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Review paper
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ASSOCIATION BETWEEN MATRIX METALLOPROTEINASE-3 GENE POLYMORPHISM AND SUSCEPTIBILITY TO CHRONIC PERIODONTITIS: A SYSTEMATIC REVIEW AND META-ANALYSIS

POVEZANOST POLIMORFIZMA GENA MATRIKS METALOPROTEINAZE-3 I OSETLJIVOSTI NA HRONIČNI PARODONTITIS: SISTEMATSKI PREGLED I META-ANALIZA

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Summary

Background: This study aimed to explore the correlation between the Matrix Metalloproteinase-3 (MMP-3) 1171 5A/6A gene polymorphism and susceptibility to Chronic Periodontitis (CP).
Methods: Following the PRISMA guidelines, a systematic search was conducted across four electronic databases (PubMed, Embase, Web of Science, and Cochrane Library) without any time or language limitations. The selection criteria included case-control studies examining the association between the MMP-3 gene polymorphism and CP. The data were independently extracted and cross-checked by two reviewers. The Newcastle-Ottawa Scale (NOS) was used to assess the quality of the studies. Statistical heterogeneity and publication bias were assessed.
Results: Five studies, published between 2004 and 2019, met the inclusion criteria for the meta-analysis. No significant association was observed between MMP-3 gene polymorphism and CP susceptibility across all subjects in the four gene models. However, subgroup analysis revealed significant differences based on genotyping methods and smoking habits. Using PCR-RFLP genotyping method, the allele and additive models showed a positive correlation with the risk of CP (5A vs 6A, OR=1.12, 95%CI (1.02~1.23); 5A5A vs 6A6A, OR=2.85, 95%CI (1.61~4.86)). In contrast, using Sanger sequencing method, the 5A muta-

Kratak sadržaj

Uvod: Ova studija je imala za cilj da istraži korelaciju između polimorfizma gena matriks metaloproteinaze-3 (MMP-3) 1171 5A/6A i osetljivosti na hronični parodontitis (CP).
Metode: U skladu sa smernicama PRISMA, sprovedena je sistematska pretraga u četiri elektronske baze podataka (PubMed, Embase, Veb of Science i Cochrane Librari) bez ikakvih vremenskih ili jezičkih ograničenja. Kriterijumi za odabir uključivali su studije slučaja i kontrole koje su ispitivale povezanost polimorfizma gena MMP-3 i CP. Podatke su nezavisno izdvojila i unakrsno proverila dva recenzenta. Za procenu kvaliteta studija korišćena je Njukasl-Otava skala (NOS). Procenjena je statistička heterogenost i pristrasnost objavljivanja.
Rezultati: Pet studija, objavljenih između 2004. i 2019. godine, ispunilo je kriterijume za uključivanje u meta-analizu. Nije primećena značajna povezanost između polimorfizma gena MMP-3 i osetljivosti na CP kod svih subjekata u četiri genska modela. Međutim, analiza podgrupa otkrila je značajne razlike na osnovu metoda genotipizacije i navika pušenja. Koristeći metodu PCR-RFLP genotipizacije, alelni i aditivni modeli su pokazali pozitivnu korelaciju sa rizikom od CP (5A naspram 6A, OR=1,12, 95% CI (1,02~1.23); 5A5A naspram 6A6A, OR=2,85, 95% CI (1,61~4,86)). Nasuprot tome, korišćenjem Sangerove metode sekvenci-

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tion appeared to reduce CP susceptibility (5A vs 6A, OR=0.77, 95%CI (0.67~0.87); 5A5A vs 6A6A, OR=0.20, 95%CI (0.09~0.42)). Moreover, smoking habits appeared to modulate the risk. Among smokers, the 5A mutation increased susceptibility to CP, while among non-smokers it decreased.

Conclusions: While no significant correlation was found in the overall population, the stratified analysis revealed nuanced relationships contingent on genotyping methods and smoking habits.

Keywords: matrix metalloproteinase-3, gene polymorphism, chronic periodontitis, meta-analysis

ranja, činilo se da mutacija 5A smanjuje osetljivost na CP (5A naspram 6A, OR=0,77, 95% CI (0,67~0,87); 5A5A naspram 6A6A, OR=0,20, 95% CI (0,09~0,42)). Štaviše, činilo se da navike pušenja moduliraju rizik. Kod pušača mutacija 5A je povećala osetljivost na CP, dok je kod nepušača smanjena.

Zaključak: Iako nije pronađna značajna korelacija u ukupnoj populaciji, stratifikovana analiza je otkrila nijansirane odnose zavisne od metoda genotipizacije i navika pušenja.

Ključne reči: matriks metaloproteinaza-3, polimorfizam gena, hronični parodontitis, meta-analiza

Introduction

Chronic periodontitis (CP) is a globally prevalent and multifactorial inflammatory disease characterized by progressive destruction of the supporting tissues of the teeth, primarily driven by bacterial infection. This condition leads to gingival inflammation, alveolar bone loss, and if untreated, eventual tooth loss (1, 2). It's clear that periodontal bacteria serve as the initial factor in the pathogenesis of CP. However, recent studies have pointed out that the progression and severity of the disease largely hinge upon the host's to these bacteria and their metabolic byproducts (3, 4). The pathogenesis of CP entails an intricate interplay of host immune responses and the microbiota in the periodontal pocket, with a plethora of cytokines and inflammatory mediators participating in the complex network of periodontal tissue inflammation and immune response (5). Within this network, Matrix Metalloproteinases (MMPs), particularly MMP-3, have been recognized to play a central role. MMPs comprise a broad family of zinc-dependent endopeptidases that govern (ECM) remodeling and degradation (6, 7). These enzymes degrade several ECM components, including but not limited to collagen, elastin, gelatin, matrix glycoproteins, and proteoglycans.

MMP-3, also known as stromelysin-1, stands as a critical member of the MMP family due to its broad substrate specificity and its unique ability to activate other MMPs. Genetic alterations, such as single nucleotide polymorphisms (SNPs), can significantly influence MMP-3 transcription levels, protein production, and therefore, the overall functioning of the MMP-3 gene (8). The MMP-3 gene, situated at chromosome 11q22.3, has an adenine nucleotide insertion at the 1171 position. This insertion leads to a promoter polymorphism of the MMP-3 gene, giving rise to two allelic variants – one with five adenine nucleotides (5A), and the other with six adenines (6A) (5). This promoter gene polymorphism has been reported to affect the expression and regulation of the MMP-3 gene and has been proposed to associate with susceptibility to CP (9).

Current research concerning the association between MMP-3 gene polymorphism and CP susceptibility is sparse and often inconclusive. The objective of the present study is to perform a comprehensive systematic review and meta-analysis of the available literature to explore this association more precisely. In doing so, our intention is to enhance the current understanding of the genetic components involved in CP's pathogenesis and their correlation with disease susceptibility. By scrutinizing the link between MMP-3 polymorphism and CP, this study endeavors to shed light on potential genetic markers for CP susceptibility, contributing to the predictive, preventive, and personalized medicine in periodontology.

Materials and Methods

Search strategy

Throughout the systematic review procedure, we upheld compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (10). Four electronic databases PubMed, Embase, Web of Science, and Cochrane Library were searched on May 9, 2023 and no time limitation was applied. Vocabulary and syntax were specifically adapted according to the database. The specific search terms of PubMed were: (»Matrix Metalloproteinase 3« (Mesh) OR »MMP-3« OR »matrix metalloproteinase-3«) AND (»Polymorphism, Genetic« (Mesh) OR »polymorphism«) AND (»Periodontitis« (Mesh) OR »periodontitis« OR »Chronic Periodontitis« (Mesh) OR »chronic periodontitis«). There were no restrictions imposed on the language used. The reference lists of pertinent articles were manually scrutinized to identify any potential additional records.

Inclusion criteria

The systematic review required that the studies included met specific criteria (11): 1) Studies investigating the association between the MMP-3 1171 5A/6A gene polymorphism and susceptibility to periodontitis, which also must be case-control studies; 2) In the case group, patients meet the diagnostic criteria for CP. The control group consists of individuals with no periodontal inflammation and no systemic

diseases; 3) The genotype experiment data for both groups are clear, complete, and obtainable, including Odds Ratio (OR) and corresponding 95% Confidence Interval (CI).

The exclusion criteria were as follows (11): 1) Studies where the case group includes individuals with severe systemic diseases that might affect periodontal status; 2) Documents that lack comprehensive or unambiguous analytical data; 3) Case reports, commentaries, expert opinion, and narrative reviews.

Data extraction

The literature screening and data extraction shall be carried out independently by two evaluators, and cross-checked, and if there are differences, the differences will be discussed and resolved. The data to be extracted included: first author's name, year of publication, geographical region, disease/condition, number of cases, genotype of case group (5A5A, 5A6A, 6A6A), genotype of control group (5A5A, 5A6A, 6A6A), genotyping method, smoking habits. When there is no data of interest in the published report, we contact the investigators of the original study.

Quality assessment

Two independent reviewers assessed the included studies quality using the Newcastle-Ottawa Scale (NOS), which comprises nine components distributed across three categories. These categories evaluate potential sources of bias, including selection, comparability, and outcome. Each study was then assigned a quality score ranging from 0 to 9. Studies scoring between 0–3 were categorized as low quality, those with a score of 4–6 were considered of medium quality, and those achieving a score of 7–9 were classified as high-quality studies. This structured quality assessment approach ensures a robust and consistent evaluation of the included studies.

Statistical analyses

Chi-square statistics and the magnitude of I^2 were utilized to gauge the degree of heterogeneity across the studies. An absence of detected heterogeneity was suggested by an I^2 value of 0%, while a value exceeding 50% signaled substantial heterogeneity. The symmetry of the funnel plot and Egger's test were employed to inspect the potential for publication bias in the meta-analyses. In the event of an asymmetric funnel plot, we introduced assumed unreported negative studies to investigate whether publication bias had a significant effect on the impact estimates. For all statistical examinations, a two-tailed P-value of less than 0.05 was deemed statistically meaningful. The analysis of data was carried out using Stata version 17 (StataCorp, College Station, TX, USA).

Results

Search results and study selection

From the initial search of the electronic databases, 750 related literatures were initially found. After removing repetitive literatures, reading titles and abstracts, and screening strictly according to the inclusion and exclusion criteria, 23 related literatures were obtained, and 18 were excluded from further reading. Finally, 5 articles were included (5, 12–15). The literature screening process and results are shown in *Figure 1*.

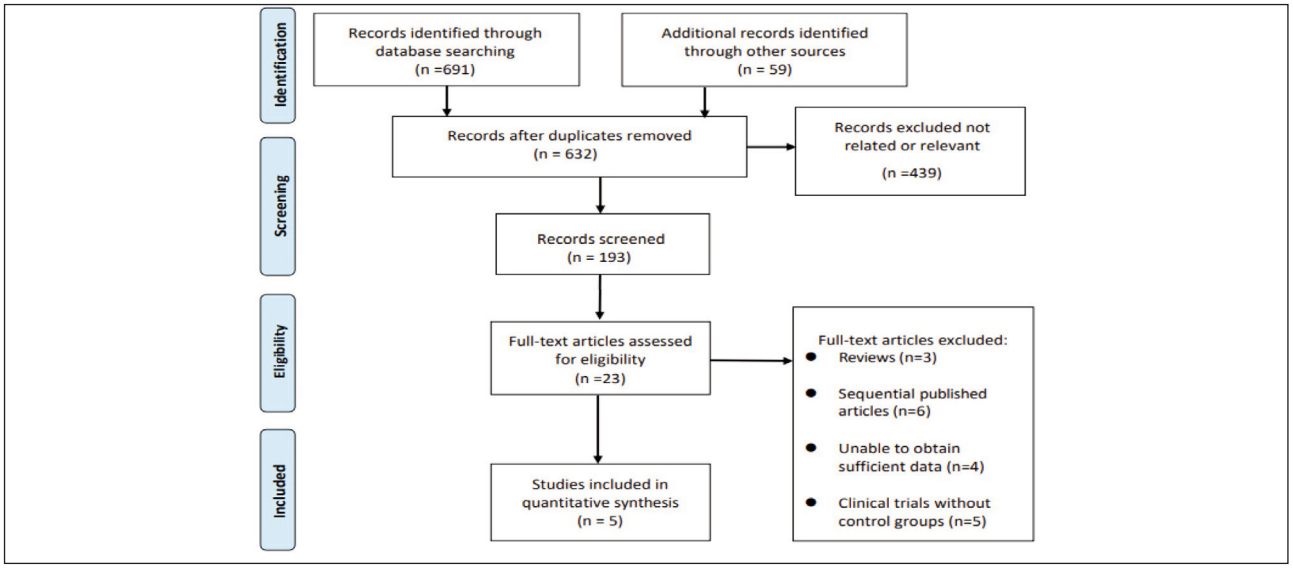

Figure 1 Selection process of included studies.

Study characteristics

The characteristics of studies included in this systematic review are presented in *Table I*. The meta-analysis under discussion incorporates five studies published over a span of 15 years (from 2004 to 2019) from various regions including Japan, Brazil, China, and India. Each study was centered on CP. The case group sizes varied across studies with the smallest group consisting of 114 individuals and the largest group containing 280 individuals. The control group sizes also differed significantly among the studies, ranging from 109 to 532 individuals. In terms of genotypes within the case groups, there were significant differences in the distribution of the 5A5A, 5A6A, and 6A6A genotypes. The studies utilized different genotyping methods; these include TaqMan PCR, PCR-RFLP, and Sanger Sequencing.

Results of quality assessment

We assessed the methodological quality of each RCT using the New Castle-Ottawa Scale (NOS). In general, two studies scored 8 points, and three studies scored 9 points. Blinding was not implemented in any of the studies, and there was a lack of indication of allocation concealment. There was no indication of funding biases in any of the studies. No studies were found to have incomplete outcome data, early stoppage bias, or baseline imbalances. *Table II* provides a summary of the potential risks associated with bias and their corresponding ratios.

Correlation between MMP-3 Gene Polymorphism and Susceptibility to Periodontitis

No significant correlation was found between MMP-3 gene polymorphism and susceptibility to CP under four gene models among all the study subjects as shown in *Figure 2*. However, subgroup analysis based on gene analysis methods and smoking habits indicated a different trend as shown in *Figure 3*. In the subgroup analysis conducted using the PCR-RFLP genotyping method, the allele and additive model showed correlation with the risk of CP onset (5A vs

Table I Characteristics of studies included in the meta-analysis.

First Author	Year	Country	Disease Type	Case Group Size	Control Group Size	Case Group Genotype	Control Group Genotype	Genotyping Method	Smoking Status
Itagaki	2004	Japan	CP	205	142	5/58/142	4/38/100	TaqMan PCR	No
Astolfi	2006	Brazil	CP	114	109	19/52/19	8/70/25	PCR-RFLP	No
Lee	2011	China	CP	280	250	154/115/11	100/135/15	PCR-RFLP	No
Li	2012	China	CP	122	532	75/44/3	213/283/36	PCR-RFLP	Mixed
Majumder	2019	India	CP	157	200	72/56/29	134/56/10	Sanger Sequencing	Mixed

SD, Standard deviation; CV, coefficient of variation

Table II The quality assessment according to NOS of each cohort study.

Study	Selection				Comparability	Outcome			Total score
	Representativeness of the exposed cohort	Selection of the non - exposed cohort	Ascertainment of exposure	Demonstration that outcome	Comparability of cohorts	Assessment of outcome	Was follow-up long enough	Adequacy of follow up of cohorts	
Majumder et al. 2019	★	★	★	★	★★	★	★	★	9
Li et al. 2012		★	★	★	★★	★	★	★	8
Lee et al. 2011	★	★	★	★	★★	★	★	★	9
Astolfi et al. 2006	★	★	★	★	★★	★		★	8
Itagaki et al. 2004	★	★	★	★	★★	★	★	★	9

NOS: New Castle-Ottawa Scale

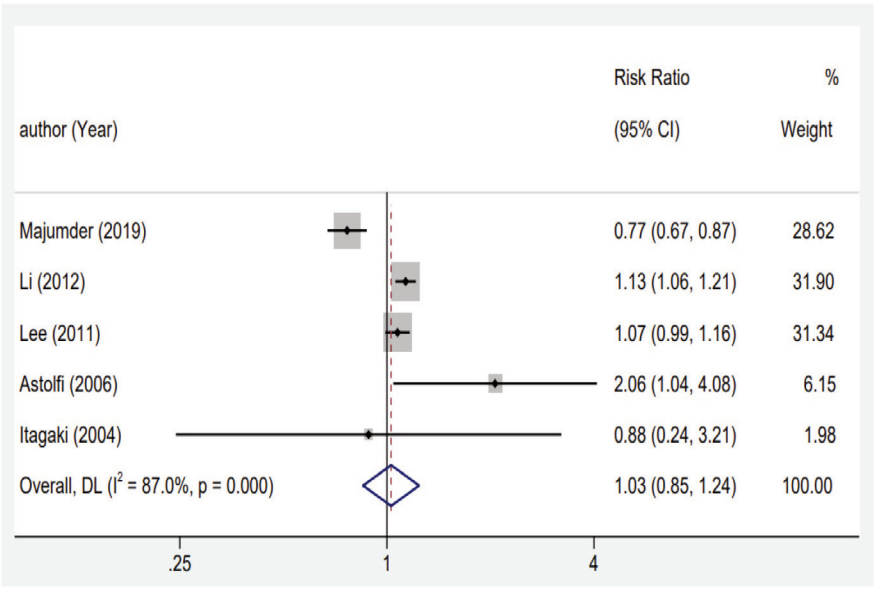

Figure 2 Forest plots of the association between MMP-3 1171 5A6A gene polymorphism and susceptibility to chronic periodontitis.

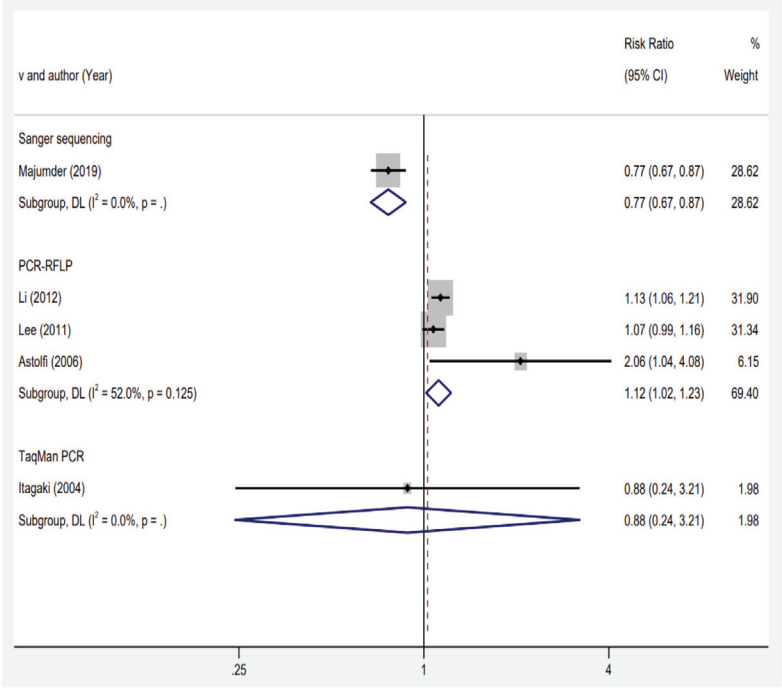

Figure 3 Subgroup analysis of the association between MMP-3 1171 5A6A gene polymorphism and susceptibility to chronic periodontitis.

6A, OR=1.12, 95%CI (1.02~1.23); 5A5A vs 6A6A, OR=2.85, 95%CI (1.61~4.86)), suggesting that carrying the 5A mutation may increase the susceptibility to CP under the PCR-RFLP genotyping method. Conversely, under the Sanger sequencing genotyping method, all four gene model groups exhibited correlation with the risk of CP onset (5A vs 6A, OR=0.77, 95%CI (0.67~0.87); 5A5A vs 6A6A, OR=0.20, 95%CI (0.09~0.42); 5A5A vs 5A6A+6A6A, OR=0.44, 95%CI (0.28~0.67); 5A5A+5A6A vs 6A6A, OR=0.24, 95%CI (0.11~0.50)). This implies that carrying the 5A mutation might reduce the susceptibility to CP under the Sanger sequencing genotyping method as shown in *Figure 3* and *Table III*.

Table III Meta-analysis of the Association between MMP-3 1171 5A6A Gene Polymorphism and Susceptibility to Chronic Periodontitis.

Gene Model	Number of Studies	Susceptibility Analysis: OR	95%CI	P-value
5A vs 6A	5	1.03	(0.85~1.24)	0.70
PCR-RFLP	3	1.12	(1.02~1.23)	<0.001
Sanger Sequencing	1	0.77	(0.67~0.87)	<0.001
Taqman	1	0.88	(0.24~3.21)	0.86
Smokers	3	1.34	(1.08~1.64)	<0.001
Non-Smokers	2	0.93	(0.21~3.95)	0.76
5A5A vs 6A6A	5	1.36	(0.40~4.40)	0.63
PCR-RFLP	3	2.85	(1.61~4.86)	<0.001
Sanger Sequencing	1	0.20	(0.09~0.42)	<0.001
Taqman	1	0.92	(0.24~3.50)	0.68
Smokers	3	2.00	(1.17~3.55)	0.01
Non-Smokers	2	0.92	(0.05~20.00)	0.89
5A5A vs 5A6A+6A6A	5	0.78	(0.24~2.45)	0.66
PCR-RFLP	3	0.94	(0.12~7.00)	0.96
Sanger Sequencing	1	0.44	(0.28~0.67)	<0.001
Taqman	1	0.90	(0.25~3.20)	0.86
Smokers	3	1.95	(1.40~2.60)	<0.001
Non-Smokers	2	0.25	(0.19~0.34)	<0.001
5A5A+5A6A vs 6A6A	5	1.05	(0.51~2.08)	0.90
PCR-RFLP	3	1.66	(1.01~2.56)	0.06
Sanger Sequencing	1	0.24	(0.11~0.50)	<0.001
Taqman	1	1.11	(0.68~1.70)	0.56
Smokers	3	1.24	(0.85~1.70)	0.68
Non-Smokers	2	1.00	(0.75~1.33)	0.86

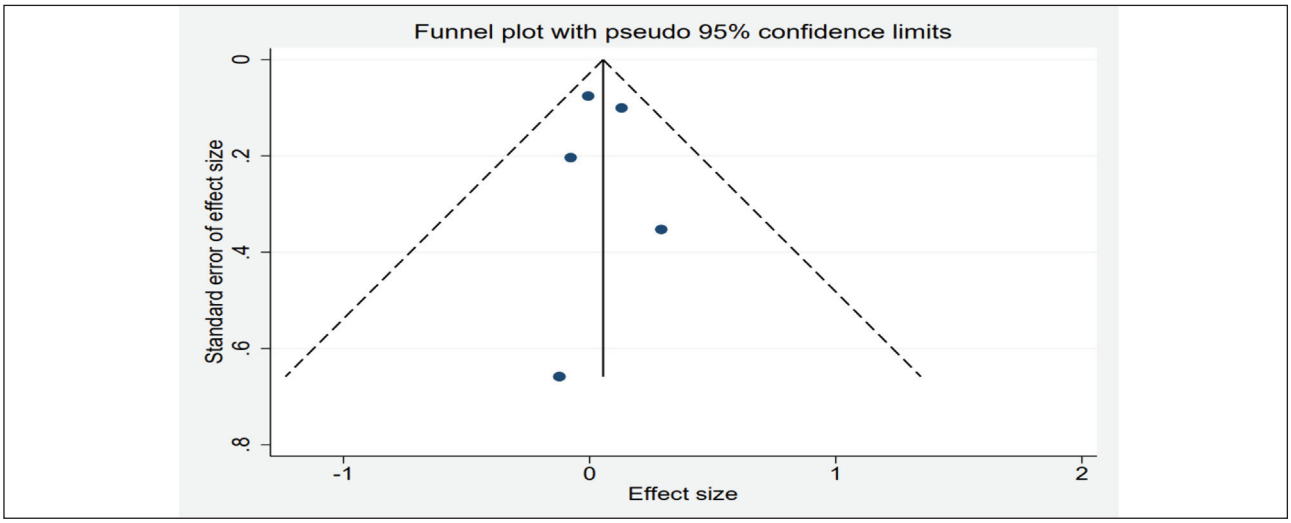

Figure 4 Funnel plot for publication bias in all included studies.

Subgroup analysis based on smoking habits revealed a correlation between all gene models, except the dominant model, and the risk of CP onset (Smokers: 5A vs 6A, OR=1.34, 95%CI (1.08~1.64); 5A5A vs 6A6A, OR=2.00, 95%CI (1.17~3.55); 5A5A vs 5A6A+6A6A, OR=1.95, 95%CI (1.40~2.60). Non-smokers: 5A5A vs 5A6A+6A6A, OR=0.25, 95%CI (0.19~0.34)). This suggests that carrying the 5A mutation may increase the susceptibility to CP among smokers, but decrease it in non-smokers. For instance, consider the analysis for 5A5A vs 6A6A as shown in *Table III*.

Publication bias

The funnel plots generated from the observed study exhibited symmetrical distribution, and no statistically significant evidence of publication bias was observed in the funnel plots (*Figure 4*).

Discussion

The MMP-3 gene polymorphism has been a subject of interest due to its potential role in the pathogenesis of CP. Previous investigations, however, have revealed conflicting findings, underscoring the need for our meta-analysis. Our study demonstrated no significant association between MMP-3 1171 5A/6A polymorphism and overall CP susceptibility. However, in a stratified analysis based on genotyping methods and smoking habits, certain interactions emerged, illuminating the nuanced nature of this relationship. MMP-3 is pivotal in degrading extracellular matrix components during inflammation, linking it to CP pathogenesis, marked by persistent inflammation and subsequent destruction of periodontal tissues. Hence, variations in the MMP-3 gene that could modulate its expression or activity might impact the inflammatory process and the susceptibility to CP. However, the precise mechanism through which MMP-3 gene polymorphism influences CP risk remains to be determined and is likely to be multifaceted.

Our findings resonate with the works of Li et al. (12) and Lee et al. (15), who observed an association between MMP-3 1171 polymorphism and CP susceptibility. This concurrence suggests that the 5A allele may reduce the risk of CP in the Chinese population. Astolfi et al. (14) also suggested that MMP-3 gene polymorphism is associated with periodontal tissue destruction in CP among Brazilians, indicating that the 5A allele could be a risk factor for CP development. Contrarily, the 6A allele has been associated with higher MMP-3 levels in patients with coronary heart disease and myocardial infarction (5), indicating the intricate relationship between gene polymorphisms, disease susceptibility, and the potential modulatory role of local and systemic conditions. The discrepancies found in various studies may be attributed to geographical factors, population migration, and genetic admixture, along with the close proximity of the MMP-1 and MMP-3 genes, which may give rise to linkage disequilibrium (16, 17).

In this meta-analysis, we did not observe a significant correlation between MMP-3 1171 5A6A gene polymorphism and the general population's susceptibility to CP. However, subgroup analysis based on genotyping methods and smoking habits suggested that carrying the 5A mutation might increase susceptibility to CP in an additive model and among smokers, whereas it could decrease susceptibility among non-smokers. One important factor influencing our results is the presence of other polymorphisms in the vicinity, such as those in the MMP-1 gene located near MMP-3 on chromosome 11q22.3 (14, 16). These neighboring polymorphisms might collectively impact the expression of MMP-3. A noteworthy point to consider is the complex regulatory mechanisms governing MMP-3 mRNA transcription (18). The regulation is so intricate that the absence of a single MMP gene, such as MMP-2, MMP-3, MMP-7, MMP-8, MMP-11, or MMP-12, does not manifest as an observable disease phenotype in mice (19). This suggests that the influence of a single MMP-3 SNP may be subtle or inconclusive in determining disease susceptibility or progression. Our systematic review and meta-analysis present a broad exploration of the association between MMP-3 gene polymorphism and CP susceptibility. However, it is essential to remember that periodontitis is a multifactorial disease, where the interplay between genes, environment, and lifestyle plays a vital role in disease pathogenesis. Furthermore, understanding the role of MMP-3 gene polymorphism in CP may have broader implications. For instance, this polymorphism has been linked to various forms of cancer, rheumatoid arthritis, and cardiovascular diseases. The further dissection of MMP-3's role could thereby contribute to our understanding of the molecular mechanisms underpinning these diseases (20–22).

Despite these insights, this study is not without limitations. The lack of raw data from individual studies limited our ability to control for potential confounders, such as age, gender, and other risk factors. Also, the sample size and the number of included studies were relatively small, which may reduce the statistical power. Furthermore, publication bias can arise from various sources, including selective reporting and unpublished studies, which can significantly impact the overall results. Selective reporting can lead to an incomplete or biased representation of the true effects of an intervention or treatment. Unpublished studies, particularly those with negative or non-significant results, may not be readily available in the literature, leading to an overestimation of the treatment effect if only published studies are considered. Therefore, the results of the present research should be mindful of these biases.

In conclusion, while our meta-analysis marks a significant step in understanding the relationship between MMP-3 gene polymorphism and CP, further investigations are warranted. The study's findings suggest that smoking habits may interact with the MMP-3 gene polymorphism to influence chronic periodontitis susceptibility. This highlights the complex interplay between genetic factors and environmental exposures in disease development, emphasizing the need for personalized approaches to prevention and treatment strategies for chronic periodontitis. The future studies should consider a comprehensive approach, examining the interplay between genetic, environmental, and lifestyle factors contributing to the risk of CP. Such research would not only enhance our understanding of CP's pathogenesis but may also offer valuable insights for personalized prevention and treatment strategies.

