error Grid i Surveillance Error Grid. Sve su zasnovane na principu konsenzusa, a razlikuju se u odnosu na definisane zone rizika. ISO 15197 (In vitro diagnostic test systems — Requirements for blood glucose monitoring for self-testing in managing diabetes mellitus) navodi da 99% pojedinačnih vrednosti glukoze izmerenih PoC uređajem kod pacijenata sa dijabetesom tip 1, treba da budu u zonama A i B (zonama niskog i umerenog rizika za pogrešnu odluku o terapiji). Mreže grešaka su takođe važne za odluke o nabavci opreme i medicinskih sredstava; nova metoda mora imati analitičku i kliničku tačnost sličnu prethodnoj. Takođe, ova procedura se može koristiti za procenu učinka KDT tokom postmarketinškog nadzora. Rezultati mreže grešaka mogu se koristiti za dobijanje uvida u kliničke performanse KDT, posebno za praćenje glukoze. Međutim, informacije iz mreže grešaka ne bi trebalo da zamene analitičku tačnost, ali mogu pružiti dodatnu vrednost novom KDT.

THE CONSTRUCTION AND INTERPRETATION OF ERROR GRID FOR QUANTITATIVE DIAGNOSTIC ASSAYS

Nataša Bogavac Stanojević

University of Belgrade – Faculty of Pharmacy, Belgrade, Serbia

The performance of quantitative diagnostic assays (QDA) can be described by analytical or clinical accuracy. Analytical accuracy is assessed by comparing two measurements in the same data sets, one performed with the QDA and the other with a reference method. A typical statistical method for assessing analytical accuracy is linear regression, and typical metrics of analytical accuracy are the mean absolute relative difference (MARD) and the agreement rate. However, analytical accuracy cannot show the clinical consequences of different treatment decisions due to differences in method results. The recommended tool for assessing clinical accuracy is the error grid analysis. It shows the potential therapeutic errors caused by incorrect treatment decisions due to erroneous QDA results. Not all measurement errors have the same clinical impact. Small errors are likely to cause less harm than large ones, and the harm caused by small ones is less serious. Error grids can be used to categorise measurement errors into zones with low, moderate or high risk for wrong therapeutic decisions. Error grids also indicate the percentage of data in each risk zone. The main users of an error grid are QDA manufacturers and regulatory bodies. The error grid zones are primarily defined for point-of-care (PoC) medical devices for blood glucose self-monitoring. Three error grids, based on a consensus approach with different zone boundaries, are recommended for regulatory use: Clarke Error Grid, Parkes Error Grid and Surveillance Error Grid. ISO 15197 (In vitro diagnostic test systems — Requirements for blood glucose monitoring for self-testing in managing diabetes mellitus) specifies that 99% of individual measured glucose values measured in type 1 diabetics patients should fall into zones A and B (zones of low and moderate risk for the wrong therapy decision). Error grids are also important for purchasing decisions; a new method must have analytical and clinical accuracy similar to the previous one. They can also be used to evaluate the performance of QDA during post-market surveillance. The results of the error grid can be used to get an overview of the clinical performance of QDA, especially in glucose monitoring. However, the information from the error grid should not replace analytical accuracy but can provide added value to the new QDA.

UPRAVLJANJE RIZICIMA U PREANALITIČKOJ FAZI

Sonja Šuput

Služba za laboratorijsku dijagnostiku, Acibadem Belmedic, Beograd, Srbija

Preanalitička faza u kliničkoj laboratoriji obuhvata sve one postupke i procese koji se dešavaju pre samog analiziranja uzoraka. Ova faza uključuje pripremu pacijenta, uzimanje uzoraka, transport, obradu i skladištenje uzoraka. Efikasno upravljanje rizikom u ovoj fazi je od ključnog značaja jer greške mogu dovesti do netačnih (neadekvatnih) rezultata merenja, pogrešnih dijagnoza i ugrožavanja bezbednosti pacijenata. Prvi korak u upravljanju rizicima u preanalitičkoj fazi je identifikovanje potencijalnih izvora grešaka. One se mogu široko kategorisati kao greške pri identifikaciji pacijenata (pomešani uzorci), greške u prikupljanju uzoraka (odnosi se na neispravan tip uzorka, nepravilne tehnike prikupljanja), greške u transportu i rukovanju (neodgovarajuća temperatura, kašnjenje), greške u pripremi i obradi (netačno obeležavanje, kontaminacija, neodgovarajuće alikvotiranje), greške skladištenja (neodgovarajući uslovi koji mogu da naruše integritet uzorka). Nakon identifikovanja potencijalnih rizika, vrši se procena njihovih mogućih efekata i verovatnoće nastanka. Ovo podrazumeva aspekte kao što su stepen ozbiljnosti događaja sa procenom potencijalnog uticaja greške na negu pacijenata, kao i učestalost pojavljivanja pojedinačnih neželjenih događaja. Istovremeno, procena verovatnoće otkrivanja greške pre nego što ona može da utiče na zdravstvenu negu i dobrobit pacijenta, predstavlja krajnji cilj procesa. Na osnovu procene rizika, laboratorija treba da definiše strategije koje se primenjuju za ublažavanje rizika u preanalitičkoj fazi. Ovo se najbolje postiže uspostavljanjem standardizovanih procedura vezanih za proces identifikacije pacijenata, prikupljanje uzoraka, obeležavanje i transport. Redovna obuka i procene kompetencija osoblja koje učestvuje u preanalitičkim procesima predstavljaju komponentu od najvećeg značaja za ovu temu, kao i upotreba automatizovanih sistema u svrhu smanjenja količine ljudskih grešaka. Obezbeđivanje odgovarajućih uslova okoline (npr. temperatura, vlažnost) tokom transporta i skladištenja uzoraka i efikasnih kanala komunikacije među zdravstvenim radnicima, laboratorijskim osobljem i učesnicima u transportu, pomaže da se minimizira količina ukupnog broja grešaka i doprinosi bržem rešavanju problema. Upravljanje rizikom je kontinuiran proces. Radi stalnog praćenja i unapređivanja celokupnog procesa neophodno je da se sprovodi prikupljanje podataka o preanalitičkim greškama i analiziranje trendova kako bi se identifikovala područja za poboljšanje. Istovremeno, poželjno je da se stvori podsticajno okruženje za prijavljivanje preanalitičkih grešaka, kako onih izbegnutih, tako i onih koje su se dogodile da bi se razumeli i otklonili njihovi uzroci. Periodična revizija i pregled protokola i procedura, neophodni su za uvođenje novih najboljih praksi i tehnologija, pri čemu je

RISK MANAGEMENT IN PREANALYTICAL PHASE

Sonja Šuput

Department for Laboratory Diagnostics, Acibadem Belmedic, Belgrade, Serbia

The preanalytical phase in a medical laboratory encompasses all the procedures and processes that occur before the actual analysis of the samples. This phase includes patient preparation, specimen collection, transportation, processing, and storage. Effective risk management in this phase is crucial as errors can lead to inaccurate test results, misdiagnoses, and compromised patient safety. The first step in managing risks in the preanalytical phase is identifying potential sources of errors. These can be broadly categorized as patient identification errors (can lead to sample mix-ups), sample collection errors (reffers to incorrect sample type, improper collection techniques), transportation and handling errors (improper temperatures, delays), preparation and processing errors (incorrect labeling,contamination, inappropriate aliquoting), storage errors (incorrect conditions that might degrade sample integrity). After identifying potential risks, the next step is to assess their impact and likelihood. This involves severity of the possible event with assessing potential impact of an error on patient care as well as frequency of occuring specific adverse events. At the same time to assess the likelihood of detecting the error before it affects patient care stands for ultimate objective of the process. Based on the risk assessment, a laboratory should define strategies to be implemented to mitigate risks in the preanalytical phase. This is best achieved through setting up standardized procedures for patient identification process, sample collection, labeling and transportation. Regular training and competency assessments for staff involved in preanalytical processes present a component of utmost significance for this topic as much as utilizing automated systems to help reduce human errors. Ensuring proper environmental conditions (e.g., temperature, humidity) during sample transportation and storage and effective communication channels among healthcare providers, laboratory staff, and couriers helps to minimize amount of errors and to address issues promptly. Risk management is an ongoing process. Continuous monitoring and improvement require collecting data on preanalytical errors and analyzing trends to identify areas for improvement, encouraging the reporting of preanalytical errors and near misses to understand and address root causes, periodic review of protocols and procedures to incorporate new best practices and technologies and creating a feedback mechanism for staff to suggest improvements based on their experiences and observations.

veoma bitan cilj i uspostavljanje mehanizama za davanje povratnih informacija od strane osoblja kojem je potrebno omogućiti način da predlaže poboljšanja na osnovu svojih iskustava i zapažanja. Efikasno upravljanje rizikom u preanalitičkoj fazi je od suštinskog značaja za obezbeđivanje tačnosti i pouzdanosti rezultata laboratorijskih ispitivanja. Sistematskim identifikovanjem, procenom i ublažavanjem rizika, medicinske laboratorije mogu poboljšati bezbednost pacijenata, povećati dijagnostičku tačnost i održati visoke standarde kvaliteta. Kontinuirano praćenje i posvećenost stalnom poboljšanju su vitalne komponente snažne strategije upravljanja rizikom u preanalitičkoj fazi.