Conclusions

Our systematic review and meta-analysis present a comprehensive evaluation of the association between MMP-3 1171 5A/6A gene polymorphism and susceptibility to CP. While no significant correlation was found in the overall population, the stratified analysis revealed nuanced relationships contingent on genotyping methods and smoking habits.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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MECHANISM OF LDH AND IL-8 INVOLVED IN PANCREATIC CANCER PAIN AND THE CORRELATION OF PAIN DEGREE

MEHANIZAM LDH I IL-8 UKLJUČENIH U BOL PANKREASNOG KANCERA I KORELACIJA SA STEPENOM BOLA

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Summary

Background: This research aimed to observe the mechanism of lactate dehydrogenase (LDH) and interleukin 8 (IL-8) in pancreatic cancer pain and their correlation with pain degree.
Methods: 126 patients with pancreatic cancer who visited our hospital from January 2021 to February 2023 were selected. The patients were divided into groups of 58 patients with low pain (1~3 points) and 68 patients with high pain (4~10 points) by visual analog scale (VAS). And 50 health examinees in the same period were selected as the healthy control group. The serum LDH and IL-8 concentrations are analyzed by enzyme-linked immunosorbent assay, and the subjective pain grading method score is analyzed. The differences in LDH and IL-8 concentrations among the three groups of patients were compared. Pearson correlation analysis was used to investigate the correlation between LDH, IL-8 concentrations, and patient pain. Binary logistic regression was used to determine independent risk factors for high pain, and ROC curves were used to analyze the diagnostic efficacy of each indicator.
Results: The serum LDH and IL-8 concentrations in the high-pain group were exceed the low-pain group's ($P<0.05$). The serum LDH and IL-8 concentrations in the low-pain group exceeded the healthy control group's ($P<0.05$). Pearson correlation analysis revealed a positive correlation between serum LDH concentration and pain

Kratak sadržaj

Uvod: Cilj ovog istraživanja je bio posmatranje mehanizma laktat dehidrogenaze (LDH) i interleukina 8 (IL-8) u vezi sa bolom povezanim sa kancerom pankreasa i njihova korelacija sa stepenom bola.
Metode: Odabrano je 126 pacijenata sa kancerom pankreasa koji su posetili našu bolnicu od januara 2021. do februara 2023. Pacijenti su podeljeni u grupu od 58 pacijenata sa niskim bolom (1~3 poena) i grupu od 68 pacijenata sa jakim bolovima (4~10 poena) prema vizuelnoj analognoj skali (VAS). Kao kontrolna grupa odabrano je 50 zdravih ispitanika u istom periodu. Koncentracije LDH i IL-8 u serumu su analizirane enzimskim imunološkim testom, a ocenjena je i subjektivna metoda procene bola. Upoređene su razlike u koncentracijama LDH i IL-8 između tri grupe pacijenata. Pearsonova korelaciona analiza je korišćena za istraživanje veze između koncentracija LDH, IL-8 i stepena bola. Za utvrđivanje nezavisnih faktora rizika za visok bol korišćena je Binarna logistička regresija, a ROC krive su korišćene za analizu dijagnostičke efikasnosti svakog pokazatelja.
Rezultati: Koncentracije LDH i IL-8 u serumu u grupi sa visokim bolom su premašile koncentracije u grupi sa niskim bolom ($P<0,05$). Koncentracije LDH i IL-8 u grupi sa niskim bolom su bile više nego kod zdrave kontrolne grupe ($P<0,05$). Pearsonova korelaciona analiza je otkrila pozitivnu korelaciju između koncentracije LDH u serumu i

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grading (r=0.736, P=0.000). The serum IL-8 positively correlates with pain grading (r=0.680, P=0.000). Serum LDH and IL-8 concentrations positively correlate (r=0.589, P=0.000). LDH and IL-8 concentrations are independent risk factors for high pain levels (OR=1.033, 1.142, P<0.05). The logistic regression prediction model formula was used: Y=constant+B1X1+B2X2+...+BnXn to set the joint diagnostic prediction model as -12.063+ 0.033×LDH+0.133×IL-8. The areas under the ROC curves of LDH, IL-8, and predictive model (LDH+IL-8) in patients with high pain were 0.925, 0.945, and 0.974, respectively. The relevant standards for LDH are >190 U/L, IL-8 is >36 pg/mL, and the relevant standards for prediction models are >5.75.

Conclusions: LDH and IL-8 participate in the pain aggravation process of pancreatic cancer and are closely related to the pain grading. The combination of LDH and IL-8 can be used as a biological indicator to evaluate the pain severity of pancreatic cancer and provide a reference for clinical diagnosis and treatment.

Keywords: lactate dehydrogenase, interleukin 8, pain, pancreatic cancer, mechanism

ocene bola (r=0,736, P=0,000). IL-8 u serumu je u pozitivnoj korelaciji sa ocenom bola (r=0,680, P=0,000). Koncentracije LDH i IL-8 u serumu su u pozitivnoj korelaciji (r=0,589, P=0,000). LDH i IL-8 su nezavisni faktori rizika za visok stepen bola (OR=1,033, 1,142, P<0,05). Formula logističke regresije je korišćena za predviđanje modela: Y=konstanta+B1X1+B2X2+...+BnXn postavljajući zajednički dijagnostički model kao -12,063+ 0,033×LDH+0,133×IL-8. Površine ispod ROC krivih za LDH, IL-8 i prediktivni model (LDH+IL-8) kod pacijenata s visokim bolom su bile 0,925, 0,945 i 0,974, redom. Relevantni standardi za LDH su >190 U/L, za IL-8 >36 pg/mL, a za prediktivne modele su >5,75.

Zaključak: LDH i IL-8 učestvuju u procesu pogoršanja bola kod kancera pankreasa i tesno su povezani sa ocenom bola. Kombinacija LDH i IL-8 se može koristiti kao biološki pokazatelj za procenu ozbiljnosti bola kod kancera pankreasa i mogu da daju referencu za kliničku dijagnozu i lečenje.

Ključne reči: laktat dehidrogenaza, interleukin 8, bol, kancer pankreasa, mehanizam

Introduction

Pancreatic cancer (PC) is a common malignant tumor of the digestive system, and its incidence rate is on the rise (1). According to statistics, there were approximately 495000 new cases of PC and approximately 420,000 deaths worldwide in 2020. In China, the 5-year relative survival rate of PCs is only 7.2% (2, 3). The main clinical manifestation of PC is abdominal pain. Severe cancer pain can have adverse effects on PC patients' life quality and is the main reason why PC patients seek medical help. Accurately determining the type and mechanism of pain is crucial for developing effective analgesic treatment plans. The research on the mechanism of PC pain mainly starts from multiple aspects, such as the tumor itself, inflammatory response, changes in the nervous system, and signaling pathways (4). At present, the MAPK pathway (5), PI3K/AKT pathway (6), and COX-2 pathway (7) play important roles in PC hyperalgesia and neuropathic pain. Lactate dehydrogenase (LDH) and interleukin-8 (IL-8) are important biomarkers involved in the occurrence and development of tumors. LDH promotes the glycolysis of PC cells by providing them with energy substances, thereby promoting tumor growth. IL-8 participates in tumor progression by promoting PC cell proliferation, angiogenesis, and infiltration. LDH and IL-8 also participate in the mechanism of PC pain and are connected to pain severity. This study's main hypothesis is that serum LDH and IL-8 concentrations significantly correlate with the degree of PC pain, which can serve as predictive factors for distinguishing different degrees of pain. To verify this hypothesis, correlation studies and ROC curve analysis were used in the experiment to determine the mechanism of action of LDH and IL-8 in PC pain. At the same time, the correlation between two biomarkers and pain severity was analyzed in the experiment. The research results can provide new ideas and directions for PC patients' diagnosis, pain assessment, and analgesic treatment.

Materials and Methods

Patients

126 PC patients who visited our hospital from January 2021 to February 2023 were selected, including 58 patients in the low-pain group (LPG) and 68 in the high-pain group (HPG). 50 healthy examinees were treated as the healthy control group.

Inclusion criteria: 1) Diagnosed as a PC patient through preoperative imaging examination. 2) 18–75 years old. 3) Karnofsky score 70, estimated survival time 3 months. 4) The patient feels pain and is able to cooperate in assessing the degree of pain.

Exclusion criteria: 1) Combined with other malignant tumors or life-threatening diseases such as severe organ failure. 2) Mental illness or inability to cooperate with research. 3) Within the past month, drugs such as immunosuppressants and glucocorticoids have been used. 4) Blood system diseases or coagulation disorders. 5) Regular follow-up or missing visits during the research process cannot be guaranteed. 6) Postoperative pathology confirmed a non-PC patient.

The health control group consists of health examination personnel aged 18–75 without any physical pain sensation. LPG has 34 males and 24 females, with an average age of (63.5±12.3), ranging from 29 to 88 years. There were 32 cases with a history of drinking alcohol. A total of 42 males and

26 females were included in the HPG, aged 44–87, averaging (65.2±10.7) years old. 38 cases have an alcohol consumption history. In the healthy controlling group, 28 males and 22 females, averaging 64.3±8.5, aged 48–82. There were 28 cases with a history of alcohol consumption. The three groups' gender, age, and alcohol consumption history are the same ($P>0.05$).

Research methods

General information survey

A general information questionnaire was self-made. Two scale investigators conducted information collection in the patient's ward. The collection content includes gender, age, smoking history, drinking history, tumor staging, chemotherapy history, and targeted treatment history.

Pain grading method

Pain assessment was conducted based on the visual analog scale (VAS) score of patient pain. A linear table with a length of nearly a certain length (10 cm) was labeled as »painless« and »most severe pain,« representing their respective endpoints. The patient is required to indicate the current level of pain felt on this straight line. VAS score was obtained by measuring and recording the distance indicated by the patient in the experiment. The range of values is 0–10, where 0 represents painless, and 10 represents the most severe pain. 1–3 points are considered mild and included in the LPG, 4–6 points are classified as moderate pain, 7–10 points are classified as severe pain, and 4–10 points are included in the HPG.

LDH and IL-8 concentration determination

Five mL of venous whole blood samples from patients were collected in the experiment, and the serum was separated by conventional centrifugation and stored at 4 °C. LDH was quantitatively detected by enzyme-linked immunosorbent assay (ELISA). First, an LDH ELISA kit was prepared, including LDH antibody, LDH standard, biotinylation LDH secondary antibody, antibiotinase, a substrate solution (including TMB), and a termination solution pre-coated on the microplate. The serum sample to be tested and the LDH standard were diluted to an appropriate concentration and added to a microporous plate pre-coated with LDH antibodies. The reaction was sustained for 30 minutes at 37 °C. The board was washed 5 times to remove unbound substances. This experiment added biotinylation LDH secondary antibody, reacting at 37 °C for 30 min. The board was washed 5 times to remove unbound substances. Antibiotinase was mixed in the experiment, and the reaction was sustained for 30 minutes at 37 °C. The board was washed 5 times to remove unbound substances. Substrate solution (TMB) was added to the experiment, and the room temperature was dark and colored for 10 to 30 minutes. A termination solution was mixed in the experiment, and its absorbance at 450 nm was tested. The LDH concentration in the sample was calculated on the foundation of the standard curve. ELISA was used to detect serum IL-8 concentration quantitatively. First, an IL-8 ELISA kit was prepared for the experiment, including a microplate precoated with IL-8 antibody, IL-8 standard, biotinylation IL-8 secondary antibody, antibiotinase, a substrate solution (containing TMB), and a termination solution. Then, the serum sample to be tested and the IL-8 standard were added to the microplate at an appropriate dilution ratio in the experiment, and the reaction was sustained for 2 hours at 37 °C. The board was washed 5 times to remove unbound substances. Biotinylation IL-8 secondary antibody was added, and the reaction was sustained for 1 hour at 37 °C. The board was washed 5 times to remove unbound substances. Antibiotinase was added in the experiment, and the reaction was sustained for 30 minutes at 37 °C. The board was washed 5 times to remove unbound substances. Substrate solution (TMB) was added to the experiment, and the room temperature was dark and colored for 30 minutes. A termination solution was mixed, and its absorbance at 450 nm was tested. IL-8 in the sample was calculated based on the standard curve.

Observation indexes

(1) Comparison of general information. The differences in gender, age, smoking history, drinking history, Karnofsky score, tumor staging, chemotherapy history, and targeted treatment history among three groups of patients were compared. (2) Pain severity. The pain grading of PC patients was recorded, and they were classified as mild to moderate to severe pain patients. (3) Serological indicators. The LDH and IL-8 concentration measurement results of each group of patients were collected.

Statistical methods

SPSS 24.0 is the analyzing tool. The measurement data belonging to the normal distribution were represented by $\bar{x}\pm s$. Otherwise, they are represented by the median (interquartile range). ANOVA analysis of variance or Mann-Whitney testing was used. These counting data were represented by example (%). χ^2 test was used. Pearson correlation analysis can identify whether LDH, IL-8 concentration, and patients' pain degree have a correlation. Binary logistic regression can identify independent risk factors resulting in high pain, and a joint diagnostic prediction model

was constructed. The receiver operating curve (ROC)can analyze LDH, IL-8, and predictive models for high pain severity. Medcalc software determined the relevant standards for each data for high pain severity, and the correction level α was set at 0.05.

Results

Clinical data comparison in two groups

In *Table I*, there was no statistical significance in tumor staging, chemotherapy history, and HPG and LPG's targeted therapy history are the same ($P>0.05$).

Comparison of serum LDH and IL-8 concentrations among three groups

The serum LDH and IL-8 concentrations in HPG were (261.46±66.55) U/L and (58.53±20.91) pg/mL, respectively, which exceed LPG's ($P<0.05$). Patients' serum LDH in LPG was (175.84±29.88) U/L, and the concentration of IL-8 was (30.31± 20.91) pg/mL, which exceeded the healthy control group's ($P<0.05$) in *Figure 1*.

The relationship between serum LDH, IL-8, and patient pain grading

Pearson correlation analysis revealed a positive correlation between serum LDH concentration and pain grading ($r=0.736$, $P=0.000$). The serum IL-8 concentration and pain grading positively correlate ($r=0.680$, $P=0.000$). Serum LDH and IL-8 concentrations positively correlate ($r=0.589$, $P=0.000$). From *Figure 2*, serum LDH and IL-8 gradually increase as pain grade increases.

Binary logistic regression results and predictive models for high pain

According to *Table II*, LDH and IL-8 concentrations are independent risk factors for high pain levels (OR=1.033, 1.142, P<0.05). In the experiment, the logistic regression prediction model formula Y=constant+B1X1+B2X2+...+BnXn was used to set the joint diagnostic prediction model = -12.063+ 0.033×LDH+0.133×IL-8.

Table I Clinical data comparison.

Group (n)	Tumor staging (case)		History of chemotherapy (case)	History of targeted therapy (case)
	I/ stage	III/ stage		
Low pain (58)	12 (20.69)	46 (79.31)	38 (65.52)	26 (44.83)
High pain (68)	9 (13.24)	59 (86.76)	54 (79.41)	37 (54.41)
t/χ^2	1.252		2.408	1.150
P	0.263		0.121	0.284

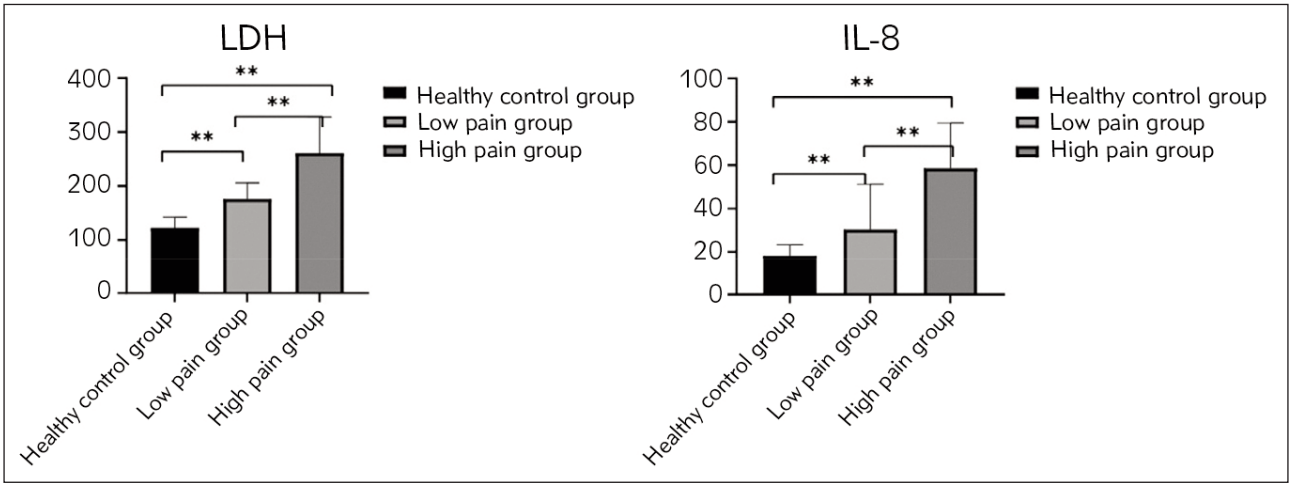

Figure 1 Serum LDH and IL-8 concentrations compared among three groups.

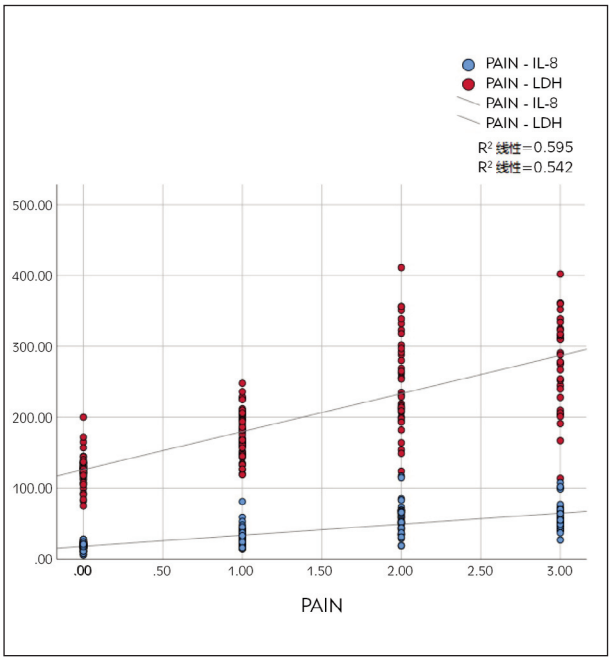

Figure 2 Trends in serum LDH and IL-8 levels in patients with different pain grades.

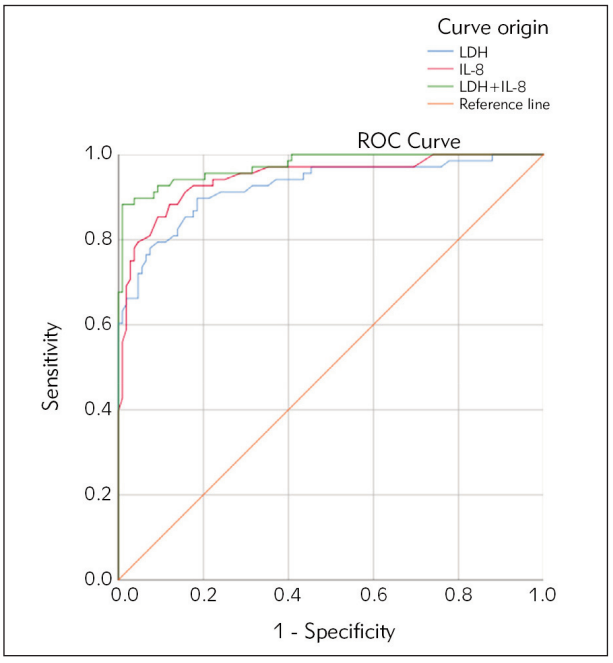

Figure 3 ROC curve with high pain.

Table II Clinical data comparison.

Factor	B	SE	Wald	P	OR	95%CI
LDH	0.033	0.008	16.969	0.000	1.033	1.017~1.049
IL-8	0.133	0.008	21.948	0.000	1.142	1.080~1.207
Constant	-12.063	2.049	34.659	0.000	0.000	

ROC analyzing

The areas under ROC curves of LDH, IL-8, and predictive model (LDH+IL-8) in patients with high pain were 0.925, 0.945, and 0.974, respectively. The relevant standard for LDH is >190 U/L, the relevant standard for IL-8 is >36 pg/mL, and the relevant standard for the prediction model is >5.75. Please refer to *Figure 3* for details.

Discussion

The mechanism of PC pain is complex and is related to tumor infiltration, compression of peripheral nerves, inflammatory reactions, and bone metastasis (7–10). Cancer pain can affect patients' quality of life, requiring accurate evaluation. Research has shown that elderly patients with digestive tract tumors still experience pain as the main symptom, accompanied by depression, anxiety, and even pain, which can lead to limited functional status (11). At present, the diagnosis and treatment of PC patients mainly rely on palliative surgery, radiotherapy and chemotherapy, pain treatment, and mixed therapy. According to Aitken et al. (12), pain treatment accounts for 16.6% of PCs. Currently, visual simulation scoring methods (13) and digital scoring methods are mainly used to evaluate PC pain. However, these scoring methods are all subjective evaluations and impact feedback on the degree of pain. Pain, as the most common clinical symptom, is a complex physiological and psychological activity (14). Ozcan et al. (15) showed that abdominal pain in PC patients is the most common symptom that affects quality of life. A good analgesic effect can improve the survival rate while worsening pain can reduce the patient's survival rate. Effective pain treatment guided by serological indicators assists in prolonging the survival of metastatic PC patients. PC pain assessment should examine both the patient's subjective score and the patient's serological indicators.

The serological indicators related to pain are not yet clear. As an enzyme, LDH expression and activity significantly increase in PC cells due to excessive metabolism and increased energy demand. Lactic acid production in the intracellular matrix is related to cell proliferation and hypoxia. The increase in LDH activity in PC cells can promote metastasis and induce hypoxia, participating in pain exacerbation (16). LDH can be regarded as a diagnostic and prognostic marker for primary pancreatic lymphoma (17). Meanwhile, LDH has been found to be associated with multiple aspects, such as tumor growth and lymph node involvement (18). High concentrations of LDH can stimulate angiogenesis, invasion, and metastasis of PC cells, expand tumor volume, compress or infiltrate surrounding tissues and nerves, and cause pain generation and aggravation. IL-8 is a cytokine that is highly expressed in the fibrotic environment of pancreatitis. It is an essential factor involved in the STAT 3/JAK2 pathway related to the inflammatory status of pancreatic ductal adenocarcinoma (19). IL-8 can promote the recruitment and activation of tumor-related bodies, releasing inflammatory and pain mediators (20, 21). In addition, IL-8 can directly act on surrounding neurons, causing the generation and transmission of pain signals (22). LDH and IL-8 are routine PC patient screening indicators and can assist in assessing pain severity.

The results of this study show that the concentrations of LDH and IL-8 are higher in the HPG than in LPG and higher in LPG than in the healthy control group. This indicates that LDH and IL-8 are closely related to the generation and exacerbation of PC pain, and there is a dose-effect relationship. These results accord with Noh et al. (23). LDH concentration is positively correlated with pain VAS score grading, with higher concentrations in the HPG. IL-8 promotes tumor angiogenesis and cell movement, significantly increasing in PC tissue and blood, and is associated with invasion and metastasis. This suggests that LDH is involved in the exacerbation of PC pain and can be used as an evaluation indicator. IL-8 is involved in the generation and exacerbation of PC pain and can serve as a biological indicator of pain severity and changes. This study conducted logistic regression and predictive models and found that LDH and IL-8 concentrations were independent risk factors for high pain levels (OR=1.033, 1.142, $P<0.05$). Joint diagnostic prediction model =-12.063+ 0.033×LDH+0.133×IL-8. The areas under the ROC curves of LDH, IL-8, and predictive model (LDH+IL-8) in patients with high pain were 0.925, 0.945, and 0.974, respectively. It is suggested that combining the detecting methods of LDH and IL-8 can determine pain severity and trend better. Furthermore, relevant threshold values were proposed to determine the severity of pain and guide clinical treatment. LDH and IL-8 connect to tumor invasion closely and participate in tumor microenvironment and even pain generation, which can be used as assessment tools.

In summary, LDH and IL-8 are involved in the generation and development of PC pain, and changes in concentration can reflect the severity of pain, which is expected to become indicators for pain assessment and prognosis. Combined applications can improve diagnostic accuracy and provide reference for pain treatment. The prediction model and related critical values constructed in this study can be used to assess the risk of pain exacerbation and the severity of pain and have guiding significance for the clinical diagnosis and treatment of PC pain. The widespread application of the model still requires expanding sample size validation. However, the research approach is innovative, and the results are relatively reliable, which has the hope of further transformation and application.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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COMPARISON OF FRIEDEWALD, MARTIN/HOPKINS, AND SAMPSON FORMULAE WITH DIRECT LDL MEASUREMENT IN HYPERLIPIDAEMIC AND NORMOLIPIDAEMIC ADULTS IN A TURKISH POPULATION

POREĐENJE FRIEDEWALDOVE, MARTIN/HOPKINSOVE I SAMPSONOVE FORMULE SA DIREKTNIM MERENJEM LDL KOD HIPERLIPIDEMIČNIH I NORMOLIPIDEMIČNIH ODRASLIH OSOBA U TURSKOJ POPULACIJI

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Summary

Background: In our study, we aimed to compare the Friedewald, Martin/Hopkins, and Sampson formulae with direct LDL-cholesterol (d-LDL-C) measurement.
Methods: The study was a retrospective investigation by the Department of Medical Biochemistry of the Ankara Training and Research Hospital between January 1, 2021, and December 31, 2022. Our study evaluated the results of 6297 patients aged 18–95 years who underwent cholesterol panel TC, TG, HDL-C, and direct LDL-C in our laboratory. The estimated LDL-C was calculated according to Friedewald, Martin/Hopkins, and Sampson formulae.
Results: All three formulae showed a stronger positive correlation with d-LDL-C (0.905, 0.897, and 0.886, respectively, for all data, $p<0.001$). In addition, when we compared the total median difference (1^{st}–3^{rd} quartile) of all formulae, it was -0.69 (-1.62 to 0.39) for Friedewald, 0.034 (-0.74 to 1.14) for Martin/Hopkins and -0.40 (-1.19 to 0.55) for Sampson. According to Passing Bablok regression analyses, the intercept was determined as -0.97 (95% CI=-1.01 to -0.93), 0.41 (95%=0.37 to 0.44) and -0.05 (-0.08 to -0. 03) and slopes were calculated as 1.083 (95% CI=1.07–1.09), 0.88 (0.88 to 0.89) and 0. 90 (95%=0.89 to 0.90) for Friedewald, Martin/Hopkins and Sampson, respectively.
Conclusions: Our findings suggest that the Martin/Hopkins formula performed better than the Friedewald and Sampson formulas. We figured out utilizing the Martin/Hopkins formula as a good alternative for estimated LDL-C in Turkish adults.

Keywords: low-density lipoprotein, Friedewald, Martin/Hopkins, Sampson

Kratak sadržaj

Uvod: Cilj našeg istraživanja je bio da uporedi formule Friedewald, Martin/Hopkins i Sampson formula sa direktnim merenjem LDL-holesterola (d-LDL-C).
Metode: Ova studija je bila retrospektivno istraživanje koje je sprovelo Odeljenje medicinske biohemije Bolnice u Ankari za obuku i istraživačke aktivnosti u periodu od 1. januara 2021. do 31. decembra 2022. Naše istraživanje je evaluiralo rezultate 6297 pacijenata uzrasta od 18 do 95 godina koji su prošli kroz panel holesterola TC, TG, HDL-C, i direktnog LDL-C u našoj laboratoriji. Procenjeni LDL-C je izračunat prema Friedewald, Martin/Hopkins i Sampson formulama.
Rezultati: Sve tri formule su pokazale jaču pozitivnu korelaciju sa d-LDL-C (0,905, 0,897 i 0,886, redom, za sve podatke, $p<0,001$). Takođe, kada smo uporedili ukupnu srednju razliku (od prvog do trećeg kvartila) svih formula, bila je -0,69 (-1,62 do 0,39) za Friedewald, 0,034 (-0,74 do 1,14) za Martin/Hopkins i -0,40 (-1,19 do 0,55) za Sampson. Prema analizama regresije Passing Bablok, presečna vrednost je određena kao -0,97 (95% CI=-1,01 do -0,93), 0,41 (95%=0,37 do 0,44) i -0,05 (-0,08 do -0,03), a nagibi su izračunati kao 1,083 (95% CI=1,07 do 1,09), 0,88 (0,88 do 0,89) i 0,90 (95%=0,89 do 0,90) za Friedewald, Martin/Hopkins i Sampson formule, redom.
Zaključak: Naši nalazi sugerišu da Martin/Hopkins formula ima bolje performarse od Friedewald i Sampson formula. Ustanovili smo da je korišćenje Martin/Hopkins formule dobra alternativa za procenjeni LDL-C kod odraslih osoba u Turskoj.

Ključne reči: niska gustina lipoproteina, Friedewald, Martin/Hopkins, Sampson

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Introduction

The level of low-density lipoprotein cholesterol (LDL-C) is an important marker for the risk of coronary heart disease. High LDL-C levels increase the risk of developing coronary heart disease, while low LDL-C levels decrease the risk of coronary heart disease (1) Routine measurement of LDL-C levels is recommended to determine the risk of coronary heart disease in both normolipidemic and hyperlipidemic individuals. In addition, LDL-C has long been an effective therapeutic target for the prevention of primary and secondary cardiovascular events (1, 2).

Both indirect estimation formulas (e.g., Friedewald, Martin/Hopkins, Sampson) and direct methods (homogeneous assay, electrophoresis, and sequential and density-gradient ultracentrifugation) are used to determine LDL-C levels. In all these formulas, LDL-C levels are estimated from triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and total cholesterol (TC) values (2). It is based on the Friedewald equation developed in 1972, which is commonly used to calculate LDL-C levels but has limited accuracy at low and very high triglyceride levels (3). Formulas such as Martin/Hopkins and Sampson have been developed more recently to overcome the disadvantages of this formula (4, 5). The Martin/Hopkins formula uses an adjustable factor instead of a fixed TG denominator. This formula is more accurate than the Friedewald formula, especially at low LDL-C levels, and has shown a much stronger agreement with LDL-C measured directly by ultracentrifugation than the Friedewald formula in terms of TG level. However, the Martin/Hopkins LDL-C formula also has the disadvantage that it tends to overestimate the LDL-C level (or direct homogeneous LDL-C [d-LDL]), especially at high TG values (4, 5).

Recently, using the United States National Institutes of Health database, Sampson et al. derived a new LDL-C formula from 18,715 samples from 8656 patients (5). They derived this formula using TG and non-HDL-C as independent variables with multiple least squares regression to calculate beta quantification and very-low-density lipoprotein cholesterol (VLDL-C) in a population with high TG. Compared to the Friedewald formula, the Sampson LDL formula provides more accurate results at higher triglyceride levels (<9.03 mmol/L).

The Friedewald, Martin/Hopkins, and Sampson formulas can be used to measure LDL-C levels in hyperlipidemic and normolipidemic adults. However, as the population-based performance of these formulas may vary, they need to be validated in different populations and compared with other laboratory techniques. In our study, we aimed to compare the d-LDL-C test with the Friedewald, Martin/Hopkins, and Sampson formulae in the Turkish population.

Materials and Methods

The study is a retrospective investigation of the Department of Medical Biochemistry of Ankara Training and Research Hospital. Our study evaluated the results of 6297 patients aged 18-95 years who underwent cholesterol panel TC, TG, HDL-C in our laboratory between January 1, 2021, and December 31, 2022 (58% female, 42% male). The study was approved by the University of Health Sciences Ankara Training and Research Hospital clinical research ethics committee (Acceptance date: 27/07/2022, No: 1007/2022) according to the principles of the Helsinki Declaration. The Laboratory Information Management System (LIS) obtained patient demographic and laboratory data. Pregnant women and patients with chronic diseases such as cancer and renal failure were excluded. Patients were divided into eight groups according to TG values (A: <1.13 mmol/L, B: 1.13–2.25 mmol/L, C: 2.26–3.38 mmol/L D: 3.39–4.50 mmol/L, E: 4.52–5.63 mmol/L, F: 5.65–6.76 mmol/L, G: 6.77–7.89 mmol/L, H: 7.90–9.02 mmol/L) and six groups according to the non-HDL values (A: <2.59 mmol/L, B: 2.59–3.34 mmol/L, C: 3.36–4.11 mmol/L, D: 4.14–4.89 mmol/L, E: 4.91–5.66 mmol/L, F ≥5.69 mmol/L)

Biochemical measurements

Only patient data where blood samples were taken between 8.00 and 10.00 a.m. were used to exclude non-fasting persons as far as possible. Serum d-LDL-C levels were determined by the homogeneous direct measurement method (Roche Diagnostic, Indianapolis, IN, USA). TC, HDL-C, and TG levels were measured using a colorimetric enzymatic reaction (Roche Diagnostic, Indianapolis, IN, USA). The Centers for Disease Control LDL Cholesterol reference method laboratory network documented the traceability of the Roche Diagnostics GmbH CFAS lipids calibrators. Friedewald, Martin/Hopkins, and Sampson formulae were used for indirect LDL-C estimations (*Table I*). Adjustable factor for Martin/Hopkins was calculated based on TG and non-high-density lipoprotein cholesterol (non-HDL-C) levels derived from a 180-cell stratification table).

Table I Friedewald, Martin/Hopkins, and Sampson formulas.

	Formula
Friedewald (1)	TC – (HDL-C+ TG/2.2)
Martin/Hopkins (4)	TC – (HDL-C+ TG/adjustable factor)
Sampson (5)	TC/0.948-HDL-C/0.971-[TG/8.56+(TG*non-HDL-C)/2140-TG^2]-9.44

Statistical analysis

Variables were represented as N (%), mean ($\bar{x}$) ± standard deviation (SD), or median (M) (25%–75% quartiles). Kolmogorov-Smirnov and Shapiro-Wilk tests were used to check the groups' normality. Comparison between groups was performed with the Student's T test or Mann-Whitney U test. The correlation of the methods was performed with the Spearman or Pearson correlation tests. The Bland–Atman approach assessed the differences between LDL-C equations and direct measurement, and the Passing-Bablok regression analysis was evaluated. A value of P <0.05 was considered statistically significant. SPSS IBM Statistics 26 (IBM SPSS, Chicago, USA) and Analyse-it (Analyse-it Software Ltd., Leeds, UK) were used for statistical analyses.

Results

A total of 6297 patients' [female: 3663 (%58.2) and male: 2634 (41.89)] results were included in the study. The age means and all the study variables data of all of the patients according to gender are shown in *Table II*. Also, for each displayed variable to gender differences, there is a statistically significant difference (*Table II*). Agreement between d-LDL-C and the formulae according to TG groups in the study population was assessed with the Bland-Altman test, and the median difference results are shown in *Table III*. For the Friedewald formula, there was a negative median difference in all TG groups. The value of negative bias was found to increase in parallel with the increase in the concentration of TG. For the Martin/Hopkins formula, a negative median difference at TG levels <4.52 mmol/L and a positive median difference were observed above this TG value. For the Sampson formula, there was a negative bias in the other TG groups except for the TG<1.13 mmol/L group. However, there was no increase in the negative bias dependent on TG concentration, and a constant bias was observed. In addition, when we compared the total median difference of all formulae, it was -0.69 (-1.62–0.39) for Friedewald, 0.034 (-0.74–1.14) for Martin/Hopkins, and -0.40 (-1.19–0.55) for Sampson for alt the patients (*Figure 1*).

The median difference was also evaluated according to non-HDL-C levels (*Table IV*). For the Friedewald formula, there was a constant negative bias in all groups. The median difference for the Martin/Hopkins formula was positive bias except for group 1. For the Sampson formula, except for group 1, the median difference was a negative bias in the other groups. The agreement between D-LDL and formulae for the total patient group was presented in the Bland-Altman plot (*Figure 1*). For both TG groups and non-HDL groups, the agreement between d-LDL-C and formulae was shown in Bland-Altman plots (*supplemental Figure 1* for TG groups, *supplemental Figure 2* for the non-HDL groups).

There was a strong significant correlation between d-LDL-C and estimated LDL-C levels from formulae according to TG levels (p<0.001) (*Table III*). Low TG levels (<4.52 mmol/L) had a relatively better correlation for all formulae (*Table III*). The non-HDL-C groups observed a strong significant correlation between d-LDL-C and formulae (p<0.001). However, the correlation coefficient value was higher

Table II The age and baseline data of all patients and according to gender differences.

	N: 6297	Female (N: 3663)	Male (N: 2634)	p-Value
Age (years)	52±13	54±13	50±13	
Friedewald (mmol/L)	2.59 (1.86–3.44)	2.74 (1.99–3.64)	2.42 (1.73–3.20)	<0.001
Martin/Hopkins (mmol/L)	3.32 (2.75–4.01)	3.41 (2.81 4.14)	3.22 (2.67–3.86)	<0.001
Sampson (mmol/L)	2.88 (2.29–3.60)	3.00 (2.37–3.77)	2.77 (2.18–3.40)	<0.001
d-LDL-C (mmol/L)	3.27 (2.59–4.06)	3.41(2.68–4.20)	3.11 (2.48–3.84)	<0.001
TC (mmol/L)	5.88 (5.15–6.75)	6.00 (5.22–6.90)	5.72 (4.99–6.54)	<0.001
TG (mmol/L)	5.06 (4.58–5.93)	5.01 (4.56–6.02)	5.10 (4.64–6.02)	0.284
HDL-C (mmol/L)	0.98 (0.83–1.14)	1.01 (0.88–1.22)	0.91 (0.78–1.06)	<0.001
Non-HDL-C (mmol/L)	4.86 (4.14–5.71)	4.94 (4.19–5.82)	4.78 (4.09–5.53)	<0.001

This study presented variables as N (%), $\bar{x}$±SD, or Median (1st–3rd quartile). d-LDL-C: measured low-density lipoprotein cholesterol, TC: Total Cholesterol, TG: Triglyceride, HDL-C: high-density lipoprotein cholesterol. Comparison between gender groups was used with the Mann-Whitney U test. The p-value shows the differences in the basic lipid parameters status by gender. P<0.05 was considered statistically significant.

Table III Comparison of LDL formulae and d-LDL-C results according to TG groups.

Triglyceride, mmol/L	Median (1st–3rd quartile)	Median Difference (Lower-Upper LoA)	r	p-Value*
Friedewald				
<1.13, n: 212	2.63 (2.08–3.25)	-0.286 (-0.85–0.15)	0.960	<0.001
1.13–2.25, n:403	3.29 (2.70–4.03)	-0.37 (-0.88–0.30)	0.948	<0.001
2.26–3.38, n:362	3.35 (2.75–4.17)	-0.63 (-1.11–(-0.10))	0.948	<0.001
3.39–4.50, n:306	2.89 (2.25–3.64)	-0.80 (-1.33–0.09)	0.943	<0.001
4.52–5.63, n:3037	2.62 (1.95–3.44)	-0.70 (-1.42–0.37)	0.909	<0.001
5.65–6.76, n:1269	2.24 (1.55–3.06)	-0.83 (-1.65–0.56)	0.867	<0.001
6.77–7.89, n:535	2.08 (1.32–2.91)	-0.85 (-1.93–0.66)	0.839	<0.001
7.90–9.02, n:173	1.67 (1.05–2.49)	-1.05 (-2.46–1.65)	0.764	<0.001
Total		-0.69 (-1.62–0.39)	0.897	<0.001
Martin/Hopkins				
<1.13	2.87 (2.22–3.52)	-0.208 (-0.82–1.00)	0.909	<0.001
1.13–2.25	3.61 (2.86–4.15)	-0.18 (-0.72–1.07)	0.896	<0.001
2.26–3.38	3.86 (3.21–4.52	-0.21 (-0.97–0.86)	0.864	<0.001
3.39–4.50	3.55 (2.99–4.29)	-0.18 (-0.88–1.00)	0.895	<0.001
4.52–5.63	3.39 (2.81–4.05)	0.08 (-0.72–1.01)	0.898	<0.001
5.65–6.76	3.15 (2.60–3.79)	0.12 (-0.75–1.26)	0.864	<0.001
6.77–7.89	3.12 (2.58–3.66)	0.14 (-0.69–1.32)	0.844	<0.001
7.90–9.02	2.92 (2.43–3.49)	0.22 (-1.08–2.62)	0.777	<0.001
Overall		0.034 (-0.74–1.14)	0.886	<0.001
Sampson				
<1.13	2.63 (2.08–3.30)	-0.27 (-0.77–0.21)	0.961	<0.001
1.13–2.25	3.39 (2.77–4.11)	-0.275 (-0.81–0.36)	0.949	<0.001
2.26–3.38	3.48 (2.91–4.26)	-0.502 (-39.20–1.75)	0.949	<0.001
3.39–4.50	3.10 (2.52–3.78)	-0.59 (-1.01–0.05)	0.943	<0.001
4.52–5.63	2.92 (2.35–3.62)	-0.39 (-1.13–0.50)	0.910	<0.001
5.65–6.76	2.65 (2.09–3.31)	-0.41(-1.36–0.69)	0.867	<0.001
6.77–7.89	2.58 (2.02–3.20)	-0.39(-1.38–0.70)	0.840	<0.001
7.90–9.02	2.33 (1.89–2.89)	-0.37 (-2.02–1.37)	0.764	<0.001
Overall		-0.40 (-1.19–0.55)	0.905	<0.001
d-LDL-C				
<1.13	2.90 (2.43–3.56)			
1.13–2.25	3.66 (3.00–4.40)			
2.26–3.38	3.98 (3.36–4.75)			
3.39–4.50	3.70 (3.00–4.43)			
4.52–5.63	3.33 (2.65–4.08)			
5.65–6.76	3.03 (2.39–3.81)			
6.77–7.89	2.89 (2.32–3.68)			
7.90–9.02	2.68 (2.13–3.56)			

In this study, variables were presented as N (%), $\bar{x} \pm$ SD, or Median (1^{st}–3^{rd} quartile). LoA: Limit of agreement. TG: Triglyceride, d-LDL-C: measured low-density lipoprotein cholesterol. Correlation between groups was used with the Spearman analysis ($P<0.001$). *P values indicate that a comparison between d-LDL-C and all formulae was used with the Mann-Whitney U test. $P<0.05$ was considered statistically significant.

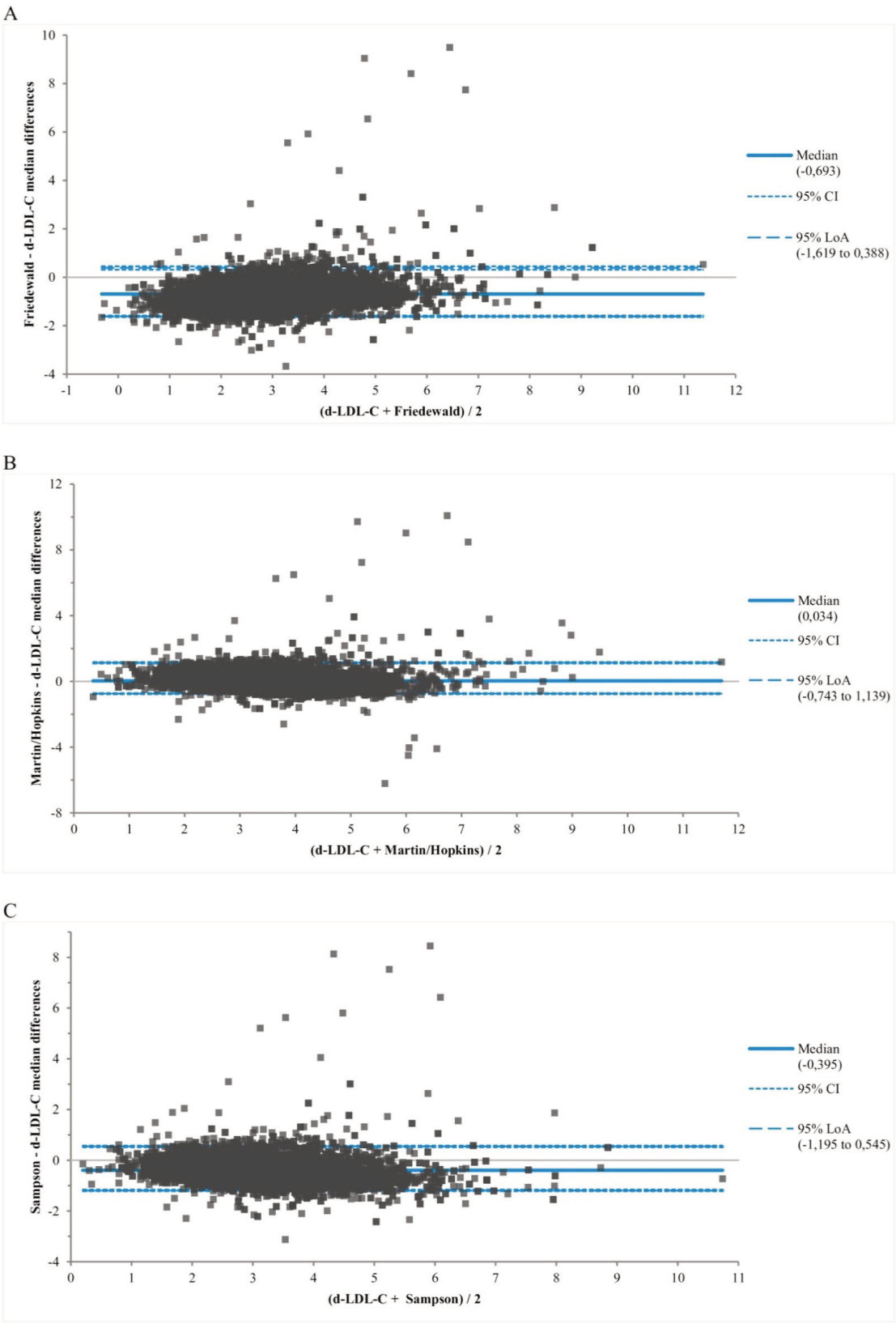

Figure 1 Differences between estimated LDL-C formulae and d-LDL-C results with Bland-Altman plots.
CI: Confident interval, LoA: limit of agreement of quartiles.

Table IV Comparison of LDL formulae and d-LDL-C results according to non-HDL-C groups.

Non-HDL-C, mmol/L	Median (1st–3rd quartile)	Median difference (Lower-Upper LoA)	r	p-Value
Friedewald				
<2.59. n: 126	1.63 (0.85–1.91)	-0.35 (-2.05–(-0.03))	0.825	<0.001
2.59–3.34. n: 357	1.11 (0.67–2.32)	-0.66 (-1.64–0.08)	0.881	<0.001
3.36–4.11, n: 1045	1.61 (1.23–2.09)	-0.78 (-1.61–(-0.02)	0.825	<0.001
4.14–4.89, n: 1691	2.18 (1.84–2.55)	-0.75 (-1.68–0.003)	0.771	<0.001
4.91–5.66, n: 1445	2.85 (2.46–3.20)	-0.70 (-1.60–0.18)	0.757	<0.001
≥5.69, n: 1633	3.89 (3.43–4.55)	-0.55 (-1.54–0.99)	0.756	<0.001
Martin/Hopkins				
<2.59	1.69 (1.40–1.99)	-0.20 (-0.51–0.78)	0.912	<0.001
2.59–3.34	2.12 (1.81–2.56)	-0.004 (-0.53–1.20)	0.841	<0.001
3.36–4.11	2.59 (2.27–2.92)	0.12 (-0.54–0.89)	0.807	<0.001
4.14–4.89	3.08 (2.78–3.38)	0.08 (-0.64–0.96)	0.743	<0.001
4.91–5.66	3.60 (3.25–3.93)	-0.01 (-0.76–1.12)	0.779	<0.001
≥5.69	4.40 (3.92–5.03)	-0.07 (-0.91–1.59)	0.755	<0.001
Sampson				
<2.59	1.66 (1.00–1.93)	-0.29 (-1.72–0.02)	0.837	<0.001
2.59–3.34	1.60 (1.28–2.36)	-0.31 (-0.88–0.25)	0.879	<0.001
3.36–4.11	2.06 (1.77–2.38)	-0.37 (-1.00–0.29)	0.829	<0.001
4.14–4.89	2.55 (2.30–2.85)	-0.38 (-1.11–0.35)	0.775	<0.001
4.91–5.66	3.12 (2.83–3.41)	-0.43 (-1.18–0.51)	0.759	<0.001
≥5.69	3.99 (3.61–4.57)	-0.45 (-1.40–0.98)	0.760	<0.001
d-LDL-C				
<2.59	1.88 (1.53–2.23)			
2.59–3.34	2.06 (1.60–2.61)			
3.36–4.11	2.49 (2.08–2.93)			
4.14–4.89	2.98 (2.58–3.45)			
4.91–5.66	3.56 (3.12–4.03)			
≥5.69	4.47 (3.84–5.09)			

This study presented variables as N (%) and Median (1st–3rd quartile). LoA: Limit of agreement, non-HDL-: non-high-density lipoprotein cholesterol, d-LDL-C: measured low-density lipoprotein cholesterol, correlation between groups was used with the Spearman analysis (P<0.001).*P values indicate that the Mann-Whitney U test compared d-LDL-C and all formulae. P<0.05 was considered statistically significant.

Table V The results of the correlation among formulae with d-LDL-C for all data sets.

	d-LDL-C r (%95 CI)	Friedewald r (%95 CI)	Martin/Hopkins r (%95 CI)
Friedewald	0.897 (0.891–0.903)	-	-
Martin/Hopkins	0.886 (0.880–0.892)	0.903 (0.897–0.909)	-
Sampson	0.905 (0.899–0.911	0.995 (0.899–1.000)	0.915 (0.909–0.921)

d-LDL-C: measured low-density lipoprotein cholesterol. CI: confident interval. Correlation between groups was used with the Spearman analysis (p<0.001). P<0.05 was considered statistically significant.

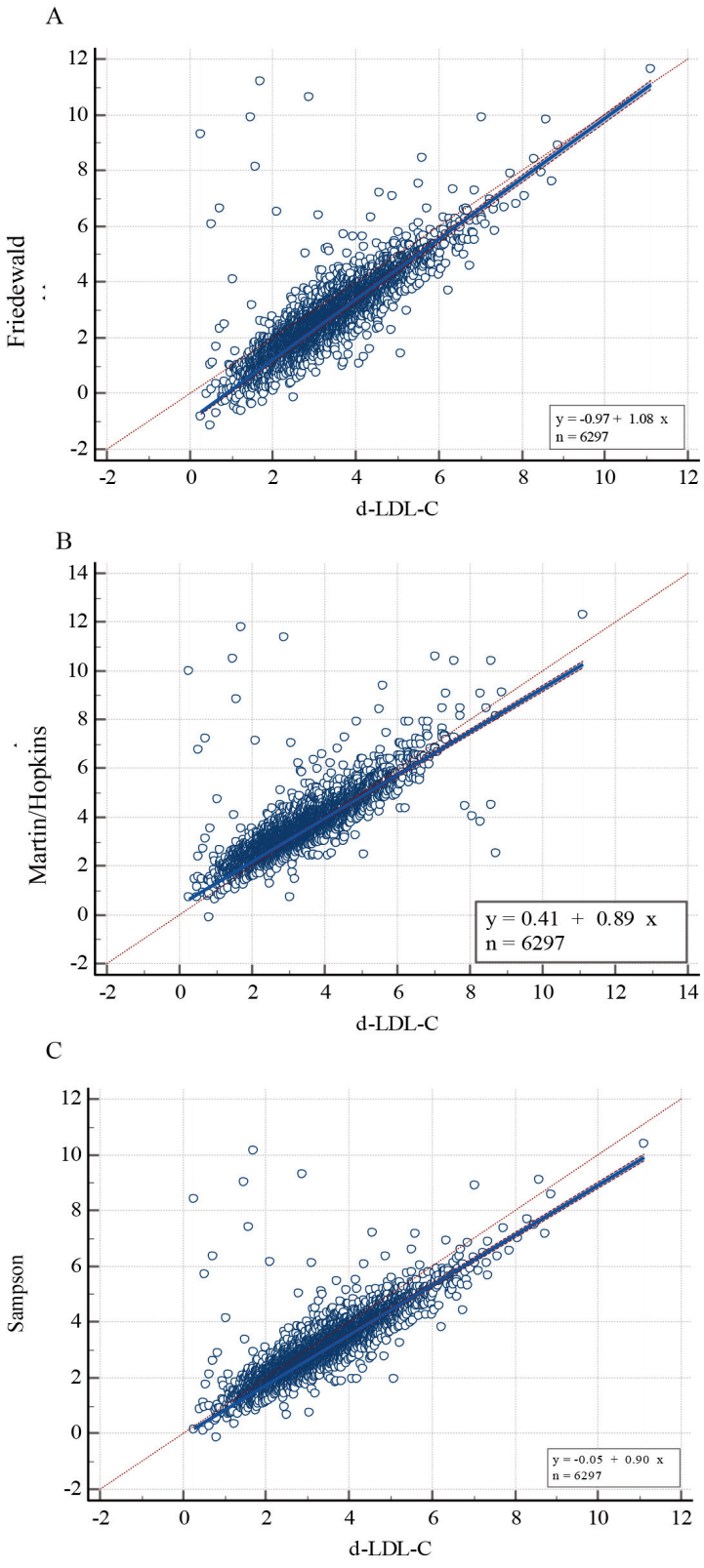

Figure 2 Passing Bablok regression analysis between estimated LDL-C formulae and d-LDL-C results for all the patients. A: Friedewald, B: Martin/Hopkins, C: Sampson.

for all formulae at non-HDL values <4.14 mmol/L. A comparison of the correlation between d-LDL-C and formulae in non-HDL groups is presented in *Table IV.* The results of the correlation of formulae with d-LDL-C for all data sets are shown in *Table V*. The Sampson formula had a better correlation than Friedewald and Martin/Hopkins formulae (0.905, 0.897, and 0.886, respectively, for all data, $p<0.001$).

According to Passing Bablok regression analyses, the intercept was determined as -0.97 (95% CI=-1.01 to -0.93), 0.41 (95%=0.37 to 0.44) and -0.05 (-0.08 to -0. 03) and slopes were calculated as 1.083 (95% CI=1.07–1.09), 0.88 (0.88 to 0.89) and 0. 90 (95%=0.89 to 0.90) for Friedewald, Martin/Hopkins and Sampson for all the patients, respectively (*Figure 2*).

The rate of risk classification based on d-LDL-C measurement using a cut-off LDL-C >2.59 mmol/L, or minimal risk concentrations according to the recommendation of NCEP ATP III (6), was 75.3% based on d-LDL-C. The percentage agreement of the formulae was 49.9%, 81.4%, and 62.9% for Friedewald, Martin/Hopkins and Sampson, respectively.

Discussion

Optimization of accurate estimation of LDL-C level is a critical and primary goal for CVD diagnosis and treatment. In this study, we compared the performance of measuring d-LDL-C with the recently developed Martin/Hopkins, Sampson, and traditional but still actively used Friedewald formulas. The present study found that the Sampson formula provided a relatively higher correlation, whereas the Martin/Hopkins formula showed a lower median difference. According to the Bland-Altman agreement, the Martin/Hopkins formula predicted slightly better LDL in both hyperlipidemic and normolipidemic subjects. In addition, there was a negative bias and underestimation in Friedewald's all TG and Sampson formulas, whereas the Martin/Hopkins formula had an overestimated situation at TG>4.52 mmol/L. In our grouping by non-HDL-C, the Martin/Hopkins formula overestimated LDL-C, while the other formulas showed the opposite.

In our current study, our data suggest that the Martin/Hopkins formula shows better agreement with d-LDL-C results than Friedewald and Sampson according to the Bland-Altman test. Similar to our study, Azimi et al. (7) found that the Martin/Hopkins formula showed better agreement and lower bias when comparing these three formulas. In a systematic review and meta-analysis by Ephraim et al. (8), the Martin/Hopkins formula showed a better correlation value. A study by Rim et al. (9) indicated that compared to other formulas, Martin/Hopkins provided the best fit in a large Asian population cohort. Similarly, a study on the Korean population also demonstrated better results with Martin/Hopkins than other formulas; Friedewald, Hatta, Puavilai (10). Although the Martin/Hopkins formula has proven to be a better assessment tool than other formulas, according to these studies and our study, it has several limitations. First, the impact of factors such as race/ethnicity, obesity, diabetes mellitus, and insulin resistance, which may affect the variance in the adjustable factor (TG/VLDL-C ratio), on the Martin/Hopkins formula has not been analyzed in all populations and situations. Secondly, it should be noted that there is a problem with standardizing the methods used in LDL-C measurement. The Martin/Hopkins formula must be validated using other LDL-C -quantification reference techniques in a larger population. Nevertheless, the Martin/Hopkins formula can be used as a remarkably accurate method for estimating LDL-C compared to the Friedewald formula.

The present study showed strong positive correlations with d-LDL-C for all formulae. The correlation intercept and slope are superior to Sampson than other formulae and in the majority of triglyceride and non-HDL subgroups and in the majority of triglyceride and non-HDL subgroups than other formulae. A study by Piani et al. (11) on a large, randomized, and blinded Italian population showed the Sampson equation provided a higher correlation with measured d-LDL-C level. Martínez-Morillo et al. (12), in Spain, suggested that the Sampson formula can be applied in clinical laboratories and provide acceptable performance. Few studies have compared the accuracy of Friedewald, Martin/Hopkins, and Sampson formulas in Turkish populations. Zararsız et al. (13) reported that the Martin/Hopkins approach is the method with the highest overall concordance according to LDL-C risk classifications. Again, in our previous study of the population of the Aegean region of our country, the Martin/Hopkins formula showed a better agreement with the direct method than the Friedewald formula. The population in this study includes the Central Anatolia region. Khan et al. (14) showed that the Teerakanchana formula had better correlation and agreement for the Pakistani population in a study comparing 13 formulae, including these formulas.

Many research studies have been conducted worldwide for these formulas for LDL-C calculation. However, no consensus has yet been reached on the most accurate and reliable formula for estimating LDL-C, especially for these two new formulas (15). Direct measurement of LDL-C is costly and not commonly performed by clinical laboratories. The Friedewald formula is the most common method for LDL-C estimation. However, the Friedewald formula gives a lower estimate above TG levels of 3.39 mmol/L. Among the treatment targets set by the NCEP Adult Treatment Panel III, the lowest risk for LDL-C was recommended to be >2.59 mmol/L. The ACC/AHA guidelines recommend an LDL-C target of <1.81 mmol/L (<70 mg/dL) for atherosclerotic car-

diovascular high-risk individuals (16). In comparison, the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) guidelines recommend more aggressive LDL-C targets [<1.42 mmol/L (<55 mg/dL)] (17). According to NCEP ATP III, our evaluation's percent agreement of the results was 75.3%, 49.9%, 81.4%, and 62.9% for d-LDL-C, Friedewald, Martin/Hopkins, and Sampson, respectively. The Martin/Hopkins formula appears least likely to misclassify patients due to the general overestimation of LDL-C values. Nevertheless, Friedewald and Sampson determined a higher misclassification and lower risk when evaluated according to d-LDL-C levels. In our study, Friedewald and Sampson's formula had a negative bias. This suggests that, depending on the performance of the different formulae, less or more risk due to misclassification can lead to problems and making treatment decisions according to these.

Our research has some limitations. First, we utilized the direct homogeneous LDL-C measurement method instead of the gold standard beta quantification LDL-C method. However, the direct LDL-C method is used in most laboratories and has the analytical accuracy specified in the NCEP guidelines. Secondly, the data for this study were obtained from the laboratory results of a large-capacity public hospital in the capital of our country. However, although our number of patients is relatively large, it should be supported by validation studies with more individuals to generalize to the whole population due to ethnic or dietary differences between regions. Our last limitation is that although the data of our patients were screened with strict exclusion criteria with the developing technology, data on conditions such as race, ethnicity, obesity, fasting situations, and insulin resistance were not available in our system. These conditions may change the type of dyslipidemia in our patients. The strength of our study is that it is one of the rare studies conducted in our country. It is crucial to evaluate the performance of formulas in both normolipidemic and hyperlipidemic patient groups.

Conclusion

In conclusion, we compared d-LDL-C measurement with formulas (Friedewald, Martin/Hopkins, and Sampson) in the Turkish population's normolipidemic and hyperlipidemic individuals. The Martin/Hopkins formula showed an order of magnitude better LDL-C prediction. Although the Friedewald formula for LDL-C estimation is easy to remember, the Martin/Hopkins formula can easily estimate LDL-C without additional software, thanks to advances in laboratory information technology. To validate the Martin/Hopkins and Sampson formula in our population, both very large populations and additional studies considering factors such as race/ethnicity, obesity, diabetes, and insulin resistance are needed.

Author contributions

MA, MFA, MŞ design the study. MA collected the data. MFA analyzed the data. MFA wrote thedraft. MA, MŞ critically reviewed the manuscript

Financial support

None.

Information on ethics committee approval

Our study protocol was approved by the Health Sciences University Ankara Training and Research Hospital Clinical Research Ethics Committee (Decision No: 1007/2022; 27/07/2022).