UPRAVLJANJE RIZICIMA U LABORATORIJAMA PRIMARNOG NIVOA ZDRAVSTVENE ZAŠTITE – OD TEORIJE DO PRAKSE

Danijela Ristovski Kornic

Dom Zdravlja Pančevo, Pančevo, Srbija

Upravljanje rizicima predstavlja važan deo sistema kvaliteta u medicinskim laboratorijama. Rutinski rad u laboratoriji iziskuje primenu praktičnih metoda za identifikovanje i procenu rizika, aktivnosti u cilju smanjenja rizika kao i dalju evaluaciju, praćenje i obaveštavanje. Primarna zdravstvena zaštita je suočena sa brojnim izazovima. Iako je u teoriji akcenat na preventivi, realnost ukazuje na veći udeo kurativnih u odnosu na preventivne aktivnosti. Pacijenti žive sa brojnim hroničnim stanjima, sa kompleksnim zdravstvenim i socijalnim uslovima i potreban im je visok nivo podrške na primarnoj zdravstvenoj zaštiti. Važna karika zdravstvenog sistema jesu službe laboratorijske dijagnostike. Kako je pacijent fokus zdravstvenog sistema, laboratorije na primarnom nivou treba da omoguće pravilnu podršku i omoguće pravovremene i pouzdane rezultate. Visoki tehnološki razvoj i automatizacija svih glavnih procesa rada u laboratorijama, implementacija laboratorijskih informacionih sistema doveli su do značajnih unapređenja kvaliteta rada. Uprkos unapređenju kvaliteta rizici i dalje postoje i ukoliko se ne kontrolišu mogu dovesti do ozbiljnih posledica. Identifikacija rizika je najznačajniji korak u uspostavljanju menadžmenta rizikom, na osnovu čega se pravi plan kontrole kvaliteta laboratorije. Plan kontrole kvaliteta mora biti individualan za laboratoriju i treba da identifikuje slabosti u preanalitičkoj, analitičkoj i postanalitičkoj fazi rada i uspostavi niz preventivnih aktivnosti sa ciljem smanjenja rizika za nastanak grešaka. Upravljanje rizicima u medicinskim laboratorijama treba posmatrati kao skup preventivnih mera sa ciljem unapređenja kvaliteta a identifikacija i umanjenje potencijalnih rizika u svim segmentima rada laboratorije je od izuzetne važnosti u pogledu bezbednosti pacijenata i zaposlenih.

Effective risk management in the preanalytical phase is essential for ensuring the accuracy and reliability of laboratory test results. By systematically identifying, assessing, and mitigating risks, medical laboratories can enhance patient safety, improve diagnostic accuracy, and maintain high standards of quality. Continuous monitoring and a commitment to ongoing improvement are vital components of a robust risk management strategy in the preanalytical phase.

RISK MANAGEMENT IN PRIMARY HEALTHCARE LABORATORIES – FROM THEORY TO PRACTICE

Danijela Ristovski Kornic

Health Centre Pančevo, Pančevo, Serbia

Risk management is an integral part of medical laboratory in assuring quality and safety. Routine laboratory work requires the use of practical methods for risk identification,assessment, evaluation, monitoring and notification. Primary health care is faced with many challenges. The current situation indicates the need for greater activities in terms of disease prevention. Patients live with numerous chronic conditions, with complex health and social situation, and they need a high level of support in primary health care. The patient is the focus of the healthcare system, and laboratories at the primary level need to provide proper support and provide timely and reliable results. High technological development and automation of all the main phases of testing in medical laboratories, implementation of laboratory information systems have led to significant improvements in quality, but despite the improvement, risks still exist and if not controlled, can lead to serious consequences. Identifying risks and establishing a quality control plan is the most important step in establishing risk management. The quality control plan must be individual for the laboratory and should identify weaknesses in the pre-analytical, analytical and post-analytical phases with the use of preventive actions to reduce the risk of errors. Risk management in medical laboratories should be implemented as a set of preventive measures with the aim of improving quality and the identification and reduction of potential risks in all phases of laboratory testing is of great importance in terms of patient and employee safety.

PLAN KONTROLE KVALITETA U UPRAVLJANJU RIZICIMA

Snežana Jovičić

Katedra za medicinsku biohemiju, Univerzitet u Beogradu-Farmaceutski fakultet, Beograd, Srbija

Medicinske laboratorije su odgovorne da osiguraju da su njihovi rezultati određivanja odgovarajućeg kvaliteta, odnosno da su pouzdani i tačni u skladu sa mogućnostima metoda i tehnologije koju koriste. Za to je neophodno razumeti rizike koji mogu da dovedu do neadekvatnog funksionisanja analitičkog sistema i adekvatno proceniti efektivnost kontrolnih procesa koji se koriste da te rizike smanje. Primenom pravilno odabranih kontrolnih procedura osigurava se da se svi rizici na odgovarajući način minimiziraju. Takođe, frekvencija sprovođenja kontrolnih procedura mora da bude proporcionalna riziku da se nanese šteta pacijentu netačnim rezultatom. Svi ovi elementi moraju da se analiziraju prilikom formiranja efikasnog plana sprovođenja kontrole kvaliteta. Adekvatan plan kontrole kvaliteta mora da se uspostavi, održava i modifikuje u skladu sa karakteristikama analitičkog sistema, a na osnovu potrebnih performansi za medicinsku primenu rezultata analitičkog procesa. Mora da bude u skladu sa regulatornim i zahtevima akreditacije, kao i sa lokalnom strukturom zdravstvenog sistema. Plan kontrole kvaliteta predstavlja dokumentovanu strategiju za otklanjanje i prevenciju grešaka u analitičkom procesu, koja opisuje izvođenje, potrebne resurse i redosled specifičnih aktivnosti kako bi se kontrolisao njegov kvalitet i zadovoljili kriterijumi neophodni za primenu dobijenih rezultata u kliničkoj praksi. Medicinska laboratorija uspostavlja plan kontrole kvaliteta da spreči pojavu grešaka i identifikuje potencijalne neusaglašenosti pre dolaska rezultata do krajnjeg korisnika i donošenja kliničke odluke. Razvoj plana kontrole kvaliteta zahteva razumevanje preanalitičkih, analitičkih i postanalitičkih procesa i identifikaciju slabosti i potencijalnih nedostataka u ovim procesima koji mogu potencijalno da dovedu do dobijanja pogrešnih rezultata koji mogu direktno da nanesu štetu pacijentu.

QUALITY CONTROL PLAN IN RISK MANAGEMENT

Snežana Jovičić

Department for Medical Biochemistry, Univesity of Belgrade-Faculty of Pharmacy, Belgrade, Serbia

Medical laboratories are responsible for ensuring that the results of their analytical processes are of appropriate quality, that is, that they are reliable and accurate in accordance with the capabilities of the methods and technology in use. For that purpose, it is necessary to understand the risks that can lead to inadequate functioning of the analytical system and adequately assess the effectiveness of the control processes used to reduce those risks. Applying properly selected control procedures ensures that all risks are appropriately minimized. Also, the frequency of performing control procedures must be proportional to the risk of harming the patient with an incorrect result. All these elements must be analyzed when defining an effective quality control implementation plan. An adequate quality control plan must be established, maintained and modified in accordance with the characteristics of the analytical system, and based on the required performances for the medical application of the results of the analytical process. It must comply with regulatory and accreditation requirements, as well as with the local healthcare structure. The quality control plan is a documented strategy for the elimination and prevention of errors in the analytical process. It describes the execution, required resources and sequence of specific activities in order to control its quality and meet the criteria necessary for the application of the obtained results in clinical practice. The medical laboratory establishes a quality control plan to prevent errors and identify potential nonconformities before the results reach the end-user and a clinical decision is made. Developing a quality control plan requires an understanding of the preanalytical, analytical, and postanalytical processes and the identification of weaknesses and potential deficiencies in these processes that could eventually lead to erroneous results that could directly harm the patient.