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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RELATIONSHIP BETWEEN SERUM THYROID HORMONE AND INTERLEUKIN-1β LEVELS AND POSTMORTEM TISSUE DEIODINASE ACTIVITY IN CRITICALLY ILL PATIENTS

ODNOS IZMEĐU NIVOA SERUMSKOG TIROIDNOG HORMONA I INTERLEUKINA-1β I AKTIVNOSTI DEJODINAZE POSTMOTREM TKIVA KOD PACIJENATA U KRITIČNOM STANJU

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Summary

Background: This study investigated the relationship between serum thyroid hormones and interleukin-1β (IL-1β) levels and postmortem tissue deiodinase activity in critically ill patients.
Methods: Serum thyroid hormones and IL-1β were measured on the 5th, 15th, and last day of 80 critically ill patients. Forty of these patients were non-survived, and liver and skeletal muscle were harvested to analyze type 1, 2, and 3 iodothyronine deiodinases (D1, D2, and D3) activity.
Results: Serum thyroid stimulating hormone (TSH), tetraiodothyronine (T4), and triiodothyronine (T3) were decreased, and reverse triiodoth while serum TSH, T4, and T3 levels decreased or remained unchanged, and rT3 and IL-1β increase yronine (rT3) and IL-1β were increased in non-survivors. From day 5 to the last day, serum TSH, T4, and T3 levels increased, and rT3 and IL-1β levels decreased with time in survivors, while serum TSH, T4, and T3 levels decreased or remained unchanged, and rT3 and IL-1β increased in non-survivors. On the last day, liver D1 activity was negatively correlated with serum rT3 and IL-1β, while liver and skeletal muscle D3 activities were positively correlated.
Conclusion: Serum thyroid hormones and IL-1β are correlated with postmortem deiodinase activity in critically ill patients.

Keywords: critical illness, prognostic markers, thyroid hormone, IL-1β, deiodinase

Kratak sadržaj

Uvod: Cilj ovog istraživanja je bio da se ispita odnos između nivoa serumskih hormona štitne žlezde i interleukina-1β (IL-1β) i aktivnosti dejodinaze postmortem tkiva kod pacijenata u kritičnom stanju.
Metode: Nivoi serumskih hormona štitne žlezde i IL-1β su mereni petog, petnaestog i poslednjeg dana kod 80 pacijenata u kritičnom stanju. Četrdeset od ovih pacijenata nije preživelo, a jetra i skeletni mišići su uzeti za analizu aktivnosti jodotironin dejodinaza tipa 1, 2 i 3 (D1, D2 i D3).
Rezultati: Nivoi serumskog hormona koji stimuliše štitnu žlezdu (TSH), tetrajodotironina (T4) i trijodotironina (T3) su bili smanjeni, dok su reverzni trijodotironin (rT3) i IL-1β bili povećani kod pacijenata koji nisu preživeli. Od petog do poslednjeg dana, nivoi serumskog TSH, T4 i T3 su se povećavali, a nivoi rT3 i IL-1β su se vremenom smanjivali kod preživelih, dok su nivoi seruma TSH, T4 i T3 opadali ili ostajali nepromenjeni. S druge strane, rT3 i IL-1β su se povećavali kod onih koji nisu preživeli. Poslednjeg dana, aktivnost jetrenog D1 je bila u negativnoj korelaciji sa serumskim rT3 i IL-1β, dok su aktivnosti D3 jetre i skeletnih mišića bile u pozitivnoj korelaciji.
Zaključak: Nivoi serumskih hormona štitne žlezde i IL-1β su povezani sa aktivnošću dejodinaze postmortem tkiva kod pacijenata u kritičnom stanju.

Ključne reči: kritična bolest, prognostički markeri, hormon štitne žlezde, IL-1β, dejodinaza

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Introduction

Critical illness causes hormonal changes that differ between acute and long-term illnesses (1). In acute critical illness, serum catecholamines, growth hormone, and cortisol levels are elevated, insulin resistance is reduced, and low triiodothyronine (T3) syndrome is observed (2). In long-term critical illness, catecholamine and cortisol levels are reduced, and thyroid stimulating hormone (TSH) and thyroid hormone levels are reduced compared to acute cases. Although no evidence has indicated the harm of acute changes, it is unclear whether endocrine changes in long-term critical illness are all beneficial, as studies have shown that some changes may lead to clinical deterioration (3).

High-dose growth hormone, glucocorticoid, or thyroid hormone have no or even negative effects on clinical outcomes of critically ill patients (4–7). Intervening with the hypothalamic releasing factor may be possible to restore pulsating secretion of pituitary hormones and normalize peripheral hormone levels (8).

A decrease in serum T3 concentration in critical illness and an increase in serum reverse triiodothyronine (rT3) are associated with the severity of the disease (9). Type 1, 2, and 3 iodothyronine deiodinases (D1-3) mediate peripheral thyroid hormone metabolism. Specifically, D1 mediates the formation of serum T3 from tetraiodothyronine (T4) and metabolite rT3 breakdown D2 converts T4 to T3 through outer ring deiodination and is important for local T3 production, while D3 catalyzes the inactivation of T4 and T3, generating rT3 and 3,3′-T2 (10, 11). D1, D2, or both reduce the deiodination of peripheral T4, significantly reducing circulating T3 levels (12, 13). D1 is the main pathway of rT3 clearance, and this mechanism may explain the increase in serum rT3 levels (14). In addition to D1 activity reduction, impairment in transporting T4 and rT3 to D1-containing tissues may be another mechanism of thyroid hormone disorders (15). However, the possibility that increased D3 activity leads to decreased serum T3 levels and elevated rT3 levels should also be considered (16).

Critical illness has been shown to be associated with disturbed metabolic and inflammatory responses (17). Interleukin-1β (IL-1β) is a pro-inflammatory cytokine that is higher in non-survivors than in critically surviving patients (18). Other studies have shown that IL-1β can cause damage or apoptosis of thyroid follicular cells and promote the onset of autoimmune thyroiditis (19–21). IL-1β is involved in autoimmune thyroiditis by inducing intercellular adhesion molecule-1 on thyroid follicular cells and interfering with the integrity of thyroid epithelium (22, 23). More importantly, postoperative serum IL-1β levels in critically ill patients after major abdominal surgery are associated with mortality (24). In addition, it has been shown that IL-1β inhibits thyroid hormone receptor-β1 gene expression (25), which inhibits hepatic D1 expression (26). Therefore, we chose IL-1β as the focus of our study to investigate its relationship with prognosis and deiodinase activity in critically ill patients.

In short, the study observed serum thyroid hormones and IL-1β in critically ill patients and analyzed the correlation between deiodinase activity with serum thyroid hormones and IL-1β.

Materials and Methods

Patients

Eighty patients who were hospitalized for more than 5 days in an intensive care unit (ICU) were included in this analysis. Among them, there were 39 patients with cardiac surgery, 17 patients with complex surgery (defined as patients with complications after abdominopelvic surgery, lung or esophageal surgery, or vascular surgery), 9 patients with organ transplantation, 7 patients with trauma, burn, or prosthetic surgery, and 8 patients with other surgeries. Blood samples were taken on the 5^{th}, 15^{th}, and last day after admission to ICU. Forty patients did not survive, and liver and skeletal muscle (rectus abdominis) were obtained within minutes of death.

Serum analysis

Treatment of ICU patients often includes systemic or local infusions of heparin to prevent vascular coagulation, which largely affects the determination of serum-free thyroid hormones (27). Heparin can lead to falsely elevated free thyroid hormone results. Specifically, heparin can activate lipoprotein esterase *in vitro* to release free fatty acids, which can displace bound thyroid hormone from thyroxine-binding globulin, resulting in falsely elevated free thyroid hormone levels (27–29). Therefore, the determination of serum-free T4 and T3 was avoided. Vitros ECi Immunodiagnostic System (Ortho-Clinical Diagnostics) tested serum total T4, total T3, and TSH. rT3 was measured by radioimmunoassay (30). IL-1β detected serum IL-1β levels in an enzyme-linked immunosorbent assay kit (R&D Systems, USA). Normal TSH, T4, T3, and rT3 values in 80 healthy subjects were measured.

Peripheral blood mononuclear cells (PBMCs)

Whole blood (2 mL) was equally diluted with phosphate-buffered saline (PBS) and transferred into a centrifuge tube containing 3 mL Ficoll Paque (G.E. Healthcare). PBMCs were collected after centrifugation at 400×g for 20 min, rinsed twice in 10 mL PBS, and re-suspended in a lysis buffer for protein extraction.

Immunoblotting

PBMCs and loading buffer (Yeasen, Shanghai, China) were heated at 99 °C for 10 min. Protein was loaded onto the sodium dodecyl sulfate-polyacrylamide gel and then imprinted onto the polyvinylidene fluoride membrane. The membrane was incubated overnight with either IL-1β (1:1000, R&D Systems) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1:1000, Abcam) primary antibody at 4 °C and then with a secondary antibody. GAPDH was used as a loading control. The protein signaling was developed via Enhanced Chemiluminescence (Solarbio). Relative protein level was analyzed using QuantityOne v4.6 (Bio-Rad) and normalized to GAPDH.

Deiodinase activity

Homogenates were produced with human liver and skeletal muscle samples homogenized in PE buffers (0.1 mol/L phosphate, 2 mol/L ethylenediamine tetraacetic acid, pH 7.2) using Polytron (Kinematica AG, Lucerne, Switzerland), frozen, and stored at -80 °C. D1 activity in liver tissues was measured by incubating 10 μg protein with 0.1 μmol/L (3′, 5′-^{125}I) rT3 (100,000 cpm) in 0.1 mL PED10 buffer (PE + 10 mmol/L dithiothreitol (DTT)) for 30 min. D2 activity in skeletal muscles was determined by incubating 200 μg protein with 1 mmol/L (3′, 5′-^{125}I) T4 (100,000 cpm) in 0.1 mL PED25 buffer (PE+25 mmol/L DTT) for 1 h. To prevent the labeled T4 substrate from being deiodized by the D3 inner ring, incubation was performed in 0.1 μmol/L unlabeled T3. When 0.1 μmol/L unlabeled T4 is present or absent, it is sufficient to saturate D2. D2 activity is equal to the deiodination of unlabeled T4 minus the deiodination of excess unlabeled T4. The procedure for further determination of ^{125}I yield is the same as the D1 determination above. D3 was detected by incubating 100 μg liver protein or 200 μg skeletal muscle protein with 1 mmol/L (3′-^{125}I) T3 (200,000 cpm) in 0.1 mL PED50 buffer (PE + 50 mmol/L DTT) for 1 h (31).

Statistical analysis

G*Power software (ver. 3.1.9.7; Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) was used for efficacy analyses and sample size calculation (32). SPSS 22.0 was utilized for data analysis. Categorical variables were compared using Fisher's exact test and presented as frequencies. Continuous variables except age and body mass index (BMI) were analyzed using non-parametric tests. Continuous variables were expressed as mean ± standard deviation or median (interquartile distance (IQR)). Differences between continuous variables were analyzed by t-test or Mann-Whitney U test, with the Spearman correlation coefficient used for correlation analysis. Serum thyroid hormone and IL-1β levels in critically ill patients on the last day of the ICU were used as independent variables. Logistic regression analysis was performed to obtain the predictive probability values, and then the predictive value of serum thyroid hormone and IL-1β levels in patient death was evaluated by receiver operating characteristic (ROC) curve analysis. $P < 0.05$ indicated a statistical difference.

Results

Baseline characteristics

No significant differences were observed in age, sex, BMI, and APACHE II score on the 5th day of the ICU between patients who survived and those who died, and patients who died had longer ICU stays (*Table I*).

Serum thyroid hormones and IL-1β differ between survivors and non-survivors

Table II shows serum thyroid hormones and IL-1β in surviving and non-survivors. Compared with the survivors, the serum TSH, T4, and T3 were decreased, and the serum rT3 and IL-1β were increased in non-survivors. Moreover, from the 5th day to the last day, TSH, T4, and T3 increased with time, and rT3 and IL-1β decreased in survivors, while TSH, T4, and T3 decreased or remained unchanged, and rT3 and IL-1β increased in non-survivors. IL-1β protein expression was increased in PBMCs of non-survivors on the last day compared with survivors (*Figure 1A, B*).

Table I Baseline characteristics of patients.

Parameters	Survivor	Non-Survivor	P
Age (yr)	61.2±15.6	61.7±15.2	0.885
Sex (male/female)	25/15	26/14	0.816
BMI (kg/m^2)	25.5±5.7	25.8±4.5	0.795
ICU stay (d)	16 (10–28)	11 (7–20)	0.003
APACHE II score on the fifth day of the ICU	11 (7–15)	12 (8–15)	0.702

Note: The Acute Physiology and Chronic Health Evaluation II (APACHE II) score reflects the severity of illness, with higher values indicating more severe illness.

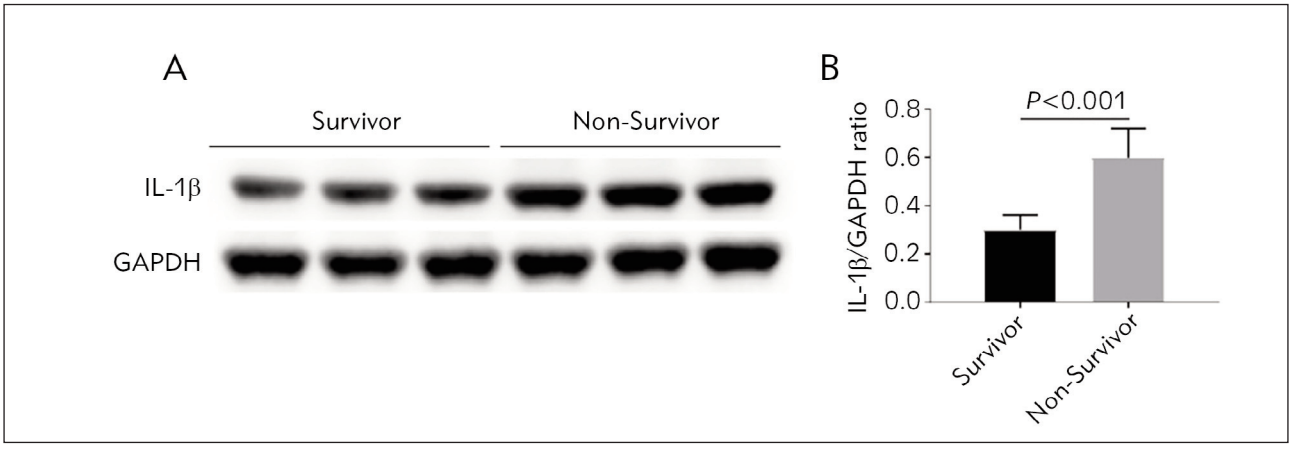

Figure 1A-B Western blot detection of IL-1β protein expression in peripheral blood mononuclear cells of critically ill patients on the last day of the ICU IL-1β/GAPDH ratio, the relative expression level of IL-1β protein was normalized to GAPDH.

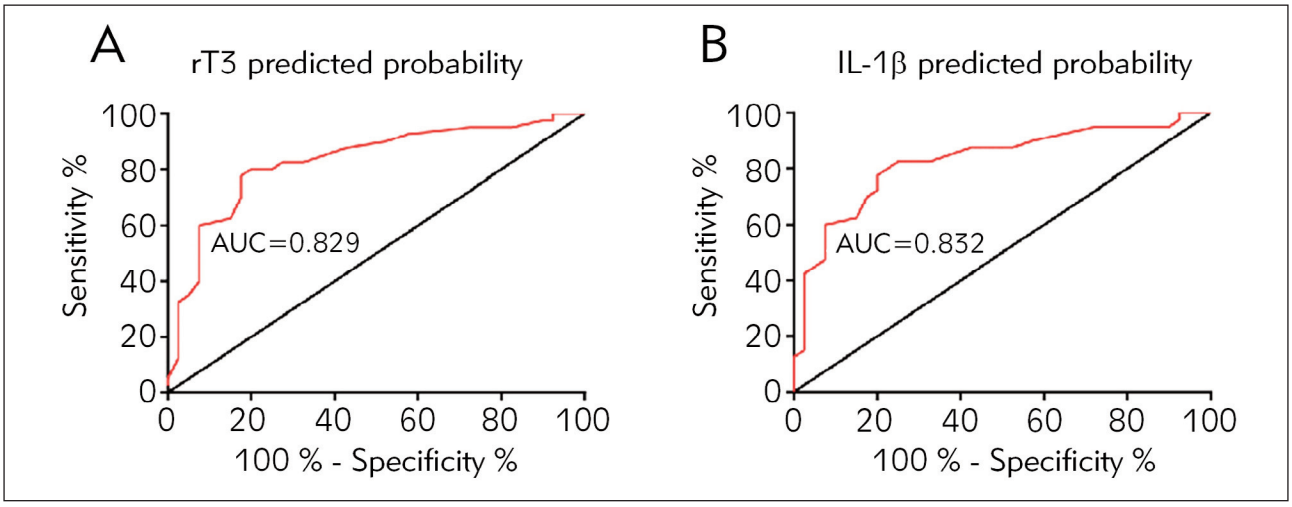

Figure 2A-B Predictive value of serum thyroid hormones and IL-1β on death in patients on the last day of the ICU.

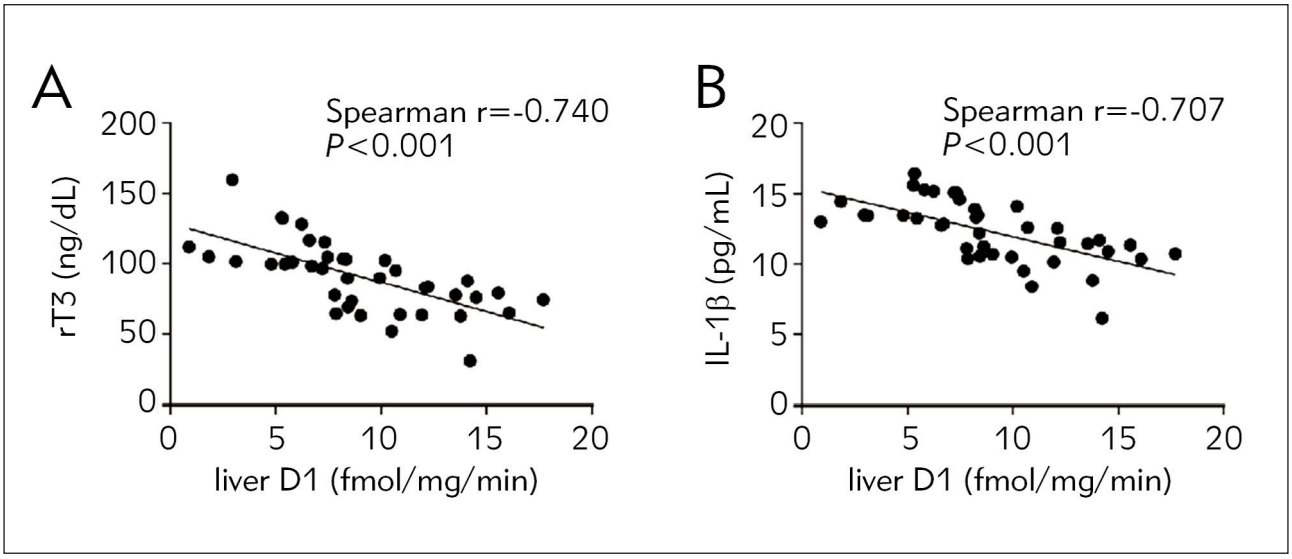

Figure 3A-B Correlation between liver D1 and serum thyroid hormones and IL-1β on the last day of the ICU.

Table II Serum thyroid hormones and IL-1β levels in survivors and non-survivors.

Parameters	Day	Survivor	Non-Survivor	P
TSH (μU/mL)	5	1.21 [0.48–2.26]	0.43 [0.12–1.34]	<0.001
	15	1.46 [0.68–2.45]	0.85 [0.26–2.39]	0.046
	Last day	1.48 [0.76–2.32]	0.45 [0.05–0.96]	<0.001
T4 (μg/dL)	5	5.65 [4.08–7.21]	3.36 [2.47–5.28]	<0.001
	15	6.71 [4.91–8.23]	3.90 [2.88–6.93]	<0.001
	Last day	7.50 [6.11–8.79]	3.39 [2.01–5.45]	<0.001
T3 (ng/dL)	5	74.1 [59.2–92.3]	53.8 [42.1–70.6]	<0.001
	15	87.1 [66.3–107.8]	60.9 [50.5–77.1]	<0.001
	Last day	94.2 [78.0–109.6]	54.4 [42.1–70.0]	<0.001
rT3 (ng/dL)	5	41.0 [28.0–65.7]	59.2 [35.1–87.5]	<0.001
	15	37.5 [25.9–63.4]	63.7 [31.9–101.3]	<0.001
	Last day	33.6 [23.3–54.5]	91.0 [40.3–135.1]	<0.001
IL-1 (pg/mL)	5	6.35 [4.56–8.07]	9.18 [7.30–11.3]	0.001
	15	5.12 [4.05–6.74]	10.6 [8.14–12.5]	<0.001
	Last day	4.07 [3.23–5.48]	12.3 [10.5–13.6]	<0.001

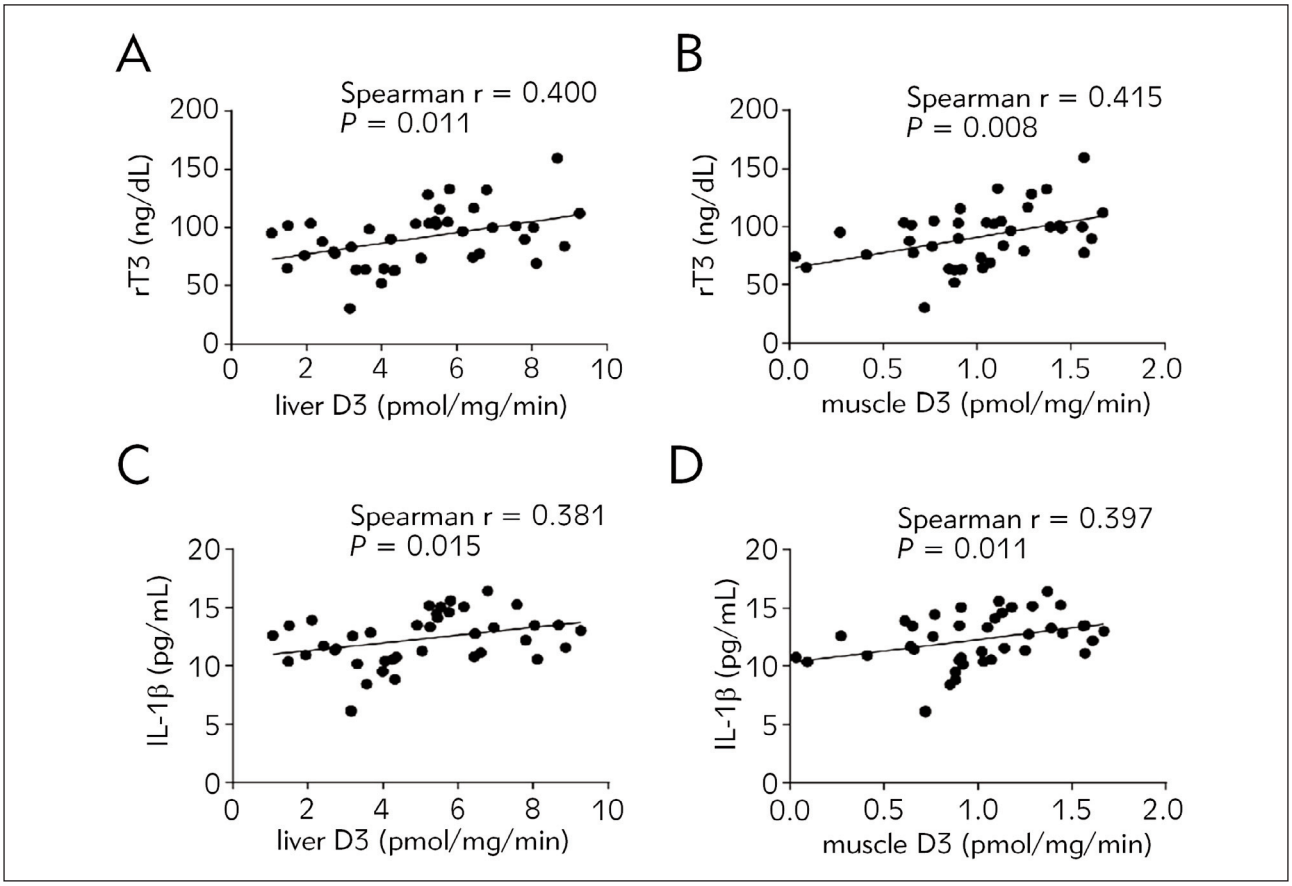

Figure 4A-B Association of liver and skeletal muscle D3 with serum thyroid hormones and IL-1β on the last day of the ICU.

Table III Correlation between tissue deiodinase activity and serum thyroid hormone and IL-1 on the last day of the ICU.

	Liver D1		Liver D3		Muscle D3	
	Spearman r	P	Spearman r	P	Spearman r	P
TSH (μU/mL)	0.212	0.128	-0.235	0.116	-0.257	0.094
T4 (μg/dL)	0.179	0.231	-0.176	0.243	-0.032	0.817
T3 (ng/dL)	0.144	0.332	-0.271	0.075	-0.154	0.309
rT3 (ng/dL)	-0.740	<0.001	0.400	0.011	0.415	0.008
IL-1 (pg/mL)	-0.707	<0.001	0.381	0.015	0.397	0.011

Predictive value of serum thyroid hormones and IL-1β on the last day of the ICU on patient death

Serum thyroid hormones and IL-1β levels of critically ill patients on the last day of the ICU were used as independent variables, and logistic regression analysis was performed to obtain the predictive probability values, and then the predictive value for the patients' deaths was evaluated by using ROC curve analysis. The results showed that serum rT3 and IL-1β had high predictive value (*Figure 2A, B*).

Correlation of liver D1 with serum thyroid hormones and IL-1β on the last day of the ICU

Spearman's correlation coefficient was used for correlation analysis. Postmortem liver D1 activity was negatively correlated with serum rT3 and IL-1β on the last day of ICU (*Figure 3A, B*) but had no correlation with serum TSH, T4, or T3 levels (*Table II*).

Association of liver and skeletal muscle D3 with serum thyroid hormone and IL-1β on the last day of the ICU

Postmortem D3 activities were positively associated with serum rT3 and IL-1β levels on the last day of ICU (*Figure 4A-D*) but not with serum TSH, T4, or T3 levels (*Table III*).

Discussion

Patients suffering from critical illnesses who require treatment in the ICU uniformly present with alterations in circulating thyroid hormone levels that are referred to by several names such as »nonthyroidal illness syndrome,« »sick euthyroid syndrome,« or »low T3 syndrome« (33, 34). Decreased serum T3 and elevated rT3 are correlated with disease severity (9), and serum T4 is inversely correlated with mortality (35). This trial found that from day 5 to the last day of ICU, serum TSH, T4, and T3 increased and rT3 levels decreased with time in survivors, while serum TSH, T4, and T3 levels decreased or unchanged, and serum rT3 levels increased in non-survivors. Serum rT3 on the last day indicated a correlation with postmortem deiodinase activity.

Patients with significant changes in serum thyroid hormones have higher mortality (36, 37). This study found significant TSH, T4, and T3 differences between survivors and non-survivors. TSH, T4, and T3 were elevated in surviving patients but not non-surviving patients. From the 5th day to the last day, T4 and T3 continued to increase, and no further TSH was observed after the 15th day, indicating that T4 and T3 both increased with the initial increase of TSH. This is consistent with previous research showing elevated serum TSH leads to elevated T4, marking the beginning of disease recovery (38, 39). Throughout the ICU period, serum rT3 levels of non-survivors continued to rise. This may be due to the short half-life of rT3 (40, 41), which is a sensitive marker for acute changes in tissue decay-mediated thyroid hormone metabolism during death.

It is estimated that D1 in the liver and kidneys contributes 15–80% of peripheral T3, and D2-containing tissues contribute to the remaining extra-thyroid T3. D1 plays the greatest role in patients with hyperthyroidism, while D2 plays a significant role in patients with hypothyroidism. The decrease in D1 activity will lead to the decrease of T3 production by T4 and the decrease of rT3 clearance (10). Another possible mechanism for lowering and increasing serum T3 levels is that D1-expressing tissues have lower uptake of T4 and rT3 (14, 15). This study found that liver D1 activity was negatively correlated with serum rT3 levels.

Under normal conditions, D3 is only present in the liver of the developing fetus and protects the fetus from overexposure to thyroid hormone, indicating that pathological conditions in adulthood may be related to deiodinase changes, especially D3 (42, 43). D3 is expressed in human skeletal muscle (44). D3 may reduce skeletal muscle local thyroid hormone levels by converting T4 to rT3 and T3 to 3,3'-T2. D3 expression in hemangiomas may lead to low T4 and T3 and high rT3 levels (16). This study found that liver D3 activity positively correlated with serum rT3.

Notably, we were unable to detect any D2 in skeletal muscle samples from these patients, whereas D2 activity was present in skeletal muscle from normal subjects (45). Elevated serum rT3 concentration may lead to D2 inactivation in critically ill patients (46). D2 in skeletal muscle promotes the production of serum T3, especially in cases of hypothyroidism (47). Therefore, skeletal muscle D2 inactivation may also lead to decreased T3 levels in critically ill patients.

Critical illness is related to metabolic and inflammatory disorders (48). Postoperative serum IL-1β levels are associated with mortality in critically ill patients after major abdominal surgery (49). In addition, it has been shown that IL-1β inhibits thyroid hormone receptor-β1 gene expression (50), which inhibits hepatic D1 expression (51). Therefore, we tested serum IL-1β levels in critically ill patients and showed that serum IL-1β levels were elevated in non-survivors compared with the survivors. From day 5 to the last day of ICU, serum IL-1β of survivors decreased with time, while it increased in non-survivors. In addition, IL-1β protein in PMBCs on the last day of non-survivors was increased compared with that of survivors. High levels of IL-1β are associated with abnormal thyroid function (52). The relationship between deiodinase activity and serum IL-1β was further analyzed. The results showed that liver D1 activity was negatively correlated with serum IL-1β, while liver and skeletal muscle D3 activity was positively correlated with serum IL-1β.

However, this study has some limitations. First, this study only explored the effect of one inflammatory factor, IL-1β, on the prognosis of critically ill patients, and more inflammatory factors need to be included in the study. Second, the present study did not delve into the potential mechanisms by which IL-1β affects deiodinase activity, and it is hoped that this can be further explored in the future.

Conclusion

This study is the first to explore the relationship between serum thyroid hormones and IL-1β and tissue deiodinase activity. Serum TSH, T4, and T3 levels were decreased compared with survivors, and rT3 and IL-1β were increased in non-survivors. Liver D1 activity was negatively correlated with serum rT3 and IL-1β, while liver and skeletal muscle D3 activities were positively correlated.

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Availability of data and materials

The data are available from the corresponding author upon request.

Ethics statement

This study was approved by the Ganzhou People's Hospital ethics committee, and the guardian of every subject signed informed consent.

Authors' contributions

Z.Z. Zhong designed the research study. Z.Z. Zhong and X.L. Xiao performed the research. X.L. Xiao provided help and advice on the experiments. Z.Z. Zhong and X.L. Xiao analyzed the data.

Z.Z. Zhong wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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IMPACT OF AN AIR BUBBLE WITHIN THE SYRINGE ON TEST RESULTS OBTAINED WITH A MODERN BLOOD GAS ANALYZER

UTICAJ VAZDUŠNOG MEHURIĆA U ŠPRICU NA REZULTATE TESTOVA DOBIJENIH SAVREMENIM ANALIZATOROM GASOVA U KRVI

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Summary

Background: Minimizing air aspiration by carefully filling blood gas syringes is crucial to prevent air contamination from causing undesirable variations in gasses and other molecules. While some previous studies investigated this aspect, these are now outdated and only analyzed a limited number of blood gas parameters. Thus, we investigated the effects air contamination in the syringe using a modern blood gas analyzer.

Methods: We sampled venous blood from 17 laboratory workers (mean age: 46±11 years; 10 women), filling two consecutive blood gas syringes. The first was filled exactly to its nominal volume (i.e., 1.0 mL), while the second was filled with 0.8 mL of blood and 0.2 mL of ambient air. Blood gas analysis was performed in each syringe using an identical analyzer.

Results: In the syringe with the air bubble, we found statistically significant increase in pH (0.1%), pO_2 (10.8%), SO_2 (11.2%), total hemoglobin (3.0%), and hematocrit (2.7%), while values of pCO_2 (-4.8%), sodium (-0.5%), and ionized calcium (-1.3%) were significantly reduced. With exception of pH, all these changes exceeded the performance specifications. Potassium, chloride, glucose, lactate, COHb and MetHb values remained unchanged.

Kratak sadržaj

Uvod: Minimiziranje aspiracije vazduha pažljivim punjenjem špriceva za gas od krvi je ključno za sprečavanje kontaminacije vazduha da izazove neželjene varijacije u gasovima i drugim molekulima. Dok su neke prethodne studije istraživale ovaj aspekt, one su sada zastarele i analizirale su samo ograničen broj parametara gasova u krvi. Stoga smo istražili efekte kontaminacije vazduha u špricu koristeći savremeni gasni analizator krvi.

Metode: Uzorkovali smo vensku krv od 17 laboratorijskih radnika (srednja starost: 46±11 godina; 10 žena), puneći dva uzastopna gasna šprica za krv. Prvi je napunjen tačno do svoje nominalne zapremine (tj. 1,0 mL), dok je drugi napunjen sa 0,8 mL krvi i 0,2 mL ambijentalnog vazduha. Analiza gasa krvi je obavljena u svakom špricu korišćenjem identičnog analizatora.

Rezultati: U špricu sa vazdušnim mehurićem utvrđeno je statistički značajno povećanje pH (0,1%), pO_2 (10,8%), SO_2 (11,2%), ukupnog hemoglobina (3,0%) i hematokrita (2,7%), dok su vrednosti pCO_2 (-4,8%), natrijum (-0,5%) i jonizovani kalcijum (-1,3%) su značajno smanjeni. Izuzev pH vrednosti, sve ove promene su premašile specifikacije performansi. Vrednosti kalijuma, hlorida, glukoze, laktata, COHb i MetHb su ostale nepromenjene.

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Conclusions: These findings confirm that air bubbles must be removed as soon as possible after sampling from blood gas syringes to prevent artifactual test results and misleading clinical judgment and inappropriate treatment. When blood gas syringes are received in the laboratory with air bubbles inside, the most vulnerable parameters (i.e., pO_2, SO_2, pCO_2, sodium, ionized calcium, hematocrit and hemoglobin) should be suppressed.

Keywords: blood gas analysis, errors, syringe, bubble, air

Zaključak: Ovi nalazi potvrđuju da se vazdušni mehurići moraju ukloniti što je pre moguće nakon uzorkovanja iz špriceva za gasove krvi da bi se sprečili artefaktički rezultati testova i pogrešna klinička procena i neodgovarajući tretman. Kada se u laboratoriji primaju špricevi za gas sa mehurićima vazduha, najugroženije parametre (tj. pO_2, SO_2, pCO_2, natrijum, jonizovani kalcijum, hematokrit i hemoglobin) treba potisnuti.

Ključne reči: gasna analiza krvi, greške, špric, mehur, vazduh

Introduction

Blood gas analysis is an important diagnostic test principally used to assess a patient's respiratory and metabolic status by measuring various parameters in anticoagulated whole blood (1). This type of testing is typically performed in acute care or short-term facilities such as emergency departments, intensive care units or operating rooms, to rapidly provide information on respiratory distress, shock or other severe metabolic disorders, but also in central laboratories where samples are shipped from regular wards for monitoring deterioration of acid-base balance or respiratory function (2). The results of blood gas analysis allow clinicians to make rapid and accurate decisions about patient management, particularly oxygen therapy and fluid resuscitation. However, the correct interpretation of test results of blood gas analysis requires not only a comprehensive understanding of the numerous and complicated processes that determine variations in acid-base balance and in some other analytes measured by modern blood gas analyzers, but also a high level of quality throughout the process of collecting and analyzing patient samples (3).

As with conventional laboratory testing, errors in blood gas analysis can occur at any step of the total testing procedure (i.e., preanalytical, analytical, and postanalytical) (4), and can significantly affect the accuracy and reliability of test results, potentially leading to misinterpretation of data and incorrect clinical management. Although preanalytical errors can typically be grouped into a few discrete categories involving patient and sample identification, specimen collection, management, transportation and preparation for testing (i.e., centrifugation, separation, aliquoting, etc.) and storage, the syringes used for blood gas testing are only susceptible to the first parts of errors of the total testing cycle, as the test is performed with whole blood and does not require specific activities for transportation, preparation and storage, except when the testing site (i.e., the central laboratory) is distant from the site for collection (i.e., the clinical ward) (5). Therefore, the more common preanalytical errors in blood gas analysis include identification errors, inappropriate sample collection (e.g. incorrect syringe filling), contamination with other exogenous fluids, air exposure (and incorporation), inappropriate management (e.g., inaccurate mixing), clotting, hemolysis, and incorrect storage time and temperature when bedside testing is not possible, and samples need to be transported to another testing site (6, 7).

Previous studies have reported that the contact of sample with (ambient) air and incorporation of air bubbles into the diagnostic sample (i.e., the blood gas syringe) can lead to significant changes in the concentration of some gasses and molecules measured by blood gas analyzers (8–12), thus compromising measurement accuracy when the air is not removed from the syringe immediately after sampling, as also clearly highlighted in the Clinical and Laboratory Standards Institute (CLSI) C46A2 approved guideline for blood gas analysis (13). This is a serious problem, in that the presence of air bubbles in blood gas syringes has been reported as high as 14% of all specimens (14). Since most previous studies on this topic are outdated and limited to a relatively small number of parameters (8–12), we examine here the effects of the presence of an air bubble in the blood gas syringe on the results of several conventional and innovative parameters measured with a modern blood gas analyzer.

Materials and Methods

We collected venous blood from 17 laboratory workers (mean age; 46±11 years; 10 women) employed in the Service of Laboratory Medicine of the Academic Hospital of Verona (Italy). An accessible vein in one of the upper arms was punctured using a 21G × 3/4" (0.8×20 mm) butterfly device (Safety Blood Collection Set, Gemtier Medical, Shangai, China), to which an evacuated 3.5 mL lithium heparin blood tube (Vacutest Kima, Padova, Italy) was first connected to remove the dead space inside the tube. Immediately thereafter, venous blood was manually aspirated within two heparinized 1.0 mL, 0.5 mm × 16 mm blood gas syringes (Arterial Blood Sampling Kit, Smiths Medical ASD IN, Minneapolis, MN, USA). The first syringe was filled exactly to its nominal volume (i.e., 1.0 mL), while the second was filled with 0.8 mL of venous blood and the remaining empty space in the 1.0 mL syringe was then filled by aspirating 0.2 mL of room/ambient air, which typical-

Table I Graphical description of the study evaluating the impact of an air bubble within the syringe on test results of blood gas analysis.

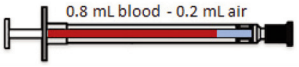
Syringe	Blood filling volume	Air bubble	Representation
1st syringe	1.0 mL (full filling)	0 mL	
2nd syringe	0.8 mL	0.2 mL	

Table II Impact of an air bubble within the syringe on test results of blood gas analysis (n=17). Results are presented with mean and standard deviation (SD), or mean and 95%CI (95% confidence interval), when appropriate. Biases beyond performance specification for each analyte are reported in bold font.

Analyte	Performance specification	Intra-assay imprecision[1]	1.0 mL full syringe	1.0 mL syringe with (0.2 mL) air bubble		
			Value	Value	P-value[2]	Bias (95%CI)*
pH	±1.5%	±0.1%	7.37±0.03	7.38±0.03	0.006	0.1% (0.0% to 0.2%)
pCO_2 (mmHg)	±2.4%	±1.4%	47.8±5.5	45.6±5.3	<0.001	-4.8% (-6.5% to -3.1%)
pO_2 (mmHg)	±1.5%	±1.7%	38.8±16.8	43.8±19.5	0.006	10.8% (5.4% to 16.3%)
sO_2 (%)	±1.5%	±1.2%	55.4±22.6	61.8±23.4	0.001	11.2% (5.3% to 17.1%)
Sodium (mmol/L)	±0.3%	±0.3%	137.3±1.4	136.6±1.2	<0.001	-0.5% (-0.7% to -0.3%)
Potassium (mmol/L)	±2.3%	±1.0%	4.26±0.26	4.19±0.22	0.069	–
Chloride (mmol/L)	±0.6%	±0.4%	104.1±1.9	104.4±1.8	0.236	–
iCa^{2+} (mmol/L)	±0.9%	±1.4%	1.25±0.05	1.23±0.05	0.006	-1.3% (-2.2% to -0.4%)
Glu (mmol/L)	±2.8%	±2.5%	5.63±0.96	5.55±0.96	0.054	–
Lac (mmol/L)	±13.6%	±7.5%	1.13±0.05	1.14±0.35	0.496	–
Hct	±1.4%	±3.6%	42.4±5.2	43.6±4.4	0.041	3.0% (0.1% to 5.9%)
tHb (g/L)	±1.4%	±2.0%	138±17	142±16	0.039	2.7% (0.0% to 5.5%)
COHb (%)	±7.5%	±43.7%	1.28±0.95	1.44±1.01	0.171	–
MetHb (%)	±11.3%	±9.3%	0.64±0.21	0.72±0.18	0.110	

[1] Intra-assay imprecision locally calculated on 10 runs [2]Compared to the reference full syringe

95%CI, 95% confidence interval; pCO_2, partial pressure of carbon dioxide; pO_2, partial oxygen pressure; sO_2, oxygen saturation; iCa^{2+}, ionized calcium; Glu, glucose; Lac, lactate; Hct, hematocrit; tHb, total hemoglobin; COHb, carboxyhemoglobin; MetHb, methemoglobin.

ly contains 78% nitrogen and 21% oxygen and minute amounts of carbon, helium, methane, argon and hydrogen (*Table I*). This specific experimental design was established because the majority of blood gas syringes contaminated with air bubbles received in the local laboratory have air bubbles (i.e., deaths space) of around 0.1–0.3 mL. The syringes were capped immediately after collection and mixed by rotation between the palms of the hands for around 20 sec, thus ensuring an accurate mix between the additive (i.e., lithium-heparin) and venous blood (and ambient air, in the case of syringes containing the death space).

Blood gas analyses of all syringes (manually transported) were always performed between 15 min after sampling, with an identical analyzer and the same test cassette (GEM Premier 5000, Instrumentation Laboratory, Monza, Italy). Before the test, 0.2 mL of venous blood were removed from the first collected syringe (i.e., that completely filled with 1.0 mL of venous blood), while the 0.2 mL of air were removed from the second drawn syringe (i.e., that containing 0.8 mL of blood plus 0.2 mL of ambient air), so that both syringes contained an identical final volume of venous blood for testing. The results of the blood gas analysis were expressed as mean and standard deviation (SD). The significance of bias obtained between the reference syringe completely filled with 1.0 mL of venous blood and that containing 0.8 mL of venous blood and 0.2 mL of ambient air was defined as percent variation exceeding the performance specifications propositioned by Kuster et al. (15), as summarized in *Table II*. Variations of analyte concentrations between the two paired syringes were evaluated with paired-sample Student's T test, Spearman's correlation, while the relative bias was assessed using Bland and Altman plot analysis. Statistical significance was set at $p<0.05$. The statistical analysis was performed using Analyse-it (Analyse-it Software Ltd, Leeds, UK).

All subjects recruited for this study gave written informed consent. The investigation was conducted in accordance with the Declaration of Helsinki and the relevant local legislation. The study was cleared by the Ethics Committee of the Hospital of Verona (approval number: 970CESC; July 20, 2016).

Results

The results of this investigation are shown in *Table II*. In the syringe with the 0.2 mL air bubble, a statistically significant increase in pH, partial pressure of oxygen (pO_2), oxygen saturation (SO_2), total hemoglobin (tHb) and hematocrit (Hct) was observed, while the values of partial pressure of carbon dioxide (pCO_2), sodium and ionized calcium (iCa^{2+}) were significantly reduced. With the exception of pH, all these changes exceeded the performance specifications (*Table II*). In contrast, no statistically significant changes were observed for potassium, chloride, glucose, lactate, carboxyhemoglobin (COHb) and methemoglobin (MetHb). A significant correlation between the baseline value in the full 1.0 mL syringe and the change recorded in the paired syringe with the air bubble was only found for sodium and hematocrit (*Table III*).

Table III Spearman's correlation between the baseline value of blood gas parameters in a full 1.0 mL syringe and variation recorded in a paired 1.0 mL syringe containing 0.8 mL of homologous blood and 0.2 mL of ambient air (i.e., air bubble) (n=17). Results are only presented for analytes significant variations (presented in *Table II*).

Analyte	Absolute variation	Spearman's Correlation (with relative p-value)
pH	0.01 (95%CI, 0.00 to 0.01)	r=-0.43 (95%CI, -0.75 to 0.07); p=0.080
pCO_2 (mmHg)	-2.2 (95%CI, -2.9 to -1.5)	r=-0.11 (95%CI, -0.56 to 0.39); p=0.662
pO_2 (mmHg)	5.0 (95%CI, 1.6 to 8.4)	r=0.42 (95%CI, -0.08 to 0.75); p=0.096
sO_2 (%)	6.5 (95%CI, 3.2 to 9.8)	r=0.22 (95%CI, -0.29 to 0.64); p=0.390
Sodium (mmol/L)	-0.7 (95%CI, -1.0 to -0.4)	r=0.91 (95%CI, 0.75 to 0.97); p<0.001
iCa^{2+} (mmol/L)	-0.02 (95%CI, -0.03 to -0.01)	r=-0.01 (95%CI, -0.49 to 0.47); p=0.977
Hct	1.2 (95%CI, 0.10 to 2.3)	r=-0.59 (95%CI, -0.83 to -0.15); p=0.013
tHb (g/L)	3.7 (95%CI, 0.2 to 7.2)	r=-0.19 (95%CI, -0.62 to 0.32); p=0.461

95%CI, 95% confidence interval; pCO_2, partial pressure of carbon dioxide; pO_2, partial oxygen pressure; sO_2, oxygen saturation; iC^{a2+}, ionized calcium; Hct, hematocrit; tHb, total hemoglobin.

Discussion

The collection of whole blood samples for blood gas analysis is a specialized procedure that necessitates technical expertise and a thorough understanding of all potential preanalytical variables that may affect the reliability of test results. To this end, there are now several lines of evidence confirming that minimizing air aspiration by carefully filling the blood gas syringe and avoiding unnecessary agitation is critical to prevent air contamination, which may cause unwarranted variations in analyte readings with blood gas analyzers. Regarding the availability of previous information on this important preanalytical aspect, the CLSI states that sample exposure to ambient (room) air can considerably impair the assessment of pH, pO_2 and pCO_2 due to direct contamination, as well as the concentration of iCa^{2+} due to increase binding to plasma proteins (13). These conclusions were mostly based on previous studies, published more than 10–15 years ago, which have investigated this aspect with relatively dated instrumentation and measuring a limited number of blood gas parameters.

The first article on this important preanalytical aspect in blood gas analysis was published by Madiedo et al. in 1980 (8). The authors first collected arterial blood into a disposable plastic syringe containing sodium heparin, and then introduced an air bubble equal to about 10% of the total volume of blood. Syringes were gently mixed, placed in ice for 15–20 min, and blood gases were measured using a Radiometer ABL-1 blood gas analyzer. The pO_2 in these samples displayed a significant mean increase of 11 mmHg (range 1.7 to 29 mmHg), which was also directly associated with the initial value of pO_2.

In a second study, Biswas et al. (9) collected venous and arterial blood samples using a blood gas syringe containing lithium-heparin. In some of these specimens, the authors introduced ambient air in the syringe (0.1 of air in a 2.0 mL syringe), representing 5% of the total syringe volume. Air bubbles were left in the syringe for 1–5 minutes before being expelled and the sample being tested on a Corning analyzer. Importantly, the value of pO_2 tended to increase after 2 min of incubation of blood with air, while that of pCO_2 displayed an inverse trend (i.e., decrease) after 3 min of incubation of blood with air, while the pH was non-significantly affected. The maximum variation after 5 min of incubation of air with blood was +10% for pO_2 and -9% for pCO_2, respectively.

In 1996, Astles et al. (10) conveyed a number of blood gas syringes with a broad range of pO_2 values through a pneumatic transport system to determine the effect of air contamination during transportation (the exact volume of air is not reported in the available text of the article). Overall, pO_2 values increase substantially after transportation, up to 160 mm Hg. The authors also reported that blood gas syringes collected from hypoxemic patients underwent pO_2 variations that might have triggered clinical misinterpretation (i.e., 50% of samples with baseline pO_2 <85 mm Hg displayed increases of 10 mm Hg when contaminated with air).

Lu et al. (11) published another interesting article in 2003. The authors filled 10 mL heparinized polypropylene syringes with pooled blood and varying volumes of ambient air (0.05 mL, 0.1 mL, 0.5 mL, and 1.0 mL, representing 0.5%, 1%, 5%, and 10% air contamination, respectively). The measurement performed on a Radiometer ABL520 evidenced a direct association between the volume of air introduced into the syringe and the increase of pO_2 in the test sample. In syringes with 10% air contamination, the pO_2 value increased by 24.2±3.4 mm Hg and 64.9±8.0 mmHg when conveyed to the testing site manually or by pneumatic transport system, respectively.

More recently, in 2011, O'Connor et al. (12) collected ten standard 1.0 mL blood gas syringes from 5 patients (two from each). Five syringes were left untreated while the other five were contaminated with 0.2 mL of room air. The results of testing conducted between 30–180 min on a Roche AVL OMNI-3 blood gas analyzer revealed a time-dependent increase in pO_2 values in all air-contaminated samples, accompanied by a slight decline of pH. Expectedly, the values of pCO_2 were also significantly lower at most time points in the air-contaminated samples.

According to our protocol, encompassing a 20% contamination of ambient air in a 1.0 mL blood gas syringe containing venous blood, and with blood gas analysis performed 15 min after sampling, a number of parameters that can be assayed with modern blood gas analyzers could be biased by the presence of air bubbles in the blood gas syringe. In agreement with previous data, we confirm that pO_2 and SO_2 increase significantly above the clinically significant deviation threshold, while pCO_2 shows an opposite trend. Because venous blood was used, the bias observed for pO_2 and SO_2 must be interpreted with caution, since their values are consistently lower than the reference ranges that one would expect for arterial blood. In addition to the findings published in other studies, we have also shown that sodium and iCa^{2+} also decreases and exceed the clinically significant variation threshold when an air bubble is present in the syringe, while hematocrit and hemoglobin increase above their respective clinically significant variation thresholds (*Table II*). In most cases, with exception of sodium and Hct, we found no significant correlation between the value of the measured parameter in the fully filled syringe and the absolute change of the same analyte in the syringe containing the air bubble (*Table III*). This means that, even if the data obtained in this study are tightly clustered, the bias in venous blood could be considered largely unpredictable, thus precluding the possibility of

"»adjusting« the value of most parameters in the air-contaminated blood gas syringe.

Although the laboratory technology beyond blood gas analysis has not undergone substantial revolutions during the past decade, the novel generation of analyzer used in this study has never been tested before for this preanalytical problem. In summary, the evidence emerged from our study confirms that air bubbles from blood gas syringes must be removed as soon as possible after sampling to prevent artifactual test results and misleading clinical judgment and inappropriate treatment. The syringe should be inverted two or three times to check for the presence of air bubbles, which should then be expelled as quickly as possible by gently tapping one side of the syringe to bring the air bubbles to the top, and then applying light pressure to the plunger until all leftover air has been removed.

Nevertheless, when blood gas syringes are received in the laboratory with large volume of air bubbles inside (e.g., 20% of the filling volume), the most vulnerable parameters (i.e., pO_2, SO_2, pCO_2, sodium, iCa^{2+}, hematocrit and hemoglobin) should be suppressed. As limitations in this study, we recognize that our results refer to a limited sample sized and to venous blood. However, this approach was chosen to avoid injury and discomfort in ostensibly healthy volunteers as typically caused by arterial punctures. Moreover, this is the very first study that has thoughtfully described the potential variation induced by ambient air contamination on a vast array of blood gas analysis parameters.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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ASSOCIATION BETWEEN CEREBRAL SMALL VESSEL DISEASE AND PLASMA LEVELS OF LDL CHOLESTEROL AND HOMOCYSTEINE: IMPLICATIONS FOR COGNITIVE FUNCTION

POVEZANOST IZMEĐU CEREBRALNE BOLESTI MALIH SUDOVA I NIVOA LDL HOLESTEROLA I HOMOCISTEINA U PLAZMI: IMPLIKACIJE NA KOGNITIVNE FUNKCIJE

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Summary

Background: Investigate the correlation between low-density lipoprotein (LDL) cholesterol, homocysteine and cognitive function in patients with cerebral small vessel disease (CSVD).
Methods: 240 patients with CSVD confirmed by head MRI in the Department of Neurology from January 2020 to December 2023 were retrospectively included in the study. All the patients had complete blood biochemical examination, and their cognitive function was evaluated by Montreal Cognitive Assessment Scale (MoCA), and after correcting for the factor of years of education, the patients were divided into a group of normal cognition (MoCA 26, 70 patients) and a group of cognitive function (MoCA 26, 70 patients) according to the scores. After correcting for the factor of years of education, the patients were divided into the normal cognitive function group (70 cases with MoCA 26) and the cognitive dysfunction group (170 cases with MoCA <26) according to their scores. The general information of the two groups and the patients' cognitive function characteristics, including visuospatial and executive ability, naming, attention and calculation, language, abstraction, delayed memory, and orientation, were compared, and the independent influences on the occurrence of cognitive dysfunction in patients with CSVD were analyzed by two-category multifactorial logistic regression.
Results: Compared with the group with normal cognitive function, the cognitive dysfunction group had lower years of education and higher homocysteine, and the differences were statistically significant ($P < 0.05$). Compared with the

Kratak sadržaj

Uvod: Cilj je bio da se istraži korelacija između holesterola lipoproteina niske gustine (LDL), homocisteina i kognitivne funkcije kod pacijenata sa bolešću malih sudova mozga (CSVD).
Metode: U studiju je retrospektivno uključeno 240 pacijenata sa CSVD potvrđenim glavnim MR na Odeljenju za neurologiju od januara 2020. do decembra 2023. godine. Svi pacijenti su imali kompletan biohemijski pregled krvi, a njihova kognitivna funkcija je procenjena Montrealskom skalom kognitivne procene (MoCA), a nakon korekcije faktora godina obrazovanja, pacijenti su podeljeni u grupu normalne kognitacije (MoCA 26, 70 pacijenata) i grupa kognitivnih funkcija (MoCA 26, 70 pacijenata) prema rezultatima. Nakon korekcije faktora godina obrazovanja, pacijenti su podeljeni u grupu sa normalnim kognitivnim funkcijama (70 slučajeva sa MoCA 26) i grupu sa kognitivnom disfunkcijom (170 slučajeva sa MoCA <26) prema rezultatima. Upoređene su opšte informacije dve grupe i karakteristike kognitivnih funkcija pacijenata, uključujući vizuelno-prostorne i izvršne sposobnosti, imenovanje, pažnju i računanje, jezik, apstrakciju, odloženo pamćenje i orijentaciju, kao i nezavisni uticaji na pojavu kognitivne disfunkcije. Kod pacijenata sa CSVD analizirane su dve kategorije multifaktorske logističke regresije.
Rezultati: U poređenju sa grupom sa normalnom kognitivnom funkcijom, grupa sa kognitivnom disfunkcijom je imala niže godine obrazovanja i viši nivo homocisteina, a razlike su bile statistički značajne ($P < 0,05$). U poređenju sa grupom sa normalnim kognitivnim funkcionisanjem,

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group with normal cognitive functioning, the cognitive dysfunction group had lower MoCA total scores, lower visuospatial and executive ability, naming, attention and calculation, language, abstraction, delayed memory, and orientation scores, and the differences were statistically significant ($P < 0.05$). Two-category multifactorial logistic regression analysis showed that low-density lipoprotein cholesterol (OR=2.756, 95% CI: 0.673–0.938, P=0.012) and homocysteine (OR=1.859, 95% CI: 1.024–1.324, P=0.016) were the independent factors influencing cognitive dysfunction in CSVD patients. The lower the risk of cognitive impairment in CSVD patients, the higher the plasma LDL cholesterol and homocysteine levels, the higher the risk of cognitive impairment in CSVD patients.
Conclusions: Plasma LDL cholesterol and homocysteine levels are associated with and may be predictors of cognitive dysfunction in patients with CSVD.

Keywords: low-density lipoprotein cholesterol, homocysteine, cerebral small-vessel disease, cognitive functions

grupa sa kognitivnom disfunkcijom imala je niže ukupne rezultate MoCA, niže vizuelno-prostorne i izvršne sposobnosti, imenovanje, pažnju i računanje, jezik, apstrakciju, odloženo pamćenje i orijentaciju, a razlike su bile statistički značajne ($P < 0,05$). Dvokategorijska multifaktorska logistička regresiona analiza pokazala je da su holesterol lipoproteina niske gustine (OR=2,756, 95% CI: 0,673–0,938, P=0,012) i homocistein (OR=1,859, 95% CI: 1,024–1,324, P=0,016) bili nezavisni faktori koji utiču na kognitivnu disfunkciju kod pacijenata sa CSVD. Što je manji rizik od kognitivnog oštećenja kod pacijenata sa CSVD, što je viši nivo LDL holesterola i homocisteina u plazmi, veći je rizik od kognitivnog oštećenja kod pacijenata sa CSVD.
Zaključak: Nivoi LDL holesterola i homocisteina u plazmi su povezani i mogu biti prediktori kognitivne disfunkcije kod pacijenata sa CSVD.

Ključne reči: holesterol lipoproteina niske gustine, homocistein, cerebralna bolest malih sudova, kognitivne funkcije

Introduction

As populations age and human life expectancy increases, age-related diseases pose significant challenges to society and health care systems. There are currently more than 40 million people living with dementia worldwide and this is expected to double every 20 years. cerebral small vessel disease (CSVD) is the most important vascular risk factor for dementia, accounting for 36% to 67% of vascular dementia (VaD). It also interacts with Alzheimer's disease (AD) and other types of dementia, increasing the risk of dementia. Cerebral small vessel disease (CSVD) is predominantly characterized by the involvement of small arteries and veins, encompassing conditions such as small vessel disease of the white matter, microvascular disease, and blood-brain barrier disruption (1, 2). Not only is CSVD a common etiology of cognitive decline and dementia in the elderly, but it is also closely associated with neurological impairments including stroke, motor disorders, and depression (3–5).

Low-density lipoprotein cholesterol (LDL-C) and homocysteine (hcy) are two biochemical markers present in the bloodstream, intimately linked to cardiovascular diseases and metabolic syndrome. Recent investigations have suggested a potential relationship between these two markers and the development of CSVD as well as cognitive decline (6). Elevated LDL-C levels primarily contribute to atherosclerosis and vascular inflammation, establishing a significant connection to the pathological processes underlying vascular diseases.

Some studies have reported a certain correlation between hypercholesterolemia and the occurrence of cerebral small vessel disease (CSVD) as well as cognitive decline. Additionally, homocysteine, a sulfur-containing amino acid, is associated with metabolic disruption, oxidative stress, and inflammatory reactions (4). Existing research has demonstrated a link between elevated homocysteine levels and cerebral vascular damage, along with cognitive decline. However, studies investigating the correlation between plasma low-density lipoprotein cholesterol (LDL-C) and homocysteine levels, and cognitive function in CSVD patients, remain relatively limited.

Cognitive impairment caused by CSVD typically exhibits an insidious onset and a slow progression, often leading to under-recognition during the early stages. By the time patients are initially diagnosed, neurological damage may be difficult to reverse (7). further contributing to the burden of the disease. Therefore, the objectives of this study are to identify relevant risk factors, particularly modifiable ones, for cognitive impairment in CSVD and to provide further evidence for the clinical management of cognitive impairment in CSVD patients. This study aims to shed light on the identification of related risk factors for cognitive impairment in CSVD and especially focus on modifiable risk factors, providing additional insights for the clinical management of cognitive impairment in CSVD patients.

Materials and Methods

Objects

Two hundred and forty patients with CSVD confirmed by head MRI in the Department of Neurology from January 2020 to December 2023 were retrospectively included in the consecutive enrollment. All patients had complete blood biochemistry examination, and cognitive function was evaluated by Montreal Cognitive Assessment Scale (MoCA), and after correcting for the factor of years of schooling, the patients were divided into cognitively normal group (MoCA 26 points, 70 cases) and cognitively impaired group (MoCA <26 points, 170 cases)

according to the scoring results. The patients were divided into the normal cognitive function group (70 cases with MoCA 26 points) and the cognitive dysfunction group (170 cases with MoCA <26 points).