UPRAVLJANJE RIZICIMA – FLEKSIBILNIJI PRISTUP TEMELJEN NA ISO 15189:2022

Domagoj Marijančević

Klinički bolnički centar Sestre milosrdnice, Klinički zavod za kemiju, Zagreb, Hrvatska

Zahtevi definisani u ISO 15189:2022 su promenjeni u smislu da je veći akcenat stavljen na upravljanje rizikom. Standard je fokusiran na pacijente i podstiče kontinuirano poboljšanje u minimiziranju rizika za pacijente koji čine okosnicu procesa upravljanja kvalitetom u laboratoriji. Laboratorija će imati proces za identifikaciju rizika od štete po pacijente i mogućnosti za poboljšanu negu pacijenata. Ishodi moraju biti u prvom planu, a poboljšani ishodi pacijenata su najviši prioritet za sve laboratorije. injenica da je upravljanje zasnovano na riziku glavna inovacija može biti potkrijepljena dokazima da se reč »rizik« pojavljuje 86 puta za razliku od verzije iz 2012. godine gdje se pojavljuje 12 puta. Ovo odražava promenu, odnosno »razmišljanje zasnovano na riziku« treba da bude svojstveno svim procesima usvojenim da bi se obezbedila dobra laboratorijska praksa. Rizik je toliko ugrađen u standard da bi trebalo da bude na vrhu procesa razmišljanja rukovodioca laboratorije. Nova verzija standarda sada zahteva da se aktivnosti koje se odnose na rizike i prilike moraju planirati i implementirati u sistem upravljanja i proceniti njihova efektivnost. Pored toga, nova verzija je manje striktna od prethodne, omogućavajući medicinskim laboratorijama veći nivo fleksibilnosti i kreativnosti u upravljanju rizikom. Standard ne zahteva implementaciju formalnog procesa upravljanja rizikom. Ovaj pristup u suštini odgovara na dva fundamentalna pitanja: 1) Koliko često nešto može poći naopako? i 2) Koje su posledice ove greške? Nova verzija ISO 15189 treba da se fokusira na povređivanje pacijenata u smislu kvantifikacije rizika. Preduzete aktivnosti moraju biti proporcionalne potencijalnom uticaju i da se prikazuju u evidenciji koju laboratorija vodi. Definisanje inovativnih zahteva omogućava laboratoriji da razvije efikasniju dokumentaciju i lakšu usklađenost sa zakonskim okvirima koji se mogu značajno razlikovati između zemalja na međunarodnom ili regionalnom nivou. Budući da analiza rizika predstavlja važnu ulogu u razvoju sistema menadžmenta kvalitetom, u skladu sa stanovištem ISO 9001:2015, novi Standard sadrži pet zahteva koji se odnose na analizu rizika umesto jednog u prethodnoj verziji. U novom Standardu, razvoj programa interne kontrole kvaliteta fokusiran je na »validnost značajnu za kliničko odlučivanje«. Učestalost se određuje analizom rizika od štete po pacijenta, a ne jednostavnim propisnim pristupom. Neophodno je izvršiti analizu rizika na rezultatima pacijenata na osnovu »kliničkog značaja« rezultata u

RISK MANAGEMENT – A MORE FLEXIBLE APPROACH BASED ON ISO 15189:2022

Domagoj Marijančević

Clinical Hospital Centre Sestre milosrdnice, Clinical Institute of Chemistry, Zagreb, Croatia

The requirements defined in ISO 15189:2022 have been changed in the sense that a greater emphasis is placed on risk management. The Standard is patient-focused and encourages continuous improvement in minimizing risks for patients forming the backbone of the laboratory's quality management process. The laboratory shall have a process for identifying risks of harm to patients and opportunities for improved patient care. Outcomes must be at the forefront, with improved patient outcomes being of the highest priority for all laboratories. The fact that risk-based management is the main innovation can be supported by evidence that the word «risk« occurs 86 times in contrast to the 2012 version where it occurs 12 times. This reflects a shift, that is, «risk-based thinking« should be inherent in all processes adopted to ensure good laboratory practice. Risk is so embedded in the Standard that it should be at the top of the laboratory manager's thinking process. The new version of the Standard now requires that actions to address risks and opportunities must be planned and implemented into the management system and their effectiveness evaluated. Additionally, the new version is less prescriptive than the previous one, allowing medical laboratories a greater level of flexibility and creativity in risk management. The Standard does not require that a formal risk management process be implemented. This approach essentially answers two fundamental questions: 1) How often can something go wrong?; 2) What are the consequences of this mistake? The new version of ISO 15189 should focus on patient harm in terms of risk quantification. The actions taken must be proportional to the potential impact and reflected in the records maintained by the facility. Defining innovative requirements enables the laboratory to develop more efficient documentation and easier compliance with legislative frameworks that may differ greatly between countries at an international or regional level. Since risk analysis represents an important role in the development of a quality management system, aligning with the viewpoint of ISO 9001:2015, the new Standard contains five requirements related to risk analysis instead of one in the previous version. In the new Standard, the development of an internal quality control program is focused on the »validity relevant to clinical decision-making«. The frequency is determined by a risk analysis of the patient's harm rather than a simple prescriptive approach. It is necessary to perform a risk analysis on the patient's results based on the «clinical signifi-

slučajevima kada eksterna kontrola kvaliteta nije zadovoljavajuća. Što se tiče laboratorijskih informacionih sistema, došlo je do velike evolucije na tržištu; stoga je najznačajniji novi rizik vezan za sajber bezbednost. U budućnosti je potrebno obratiti posebnu pažnju na izbor, implementaciju i nadogradnju ovakvog sistema. Dve ključne modifikacije u upravljanju rizikom i mogućnostima su da se moraju ažurirati kada dođe do neusaglašenosti, a pregledi rukovodstva sada moraju uzeti u obzir rezultate identifikacije rizika.

cance« of the results in cases where the external quality control is not satisfactory. Regarding laboratory information systems, there has been a major evolution in the market; therefore, the most significant new risk is related to cybersecurity. In the future, it is necessary to pay special attention when selecting, implementing, and upgrading such a system. Two key modifications in risk and opportunity management are that they must be updated, when a nonconformity occurs, and management reviews must now take into account the results of risk identification.

INFLAMATORNE BOLESTI CREVA: EPIDEMIOLOGIJA, ETIOPATOGENEZA, KLINIČKA SLIKA, POSTAVLJANJE DIJAGNOZE I SAVREMENA TERAPIJA

Nikola Panić, Zoran Milenković

KBC »Dr Dragiša Mišović-Dedinje«, Beograd, Srbija

Zapaljenske bolesti creva su hronične zapaljenske bolesti digestivnog trakta i uključuju dva dominantna entiteta – Kronovu bolest i ulcerozni kolitis. Ove bolesti nastaju kod genetski predisponiranih osoba, u sklopu prenaglašenog imunskog odgovora na uobičajene stimuluse kao što su antigeni hrane ili crevnog mikrobioma. Karakterišu ih epizode ponavljane inflamacije koja vodi trajnom oštećenju digestivnog trakta što i određuje prirodnu istoriju ovih bolesti. Zapaljenske bolesti creva imaju sistemski karakter i često su praćene ekstraintestinalnim, vancrevnim manifestacijama i kliničkim poremećajima koji najčešće zahvataju koštano – zglobni sistem, jetru i žučne puteve, kožu i oči. U osnovi etiopatogeneze ovih bolesti je presudan izmenjen imunološki odgovor, aktivacijom imunskog sistema i dominantnim uticajem proinflamatornih medijatora se uspostavljaju putevi inflamacije i oštećenja tkiva. Kronova bolest može zahvatati bilo koji deo digestivnog trakta dok ulcerozni kolitis zahvata debelo crevo, kolon. U inicijalnom dijagnostičkom pristupu, osim prirode simptoma i laboratorijskih parametara, presudna je endoskopska dijagnostika digestivnog trakta kao i morfološka ispitivanja (intestinalni ultrazvuk, MSCT i MR enterografija). U sklopu postavljanja dijagnoze definišu se i karakteristike bolesti kao što su aktivnost i težina, a na osnovu ovih parametara se određuje i terapijski pristup. Osnovu terapije u zapaljenskim bolestima creva čine lekovi koji suprimiraju imunski sistem (kortikosteroidi, imunomodulatori) te lekovi različitih mehanizama delovanja na odgovarajuće komponente patofiziološkog procesa (antitela na proinflamatorne medijatore – anti TNF, lekovi koji