Inclusion criteria: (1) Participants must be 18 years of age or older, with no gender restrictions; (2) Participants must meet the diagnostic criteria for CSVD as outlined in the 2021 Chinese Expert Consensus on the Diagnosis and Treatment of Cerebral Small Vessel Disease and must have received confirmation through head MRI imaging.

Exclusion criteria: (1) Patients with alternative causes of stroke such as atherosclerotic cerebral infarction, cardiac cerebral embolism, traumatic brain injury, cerebral hemorrhage, intracranial space-occupying lesions, or other intracranial abnormalities will be excluded; (2) Patients with multiple organ failure, hematological diseases, or advanced systemic malignant tumors will be excluded; (3) Patients unable to undergo or cooperate with necessary study procedures, including head MRI and cognitive function assessments, will be excluded. The study protocol was approved by the hospital's Ethics Committee.

Methods

Blood tests (morning blood after at least 8 hours of fasting): Renal function assessment includes creatinine (normal reference range: 44–133 μmol/L), estimated glomerular filtration rate (eGFR; normal reference range: 80–120 mmol/L – 1.73 m^2), urea (normal reference range: 1.8–1.73 m^2), and blood glucose (normal reference range: 1.5–1.5 m^2), reference range: 80–120 mmol/L -1.73 m^2), urea (normal reference range: 1.8–7.1 mmol/L) and uric acid (normal reference range: 150–420 μmol/L), fasting glucose (normal reference range: 3.61–6.11 mmol/L), and ultrasensitive C-reactive protein (normal reference range: 3.61–6.11 mmol/L).Lipids include total cholesterol (normal reference range: 3.4–5.2 mmol/L), HDL cholesterol (normal reference range: 0.9–1.4 mmol/L), LDL cholesterol (normal reference range: 2.1–3.1 mmol/L), and triacylglycerol (normal reference range: 0.56–1.5 mmol/L). Reference range: 0.56–1.7 mmol/L), homocysteine (normal reference range: 6–17 μmol/L); current statin use.

The diagnostic criteria for hypertension, diabetes mellitus and hyperlipidemia were referred to the Chinese Guidelines for the Prevention and Control of Hypertension (2018 Revision) (8), the Chinese Guidelines for the Prevention and Control of Type 2 Diabetes Mellitus (2020 Revision) (9) and the Chinese Guidelines for the Prevention and Control of Dyslipidemia in Adults (2016 Revision) (10), respectively. Smoking history was defined as continuous smoking for more than 6 months with an average of more than 10 cigarettes/d, and drinking history was defined as drinking for more than 6 months with an average alcohol intake of more than 30 g/d or 210 g/week (11). The eGFR was calculated according to the Cockcroft-Gault formula (12): eGFR = (140-age) x body mass (kg)/[0.818 x blood creatinine (μmol/L)] for men and Female Ccr= (140-age) × Body weight (kg) × 1.03/ serum creatinine (μmol/L).

Cognitive assessment

Cognitive assessment was conducted using the Montreal Cognitive Assessment (MoCA) tool (13), which comprises 7 subcognitive domains: visuospatial and executive ability, naming, attention and calculation, language, abstraction, delayed memory, and orientation. The total MoCA score ranges from 0 to 30. To account for educational differences, an additional point was added to the total score for patients with less than 12 years of education. Patients were categorized into two groups based on cognitive function: a cognitively normal group (MoCA score 26 points) and a cognitively impaired group (MoCA score <26 points). However, the specific criteria used for categorization and the detailed process of cognitive assessment were not clearly defined in the methods section. Including more detailed information on these aspects would improve the methodological rigor and reproducibility of the study.

Statistical Methods

The study employed several statistical methods to investigate the correlation between LDL cholesterol, homocysteine, and cognitive function in patients with cerebral small vessel disease (CSVD). General information and cognitive function characteristics of the two groups were compared using descriptive statistics to provide an overview of the patient population. Statistical tests such as t-tests or non-parametric equivalents were used to compare the differences in years of education, homocysteine levels, and MoCA scores between the group with normal cognitive function and the cognitive dysfunction group. Two-category multifactorial logistic regression analysis was performed to identify independent factors influencing cognitive dysfunction in CSVD patients. This analysis assessed the relationship between LDL cholesterol, homocysteine levels, and the occurrence of cognitive dysfunction while controlling for other potential confounding variables. Odds ratios (OR) and 95% confidence intervals (CI) were reported to quantify the strength of association between LDL cholesterol, homocysteine levels, and cognitive dysfunction. Statistical significance was determined using P-values, with values less than 0.05 considered significant.

Results

General Information

Compared with the patients in the group with normal cognitive function, the patients in the cognitive dysfunction group had lower years of education and higher levels of low-density lipoprotein cholesterol and homocysteine, and the differences were statistically significant ($P < 0.05$). See *Table I*.

Comparison of cognitive function between the two groups of patients

Compared with the group with normal cognitive function, the cognitive dysfunction group had a lower MoCA total score and its various sub-cognitive domain scores, and the differences were statistically significant (all $P < 0.05$). See *Table II*.

Dichotomous multifactorial logistic regression analysis

Binary Logistic regression analysis showed that years of education, low density lipoprotein cholesterol and homocysteine were independent factors affecting cognitive dysfunction in CSVD patients. The higher the levels of low-density lipoprotein cholesterol and homocysteine, the higher the risk of cognitive impairment in CSVD patients; The higher the number of years of education, the lower the risk of cognitive impairment in CSVD patients. See *Table III*.

Table I Comparison of general data of patients.

	Cognitively normal group (n=70)	Cognitive dysfunction group (n=170)	χ^2/F value	P value
Age ($\bar{x}\pm s$, year)	65.2±14.4	66.8±11.3	-1.784[a]	0.156
Masculinity [n(%)]	50(71.4)	104(61.2)	1.679[b]	0.304
Education years [M(P25,P75), year]	13(12, 17)	10(8,12)	-3.167[e]	0.042
BMI ($\bar{x}\pm s$, year)	26.7±4.26	27.1±4.79	1.673[a]	0.278
Hypertension [n(%)]	60(85.7)	122(71.8)	3.157[b]	0.127
Diabetes [n(%)]	24(34.3)	44(25.9)	0.486[b]	0.158
Hyperlipemia [n(%)]	54(77.1)	122(71.8)	0.782[b]	0.356
Statins [n(%)]	52(74.3)	112(65.9)	0.892[b]	0.429
Smoking history [n(%)]	44(62.9)	82(48.2)	2.178[b]	0.126
Systolic pressure ($\bar{x}\pm s$, mmHg)	134.7±17.2	142.6±22.6	-1.782[a]	0.172
Diastolic pressure ($\bar{x}\pm s$, mmHg)	82.7±16.8	81.6±9.2	0.562[a]	0.479
Heart rate ($\bar{x}\pm s$/min)	75.3±10.6	75.9±11.2	0.765[a]	0.705
Creatinine [M(P25,P75), μmol/L]	78.0(67.9, 92.6)	79.2(68.5, 91.7)	-0.502[e]	0.809
Egfr [M(P25,P75]	85.06(70.67, 94.27)	82.78(75.02, 90.89)	-0.506[e]	0.708
Urea [M(P25,P75), μmol/L]	5.46(4.56, 6.74)	5.28(4.37, 6.83)	-0.573[e]	0.673
UA [($\bar{x}\pm s$, μmol/L)]	345.7±67.89	309±89.56	-0.783[a]	0.302
FBG [($\bar{x}\pm s$, mmol/L)]	6.26±1.67	5.78±1.89	-0.908[a]	0.152
hs-CRP [M(P25,P75), mg/L]	1.56(0.67, 2.18)	1.07(0.63, 2.37)	-0.987[e]	0.275
TCHO [($\bar{x}\pm s$, μmol/L)]	3.87±1.26	4.04±1.56	-1.562[a]	0.106
TG [M(P25, P75)]	1.16(0.83, 1.95)	1.19(0.92, 1.89)	-0.705[e]	0.605
HDL-C [M(P25, P75)]	1.06(0.83, 1.25)	1.08(0.87, 1.45)	-2.062[e]	0.221
LDL-C [($\bar{x}\pm s$)]	2.07±0.45	2.25±0.38	-2.806[a]	0.012
Hcy [M(P25, P75), μmol/L]	12.78(9.67, 18.27)	1.56(11.6, 18.56)	-2.408[e]	0.023

Note: BMI is body mass index, eGFR is estimated glomerular filtration rate; [a] is t value and [b] is 2 is the value, and [c] is the z value

Table II Comparison of cognitive function in patients with small cerebral vascular disease.

	Cognitively normal group (n=70)	Cognitive dysfunction group (n=170)	*Z* value	*P* value
MoCA score [M(P25, P75), score]	27.0(26.0,29.0)	21.0(17.0,24.0)	-9.792	0.001
Visual space and executive ability [M(P25, P75), score]	5.0(4.0,5.0)	3.0(2.0,4.0)	-7.351	0.002
Namespace [M(P25, P75), score]	3.0(3.0,3.0)	3.0(2.0,3.0)	-3.026	0.012
Attention and numeracy [M(P25, P75), score]	6.0(6.0,6.0)	6.0(4.0,6.0)	-4.056	0.025
Language [M(P25, P75), score]	3.0(2.0,3.0)	2.0(1.0,2.0)	-4.682	0.017
Abstraction [M(P25, P75), score]	2.0(2.0,2.0)	1.0(1.0,2.0)	-5.726	0.019
Delayed Memory [M(P25, P75), score]	4.0(3.0,5.0)	1.0(0.0,2.0)	-2.783	0.025
Orientation [M(P25, P75), score]	6.0(6.0,6.0)	5.0(5.0,6.0)	-2.056	0.021

Note: MoCA is the Montreal Cognitive Assessment Scale

Table III Multivariate Logistic regression analysis of cognitive dysfunction.

Factor	value	*SE*	Wald χ^2	*P* value	*OR* value	95%*CI*
LDL-C	0.879	0.372	5.891	0.012	2.756	0.673–0.938
HCY	0.125	0.057	4.268	0.016	1.859	1.024–1.324

Note: LDL-C is low-density lipoprotein cholesterol; HCY is Homocysteine.

Discussion

Cerebral small-vessel disease refers to diseases with clinical, cognitive, imaging and pathological manifestations caused by small-vessel lesions in the brain. Studies in China have shown that lacunar cerebral infarction accounts for 42.3% of all ischemic stroke causes (14). Among them, cognitive decline is the most common and important clinical manifestation of cerebral small vessel disease. Therefore, early identification and intervention at the VCIND or VaMCI stage is a key target for the prevention and treatment of VaD and is of great clinical significance. Currently, it is believed that CSVD is caused by various factors, and lipid metabolism disorder is one of the risk factors for CSVD, and among the lipid metabolism disorders, the increase of LDL level is the most harmful one, and CSVD can be prevented by controlling the level of LDL in clinical practice (15). The pathology of cerebral small vessel disease is characterized by atherosclerosis of small arteries, i.e., loss of smooth muscle in the vessel wall, diffuse lipid deposition, infiltration of plasma proteins and inflammatory factors, and formation of lipid hyalinosis, and LDL-C, the main component of cholesterol, is an important factor in the formation of atherosclerosis.

Cerebral small vessel disease is a common risk factor for stroke and a major cause of vascular cognitive impairment. Cerebral microangiopathy and neurodegeneration are closely associated with cognitive decline and dementia in the elderly (16). Relevant studies have found that CSVD is a dynamic disease, and the degree of cognitive decline caused by cerebral microangiopathy depends on the progression of microangiopathy (17). VHyperhomocysteinemia (HHcy) is also recognized as a risk factor for Vascular cognitive impairment (VCI). Wang et al. (18) found that Hcy levels are strongly associated with cerebral small vessel disease and are considered an independent risk factor for CSVD. Hcy is an endothelial toxin that causes vascular injury by promoting oxidative damage in arteries, destroying vascular matrix, and increasing the proliferation of vascular smooth muscle cells, and also alters blood coagulation properties and disrupts endothelium-dependent diastolic regulation of the vasculature, which further causes cognitive dysfunction (19).

The present study focused on exploring the association of Hcy levels and LDL-C with cognitive impairment in CSVD. Hcy has received widespread attention as a risk factor for cognitive impairment (20). The results of this study showed that Hcy and LDL is an independent risk factor for cognitive dysfunction in CSVD, and age, gender, smoking history, drinking history, hypertension, diabetes mellitus, uric

acid, fasting glucose, triglycerides, total cholesterol, and low-density lipoprotein (LDL-C), which were mentioned above, did not affect the level of Hcy. Recent studies have demonstrated that Hcy acts as a neurotoxin that promotes neurodegeneration through apoptosis caused by DNA breaks (21). HHcy has been reported to be a risk factor for VCI in patients with cerebral infarction and an independent risk factor for MCI in the Xinjiang Uyghur population (22, 23). Wang et al. (16) demonstrated that serum Hcy levels correlated with the occurrence of vascular MCI , and that cognitive impairment may be caused by increased cerebrovascular disease-associated cortical or hippocampal atrophy as a result of the toxic injury of a high Hcy In addition, serum Hcy levels were positively associated with vascular MCI in patients with CSVD and may serve as a predictor of vascular MCI (16). Feng et al. (24) demonstrated that Hcy is more closely associated with small vessel disease than large vessel disease. Jakubowski et al. et al. (25) demonstrated that Hcy in general is an independent risk factor for CSVD, and that Hcy toxic effects may include direct endothelial injury or triggering endothelial inflammation. The toxic effects of Hcy may include direct endothelial damage or triggering endothelial inflammatory responses. The results of the present study are consistent with these studies and suggest that elevated levels of Hcy lead to cognitive impairment in CSVD, and that the pathogenesis of CSVD is based on the following mechanisms: 1) Hcy is endotoxic and neurotoxic to the vascular endothelium, and 2) Hcy has a post-translational modification of proteins known as homocysteinylation, which is toxic to the nerves (25, 26).

Meanwhile, the results of this study showed that LDL cholesterol levels were higher in the cognitive dysfunction group than in the cognitive function group, and logistic regression analysis showed that LDL cholesterol was an independent risk factor for cognitive dysfunction in patients with CSVD, suggesting that LDL cholesterol may be involved in the development of cognitive dysfunction in CSVD. Todate et al. (8) showed that the prevalence of periventricular white matter high signal was significantly higher than that of healthy controls in patients with familial hypercholesterolemia. Todate et al. (8) showed that the prevalence of periventricular white matter hyperintensities in patients with familial hypercholesterolemia was significantly higher than that in healthy controls, and the prevalence of deep white matter hyperintensities was also on the rise in familial hypercholesterolemia. Imamura et al. (27) followed up 2,351 residents aged 40 years of a certain community for 19 years, and found that the patients with high levels of LDL-cholesterol had a higher risk of cavernous cerebral defects. It was found that patients with high levels of LDL cholesterol had a higher incidence of lacunar cerebral infarction. According to national and international guidelines, CSVD may cause cognitive decline (3, 28). The present study also supports that high levels of LDL cholesterol are associated with cognitive impairment in CSVD.

There is a positive association between LDL-C and small cerebral vessel disease: high levels of LDL-C may be an important risk factor for small cerebral vessel disease. Long-term high levels of LDL-C can lead to vascular endothelial damage, atherosclerosis and small brain vessel damage, thereby increasing the risk of small brain vessel disease. There is a positive association between homocysteine and small cerebral vessel diseases: high levels of homocysteine are closely related to the occurrence and development of small cerebral vessel diseases. Accumulation of homocysteine can cause endothelial cell damage, inflammatory responses, and changes in blood vessel walls, thereby increasing the risk of brain small vessel disease. High levels of LDL-C and homocysteine are associated with cognitive decline: Brain small vessel disease is strongly associated with cognitive decline. High levels of LDL-C and homocysteine may further impair brain function by promoting mechanisms such as cerebrovascular injury, ischemia, and inflammatory response, leading to cognitive decline. Based on the above conclusions, rational control of plasma LDL-C and homocysteine levels may help reduce the risk of brain small vessel disease and may improve cognitive function. LDL-C and homocysteine levels can be effectively controlled through appropriate lifestyle interventions, such as rational diet, active exercise and drug therapy, thereby preventing the development of brain small vessel disease and improving cognitive performance.

In this study, there are several limitations that should be acknowledged. Firstly, the retrospective design of the study may introduce biases and limit the ability to establish causal relationships between low-density lipoprotein (LDL) cholesterol, homocysteine, and cognitive function in patients with cerebral small vessel disease (CSVD). Additionally, the sample size of 240 patients from a single department may not be representative of the broader population with CSVD, potentially affecting the generalizability of the findings. Furthermore, the use of the Montreal Cognitive Assessment Scale (MoCA) for evaluating cognitive function, while widely used, may not capture the full spectrum of cognitive abilities, potentially overlooking certain aspects of cognitive impairment in patients with CSVD. The study also lacks detailed information on other potential confounding factors, such as lifestyle habits, comorbidities, or medication use, which could influence the relationship between LDL cholesterol, homocysteine, and cognitive function. Moreover, the study did not address the longitudinal changes in LDL cholesterol and homocysteine levels over time or their potential impact on cognitive function in CSVD patients. Therefore, further prospective investigations are needed to better understand the causal mechanisms and long-term implications of these biomarkers on cognitive impairment in CSVD.

In conclusion, MoCA examination of CSVD patients for early identification of cognitive dysfunction, detection of plasma LDL cholesterol and homocysteine levels in CSVD patients, and timely diagnosis and treatment may have a predictive effect on the occurrence and development of cognitive dysfunction in CSVD patients. However, the shortcoming of this study is that it is a single-center, small-sample retrospective analysis, and the degree of cognitive dysfunction of CSVD patients was not graded, which needs to be further explored and verified by subsequent multi-center, large-sample, and more detailed analysis data.

Ethical compliance

This study was approved by the ethics committee of Affiliated Hospital of Gansu Medical College.

Author contributions

YC and GX designed the study and performed the experiments, LL and YL collected the data, LZ and WC analyzed the data, YC and GX prepared the manuscript. All authors read and approved the final manuscript.

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Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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SIGNIFICANCE OF PLASMA TGF-β1 LEVEL DETECTION IN PATIENTS WITH T2DM WITH HEART FAILURE

ZNAČAJ DETEKCIJE TGF-β1 NIVOA PLAZME KOD PACIJENATA SA T2DM I SRČANOM INSUFICIJENCIJOM

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Summary

Background: The aim of the study was to examine the significance of plasma Transforming Growth Factor-1/TGF-β1 (TGF-β1) level testing in patients with Type 2 Diabetes Mellitus (T2DM) and heart failure.
Methods: A sample of T2DM patients who were hospitalised for dyspnea was chosen between June 2021 and June 2023. Based on the convenience sample approach, 150 cases were screened for the study, and 50 healthy non-diabetic people without cardiac problems who completed physical examinations over the same period were included as a control group. All study participants had their serum NT-proBNP and plasma TGF-I levels checked, and the values between the two groups were compared. Then, the patients with T2DM with heart failure were grouped according to whether they were accompanied by heart failure or not and the grading of cardiac function, and then the serum NT-proBNP and plasma TGF-β1 levels were compared between the different groups of patients. The diagnostic value of plasma TGF-β1 in the occurrence of heart failure in patients with T2DM was analysed.
Results: There were 54 patients without heart failure and 96 people with heart failure among the 150 T2DM patients. The cut-off point was 44.50 g/L. At this time, the sensitivity and specificity for diagnosing concomitant heart failure in T2DM were 79.63% and 52.51%, respectively. 96 individuals with T2DM and heart failure showed greater serum and plasma levels of NT-proBNP and TGF-β1 compared to the other two groups (P=0.05). ProBNP and plasma TGF-β1 levels had a positive and significant relationship (P=0.05).

Kratak sadržaj

Uvod: Cilj ovog istraživanja je bio da se ispita značaj testiranja nivoa plazme Transformišućeg faktora rasta-1/TGF-β1 (TGF-β1) kod pacijenata sa dijabetesom melitusom tip 2 (T2DM) i srčanom insuficijencijom.
Metode: Odabran je uzorak pacijenata sa T2DM koji su hospitalizovani zbog dispneje između juna 2021. i juna 2023. godine. Na bazi uzorka pogodnosti, pregledano je 150 slučajeva za studiju, a 50 zdravih osoba koje nisu dijabetičari i koje nemaju srčane probleme, a koje su završile fizikalne preglede u istom periodu, je uključeno u studiju kao kontrolna grupa. Svim učesnicima studije provereni su nivoi serumskog NT-proBNP i plazme TGF-I, a vrednosti između dve grupe su upoređene. Zatim su pacijenti sa T2DM i srčanom insuficijencijom grupisani prema tome da li su imali srčanu insuficijenciju ili ne, kao i prema stepenu srčane funkcije, i zatim su nivoi serumskog NT-proBNP i plazme TGF-β1 upoređeni između različitih grupa pacijenata. Analizirana je dijagnostička vrednost plazme TGF-β1 u pojavi srčane insuficijencije kod pacijenata sa T2DM.
Rezultati: Među 150 pacijenata sa T2DM, 54 pacijenta nisu imala srčanu insuficijenciju, dok je 96 osoba imalo srčanu insuficijenciju. Granična vrednost bila je 44,50 g/L. U ovom trenutku, senzitivnost i specifičnost za dijagnostikovanje srčane insuficijencije uz T2DM su bile 79,63% i 52,51%, respektivno. 96 osoba sa T2DM i srčanom insuficijencijom pokazalo je veće serumske i plazmatske nivoe NT-proBNP i TGF-β1 u poređenju sa ostale dve grupe (P=0,05). Nivoi ProBNP i plazmatskog TGF-β1 su imali pozitivnu i značajnu korelaciju (P=0,05).

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Conclusions: Plasma TGF-β1 levels were much higher in T2DM patients than in the general population, and the increase in this index was more pronounced in patients who also had heart failure, a diagnostic indicator for T2DM and heart failure.

Keywords: heart failure, NT-proBNP, type 2 diabetes mellitus, TGF-β1

Zaključak: Nivoi plazme TGF-β1 su bili znatno viši kod pacijenata sa T2DM nego u opštoj populaciji, a porast ovog indeksa je bio izraženiji kod pacijenata koji su takođe imali srčanu insuficijenciju, što predstavlja dijagnostički pokazatelj za T2DM i srčanu insuficijenciju.

Ključne reči: srčana insuficijencija, NT-proBNP, dijabetes melitus tip 2, TGF-β1

Introduction

Heart failure (HF) is a complicated clinical syndrome brought on by abnormal ventricular systolic function. It occurs at the end stage of the progression of all types of heart disease, with dyspnea as the primary clinical symptom. Morbidity, readmission, and mortality rates are high, and the 5-year survival rate is similar to that of patients with malignant tumours, which is around 50%. As such, it has emerged as a significant risk factor for human health (1–4). The HF Section of the European Heart Association noted in 2018 that Type 2 diabetes mellitus (T2DM) is a significant risk factor for the development of HF and that it is the cause of HF in about 30% to 40% of patients (5, 6). Patients with T2DM are also 2 to 4 times more likely to develop HF than those without the condition. The clinical symptoms, cardiac function, and even quality of life of T2DM patients are all adversely affected by complicated HF, and the mortality risk is increased by 10–12 times (5). Therefore, reducing the risk of HF in T2DM patients has always been a key direction of clinical research, and there are more clinical studies about risk prediction model analysis at home and abroad, but how to quickly detect and diagnose the occurrence of HF is also one of the key research contents while preventing the risk of HF for T2DM patients. Transforming growth factor-β1 (TGF-β1), one of the pro-fibrotic cytokines, promotes the onset of tissue fibrosis and assumes a crucial role in the physiological changes of myocardial fibrosis, triggering the accumulation of Periostin protein, which reduces the adhesion between cardiomyocytes and myocardial fibroblasts and induces cardiac dilatation, resulting in the development of HF (7–9). Based on the above theory, TGF-β1 was selected as an evaluation index in this study to analyse its diagnostic value in T2DM with HF, and the results are as follows:

Materials and Methods

Research object

Sampling was done among T2DM patients treated at the hospital from June 2021 to June 2023 due to dyspnea, and 150 cases were screened for the study based on the convenience sampling method as the study group. Inclusion criteria: (1) aged 41 years; (2) satisfied the Chinese Guidelines for the Prevention and Treatment of T2DM (2017 edition) (10) diagnostic criteria for T2DM, with a disease duration of more than two years; (3) all complained of the presence of dyspnoea symptoms and suspected HF at the time of admission; (4) had complete clinical data; and (5) signed a written informed consent form for this study. Exclusion Criteria: (1) concomitant renal impairment, acute and chronic inflammation, and tumours; (2) type 1 diabetics; (3) admitted to hospital for acute myocardial infarction; (4) treated with cardiopulmonary resuscitation (CPR) after admission to the hospital; and (5) respiratory distress due to chest trauma. The control group comprised 50 healthy persons who had undergone physical examinations over the same period, were not diabetic, and showed no abnormalities in heart function. The study group included 99 males and 51 females. There were 21 girls and 29 males in the control group, all of whom were 61.78 10.08 years old.

Research methods

Blood samples: Before starting medication, 5 mL of fasting venous blood was drawn from patients. It was centrifuged into two parts – one with anticoagulant and the other without. The supernatant was then obtained to determine the concentrations of 1 NT-proBNP and TGF-β1 in the serum and plasma, respectively.

Diagnostic criteria for HF: The diagnostic criteria for HF from the Chinese Guidelines for Diagnosis and Treatment of HF 2018 (11) were used to assess HF: the presence of HF symptoms, a lowered or normal LVEF, but imaging-detected diastolic dysfunction of the heart, and serum concentrations of NT-proBNP (brain natriuretic peptide precursor) >300 pg/mL.

Grouping: According to the existence or absence of HF, T2DM patients were divided into groups, and the serum NT-proBNP and plasma TGF-β1 levels were compared. Patients were also classified according to the Functional Classification of the New York Heart Association (NYHA) (12). The serum NT-proBNP and plasma TGF-β1 levels of patients with various cardiac function classes were then compared. Patients with T2DM with HF were then categorised according to cardiac function class.

Statistical analysis

The data were statistically analysed using SPSS22.0. The effect of plasma TGF-β1 levels on T2DM patients with HF was analysed using the ROC curve. In T2DM patients with HF, the relationship between plasma TGF-β1 level and serum NT-proBNP was investigated by Pearson, and the diagnostic value of plasma TGF-β1 level in T2DM patients with concurrent HF was investigated using the ROC curve.

Results

Patients with and without HF and HF+T2DM were compared for plasma TGF-β1 levels.

Among the 150 patients with T2DM, there were 96 patients with HF and 54 patients without HF. Among the 96 patients with T2DM with HF, the causes of HF were 64 cases of ischemic heart disease, 13 cases of dilated cardiomyopathy, 9 cases of hypertension, 7 cases of valvular heart disease, 1 of atrial fibrillation, 1 of obesity, and 1 of pulmonary heart disease, respectively, as shown in *Figure 1*.

Plasma TGF-β1 levels in T2DM with HF: diagnostic value

ROC curve analysis of 96 patients with T2DM with HF compared with 54 patients with T2DM without HF within the Research group showed that its cut-off point was 44.50 μg/L, at which time its sensitivity and specificity for diagnosing patients with T2DM. The sensitivity and specificity of concurrent HF were 79.63% and 52.51%, respectively. For details, see *Figure 2* and *Table II*.

Table I Comparison of serum NT-proBNP and plasma TGF-β1 levels in different groups of study subjects.

Group		number of examples	NT-proBNP (pg/mL)	TGF-β1 (mg/L)
Research group	With HF	96	1050.82±369.42*#	49.18±11.89*#
	Without HF	54	196.63±50.20*	35.70±9.11*
	Total	150	725.69±496.40*	44.33±12.72*
Control group		50	80.56±14.08	30.50±8.68

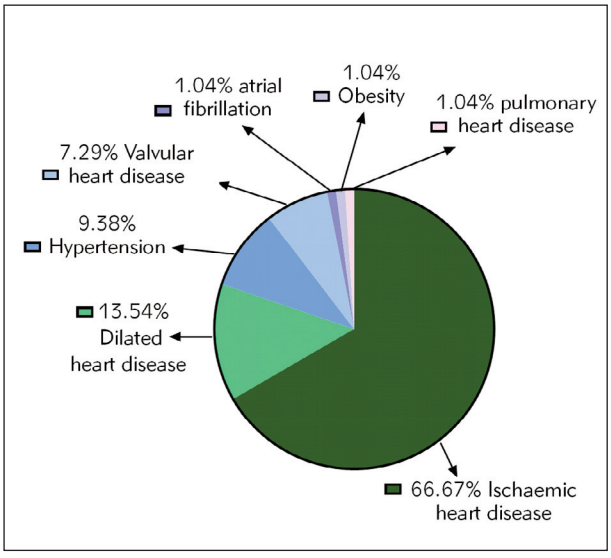

Figure 1 Etiological composition of HF in patients with T2DM with HF.

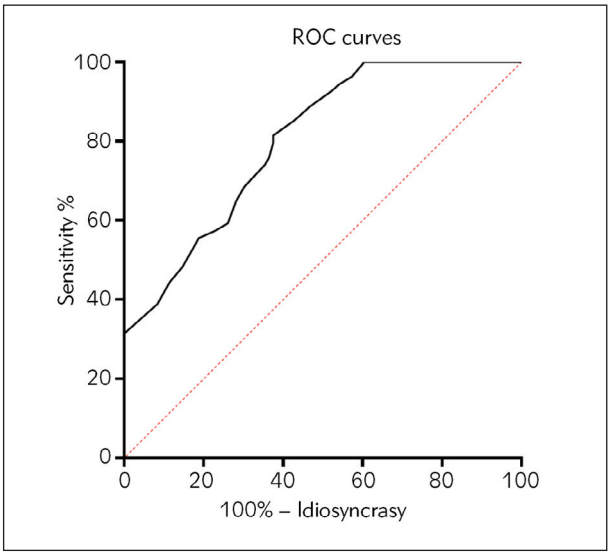

Figure 2 ROC of plasma TGF-b1 levels for the diagnosis of T2DM with HF.

Table II Predictive efficacy of serum Gal-3 levels in the diagnosis of heart failure.