INFLAMMATORY BOWEL DISEASES: EPIDEMIOLOGY, ETIOPATHOGENESIS, CLINICAL PRESENTATION, DIAGNOSIS AND CONTEMPORARY TREATMENT

Nikola Panić, Zoran Milenković

CHC »Dr Dragiša Mišović-Dedinje«, Belgrade, Serbia

Inflammatory bowel diseases (IBD) are chronic inflammatory conditions of the digestive tract, encompassing two dominant entities – Crohn's disease and ulcerative colitis. These diseases occur in genetically predisposed individuals, as part of an exaggerated immune response to common stimuli such as food antigens or the intestinal microbiome. They are characterized by episodes of recurrent inflammation leading to permanent damage to the digestive tract, which determines the natural history of these diseases. IBDs have a systemic nature and are often accompanied by extraintestinal, non-digestive manifestations and clinical disorders, most commonly affecting the musculoskeletal system, liver and biliary tract, skin, and eyes. The altered immune response is crucial in the etiopathogenesis of these diseases, establishing pathways of inflammation and tissue damage through the activation of the immune system and the dominant influence of proinflammatory mediators. Crohn's disease can affect any part of the digestive tract, while ulcerative colitis affects the colon and rectum. In the initial diagnostic approach, besides the nature of symptoms and laboratory parameters, endoscopic diagnosis of the digestive tract and morphological investigations (intestinal ultrasound, MSCT, and MR enterography) are crucial. Disease characteristics such as activity and severity are defined as part of the diagnostic process, guiding therapeutic approaches based on these parameters. The foundation of therapy in inflammatory bowel diseases consists of drugs that suppress the immune system (corticosteroids, immunomodulators) and drugs with various mechanisms of action

blokiraju aktivaciju i migraciju zapaljenskih ćelija ili inhibiraju odgovarajuće receptore u procesu produkcije proinflamatornih medijatora). U mnogim slučajevima, komplikovanim oblicima ovih bolesti, indikovano je i hirurško lečenje. Cilj terapije je uspostavljanje i održavanje remisije i usporavanje i zaustavljanje progresije oštećenja digestivnog trakta. I pored velikog broja potentnih terapijskih agenasa, uspešnost lečenja ovih bolesti je generalno samo nešto veća od 50%. S obzirom da se radi o dinamičnim bolestima, bolestima kod kojih se u toku menjaju putevi i medijatori inflamacije, noviji terapijski pristupi teže bazičnijim ciljevima i delovanju na bolest pre pojave kliničkih i fenotipskih manifestacija. Ovo odredjuje i najvažniju karakteristiku pristupa ovim bolestima – rana dijagnoza, definisanje faktora progresivne i komplikovane bolesti, pravovremena, adekvatna i individualizovana terapija kao i redovan nadzor, što omogućava postizanje glavnih ciljeva – izlečenje sluznice digestivnog trakta i sprečavanja trajnih oštećenja i invalidnosti.

on the corresponding components of the pathophysiological process (antibodies to proinflammatory mediators – anti-TNF, drugs that block the activation and migration of inflammatory cells or inhibit appropriate receptors in the production process of proinflammatory mediators). In many cases, surgical treatment is indicated for complicated forms of these diseases. The goal of therapy is to establish and maintain remission and to slow down or halt the progression of digestive tract damage. Despite the large number of potent therapeutic agents, the success rate of treating these diseases is generally only slightly higher than 50%. Since these are dynamic diseases, with changing pathways and mediators of inflammation, newer therapeutic approaches aim for more fundamental goals and action on the disease before clinical and phenotypic manifestations occur. This also determines the most important characteristic of approaching these diseases - early diagnosis, defining factors of progressive and complicated disease, timely, adequate, and individualized therapy, as well as regular monitoring, allowing the achievement of main goals - healing of the digestive tract mucosa and preventing permanent damage and disability.

LABORATORIJSKA PROCENA INFLAMATORNIH BOLESTI CREVA

Sanja Obradović

Služba za laboratorijsku dijagnostiku, Kliničko bolnički centar »Zvezdara«, Beograd, Srbija

Inflamatorne bolesti creva obuhvataju autoinflamatorne bolesti digestivnog trakta – Kronovu bolest, ulcerozni kolitis i nedeterminisani kolitis. Ove bolesti se klinički manifestuju hroničnim zapaljenjem određenog dela digestivnog trakta, uz povremenu akutizaciju, što je praćeno različitim simptomima i nepredvidivim tokom. U dijagnostici inflamatornih bolesti creva, osim kliničke evaluacije, endoskopije i radioloških metoda, važnu ulogu ima i laboratorijsko testiranje. U tu svrhu kao uzorci se koriste krv i feces. Pomoću analiza iz krvi procenjuje se prisustvo sistemske inflamacije (određuju se koncentracije CRP i fibrinogena, sedimentacija eritrocita, broj leukocita), malapsorpcije i anemije (određuju se koncentracije albumina, vitamina B12, gvožđa i hemoglobina) i vrši pomoćna diferencijalna dijagnostika Kronove bolesti i ulceroznog kolitisa određivanjem seroloških markera, poput ASCA i pANCA antitela. U fecesu se određuje koncentracija kalprotektina za procenu lokalizovane inflamacije, ispituje prisustvo okultnog krvarenja i vrše mikrobiološka ispitivanja za utvrđivanje prisustva infekcije. Značajnu ulogu ima određivanje fekalnog kalprotektina, kao vodećeg nein-

LABORATORY ASSESSMENT OF INFLAMMATORY BOWEL DISEASES

Sanja Obradović

Department of Laboratory Diagnostic, Clinical Medical Center »Zvezdara«, Belgrade, Serbia

Inflammatory bowel diseases include autoinflammatory diseases of the digestive tract – Crohn's disease, ulcerative colitis, and indeterminate colitis. Clinically, these diseases are manifested by chronic inflammation of a specific part of the digestive tract, with occasional exacerbations, followed by various symptoms and an unpredictable course. In addition to clinical assessment, endoscopy and radiological procedures, laboratory tests also play an important role in the diagnosis. Both blood and feces are used as samples. Blood analyses serve to evaluate the presence of systemic inflammation (CRP and fibrinogen, ESR, leukocytes count are determined), malabsorption and anemia (albumin, vitamin B12, iron and hemoglobin are determined), as well as for differential diagnosis of Crohn's disease and ulcerative colitis by determination of serological markers such as ASCA and pANCA. In feces, the concentration of calprotectin is determined to assess localized inflammation, the presence of occult bleeding is examined, and microbiological tests are performed to determine the presence of infection. The determination of faecal calprotectin plays an important role, as a leading

vazivnog markera inflamacije digestivnog trakta. Ovaj biomarker omogućava diferencijaciju organskih od funkcionalnih bolesti creva, praćenje kliničke aktivnosti bolesti, jer dobro korelira sa endoskopskom i histološkom aktivnošću bolesti, omogućava predviđanje relapsa, praćenje efekta terapije (pomaže u proceni vremena trajanja ili prekida terapije), kao i selekciju pacijenata za endoskopiju. Međutim, postoji velika varijabilnost između rezultata različitih metoda za određivanje fekalnog kalprotektina i ne postoji globalno prihvaćena cut off vrednost. Prema tome, treba pažljivo interpretirati rezultate, raditi na standardizaciji određivanja kalprotektina i uvođenju novih biomarkera.

RAZVOJ I IMPLEMENTACIJA LABORATORIJSKOG PRAĆENJA BIOLOŠKE TERAPIJE U INFLAMATORNIM BOLESTIMA CREVA

Iva Perović Blagojević

KBC »Dr Dragiša Mišović-Dedinje«, Beograd, Srbija

Inflamatorne bolesti creva (IBC) su idiopatska hronična zapaljenja creva, u čijem nastanku ulogu igra neadekvatan imunološki odgovor uz uticaj kompleksne interakcije genetskih faktora, faktora sredine i crevne mikrobiote. U grupu IBC spadaju: Kronova bolest (KB), ulcerozni kolitis (UK) i nedeterminisani kolitis. Kod približno 10–20% pacijenata sa IBC nisu ispunjeni histološki kriterijumi za razlikovanje KB od UK što ih svrstava u grupu pacijenata sa nedeterminisanim kolitisom. Najefikasniji medikamenti u lečenju KB i UK su antiinflamatorni lekovi (kortikosteroidi, aminosalicilati i imunosupresivi), kao i savremeni tip lečenja za teže oblike – biološka terapija. Biološka terapija se koristi u lečenju najtežih formi KB i UK. Biološka terapija predstavlja antitela koja napadaju/blokiraju/zadržavaju zapaljenske faktore i tako zaustavljaju zapaljensku reakciju. Ova vrsta terapije može da blokira na sistemskom nivou (osnovne i glavne faktore zapaljenja u celom organizmu, i samim tim slabi čitav imuni sistem), ili sve specifičnija antitela koja danas postoje (blokiraju imuni sistem i zapaljenje samo u crevima). Biološka terapija je od ključne važnosti u lečenju obolelih od UK i KB jer smanjuje procenat operisanih pacijenata što je cilj svake terapije kod ovako agresivnih bolesti. Koristi se i u fazi održavanja bolesti uz aminosalicilate, uz mogućnost isključivanja kada apsolutne indikacije

non-invasive marker of inflammation of the digestive tract. This biomarker enables the differentiation between organic and functional intestinal diseases, monitoring clinical activity of the disease, as it correlates well with the endoscopic and histological activity of the disease, the prediction of relapses, monitoring of the effect of therapy (it helps to evaluate the duration or interruption of therapy), as well as the selection of patients for endoscopy. However, the results of the different methods for the determination of faecal calprotectin vary widely and there is no globally accepted cut off value. Therefore, the results should be interpreted carefully, and work on standardizing calprotectin determination and introducing new biomarkers should be continued.

DEVELOPMENT AND IMPLEMENTATION OF LABORATORY MONITORING OF BIOLOGICAL THERAPY IN INFLAMMATORY BOWEL DISEASES

Iva Perović Blagojević

CHC »Dr Dragiša Mišović-Dedinje«, Belgrade, Serbia

Inflammatory bowel diseases (IBD) are idiopathic chronic inflammations of the intestines, where inadequate immune response plays a role alongside the complex interaction of genetic factors, environmental factors, and intestinal microbiota. The IBD group includes Crohn's disease (CD), ulcerative colitis (UC), and indeterminate colitis. In approximately 10–20% of patients with IBD, histological criteria for distinguishing between CD and UC are not met, placing them in the group of patients with indeterminate colitis. The most effective medications in treating CD and UC are anti-inflammatory drugs (corticosteroids, aminosalicylates, and immunosuppressants), as well as advanced treatment options for severe cases – biological therapy. Biological therapy is used in treating the most severe forms of CD and UC. Biological therapy involves antibodies that attack/block/neutralize inflammatory factors, thereby halting the inflammatory reaction. This type of therapy can either block inflammation systemically (targeting fundamental and major inflammatory factors throughout the body, thus weakening the entire immune system) or employ more specific antibodies that target inflammation only in the intestines. Biological therapy is crucial in treating patients with CD and UC as it reduces the percentage of patients requiring surgery, which is the goal of therapy for such aggressive diseases. Biological therapy involves antibodies that attack/block/neutralize inflammatory factors, thereby halting the

ukazuju da pacijent može da ostane samo na aminosalicilatima. Naravno, ukoliko kasnije dođe do relapsa, biološka terapija se može ponovo uključiti. Trenutno su za lečenje IBC u Srbiji u upotrebi inhibitori TNF-alfa (tumor nekrotizirajućeg faktora alfa): Infliksimab, Adalimumab, Vedolizumab i Golimumab. Danas se, zahvaljujući RFZO-u, biološka terapija prima godinama, a dodatno se produžuje u slučaju želje za ostvarivanjem potomstva. Uloga laboratorije u praćenju efikasnosti biološke terapije kod pacijenata sa IBC sastoji se u merenju/određivanju koncentracije leka– terapijsko praćenje leka (eng. Terapeutic Drug Monitoring, TDM), kao i antitela na lek u referentnim IBC centrima. Danas je dostupno merenje koncentracije sledećih lekova i antitela na lek: Infliksimab, Adalimumab, Vedolizumab, odn. anti-Infliksimab, anti-Adalimumab i anti-Vedolizumab antitela. Prva linija biološke terapije, tzv. anti-TNF agensi (Infliksimab, Adalimumab), su lekovi koji se primenjuju parenteralno (intravenski ili subkutano) i njihova karakteristika je da ispoljavaju imunogeni potencijal. To znači da organizam može reagovati stvaranjem antitela na biološki lek što može usloviti smanjenu efikasnost terapije. U kliničkoj praksi je neophodan adekvatan monitoring dinamike primene leka. Osim praćenja kliničkog i laboratorijskog odgovora na primenjenu terapiju, danas se sprovodi pravovremeni pristup u monitoringu – laboratorijsko merenje/određivanje koncentracije leka u krvi i eventualnog prisustva antitela na lek. Ove laboratorijske analize se rade nakon primene indukcionog perioda terapije (tri ciklusa: 0, 2 i 6 nedelja) u kojem se očekuje farmakodinamski i terapijski efekat, i u 14. nedelji od početka primene biološkog leka. Danas se u laboratorijama koriste različite, komercijalno dostupne metode za TDM: ELISA, RIA kao i HPLC. U zavisnosti od laboratorijskih rezultata (npr. smanjena koncentracija leka u krvi, postojanje antitela na lek) vrši se tzv. optimizacija terapije. Laboratorijski rezultati analize nivoa leka i prisustva antitela na lek u krvi, mogu još u ranoj fazi lečenja kliničaru ukazati na potrebu promene terapije i neadekvatan odgovor na primenu biološkog leka, odn. pružiti informacije da li je mehanizam delovanja leka odgovarajući za imunološki fenotip bolesti i omogućiti nastavak lečenja bolesti biološkim lekom drugog mehanizma delovanja (druga terapijska linija). Ukoliko se optimizacijom postigne adekvatan odgovor i koncentracija leka u krvi, u daljem toku laboratorijske analize se ponavljaju, u zavisnosti od kliničkog i endoskopskog odgovora, a radi nastavka terapije optimalnim nivoom leka. Osim postojanja antitela na lek, koncentracija leka u krvi može biti snižena i u slučajevima izražene aktivnosti bolesti (gubitak leka preko oštećene sluzokože creva), povećanog klirensa leka, oštećene bubrežne funkcije. Isto tako, farmakodinamika zavisi i od pola, starosti, telesne težine bolesnika, kataboličkih

inflammatory reaction. This type of therapy can either block inflammation systemically (targeting fundamental and major inflammatory factors throughout the body, thus weakening the entire immune system) or employ more specific antibodies that target inflammation only in the intestines. It is also used for maintenance therapy alongside aminosalicylates, with the possibility of discontinuation when absolute indications suggest that the patient can remain solely on aminosalicylates. Of course, if relapse occurs later on, biological therapy can be reintroduced. Currently, in Serbia, TNF-alpha inhibitors (tumor necrosis factor alpha inhibitors) are used for treating IBD: Infliximab, Adalimumab, Vedolizumab, and Golimumab. Thanks to the National Health Insurance Fund (NHIF), biological therapy has been available for years and is further extended in case of the desire for offspring. The role of the laboratory in monitoring the effectiveness of biological therapy in patients with IBD involves measuring/determining drug concentration – therapeutic drug monitoring (TDM), as well as antibody levels to the drug in reference IBD centers. Today, it is possible to measure the concentration of the following drugs and drug antibodies: Infliximab, Adalimumab, Vedolizumab, and respective anti-Infliximab, anti-Adalimumab, and anti-Vedolizumab antibodies. The first-line biological therapy, called anti-TNF agents (Infliximab, Adalimumab), are drugs administered parenterally (intravenously or subcutaneously) and are characterized by their immunogenic potential. This means that the body may react by producing antibodies to the biological drug, which can result in reduced therapy effectiveness. Adequate monitoring of drug administration dynamics is essential in clinical practice. Besides monitoring clinical and laboratory responses to therapy, timely monitoring is conducted today – laboratory measurement/ determination of drug concentration in the blood and the potential presence of antibodies to the drug. These laboratory analyses are performed after the induction period of therapy (three cycles: 0, 2, and 6 weeks), during which pharmacodynamic and therapeutic effects are expected, and at week 14 of starting biological drug therapy. Various commercially available methods for TDM are used in laboratories: ELISA, RIA, as well as HPLC. Depending on laboratory results (e.g., decreased drug concentration in the blood, presence of antibodies to the drug), therapy optimization is performed. Laboratory results of drug levels and antibody presence in the blood can indicate to clinicians, even in the early stages of treatment, the need for therapy modification and inadequate response to biological drug administration, or provide information on whether the drug's mechanism of action is appropriate for the disease's immunological phenotype, enabling the continuation of treatment with a biological drug of a different mechanism of action (second-line therapy). If adequate response and drug concentration in the blood are achieved through optimization, subsequent laboratory analyses

procesa (npr. hipoalbuminemija u sklopu izražene aktivnosti bolesti). Smanjena ili neadekvatna komplijansa bolesnika, neredovna ili neadekvatna primena imunosupresiva, takođe može dovesti do sniženja koncentracije leka u krvi.

are repeated, depending on clinical and endoscopic responses, to maintain therapy at an optimal drug level. In addition to the presence of antibodies to the drug, drug concentration in the blood may be reduced in cases of severe disease activity (loss of drug through damaged intestinal mucosa), increased drug clearance, impaired renal function. Similarly, pharmacodynamics also depend on the patient's gender, age, body weight, catabolic processes (e.g., hypoalbuminemia as part of severe disease activity). Reduced or inadequate patient compliance, irregular or inadequate use of immunosuppressants, can also lead to decreased drug concentration in the blood.