Projects	AUC	P	95%CI	cut-off point (mg/L)	Sensitivity (%)	Idiosyncrasy (%)
TGF-β1	0.803	<0.0001	0.734~0.871	44.50	79.63	52.51

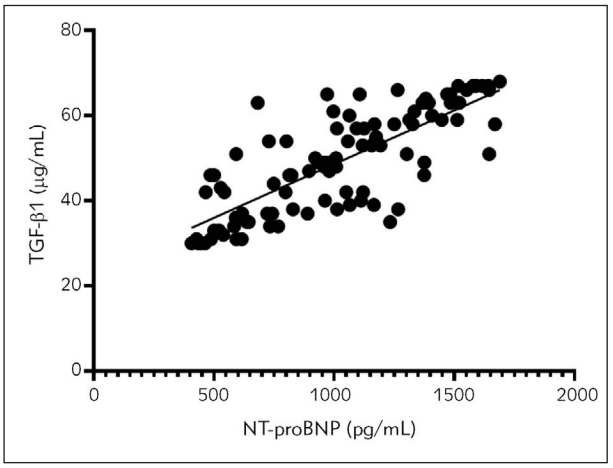

Figure 3 Patients with T2DM and HF were shown a scatter plot of their blood NT-proBNP and plasma TGF-β1 levels.

Comparison of plasma TGF-β1 and serum NT-proBNP levels in T2DM patients with HF with different cardiac functions

31 individuals had NYHA cardiac function Level II, 40 had Level III, and 25 had Level IV among the 96 patients with T2DM with HF. As shown in *Table III*, serum NT-proBNP and plasma TGF-β1 levels were higher in patients with Level III cardiac function compared to those with Level II cardiac function.

Analysis of the relationship between plasma TGF-β1 and serum NT-proBNP in patients with T2DM and HF

Comparing the levels of plasma TGF-β1 and serum NT-proBNP in people with T2DM who had HF and different cardiac functions

Discussion

The study revealed significant findings regarding the association between heart failure and type 2 diabetes mellitus (T2DM) among the study participants. Using a cut-off point of 44.50 g/L, the sensitivity and specificity for diagnosing heart failure in T2DM patients were found to be 79.63% and 52.51%, respectively. Furthermore, individuals with both T2DM and heart failure exhibited notably elevated serum and plasma levels of NT-proBNP and TGF-β1 compared to those without heart failure. Additionally, a positive and significant correlation was observed between proBNP levels and plasma TGF-β1 levels, indicating a potential interplay between these biomarkers in the context of heart failure and T2DM.

Additionally, our study explored the association between circulating biomarkers and HF severity, as assessed by NYHA cardiac function classification. Notably, patients with higher NYHA functional classes

Table III Comparison of plasma TGF-β1 and serum NT-proBNP levels in individuals with T2DM who have HF with various cardiac functions (±s, score).

NYHA Cardiac function classification	Number of examples	NT-proBNP (pg/mL)	TGF-β1 (mg/L)
Level	31	701.32±219.62[ab]	37.65±7.77[ab]
Level	40	1010.40±377.63[b]	49.95±7.86[b]
Level	25	1443.12±192.04	62.24±5.75

Table IV Patients with T2DM and HF who had their plasma TGF-β1 levels and serum NT-proBNP levels examined.

variant	NT-proBNP	
	r	P
TGF-β1	0.784	<0.0001

exhibited elevated serum NT-proBNP and plasma TGF-β1 levels, indicating their potential prognostic significance in gauging HF severity and progression in T2DM patients. These observations are consistent with prior studies demonstrating the prognostic value of NT-proBNP and TGF-β1 in predicting adverse cardiovascular outcomes and mortality in HF patients (13, 14).

Cardiomyopathy, acute and chronic myocardial infarction and other heart-related diseases can cause myocardial injury, inducing abnormal changes in the structure and function of the myocardium, which reduces the contractile function of the patient's heart, decreases the filling function of the ventricle, and ultimately causes the occurrence of chronic HF, leading to severe threats to the patient's physical health (15–17). In addition to direct cardiac disease triggering, T2DM is also an important factor in damaging myocardial function to trigger HF. The incidence of HF in diabetic patients is about 9% to 22% (18–20). In clinical studies in relevant animal models, diabetes causes myocardial steatosis, increasing the thickness of the left ventricular wall, which in turn induces centripetal remodelling of the left ventricle. This process is one of the physiological processes contributing to the development of HF (21, 22).

Our study found that NT-proBNP levels were significantly higher in T2DM patients with HF than those without. This is consistent with other studies. For instance, a study published in the American College of Cardiology found that NT-proBNP levels increase significantly in HF patients (23). Another study found that each doubling of baseline NT-

proBNP was associated with a hazard ratio of 1.17 for CV death or HF hospitalisation (24).

This study showed that TGF-β1 levels were significantly higher in T2DM patients with HF. While limited studies specifically investigate TGF-β1 levels in T2DM patients with HF, TGF-β1 has been studied in other contexts. For example, a study found that serum TGF-β1 levels were significantly lower in patients with coronary artery ectasia than in controls (25). TGF-β1 had a good predictive efficacy for diagnosing concurrent HF in patients with T2DM, with an AUC of 0.803. This is similar to a study on coronary artery ectasia patients, where the AUC value of serum TGF-β1 levels for predicting CAE was 0.64 (25).

TGF-β1 produces reactive oxygen species and the inflammatory response through autocrine pathways, among other physiological processes. Even though all three of TGF-'s isoforms in the human body share many biological similarities, TGF-β1 is the most active and contributes the most to controlling different cell physiological activities (26, 27). TGF-β1 can play a role in the beginning and progression of fibrosis in diabetic nephropathy by causing epithelial mesangialization of renal tissue, which in turn causes glomerular mesangial fibrosis, according to previous clinical research (28, 29). In addition to diabetic nephropathy, cardiovascular complications also have a high incidence in diabetic patients, mainly due to metabolic function abnormalities leading to impaired myocardial function in diabetic patients. Qin Chaoshi et al. (30) showed that TGF-β1 overexpression could activate myocardial oxidative stress and inflammatory response, increase cardiomyocyte apoptosis, and induce myocardial injury in a T2DM cardiomyopathy mouse model. As a result, the current study concluded that TGF-β1 expression is crucial in T2DM with HF.

In this study, among 150 T2DM patients who visited the clinic for dyspnoea, the detection rate of HF was 64.0% (96/150), which shows that HF is more common in T2DM patients, but it is difficult to accurately assess the occurrence of HF solely based on the symptom of dyspnea. Serum TGF-β1 levels in the current study's T2DM patients were higher than those in healthy individuals who did not have diabetes or cardiac impairment, indicating that TGF-β1 is more active in T2DM patients. The current study's T2DM patients had serum TGF-β1 levels that were higher than those of healthy people who did not have diabetes or cardiac impairment, showing that TGF-β1 is more active in T2DM patients. The sensitivity and specificity for diagnosing concomitant HF in patients with T2DM were 79.63%, respectively, and 52.51%. It is suggested that plasma TGF-β1 level has the application value of predicting HF in T2DM patients.

A strong positive correlation ($r=0.784$, $p<0.0001$) between plasma TGF-β1 and serum NT-proBNP levels in patients with T2DM and HF. While no specific studies investigate the correlation between NT-proBNP and TGF-β1 in T2DM patients with HF, both markers have been independently associated with HF in various studies (31–33).

In conclusion, plasma TGF-β1 level has some diagnostic use in T2DM with HF, and patients' plasma TGF-β1 level steadily rises as they experience HF symptoms. Deficiencies in this study: Throughout the course of the study, it was discovered that this index is not only involved in the development of the disease but also in the occurrence of HF. It is, therefore, implied that the reformulation of the representative has the same effect of predicting the prognosis of patients with T2DM with HF.

Prognostic effect

However, since the patients were not followed up in this study, verifying the reformulation conjecture was impossible.

Suggestion for improvement

It is suggested that follow-up studies could be conducted for patients with T2DM with HF diagnosed for the first time and verify the value of plasma TGF-β1 levels in the prognosis of patients with T2DM with HF.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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ASSESSMENT OF THE DIAGNOSTIC VALUE OF SERUM CATHEPSIN S AND ITS CORRELATION WITH HDL SUBCLASSES IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA

PROCENA DIJAGNOSTIČKE VREDNOSTI SERUMSKOG KATEPSINA S I NJEGOVE KORELACIJE SA HDL PODKLASAMA KOD PACIJENATA SA NE-HODGKINOVIM LIMFOMOM

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Summary

Background: Recent findings point to the key role of cathepsin S (CTSS) in the survival of malignant cells, as well as the significance of the anti-apoptotic properties of high-density lipoprotein (HDL) that contribute to enhanced cell survival. The purpose of this study is to analyse CTSS as a potential biomarker in lymphoma. Also, in order to better understand the role of CTSS in the origin and development of lymphoma, its association with cystatin C (Cys C), lipids, and inflammatory markers was analysed.
Methods: The study included 90 subjects: 11 Hodgkin (HL) and 44 B-cell non-Hodgkin lymphoma (NHL) patients, as well as 35 healthy subjects. CTSS was determined using the Invitrogen ELISA kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The non-denaturing 3%–31% polyacrylamide gradient gel electrophoresis method was used to separate plasma HDL particles.

Kratak sadržaj

Uvod: Novija otkrića ukazuju na ključnu ulogu katepsina S (CTSS) u preživljavanju malignih ćelija, kao i na značaj antiapoptotičkih osobina lipoproteina visoke gustoće (HDL) koje doprinose većem preživljavanja ćelija. Cilj ove studije je analizirati CTSS kao potencijalni biomarker kod limfomu. Takođe, kako bi se bolje razumela uloga CTSS-a u nastanku i razvoju limfoma, analizirana je njegova povezanost sa cistatinom C (Cys C), lipidima i upalnim markerima.
Metode: U istraživanje je bilo uključeno 90 ispitanika: 11 bolesnika sa Hodgkinovim (HL) i 44 bolesnika sa B-ćelijskim ne-Hodgkinovim limfomom (NHL), te 35 zdravih ispitanika. Za merenje serumskog CTSS je korišćen Invitrogen ELISA kit (Thermo Fisher Scientific, Inc., Waltham, MA, SAD). HDL subfrakcije su razdvojene metodom vertikalne elektroforeze na gradijentu poliakrilamida.

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List of abbreviations: HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; SLL, small lymphocytic lymphoma; LPL, lymphoplasmacytic lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; CTSS, cathepsin S; Cys C, cystatin C; LDH, lactate dehydrogenase; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP, C-reactive protein; IL-6, interleukin-6; AUC, area under the receiver operating characteristic; ROC, receiver operating characteristic.

Results: The level of CTSS was significantly higher in NHL patients than in control subjects: 12.20 (9.75–14.57) vs 9.97 (8.44–10.99), P<0.001. In NHL patients, there was a positive correlation between CTSS and the proportions of HDL3a, HDL3b, and the sum of the HDL3 subclasses (r=0.506, P<0.001; r=0.411, P=0.006, r=0.335, P=0.026, respectively). In addition, the area under the receiver operating characteristic curve (AUC curve) of CTSS was 0.766 (CI: 0.655–0.856) for NHL patients. There was no significant difference in CTSS values between the control group and patients with HL, nor significant correlations between CTSS and HDL subclasses in the HL group.
Conclusions: CTSS is significantly elevated in patients with NHL and has the potential to be a new diagnostic biomarker for the detection of NHL. Also, this study was the first to unveil the association between serum CTSS levels and the proportions of anti-apoptotic HDL3a and HDL3b subclasses in NHL patients.

Keywords: cathepsin S, cystatin C, HDL3a and HDL3b subclasses, apoptosis, Hodgkin and non-Hodgkin lymphoma

Rezultati: Nivo CTSS bio je značajno viši kod pacijenata sa NHL-om nego kod ispitanika kontrolne grupe: 12,20 (9,75–14,57) naspram 9,97 (8,44–10,99), P<0,001. Takođe, kod pacijenata s NHL-om postojala je pozitivna korelacija između CTSS i proporcije HDL3a, HDL3b i HDL3 potklasa (r=0,506, P<0,001; r=0,411, P=0,006, r=0,335, P =0,026). Osim toga, površina ispod ROC krive (AUC) CTSS bila je 0,766 (CI: 0,655–0,856) za NHL pacijente. Nije bilo značajne razlike u vrednostima CTSS između kontrolne grupe i pacijenata sa HL-om, kao niti značajnih korelacija između CTSS i HDL podklasa.
Zaključak: CTSS je bio značajno povišen kod pacijenata sa NHL što pokazuje da CTSS ima potencijal novog dijagnostičkog biomarkera za otkrivanje NHL. Takođe, ovo istraživanje je prvo koje je pokazalo da postoji korelacija između serumskog CTSS i udela anti-apoptotskih podklasa HDL3a i HDL3b kod pacijenata s NHL-om.

Ključne reči: katepsin S, cistatin C, HDL3a i HDL3b subklase, apoptoza, Hodgkinov i ne-Hodgkinov limfom

Introduction

Lymphomas are widely distributed hematologic malignancies that originate from malignantly transformed B and T lymphocytes, as well as NK cells. According to the World Health Organization classification, lymphomas represent a heterogeneous group with greater than 80 subtypes (1). These cancers are categorised into two main groups, the most common of which is non-Hodgkin lymphoma (NHL) and the less frequent group of Hodgkin lymphoma (HL). The diagnosis of lymphomas at an initial stage is essential; therefore, looking for a new, good serum marker is important to improve lymphoma diagnosis, monitoring, and treatment (2).

Review articles point out the involvement of cathepsin S (CTSS) in various processes, such as tumour microenvironment remodelling, angiogenesis promotion, tumour migration and growth (3, 4). Given that CTSS released from lysosomes triggers apoptosis by activating pro-apoptotic Bid and degrading anti-apoptotic Bcl-2 family, cathepsins are involved in the control of cell death or survival (3).

CTSS is a lysosomal protease that plays an important role in the catabolism of intracellular proteins, commonly with an activity optimum at an acidic pH (5). Both acidic tumour microenvironment and glycosaminoglycans accelerate the autocatalytic activation of cathepsins and enhance their stability in the extracellular matrix (ECM) (6). CTSS plays a key role in cancer progression, angiogenesis, and metastasis, particularly through its ability to degrade the ECM (7). Altogether, the tumour cells overexpress cysteine cathepsins to increase opportunities for survival, proliferation, motility, and invasion. Recent studies have shown that inhibition of CTSS reduces angiogenesis, increases apoptosis, and reduces tumour volume and invasion. Therefore, CTSS could be a viable target for cancer treatment (8).

Besides that, recent evidence suggests that during lymphomagenesis, a decrease in circulating high-density lipoprotein cholesterol (HDL-C) may exist (9, 10). However, some studies indicated that CTSS could potentially affect not only HDL but also low-density lipoprotein (LDL) (11). The cathepsin-secreting cells induce rapid depletion of lipid-poor (pre-beta-HDL) and lipid-free apoA-I while, on the other hand, inhibiting cellular cholesterol efflux (12). Namely, the transport of HDL-mediated cholesterol is closely associated with malignant cell survival and tumour development (13). In that way, previous studies demonstrated that HDL particles stimulated the growth and proliferation of breast carcinoma, primarily HDL3 particles (13, 14). Also, the knowledge about the anti-apoptotic properties of HDL particles in atherosclerosis is also increasingly strengthening (15), but there is a lack of such studies in the field of lymphoma.

Cysteine proteinase inhibitor, cystatin C (Cys C), is necessary for regulating intracellular and extracellular protein degradation. Previous research has demonstrated that Cys C is involved in altering the proteolytic system in cancer in such a way that elevated levels of plasma Cys C are associated with a poor prognosis in these patients (16, 17).

Additionally, regarding the inflammatory aspect, several studies suggested that CTSS might contribute to the inflammation process in various diseases, including cancer (4, 18).

The aim of the study was to analyse the association of CTSS with Cys C, HDL subclasses, cholesterol, and inflammatory markers in lymphoma patients in order to better understand the mechanisms of lymphomagenesis and lymphoma progression. Additionally, the objective was to evaluate the CTSS as a potential biomarker in lymphoma.

Materials and Methods

Administrative study procedures

The study was conducted at the University Clinical Centre of the Republic of Srpska, Banja Luka, Bosnia and Herzegovina in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the University Clinical Centre of the Republic of Srpska, Banja Luka (No 01-19-51-2/20), and at the Faculty of Medicine at the University of Banja Luka (No 18/4.3.95/2020). Informed consent was obtained from all subjects involved in the study.

Subjects

The study enrolled 55 newly diagnosed lymphoma patients between July 2020 and April 2022. Of the 55 patients, 11 patients had HL, 44 had NHL, and all 44 patients were B-cell NHL. Inclusion and exclusion criteria for the study group, along with those for the control group, as well as lymphoma classification, therapy, and monitoring of therapy success, are described in detail in a recently published paper by Mirjanic-Azaric et al. (18). Only 25 patients were evaluated in the middle of treatment therapy (point of reassessment). Demographic and clinical data of the participants, such as age, sex, and clinical stage, were obtained from their medical history.

Laboratory analyses

The patient's blood samples were obtained before the 18F-2-fluoro-2-deoxy-D-glucose-positron 415 emission tomography/computed tomography (FDG-PET/CT), after overnight fasting (first time-point) and at the middle of the treatment immunochemotherapy (point of reassessment, second time-point) also after fasting, the same day of (and prior to) the FDG-PET/CT diagnostics. The blood was collected into one EDTA sample tube (for plasma) and one serum sample tube (for serum) before immediate centrifugation at 1500×g for 10 min at 4 °C for plasma and 3000×g for 10 min for serum and then stored at -80 °C until required.

CTSS levels were measured using the Invitrogen ELISA kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA), following the manufacturer's protocol. In brief, serum diluted 1:100 was added to microtiter plate wells pre-coated with the monoclonal antibody for CTSS capture. The minimum detectable dose of human CTSS was 4 pg/mL. The analysis of the standard curve and triplicate samples confirmed that the coefficient of variation (CV) was <10% for intra-assay and <12% for inter-assay, as instructed by the manufacturer.

The non-denaturing 3%–31% polyacrylamide gradient gel electrophoresis method was employed to separate plasma HDL particles (19).

Cys C was measured using the Cobas e 801 analyser (Roche Diagnostics GmbH, Mannheim, Germany), interleukin-6 (IL-6) and ferritin levels were assessed using the ADVIA Centaur XP system (Siemens Healthineers USA, United States). Total cholesterol (TC), LDL-C, HDL-C, LDH, and CRP were quantified using standard procedures on an Alinity Abbott analyser (Abbott Laboratories, IL, USA).

FDG -PET/CT scan

Baseline and posttherapy FDG- PET/CT were performed on the same scanner (Discovery 610, GE Healthcare, Milwaukee, WI, USA). The response to therapy was evaluated using the Deauville five-point scale (Deauville criteria) (20). Patients were categorised into three groups: complete metabolic responders (score 1 or 2), partial metabolic responders (score 3 or 4), and non-responders/progressive metabolic disease (score 5).

Statistical analysis

Data are presented as mean ± standard deviation for normally distributed continuous variables, median with interquartile range for skewed data and relative and absolute frequencies for categorical variables. Continuous variables were compared between three groups using ANOVA with Tukey's post hoc test for subgroup differences or Kruskal Wallis with Mann Whitney as a post hoc test. Analysis of covariance (ANCOVA) and Quade's test were applied to investigate the influence of age and gender as confounders on the difference in normally distributed and skewed variables, respectively. Categorical variables were tested using the Chi-squared test. The correlations between the variables were estimated using Spearman's correlation coefficient (r). Discriminative abilities of investigated parameters for lymphoma detection were assessed by receiver operating characteristic (ROC) curve analysis, and accuracy was presented as the area under the receiver operator characteristic curve (AUC) (21). The statistical analyses were performed with PASW Statistics, v. 27, software (Chicago, Illinois, USA).

Results

Baseline patient characteristics and differences in examined parameters in the study

The median age of control subjects with an interquartile range was 49 (36–55), and they were significantly younger than NHL patients whose median age was 65 (55–72), P<0.001. The HL

Table I Main clinical characteristics of HL and NHL patients.

	HL-patients, n=11	NHL-patients, n=44	P-value
Stage of cancer			
Stage I, n (%) Stage II, n (%) Stage III, n (%) Stage IV, n (%)	/ 3 (27.27) 2 (18.18) 6 (54.54)	4 (9.09) 6 (13.63) 10 (22.73) 24 (54.55)	0.615
Subtype of NHL			
DLBCL, n (%) Follicular, n (%) Burkitt, n (%) SLL, n (%) LPL, n (%) MCL, n (%) MZL, n (%)	/	14 (31.81) 21 (47.73) 1 (2.27) 4 (9.10) 1 (2.27) 2 (4.55) 1 (2.27)	/

HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; DLBCL, diffuse large B- cell lymphoma; SLL, small lymphocytic lymphoma; LPL, lymphoplasmacytic lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; Compared by Chi square test.

Table II Comparison of serum biomarkers of healthy subjects and patients with HL and NHL.

	Controls n=35	HL patients n=11	NHL patients n=44	P-value
CTSS (μg/L)	9.97 (8.44–10.99)	10.83 (9.23–12.40)	12.20* (9.75–14.57)	0.002
Cys C (mg/L)	0.82 (0.74–0.90)	0.81 (0.74–1.13)	1.03* (0.88–1.24)	<0.001
LDH (U/L)	118 (103–153)	195* (137–218)	164* (137–203)	<0.001
TC (mmol/L)	5.43±0.95	4.08±0.62*	5.12±1.25 †	<0.001
HDL-C (mmol/L)	1.47±0.30	1.07±0.25*	1.29±0.39*	<0.001
LDL-C (mmol/L)	3.45±0.92	2.51±0.73*	3.37±1.00†	0.009
HDL 2b (%)	38.9±11.1	48.7± 10.6	39.8±13.2	0.062
HDL 2a (%)	21.2±7.7	22.3±4.5	20.3±4.2	0.564
HDL 2 (%)	60.1±14.4	71.0±15.1	60.1±14.3	0.356
HDL 3a (%)	15.2±5.5	12.9±2.5	14.1±4.0	0.300
HDL 3b (%)	10.6±4.5	7.0±3.3*	9.4±3.6	0.022
HDL 3c (%)	13.8±8.0	9.1±5.1	16.54±11.2	0.074
HDL 3 (%)	39.6±14.4	29.0±9.5	39.9±14.3	0.058
CRP (mg/L)	0.80 (0.40–2.80)	29.05* (3.53–51.95)	2.70* (0.85–5.85)	<0.001
Ferritin (μg/L)	27.0 (13.0–64.0)	227.0* (150.5–394.5)	78.5*† (29.0–238.0)	<0.001
IL-6 (ng/L)	1.1 (0.7–2.4)	6.8* (2.1–14.0)	2.0* (1.2–3.9)	<0.001

Compared by ANOVA or Kruskal-Wallis test, *significantly different from the control group by Tukey test ($p<0.05$) or Mann-Whitney test; †significantly different from HL group by Tukey test or Mann-Whitney test ($p<0.05$). Abbreviations: CTSS, cathepsin S; Cys C, cystatin C; LDH, lactate dehydrogenase; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP, C-reactive protein; IL-6, interleukin-6.

Table III Markers significantly different between the control group and the group of patients with follicular lymphoma and DLBCL before therapy.

	Controls n=35	Follicular lymphoma (indolent) n=21	DLBCL (aggressive) n=14	P-value
CTSS (μg/L)	9.97 (8.44–10.99)	11.8* (8.35–14.28)	13.64* (11.85–15.58)	<0.001
Cys C (mg/L)	0.82 (0.74–0.90)	1.03* (0.89–1.36)	1.03* (0.88–1.19)	<0.001
LDH (U/L)	118 (103–153)	168* (147–198)	154* (122–190)	0.002

Compared by ANOVA or Kruskall Wallis test, *significantly different from control group by Tukey test ($p < 0.05$) or Mann Whitney test; Abbreviations: DLBCL, diffuse large B- cell lymphoma, CTSS, cathepsin S; Cys C, cystatin C; LDH, lactate dehydrogenase.

Table IV Significant correlations between CTSS and proportion of the HDL subclasses, HDL-C and CRP in NHL before therapy.

Laboratory parameters	HDL2b (%) r (P)	HDL3a (%) r (P)	HDL3b (%) r (P)	HDL3 (%) r (P)	HDL-C (mmol/L) r (P)	CRP (mg/L) r (P)
CTSS (μg/L)	-0.328 (0.030)	0.506 (<0.001)	0.411 (0.006)	0.335 (0.026)	-0.424 (0.004)	0.351 (0.019)

r, Spearman's correlation coefficient; P, level of significance. CTSS, cathepsin S; HDL high-density lipoprotein; HDL-C, high-density lipoprotein-cholesterol; CRP, C-reactive protein.

group consisted of the younger subjects [34 (24–71)], but their age was not significantly different from that of the previous groups. The distribution of genders was significantly different across the groups. Males were more prevalent in HL and NHL patient groups [6 (54.5%) and 22 (50.0%), respectively] than in controls [9 (25.7%), P=0.025]. The results showed that patients with HL and NHL were at similar disease stages at the baseline (*Table I*). Among NHL subtypes, the follicular subtype of the disease was the most prevalent (47.73%), followed by diffuse large B-cell lymphoma (DLBCL) (31.81%) and small lymphocytic lymphoma (SLL) (9.10%) (*Table I*).

The Deauville score was analysed in 25 lymphoma patients before and after therapy (6 HL and 19 NHL patients). The most frequent Deauville scores were 1 (33.33%) and 5 (33.33%) in HL patients, and scores 1 (31.58%) and 3 (36.84%) in the HNL group. Of the 25 patients, 32% had a Deauville score of 1, a score of 2 had 4%, 28% had a score of 3, 16% had a score of 4, and 24% had a score of 5.

In *Table II*, the laboratory parameters are examined. Levels of CTSS and CyS were significantly higher in NHL patients than in control subjects. As expected, LDH was significantly higher in both lymphoma groups than in controls.

We additionally evaluated the difference in normally distributed parameters (TC, HDL-C and LDL-C) between healthy, HL and NHL subjects using ANCOVA, with age and gender included as covariates. Differences in TC, HDL-C and LDL-C between groups were not confounded by age and gender (P=0.004, P=0.012, and P=0.013, respectively). However, CTSS, Cys C, LDH, and inflammatory parameters were compared using Quade's test, which had the same confounders. Age and gender did not influence a difference in CTSS (P=0.036), CyS C (P=0.041), LDH and inflammatory parameters (P<0.001) between groups.

In view of its heterogeneity, NHL patients were divided into the two most represented lymphoma subtypes in our research: follicular lymphomas and DLBCL. Accordingly, we compared follicular lymphoma and DLBCL patients with the control group, and the results are presented in *Table III*. All significantly different parameters were higher in both groups compared to the control subjects. There was no significant difference for CTSS, Cys C and LDH between follicular and DLBCL.

The correlation of CTSS with a proportion of the HDL subclasses and inflammatory parameters in NHL patients

In HL patients, only a correlation between CTSS and Cys C was shown (r=0.685, P=0.014). In NHL patients, serum CTSS showed a negative correlation with HDL-C and the HDL2b subclasses but a positive correlation with the proportion of the HDL3a, HDL3b, and the sum of the HDL3 subclasses (r=0.506, P<0.001; r=0.411, P=0.006; r=0.335, P=0.026, respectively) (*Table IV*). Regarding the inflammatory biomarkers, there was a positive correlation between CRP and CTSS.

The results of diagnostic values of serum biomarkers in NHL patients

In further analyses, the receiver operating characteristic (ROC) curve was applied to investigate the accuracy of the analysed parameters in discriminating between NHL and control subjects. The highest discriminatory ability was found for CTSS with AUC=0.766, indicating a moderate diagnostic value for the detection of NHL. The optimal cut-off value for CTSS was 10.56 μg/L with a sensitivity and specificity of 73% and 74%, respectively. *Figure 1* presented only parameters with an accuracy higher than 0.7.