UTICAJ MIKROBIOTE NA ZDRAVLJE LJUDI

Brankica Filipić

Katedra za mikrobiologiju i imunologiju, Univerzitet u Beogradu-Farmaceutski fakultet, Beograd, Srbija

Telo čoveka sadrži milione mikroorganizama koji čine kompleksnu zajednicu i mogu kolonizovati različite delove tela poput creva, usne duplje, respiratornog trakta, vagine i kože, čineći humanu mikrobiotu. Sam termin mikrobiota označava zajednicu živih mikroorganizama koji se mogu naći na/u ljudskom organizmu i žive u simbiotskom odnosu sa domaćinom, pri čemu ga treba razlikovati od termina mikrobiom koji predstavlja zajednicu mikroorganizama i njihovih gena, usled čega mikrobiom obuhvata širi spektar elemenata od mikrobiote. Humana mikrobiota, poznata i pod nazivom »prikriveni organ«, sastavljena je od različitih (uglavnom nepatogenih) mikroorganizama poput bakterija, gljiva, protista, arhea i virusa i sadrži oko 150 puta više gena nego ceo humani genom. Karakteristika mikrobiote je visok diverzitet mikroorganizama koji ulaze u njen sastav, ali distribucija mikroorganizama je jedinstvena kod različitih pojedinaca i pored toga, podložna je promenama u sastavu kod iste individue. Oko 100 triliona mikroorganizama naseljava samo gastrointestinalni trakt i utiče na brojne fiziološke aktivnosti organizma (ekstrakcija nutrijenata iz hrane, homeostaza, inflamacija itd.) i smatra se da mikrobiota creva ima najveći uticaj na naše zdravlje, jer su crevne bakterije uključene u fermentaciju hrane, zaštitu od patogenih mikroorganizama, proizvodnju vitamina, stimulaciju imunskog odgovora… Crevna mikrobiota prepoznata je kao ključni faktor u regulaciji crevno-moždane ose, pri čemu je proces dvosmeran – crevna mikrobiota može uticati na različite mehanizme u mozgu, a sa druge strane, brojni procesi u mozgu mogu dovesti do poremećaja u sastavu crevne mikrobiote. Disbioza crevne mikrobiote povezuje se sa promenama u sas-

THE IMPACT OF MICROBIOTA ON HUMAN HEALTH

Brankica Filipić

Department of Microbiology and Immunology, University of Belgrade-Faculty of Pharmacy, Belgrade, Serbia

The human body has millions of microorganisms that define a complex microbial community inhabiting different parts of the body, and depending on localized regions, microbiota can be divided into gut, oral, respiratory, skin and vaginal microbiota. The term microbiota, itself, describes living microorganisms found in/on the body and have symbiotic host relationships, and should be distinguish from the term microbiome that represents collection of these microorganisms and their genomes, meaning that microbiome encompasses broader spectrum of elements than microbiota. The human microbiota, also known as »the hidden organ«, consists of various microorganisms (mostly non-pathogenic) such as bacteria, fungi, protists, archea and viruses and carries about 150 times more genes comparing to all human genomes. The microbiota has high taxonomic diversity of microorganisms, but distribution of these microorganisms is unique between individuals and, in addition, may undergo changes within the same individual. Around a 100 trillion microorganisms inhabit gastrointestinal tract and impact numerous physiological activities (nutrient extraction from food, homeostasis, inflammation etc.), and it is considered that gut microbiota has the most significant impact on our health, as gut bacteria are involved in the fermentation of food, protection against pathogens, vitamin production, stimulation of immune response… Gut microbiota has been recognized as a key regulator of gut-brain axis, which is bidirectional – gut microbiota could influence pathways within brain, while, on the other hand, these pathways could impact disorders of gut microbiota. Dysbiosis of gut microbiota is associated with alternations in gut

tavu i funckiji mikrobiote creva i može se manifestovati kao smanjen diverzitet mikroorganizama koji ulaze u sastav mikrobiote, gubitak korisnih mikroorganizama i povećan rast štetnih mikroorganizama. Kada je poremećen balans zajednice mikroorganizama mikrobiote, može doći do razvoja različitih bolesti, poput bolesti kardiovaskularnog sistema (hipertenzija, ateroskleroza), dijabetesa (tip 1, tip 2 i gestacionog dijabetesa), bolesti jetre, inflamatorne bolesti creva, poremećaja u mozgu (Parkinsonova bolest, Alchajmerova bolest, depresija), karcinoma i sl. Sve bolje razumevanje odnosa domaćina i mikrobiote omogućilo je razvoj terapije zasnovane na primeni mikrobiote, poput fekalne transplantacije mikrobiote i modulacije mikrobiote, a brojni napori se ulažu u razvoj strategija za terapiju infekcija uzrokovanih Clostridioides difficile, terapiju dijabetesa i drugih bolesti povezanih sa disbiozom mikrobiote. Može se zaključiti da su brojna istraživanja u oblasti mikrobiote doprinela boljem razumevanju kako tretirati određene bolesti i unaprediti zdravlje modifikacijom sastava mikrobiote.

PROBIOTICI KAO PRIRODNA INOVATIVNA I JEDINSTVENA STRATEGIJA ZA USPORAVANJE STARENJA ĆELIJA

Nataša Golić, Nikola Popović, Miroslav Dinić, Katarina Veljović, Amarela Terzić-Vidojević

Grupa za interakcije probiotika i mikrobiote sa domaćinom, Institut za molekularnu genetiku i genetičko inženjerstvo, Univerzitet u Beogradu, Beograd, Srbija

Prema podacima Svetske zdravstvene organizacije (SZO) broj ljudi širom sveta starijih od 60 godina procenjen je na 1 milijardu 2019. godine, sa očekivanim porastom na 2,1 milijarde do 2050. godine. Ovaj globalni fenomen je opisan metaforom »srebrni cunami«, koju su prvi upotrebili Forbes.com i The Economist, koji slikovito povezuje starenje stanovništva sa ogromnim uticajem na ekonomiju, zdravstveni sistem i kvalitet života ljudi. Stoga je jedan od ciljeva Inicijative Ujedinjenih nacija Dekada zdravog starenja (2021–2030) da se unapredi istraživanje o »zdravom starenju« i spreči nastanak bolesti povezanih sa starenjem kod starije populacije. Savremeni način života i ishrane, koju karakteriše visok dnevni unos zasićenih masti i rafinisanih ugljenih hidrata, dovodi do poremećaja mikrobiote creva i hroničnih upalnih procesa u organizmu, povezanih sa kognitivnim oštećenjem, emocionalnim poremećajima i demencijom, ali i sa inflamatornom bolešću creva (10 miliona ljudi širom sveta, CDC), sindromom iritabilnog creva (između 25 i 45 miliona ljudi u SAD, 11% svetske populacije, CDC), kardio-

microbiota composition and function and it can be manifested as decreased diversity of microorganisms, loss of beneficial microbiota or an overgrowth of harmful microorganisms. When the balance of microbiota community is affected, it can lead to dysregulation of body functions and wide spectrum of diseases, such as cardiovascular diseases (hypertension, atherosclerosis), diabetes (type 1, type 2, gestational), liver disease, inflammatory bowel disease, brain disorders (Parkinson¢s disease, Alzheimer's disease, depression), cancer, etc. The greater understanding of host-microbiota relationship enabled development of microbiota-based therapy such as faecal microbiota transplantation and microbiota modulation, and great effort goes to development of strategies in the treatment of Clostridioides difficile infections, diabetes, brain disorders and other diseases connected with dysregulation of microbiota. It can be concluded that wide research in the field of microbiota has contributed to better understanding how to treat diseases and foster health via manipulation of the microbiota composition.

PROBIOTICS AS NATURAL INNOVATIVE AND UNIQUE STRATEGY FOR CELL-AGING DECELERATION

Nataša Golić, Nikola Popović, Miroslav Dinić, Katarina Veljović, Amarela Terzić-Vidojević

Group for Probiotics and Microbiota – Host Interaction, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia

According to the World Health Organization (WHO), the number of people worldwide over 60 years of age was estimated to be 1 billion in 2019, with an expected rise to 2.1 billion by 2050. This global phenomenon is described with the metaphor «silver tsunami« used by Forbes.com and The Economist, which vividly connects population ageing with its huge impact on the economy, healthcare systems and people's quality of life. Therefore, one of the goals of the United Nations Decade of Healthy Ageing Initiative (2021-2030), is to prevent the onset of age-related diseases in the elderly population by improving the research on «healthy ageing«. The Western-type diet characterized by a high daily intake of saturated fats and refined carbohydrates leads to disturbance in gut microbiota and prolonged low-grade inflammation, linked with cognitive impairment, emotional disorders, and dementia, but also with inflammatory bowel disease (10 million people worldwide, CDC), irritable bowel syndrome (between 25 and 45 million people in USA, 11% of global population, CDC), cardiovascular diseases (the leading

vaskularnim bolestima (vodeći uzrok smrti u svetu, koji godišnje oduzimaju oko 17,9 miliona života, SZO), kancerom (globalno 18 miliona slučajeva dijagnostikovanih 2020. godine, VCRF), i ubrzanim starenjem. Novi podaci sugerišu da bi modulacija mikrobioma creva sastavljenog od složenih mikrobnih zajednica mogla uticati na starenje organizma. Metagenomske studije ukazuju na razlike u sastavu crevne mikrobiote između mladih i starih osoba koji pokazuju disbiozu povezanu sa uzrastom koja se ogleda u smanjenju kratkolančanih masnih kiselina i proizvođača γ-amino buterne kiseline (GABA). Testirali smo nekoliko pažljivo odabranih prirodnih izolata bakterija mlečne kiseline, poreklom od tradicionalnih mlečnih proizvoda proizvedenih u domaćinstvima na specifičnim geografskim lokalitetima na Balkanskom poluostrvu na sposobnost usporavanja procesa starenja ćelija. Naši rezultati su pokazali da ovi sojevi poseduju izuzetne probiotičke karakteristike: i) ojačavaju epitelnu crevnu barijeru putem stimulacije autofagije, ii) regulišu tesne veze između epitelnih ćelija creva koje sprečavaju prolazak štetnih supstanci iz creva u druge organe, iii) aktiviraju antimikrobnu odbranu, iv) pokazuju visoku sposobnost adhezije na crevne ćelije, bez štetnog uticaja na njihovu vitalnost, v) pokazuju odličnu antioksidativnu aktivnost, kao i vi) izuzetne antiinflamatorne efekte koji se ogledaju u smanjenom nivou LPS-indukovanih proinflamatornih citokina u ćelijskoj kulturi, i vii) produžavaju životni vek Caenorhabditis elegans putem aktivacije autofagije, što ih čini odličnim kandidatima za probiotičke starter kulture za funkcionalne mlečne proizvode.

cause of death globally, taking an estimated 17.9 million lives each year, WHO), cancer (globally, 18 million cases diagnosed in 2020, WCRF), and finally accelerated ageing. New data suggest that modulation of gut microbiome composed of complex microbial communities could influence host ageing. Metagenomics studies report the differences in the composition of gut microbiota between young and old subjects showing age-related dysbiosis reflecting a decrease in the short-chain fatty acids and γ-amino butyric acid (GABA) core producers. We have tested several carefully selected natural isolates of lactic acid bacteria, originating from artisanal dairy products from specific geographical locations in the Balkan peninsula for the ability to decelerate cell-aging process. Our results revealed that these strains possess exciting probiotic features: i) strengthen the epithelial intestinal barrier through stimulation of autophagy, ii) upregulate the tight junctions between the epithelial cells of the intestine which prevent the passage of harmful substances from the intestine to other organs, iii) activate the antimicrobial defense, iv) show high adhesion capability to intestinal cells, without producing harmful effects on their viability, v) exhibit excellent antioxidant activity, as well as vi) exceptional anti-inflammatory effects reflected in reduced levels of LPS-induced pro-inflammatory cytokines in cell culture, and vii) extend the lifespan of Caenorhabditis elegans via autophagy activation, making them great candidates for probiotic starter cultures for functional dairy food.

ANTIDEPRESIVNI POTENCIJAL POSTBIOTIKA U ANIMALNOM MODELU DEPRESIJE

N. Todorović Vukotić[1], N. Đorđević[1], S. Soković Bajić[2], H. Mitrović[2] , E. Brdarić[2] , M. Tolinački[2], J. Đokić[2], M. Dinić[2], M. Živković[2], D. Radojević[2], A. Bisenić[2], S. Jakovljević[2], S. B. Pajović[1], N. Golić[2]

[1]Laboratorija za molekularnu biologiju i endokrinologiju, Institut za nuklearne nauke »Vinča«, Institut od nacionalnog značaja za Republiku Srbiju, Univerzitet u Beogradu, Beograd, Srbija
[2]Grupa za interakcije probiotika i mikrobiote sa domaćinom, Laboratorija za molekularnu mikrobiologiju, Institut za molekularnu genetiku i genetičko inženjerstvo, Univerzitet u Beogradu, Beograd, Srbija

Depresija je najčešće psihijatrijsko oboljenje od kog boluje više od 264 miliona ljudi širom sveta. Upotreba antidepresiva se suočava sa velikim izazovi-

THE ANTIDEPRESSANT POTENTIAL OF POSTBIOTIC IN RAT MODEL OF DEPRESSION

N. Todorović Vukotić[1], N. Đorđević[1], S. Soković Bajić[2], H. Mitrović[2] , E. Brdarić[2] , M. Tolinački[2], J. Đokić[2], M. Dinić[2], M. Živković[2], D. Radojević[2], A. Bisenić[2], S. Jakovljević[2], S. B. Pajović[1], N. Golić[2]

[1]Department of Molecular Biology and Endocrinology, »Vinča« Institute of Nuclear Sciences, National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia
[2]Group for Probiotics and Microbiota-Host Interaction, Laboratory for Molecular Microbiology, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia

Depression is the most common psychiatric disorder that affects more than 264 million people worldwide. Antidepressants struggle with great challenges such

ma poput visoke stope relapsa, odloženog kliničkog odgovora i brojnih neželjenih efekata. Postbiotici, bioaktivne komponente produkovane od strane psihobiotika, probiotika koji imaju blagotvorni efekat na mentalno zdravlje, pokazali su obećavajuće rezultate u ublažavanju poremećaja raspoloženja. Ova studija ima za cilj da proceni antidepresivni potencijal postbiotika Phocaeicola vulgatus NGB218. P. vulgatus NGB218 je izolovan iz fecesa zdravog donora i kultivisan u Pyg medijumu u anaerobnoj komori. P. vulgatus NGB218 je odabran među 35 izolovanih sojeva na osnovu najveće produkcije GABA, antiinflamatornog efekta uočenog na Caco2 ćelijama, kao i zbog uticaja na nervni sistem koji je zabeležen u eksperimentima na modelu Caenorhabditis elegans. Pacovi podvrgavani hroničnom nepredvidivom blagom stresu su korišćeni kao životinjski model depresije. Pacovi stari tri nedelje su podeljeni na tri grupe (n=16): (1) kontrolni, netretirani pacovi; (2) pacovi tretirani Pyg medijumom i (3) pacovi tretirani P. vulgatus NGB218 postbiotikom u trajanju od 8 nedelja. Posle 4 nedelje tretmana, polovina pacova iz svake grupe (n=8) je podvrgnuta CUMS-u naredne 4 nedelje. Test zainteresovanosti za zaslađen rastvor i test prskanja (engl.splash test) korišćeni su za merenje anhedonije, glavnog simptoma depresije, a test zakopavanja klikera i izdignutog lavirinta za procenu anksioznosti. Koncentracija kortikosterona i proinflamatornih citokina TNF-α, IL-1 i IL-6 u serumu pacova merena je odgovarajućim ELISA kitovima. Tretman postbiotikom P. vulgatus NGB218 pokazao je antidepresivno i anksiolitičko dejstvo u CUMS životinjskom modelu depresije.

MODIFIKACIJA MIKROBIOTE KAO PRISTUP LEČENJU MULTIPLE SKLEROZE

Jelena Đokić[1], Aleksandar Bisenić[1], Sergej Tomić[2], Marina Bekić[2], Luka Pavlović[2], Miroslav Dinić[1], Amarela Terzić-Vidojević[1], Dušan Radojević[1], Svetlana Soković Bajić[1], Hristina Mitrović[1], Stefan Jakovljević[1], Nevena Vukotić Todorović[3], Nataša Golić[1]

[1]Institut za molekularnu genetiku i genetičko inženjerstvo, Univerzitet u Beogradu; Beograd, Srbija
[2]Institut za primenu nuklearne energije, Univerzitet u Beogradu; Beograd, Srbija
[3]Institut za nuklearne nauke »Vinča«, Institut od nacionalnog značaja za Republiku Srbiju, Univerziteta u Beogradu, Srbija

Napredak u metodama sekvenciranja nove generacije i analizi velikih podataka u velikoj meri je doprineo

as high rate of relapse, delayed clinical response and numerous side-effects. Postbiotics, bioactive compounds produced by psychobiotics, the emerging group of probiotics that have beneficial effects on mental health, have already shown promising results in mood disorder alleviation. This study aimed to evaluate the antidepressant potential of postbiotic Phocaeicola vulgatus NGB218. P. vulgatus NGB218, isolated from the fecal samples of a healthy donor, was cultivated in PYG medium within an anaerobic chamber. Among 35 isolated strains, P. vulgatus NGB218 was selected for its pronounced production of GABA and its observed anti-inflammatory effects in Caco2 cell cultures, as well as its neural impact on the Caenorhabditis elegans model. We used rats exposed to chronic unpredictable mild stress (CUMS) as an animal model of depression. Three-weeks old rats were divided in 3 groups (n=16): control, non-treated rats; rats treated with Pyg medium and rats treated with P. vulgatus NGB218 postbiotic for 8 weeks. After 4 weeks half of the animals from each group (n=8) was subjected to CUMS for 4 weeks. Anhedonia, a core symptom of depression, was monitored using sucrose preference and splash test, while marble burying test and elevated plus maze were used to score anxiety. The levels of corticosterone and pro-inflammatory cytokines TNF-α, IL-1 and IL-6 in serum were measured using ELISA kits. Treatment with postbiotic P. vulgatus NGB218 demonstrated both antidepressive and anxiolytic effects in CUMS rats.

MICROBIOTA MODIFICATION AS AN APPROACH TO MULTIPLE SCLEROSIS TREATMENT

Jelena Đokić[1], Aleksandar Bisenić[1], Sergej Tomić[2], Marina Bekić[2], Luka Pavlović[2], Miroslav Dinić[1], Amarela Terzić-Vidojević[1], Dušan Radojević[1], Svetlana Soković Bajić[1], Hristina Mitrović[1], Stefan Jakovljević[1], Nevena Vukotić Todorović[3], Nataša Golić[1]

[1]Institute of Molecular Genetics and Genetic Engineering, University of Belgrade; Belgrade, Serbia
[2]Institute for the Application of Nuclear Energy, University of Belgrade; Belgrade, Serbia
[3]Vinča, Institute of Nuclear Sciences, National Institute of the Republic of Serbia, Serbia

The advancement in new-generation sequencing and big-data analysis has contributed greatly to our

našem razumevanju ključne uloge crevne mikrobiote kako u razvoju zdravog organizma, tako i u raznim patološkim stanjima. Sojevi bakterija koji žive u anaerobnom okruženju debelog creva, posebno oni koji su sposobni da proizvode masne kiseline kratkog lanca (SCFA), igraju značajnu ulogu u održavanju homeostaze barijere i imunskog sitema creva. Smanjenje bakterija koje proizvode SCFA je povezano sa nižim nivoima ovih kiselina kod pacijenata sa multiplom sklerozom (MS). Takođe je pokazano da ove kiseline imaju izražene imunomodulatorne efekte na različite populacije limfocita i ćelija mijeloidnog porekla. Stoga je cilj naše studije bio da se iz fecesa zdravih davalaca, korišćenjem različitih medijuma u anaerobnim uslovima, izoluju bakterijski sojevi sa visokim kapacitetom za proizvodnju SCFA. U nastavku je analiziran efekat ovih izolata u kulturama intestinalnih epitelnih ćelija Caco-2 i mononuklearnih ćelija periferne krvi (PBMC) u in vitro modelu zapaljenja creva, u modelu Caenorhabditis elegans za ispitivanja neuromodulatornih efekata, i na mišijem modelu MS-a. Obzirom na osetljivost ovih bakterija na kiseonik, u eksperimentima su korišćene kulture sa metabolitima koje su bakterije proizvele tokom noći (postbiotici). Na osnovu visoke proizvodnje BA (15 mM), anti-inflamatornog efekta u Caco-2/PBMC kokulturi i neuromodulatornih efekata u modelu C. elegans, Faecalimonas sp. NGB245 je odabran za dalju procenu efekta u mišijem modelu MS-a. Kao model MS-a korišćeni su C57BL6 miševi kojima je indukovan eksperimentalni autoimunski encefalomijelitis (EAE) aplikacijom peptida mijelinskog oligodendrocitnog glikoproteina, kompletnog Frojndovg adjuvansa i toksina pertusisa. EAE miševi su pili NGB245-postbiotik tokom 15 dana u režimu od 16 sati dnevno, ad libitum. Kontrolna grupa EAE miševa je pila PYG medijumom obogaćen celobiozom i skrobom, koji je korišćen za kultivaciju NGB245, u istom režimu. Primena NGB245-postbiotika je kod EAE-miševa dovela do razvijanja blažih dnevnih kliničkih rezultata, maksimalnih kliničkih rezultata i kraćeg trajanja EAE u poređenju sa kontrolnom grupom. Ovi efekti NGB245-postbiotika na simptome EAE bili su praćeni nižom učestalošću Th1 i Th17 ćelija, kao i različitih proinflamatornih mijeloidnih ćelija, zajedno sa povećanjem nivoa supresorskih ćelija mijeloidnog porekla u centralnom nervnom sistemu. Mikrobiota u debelom crevu životinja koje su pile postbiotik imala je veći diverzitet od kontrolne grupe životinja. Rezultati ove studije ukazuju na potencijal terapijskih pristupa baziranih na primeni postbiotika anaerobnih bakterija koje produkuju butirat kako bi se očuvala homeostaza mikrobiote i ublažio razvoj autoimunskih procesa. Istraživanje je finansirano od strane Ministarstva nauke, tehnološkog razvoja i inovacija, ugovori broj: 451-03-66/2024-03/200042, 451-03-66/2024-03/200019, i Fonda za nauku Republike Srbije, program IDEJA, #7744507, NextGenBiotics.

understanding of the crucial role of gut microbiota in both healthy organism development and various pathological conditions. Bacterial strains residing in the anaerobic environment of the colon, particularly those capable of producing short-chain fatty acids (SCFAs), play a significant role in maintaining gut homeostasis and consequently, the overall well-being of the host. Notably, a decrease in butyric acid (BA)-producing bacteria has been linked to lower BA levels observed in patients with multiple sclerosis (MS). Moreover, the immunoregulatory properties of BA have been demonstrated on various immune cells of lymphoid and myeloid origin in vitro. Hence, the study aimed to use different media in anaerobic conditions to isolate bacterial strains with high BA production capacity from the feces of healthy donors, and to assess the effects of isolates in Caco-2/peripheral blood mononuclear cells (PBMC) in vitro model of gut inflammation, Caenorhabditis elegans model for neurodegenerative studies, and in mice model of MS. Considering the sensitivity of these bacteria to oxygen, the cultures with metabolites produced by these bacteria during the night (postbiotic), were used in experiments. Based on the high BA production (15 mM), the anti-inflammatory effects in Caco-2/PBMC co-culture, and neuromodulatory effects in C.elegans model, Faecalimonas sp. NGB245 was selected for further assessment in the mice model of MS. Myelin oligodendrocyte glycoprotein peptide/complete Freund's adjuvant/pertussis toxin-induced experimental autoimmune encephalomyelitis (EAE) in C57BL6 mice was used as a model of MS. The EAE mice consumed NGB245-postbiotic over 15 days in a 16-hour/day regime, ad libitum. The control group of EAE mice received supplementation with PYG medium enriched with cellobiose and starch, which was used for NGB245 cultivation, in the same regime. The supplementation with NGB245-postbiotic resulted in alleviation of daily clinical scores, maximal clinical scores, and duration of EAE compared to the control group. These effects on EAE symptoms were accompanied by a decrease in the abundance of Th1 and Th17 cells, as well as different proinflammatory myeloid cells, along with an increase in the level of myeloid-derived suppressor cells in the central nervous system of NGB245-postbiotic-supplemented EAE mice. This was associated with a higher diversity of microbiota in the colon. These findings underscore the potential of using the postbiotics of BA-producing anaerobic bacteria to preserve immune-microbiota homeostasis and mitigate the development of autoimmune processes. This work was supported by the Minister of Science, Technological Development and Innovation, 451-03-66/2024-03/200042, 451-03-66/2024-03/200019; and by the Science Fund of the Republic of Serbia, #7744507, NextGenBiotics.