The changes in CTSS between two follow-up points in patients with lymphoma

Figure 2A shows significant changes in the CTSS after therapy in patients divided according to disease stages. CTSS levels significantly increased by

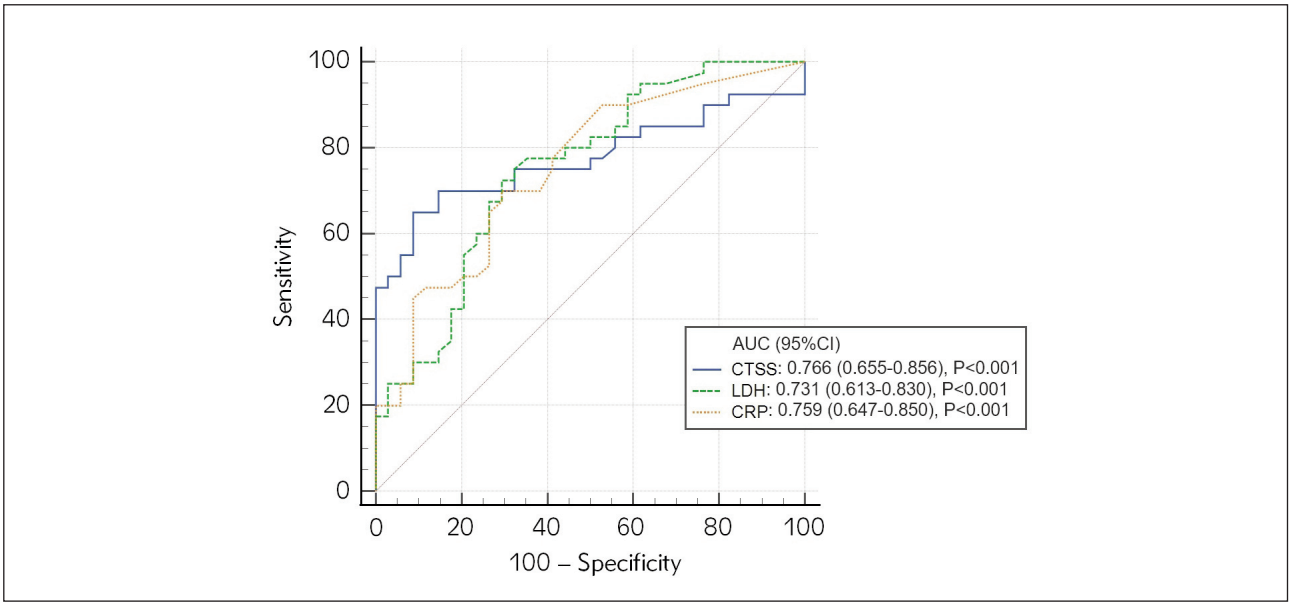

Figure 1 AUCs for CTSS in discrimination NHL patients from healthy subjects.
Abbreviations: AUC, the area under the ROC curve; CTSS, cathepsin S; LDH, lactate dehydrogenase; CRP, C-reactive protein

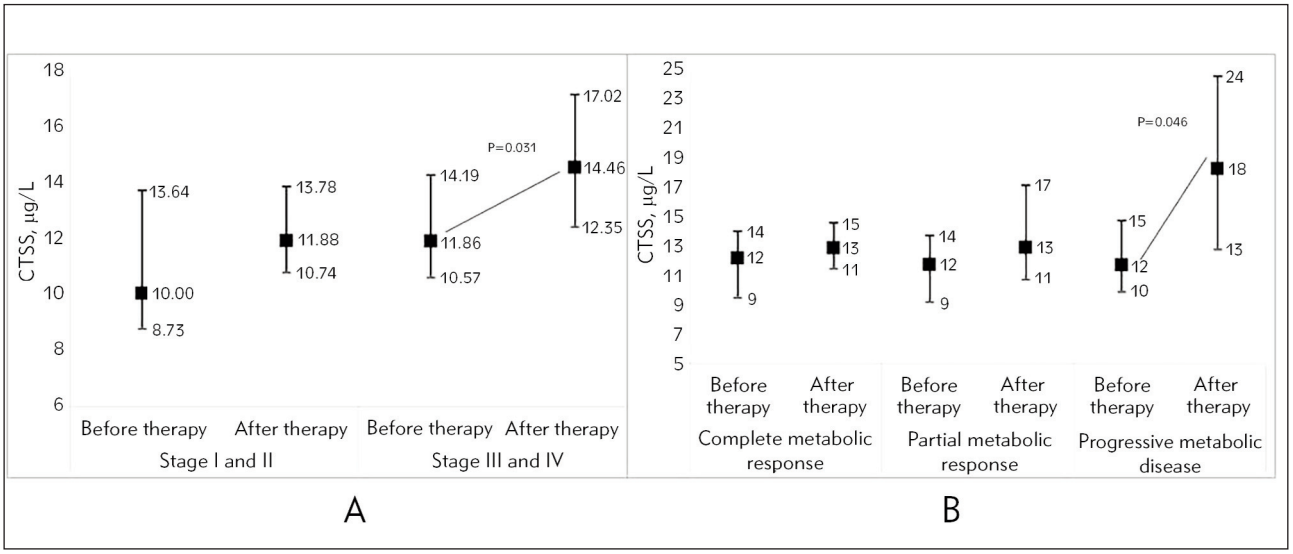

Figure 2 CTSS changes after therapy.
Figure 2 A, according to disease stages; Figure 2 B according to response to therapy Abbreviation: CTSS, cathepsin S.

approximately 12% in patients with disease stages III and IV. Additionally, patients were divided according to response to therapy (*Figure 2B*). Patients with progressive metabolic disease (Deauville score 5, n=6) had increased CTSS levels by 25% after therapy. After therapy in patients with partial metabolic response (n=11, Deauville scores 3 and 4) and patients with complete metabolic response on therapy (n=8, Deauville scores 1 and 2), CTSS changes were without statistical significance.

Discussion

The presented results of the study showed that the serum CTSS and Cys C levels were significantly elevated in NHL patients compared to healthy subjects. These results are concordant with similar cross-sectional studies, which suggested that CTSS may have several potential implications in the development and progression of carcinoma (22–24).

Increased values of CTSS in NHL could lead to an impaired apoptosis control mechanism and increased cell survival. Furthermore, extensive research has indicated that CTSS can be a therapeutic target (8), as supported by the results of our study. The elevated CTSS levels could be elucidated by a mechanism suggested by Dheilli et al., (25) namely, cancer cells interact with immune cells called CD4+ T-cells when CTSS is active, which helps tumours to grow. At the same time, they maintain distance from CD8+ T-cells, which would attack and kill cancer cells. Contrary to a study by Ma et al. (26), we did not observe a high level of CTSS in the HL group. This finding may result from different tumour microenvironments in HL characterised by a minority of neoplastic cells and an extensive inflammatory milieu (27). Also, NHL generally involves a wider range of cells in the lymphatic system compared to HL and CTSS derived from tumour cells, and cells within the tumour microenvironment contribute to tumorigenesis.

The role of Cys C has been proposed in relation to the modification of the proteolytic system in cancer. The findings that Cys C levels were higher in NHL patients than in healthy subjects are in accordance with previous studies (16, 28, 29). Cystatins effectively inhibit a small amount of catalytically active proteases. Therefore, it may be assumed that the disturbance of signalling pathways, as seen in cancer, probably disables Cys C from inhibiting large amounts of CTSS, and that is probably why there was not a correlation between these two biomarkers.

When considering lipid parameters, only LDL-C values were notably lower in HL patients than in the control group. During an inflammatory state, also increased levels of reactive oxygen species can lead to the oxidation of LDL into oxidised LDL (ox-LDL) and a decrease in circulating LDL because oxidised LDL is taken up by macrophages at the site of inflammation. Furthermore, this is likely due to significantly elevated IL-6 values within this group of patients; it is widely recognised that cytokines can contribute to dyslipidemia (30). Additionally, *in vitro* studies demonstrated the increased uptake of cholesterol from plasma by malignant cells to meet their own proliferation (31).

Low levels of HDL-C in haematological cancers indicate that HDL particles may play a crucial role in maintaining strict control over the proliferation and homeostasis of the hematopoietic system, potentially impeding malignant transformation (9, 10, 30). In this study, significantly lower values of HDL-C were found in both types of lymphoma. These results are similar to those obtained in the study by Pedersen et al., (32), which showed that patients with low HDL-C have an increased risk for haematological cancers. The Spearman correlation analysis showed that circulating CTSS was negatively correlated with HDL-C in NHL. Increased cell proliferation and reduced apoptosis in lymphoma, which could be associated with elevated levels of CTSS, have led to higher cholesterol consumption and, consequently, reduced circulating HDL-C levels. This is also an explanation for the negative correlation of CTSS with HDL2b, considering that the HDL2 subclasses are significantly richer in cholesterol compared to the HDL3 subclasses. In NHL patients, we revealed a positive association of CTSS with HDL3a, HDL3b, and HDL3 lipoprotein particles. HDL3 subclasses are small, dense, protein-rich subclasses that exhibit pronounced anti-apoptotic, anti-oxidative, and anti-inflammatory properties (33, 34). Molecules with anti-apoptotic effects can block specific enzymes or proteins in apoptotic signalling pathways, thereby disrupting or preventing programmed cell death. The strong connection between CTSS and these particles could be attributed precisely to the anti-apoptotic characteristics of these subclasses. As far as we know, this is the first study that has shown an association between CTSS and the HDL3a and HDL3b subclasses, and the reduced apoptosis present in cancer could also be a consequence of this association. Moreover, the first demonstrated a positive correlation between CTSS levels and the proportion of anti-apoptotic subclasses HDL3a and HDL3b, which will enhance the understanding of disease mechanisms in NHL, potentially contributing to the development of new therapeutic strategies for this cancer.

This study supports the hypothesis that inflammation contributes to the development of cancer (35–37). Contrary to the study conducted by Preti et al. (35), IL-6 did not prove to have diagnostic potential in patients with NHL.

Finding new and non-invasive biomarkers for early cancer detection has become crucial nowadays.

ROC curve analysis demonstrated that CTSS can serve as a diagnostic marker for NHL. The AUC value for CTSS was higher than that of LDH, although LDH is still considered a valuable marker in the diagnosis and monitoring of NHL therapy.

This study demonstrates that the progression of cancer to later stages (stages III and IV) is associated with higher levels of serum CTSS compared to the early stages (stages I and II). Furthermore, the rise of CTSS levels after therapy with a Deauville score of 5 indicated that CTSS may be important in monitoring the success of lymphoma treatment. Observing lower levels of CTSS in patients with Deauville score 1 or 2, we emphasise that CTSS can indicate disease remission status.

It is important to note that this study has a relatively small number of patients. Due to vis major and unforeseen consequences of the COVID-19 pandemic on travelling restrictions and diagnostics availability, a significant number of our patients were unable to return for a reassessment of FDG-PET/CT after treatment. We consider this to be a significant limitation of our research. Based on these findings, further research will be customised to specific patient groups, taking into account distinctions in lymphoma type, severity, and prognosis. We strongly believe that some other studies with a larger number of patients will confirm these results.

Conclusions

CTSS is significantly elevated in patients with NHL but not in HL and has the potential to be a new diagnostic biomarker for the detection of NHL. New biomarkers are significant not only for diagnosis but also for their ability to uncover new avenues for therapeutic intervention. Additionally, this study was the first to unveil the association between CTSS levels and the proportions of HDL3a and HDL3b subclasses in NHL patients, which could play a pivotal role in the enhanced survival of cancer cells. Further studies with more patients are needed to confirm the diagnostic potential of serum CTSS in NHL.

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Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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RELATIONSHIP BETWEEN HBV RNA LEVEL AND PREGNANCY OUTCOMES AMONG HEPATITIS B CARRIERS

ODNOS IZMEĐU NIVOA HBV RNK I ISHODA TRUDNOĆE KOD NOSILACA HEPATITISA B

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Summary

Background: This study aims to investigate the relationship between hepatitis B virus (HBV) RNA level and pregnancy outcomes among hepatitis B carriers.
Methods: This study collected pregnant women who attended the Affiliated Hospital of Guizhou Medical University (Guizhou, China) from June 2020 to June 2023. The levels of HBV DNA, HBV RNA, and HBeAg status in HBV carriers were detected. Pregnancy outcomes including intrahepatic cholestasis of pregnancy (ICP), gestational hypertension (GH), pre-eclampsia, gestational diabetes mellitus (GDM), preterm prelabour rupture of membranes (PPROM), mode of delivery, preterm birth, low birth weight (LBW) and macrosomia.
Results: A total of 562 pregnant women were collected, 203 (36.12%) were infected with HBV. Compared with HBsAg negative, HBsAg positive pregnant women had a higher risk of ICP. There were no significant differences in the rates of GDM, GH, pre-eclampsia, PPROM, preterm birth, LBW, macrosomia, and mode of delivery among women in the two groups. Multivariate logistic regression analysis showed that maternal HBV RNA level (OR = 3.814, 95% CI: 2.036~7.142, P< 0.001) was an independent risk factor for ICP in HBsAg-positive pregnant women. The receiver operating characteristics (ROC) curve revealed that the areas under the curve of HBV RNA for prediction of ICP was 0.8652(95% confidence interval 0.7636–0.9669, P< 0.001).
Conclusions: The HBV RNA level has a significant negative impact on pregnancy outcomes. It may serve as an indica-

Kratak sadržaj

Uvod: Ova studija ima za cilj da istraži vezu između nivoa RNK virusa hepatitisa B (HBV) i ishoda trudnoće kod nosilaca hepatitisa B.
Metode: Ova studija je prikupila trudnice koje su pohađale pridruženu bolnicu Medicinskog univerziteta Guizhou (Guizhou, Kina) od juna 2020. do juna 2023. Otkriveni su nivoi HBV DNK, HBV RNK i HBeAg statusa kod HBV nosilaca. Ishodi trudnoće uključujući intrahepatičnu holestazu trudnoće (ICP), gestacijsku hipertenziju (GH), preeklampsiju, gestacijski dijabetes melitus (GDM), prevremeno preporođajnu rupturu membrana (PPROM), način porođaja, prevremeni porođaj, nisku porođajnu težinu (LBV) i makrozomiju.
Rezultati: Prikupljeno je ukupno 562 trudnice, 203 (36,12%) su inficirane HBV-om. U poređenju sa HBsAg negativnim, HBsAg pozitivne trudnice su imale veći rizik od ICP. Nije bilo značajnih razlika u stopama GDM, GH, preeklampsije, PPROM, prevremenog porođaja, LBV, makrozomije i načina porođaja među ženama u dve grupe. Multivarijantna logistička regresiona analiza je pokazala da je nivo HBV RNK kod majke (OR = 3,814, 95% CI: 2,036~7,142, P< 0,001) bio nezavisan faktor rizika za ICP kod HBsAg pozitivnih trudnica. Kriva radnih karakteristika prijemnika (ROC) je otkrila da su površine ispod krive HBV RNK za predviđanje ICP-a bile 0,8652 (95% interval poverenja 0,7636–0,9669, P< 0,001).
Zaključak: Nivo HBV RNK ima značajan negativan uticaj na ishod trudnoće. Može poslužiti kao indikator za prevenciju ICP-a i poboljšanje zdravlja majki.

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tor to guide the prevention of ICP and improve maternal health.

Keywords: hepatitis B virus, serum marker, HBV RNA, pregnancy outcomes, intrahepatic cholestasis of pregnancy

Ključne reči: virus hepatitisa B, serumski marker, HBV RNA, ishodi trudnoće, intrahepatična holestaza trudnoće

Introduction

HBV infection is a significant global public health issue, with approximately 296 million people chronically infected with HBV worldwide and more than 800,000 deaths a year from the disease (1). Traditional HBV serum markers such as HBsAg, HBeAg, and HBV DNA still have limitations in terms of their effectiveness in predicting clinical outcomes, then several new serological markers are being investigated (2). In recent years, several studies have found that serum HBV RNA can reflect the level and transcriptional activity of HBV covalently closed circular DNA (cccDNA) in the liver, and may be used to monitor the disease progression and predict the prognosis of patients with chronic HBV infection (3).

For a long time, the impact of hepatitis B virus infection on pregnant women's pregnancy results has been a problem of concern to the majority of patients. The impact of HBV infection on the occurrence of pregnancy complications in pregnant women remains controversial. Some studies suggest that HBV infection is associated with pregnancy complications, while others argue that there is no such relationship.

This study collected blood samples from 203 pregnant women with HBV during pregnancy, HBV DNA, HBV RNA level, and HBeAg status were detected. GH, pre-eclampsia, GDM, delivery mode, preterm birth, LBW, and macrosomia were recorded in hepatitis B carriers. To investigate the relationship between HBV RNA, a novel clinical marker, and pregnancy complications.

Materials and Methods

Study design and populations

This study collected pregnant women admitted to the Affiliated Hospital of Guizhou Medical University from June 2020 to June 2023. The diagnosis of hepatitis B carriers was consistent with the 2022 update of the Guideline of Prevention and Treatment for Chronic Hepatitis B in China (4).

Inclusion Criteria: 1) patients >18 years; 2) patients able to attend follow-up visits as required and signed an informed consent form.

Exclusion Criteria: 1) patients with HIV infection and hepatitis C virus infection and other infectious diseases; 2) patients with non-alcoholic steatohepatitis, autoimmune hepatitis, and cirrhosis and other hepatobiliary diseases; 3) patients with malignant tumors and hematological diseases; 4) patients who have experienced miscarriage, termination of pregnancy, stillbirth, or multiple pregnancies.

Informed consent was obtained from all subjects and this study protocol was approved by the Ethics Committee of the Affiliated Hospital of Guizhou Medical University (Guizhou, China) and performed in accordance with the relevant provisions of the Helsinki Declaration.

Data collection

The general data and clinical information, including Age, ethnicity, parity status, HBeAg status, HBV DNA, and HBV RNA were collected. All blood samples were collected during pregnancy. The OMEGA Viral RNA Kit (Omega Bio-Tek, Biotek Winooski, VT, USA) was used for the extraction of HBV RNA from serum. Serum RNA was measured by quantitative reverse transcription-polymerase chain reaction (RT-qPCR). The specific primers (including HBV RNA RT primer 5-ACC ACG CTA TCG CTA CTC AC (t17) GWA GCT C) used were designed according to van Bömmel et al. (5) HBV DNA was measured using a 7,500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The HBeAg status was examined with HBeAg ELISA Kit (Jingmei Biotechnology, Jiangsu, China) using the Synergy™ H4 Multi-function microplate reader (BioTek, Biotek Winooski, VT, USA). HBV DNA <100 IU/mL, HBeAg <0.5 PEIU/mL was reported as negative.

Statistical analysis

Statistic Package for Social Science (SPSS) version 27.0 (SPSS Inc., Chicago, IL, USA) was used to perform statistical analyses. Rates (n, %) indicate categorical variables and were analyzed by chi-square test. Logistic regression analysis was used to determine risk factors of ICP. The ROC curve was constructed to determine the clinical diagnostic value of ICP. Serum HBV RNA levels and HBV DNA levels were log-Transformed. A two-sided $P<0.05$ was considered a statistically significant difference.

Results

Patient population

A total of 562 pregnant women were collected, and 203 (36.12%) were infected with HBV. The basic characteristics of patients are shown in *Table I*.

Comparison of pregnancy outcomes between HBsAg positive and negative pregnant women

The rates of cholestasis during pregnancy in HBsAg-positive pregnant women were significantly higher than those in HBsAg-negative pregnant women (χ^2=10.200, P=0.001, *Table II*). There was no significant difference in other pregnancy outcomes between the two groups (P > 0.05, *Table II*).

Factors related to ICP

Table III shows that by univariate logistic regression analysis, risk factors affecting ICP include: HBV RNA level (OR =3.449, 95% CI: 2.005~5.932, P< 0.001) and HBV DNA level (OR =2.066, 95% CI: 1.338~3.188, P< 0.001). Similarly, HBV RNA level (OR = 3.814, 95% CI: 2.036~7.142, P< 0.001) and HBV DNA level (OR = 2.392, 95% CI: 1.345~4.256, P=0.003) were independently risk factors for ICP by multivariate logistic regression analysis (*Table III*). HBsAg-positive pregnant women whose HBV DNA and HBV RNA were higher than 6 log10 IU/mL were found to be at risk of ICP. The results are summarized in *Table IV*.

Table I Basic maternal demographics.

Parameters	Whole cohort N=562	HBsAg positive N=203	HBsAg negative N=359	*P*
Age, years				<0.001
<35, n (%)	429	181 (32.21)	248 (44.13)	
≥35, n (%)	133	22 (3.91)	111 (19.75)	
Ethnic groups, n (%)				0.116
Han	445	168 (29.89)	277 (49.29)	
Others	117	35 (6.23)	82(14.59)	
Parity status, n (%)				<0.001
1	382	120 (21.35)	262 (46.62)	
2	180	83 (14.77)	97 (17.26)	
in Vitro Fertilisation				0.845
yes	116	41 (7.30)	75 (13.34)	
no	446	162 (28.83)	284 (50.53)	

Table II Comparison of the pregnancy outcomes HBsAg positive and non-HBV controls.

Parameters	HBsAg positive N=203	HBsAg negative N=359	χ^2	P
ICP, n (%)	15	7	10.200	0.001
GDM, n (%)	32	75	2.212	0.137
GH, n (%)	5	3	2.447	0.145
Pre-eclampsia, n (%)	8	15	0.019	0.891
PPROM, n (%)	27	42	0.309	0.578
Preterm birth, n (%)	18	51	3.432	0.064
Vaginal delivery, n (%)	155	292	1.978	0.160
Birth weight, (grams), n (%)				
≤2500 g, n (%)	8	29	3.642	0.056
≥4000 g, n (%)	4	8	0.076	0.782

Pregnancy outcomes including intrahepatic cholestasis of pregnancy (ICP), gestational hypertension (GH), pre-eclampsia, gestational diabetes mellitus (GDM), preterm prelabour rupture of membranes (PPROM)

Table III Logistic regression analysis.

	Univariable			Multivariable		
	OR	CI-95%CI	*p*	OR	CI-95%CI	*p*
Age	1.013	(0.892~1.150)	0.846			
Parity status	1.261	(0.439~3.623)	0.666			
Ethnic groups	1.757	(0.381~8.106)	0.470			
HBV RNA	3.449	(2.005~5.932)	<0.001	3.814	(2.036~7.142)	0.001
HBV DNA	2.066	(1.338~3.188)	<0.001	2.392	(1.345~4.256)	0.003
HBeAg						
HBeAg pos	1.107	(0.386~3.177)	0.850			
HBeAg neg	reference					

Table IV Pregnancy outcomes with different maternal HBV RNA levels.

	n	ICP, n (%)	p
HBV RNA (log10 IU/mL)			<0.001
≤3	12	0 (0%)	
3~6	138	4 (2.90%)	
≥6	53	11 (20.75%)	
HBV DNA (log10 IU/mL)			0.004
≤3	22	0 (0%)	
3~6	125	5 (4%)	
≥6	56	10 (17.86%)	

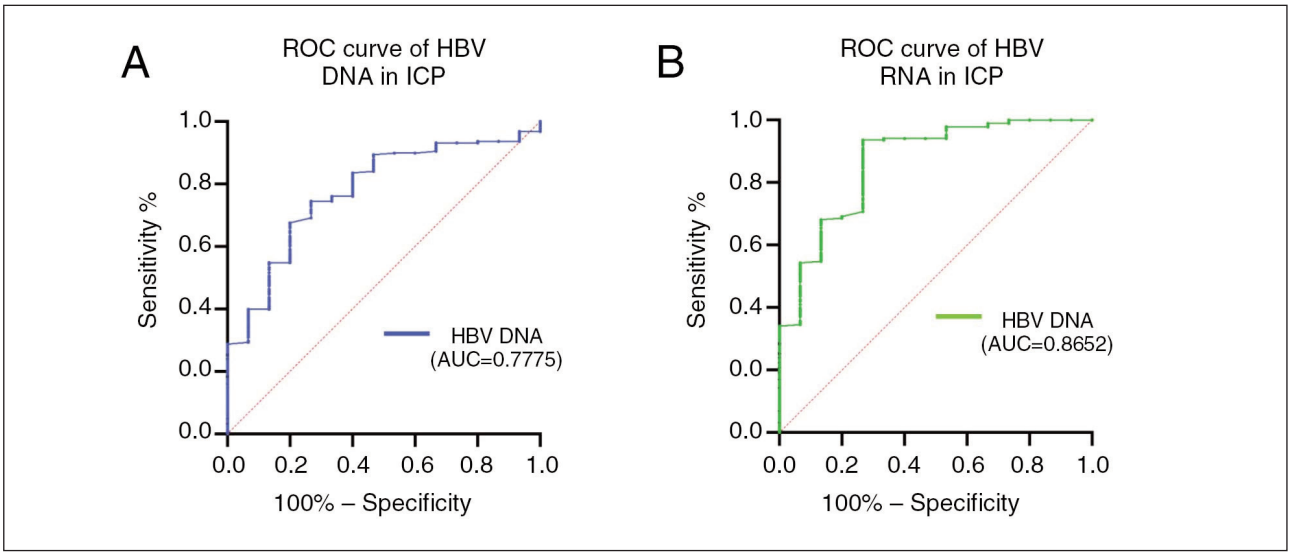

Figure 1 Receiver operating characteristics (ROC) curves of HBV RNA and HBV DNA discriminating HBsAg positive from ICP patients. (A) HBV DNA level manifested an area under the curve (AUC) value of 0.7775 which was lower than 0.8 and $P < 0.05$. (B) HBV RNA level manifested an area under the curve (AUC) value of 0.8652 which was greater than 0.8 and $P < 0.05$, suggesting HBV RNA maybe a potential diagnostic candidate for ICP. ICP, Pregnancy outcomes including intrahepatic cholestasis of pregnancy.

Diagnosis Value of HBV RNA in ICP

Figure 1 shows the performance of the HBV RNA and HBV DNA in predicting ICP. For the prediction of HBV infection, the areas under the curves (AUCs) of HBV RNA and HBV DNA for prediction of ICP were 0.8652 (95% CI = 0.7636~0.9669, $P < 0.001$) and 0.7775 (95% CI = 0.6672~0.8877, $P < 0.001$), respectively.

Discussion

HBV cccDNA is a unique intermediate that forms during HBV replication and serves as a transcriptional template for various mRNAs of different sizes, including pre-genomic RNA and pre-core RNA. Understanding HBV cccDNA is crucial for a comprehensive understanding of HBV replication and infection status (6, 7). Increasingly, studies have shown that HBV RNA can be used as an alternative marker for HBV cccDNA to guide CHB treatment and assess disease progression. However, its role has not been systematically studied in pregnant women with HBV infection (8, 9).

Wan et al. (10) found that gestational hypertension (GH), cesarean section, macrosomia, and preterm birth risk were associated with maternal HBsAg. Cai et al. reported that the increased risk of premature rupture of membranes (PROM) and intrahepatic cholestasis of pregnancy (ICP) are related to hepatitis B carriers during pregnancy (11). However, study did not find a relationship between HBV infection and adverse pregnancy outcomes (12, 13). Currently, studies on pregnancy outcomes in women with hepatitis B infection primarily focus on analyzing HBsAg, HBV DNA, and HBeAg status. Few studies have used HBV RNA as a parameter to evaluate pregnancy outcomes. As a new virological marker, HBV RNA can reflect the replication of the virus, and its detection may guide managing pregnant women infected with hepatitis B. In this study, we compared the pregnancy outcomes of 203 HBsAg-positive and 359 HBsAg-negative pregnant women, examining outcomes such as ICP, GH, preeclampsia, gestational diabetes mellitus (GDM), PROM, mode of delivery, preterm birth, low birth weight (LBW), and macrosomia. The results indicate that HBV RNA has predictive value for ICP in pregnant women infected with hepatitis B.

ICP is an idiopathic condition in pregnancy characterized by pruritus and/or abnormal liver function in pregnant women, along with elevated total bile acid levels and the absence of other liver and bile diseases. This condition primarily threatens newborn outcomes, including amniotic fluid contamination, fetal distress, preterm delivery, and even sudden fetal death in utero (14). The concentration of bile acid is used to diagnose and monitor the general condition of pregnant women with intrahepatic cholestasis. The etiology of ICP is not clear but is likely due to a combination of genetic predisposition factors (such as hepatobiliary transporter variants), hormonal factors, and environmental factors (15–18). Some studies have identified sodium-taurocholate cotransporting polypeptide (NTCP) as a specific receptor for HBV-infected hepatocytes, and changes in hormone levels during pregnancy may increase the risk of ICP in women infected with HBV (19). Our study found that HBV RNA is a significant risk factor for ICP (OR = 3.814, 95% CI: 2.036–7.142, $P < 0.001$). The area under the curve (AUC) for HBV RNA was 0.8652 (95% CI: 0.7636–0.9669, $P < 0.001$), indicating its potential to predict the occurrence of ICP in HBV-infected pregnant women.

However, several limitations are present in this study. Firstly, the sample size was small, underscoring the need for larger studies to validate our findings. Secondly, the study population consisted entirely of Chinese individuals, raising questions about the generalizability of our results to other countries and ethnicities. Thirdly, quantitative methods for HBV RNA are still evolving, which could potentially influence our experimental results. Therefore, the development of more standardized detection methods and reagents is urgently warranted to enhance the accuracy and reliability of future studies.

Conclusion

This study revealed the HBV RNA level has a significant negative impact on pregnancy outcomes. Based on the results of the study, we can conclude that high levels of HBV RNA during pregnancy may be a risk factor for the ICP and that may predict the ICP.

Ethics Statement

This study protocol was approved by the Ethics Committee of the Affiliated Hospital of Guizhou Medical University (Guizhou, China) and performed in accordance with the relevant provisions of the Helsinki Declaration.

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Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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Međunarodni XXIII srpski kongres medicinske biohemije i laboratorijske medicine

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Plenarne sekcije/Plenary Sessions
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?

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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

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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

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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

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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

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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

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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?

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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?

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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.

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P001
PREVALENCA INTOLERANCIJE LAKTOZE U SEVERNOJ MAKEDONIJI

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Čest uzrok gastrointestinalnih tegoba kod ljudi je abnormalna probava mlečnog šećera laktoze. Intolerancija na laktozu se javlja kada gastrointestinalni sistem nije u stanju da svari šećer laktozu. Primarna hipolaktazija je jedan od glavnih uzroka netolerancije na laktozu. Kod primarne intolerancije na laktozu koriste se genetski testovi. Intolerancija je povezana sa dva polimorfizma: 13910 C>T i 22018 G>A koji se nalaze u genu laktaze florizin hidrolaze (LPH). Gen se nalazi na lokusu 2q21. Polimorfizmi se otkrivaju testiranjem DNK-a (izolovanih iz belih krvnih ćelija), ekstrahovanih iz uzoraka krvi ili pljuvačke. Zbog toga je cilj ovog istraživanja bio da se utvrdi prisustvo 13910 T>C polimorfizma kod osoba Severne Makedonije sa abdominalnim simptomima. Analizirani su rezultati sto šezdeset pacijenata sa abdominalnim simptomima, koji su testirani na najčešću mutaciju 13910 T>C u promotoru LPH gena. Test je izveden korišćenjem PCR u realnom vremenu (FLASH tehnika). Rezultati su pokazali da je prevalencija CC (LCT-13910C/T) genotipa povezanog sa hipolaktazijom bila veoma visoka među makedonskom populacijom. Intolerancija na laktozu je najčešća kod ljudi istočnoazijskog porekla. U evropskoj populaciji ovo stanje se razlikuje između severnih zemalja sa nivoom tolerancije od preko 70% i ostalih delova Evrope, posebno južne Evrope gde Severna Makedonija geografski pripada, kao i Turske, Grčke i Italije gde je veći procenat ljudi pogođen.

P001
LACTOSE INTOLERANCE PREVALENCE IN NORTH MACEDONIA

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A common cause of gastrointestinal complaints in humans is abnormal digestion of the milk sugar lactose. Lactose intolerance occurs when the gastrointestinal system is unable to digest the milk sugar lactose. Primary hypolactasia is one of the main causes of lactose intolerance. Genetic testing is used for primary lactose intolerance. It is associated with two polymorphisms: 13910 C>T and 22018 G>A in the lactase phlorizin hydrolase gene (LPH). The gene is located in locus 2q21 and the polymorphism is detected by testing DNA (white blood cell isolate) obtained from blood or saliva samples. The aim of this study was therefore to determine the presence of the 13910 T>C polymorphism in North Macedonian individuals with abdominal symptoms. We analyzed the results of one hundred and sixty patients with abdominal symptoms who had been tested for the most common mutation 13910 T>C in the promoter of the LPH gene. The test was performed using real-time PCR (FLASH technique). The results showed that the prevalence of genotype CC (LCT-13910C/T), which is associated with hypolactasia, is very high in the Macedonian population. Lactose intolerance is most common in people of East Asian descent. In the European population, this condition differs between the northern countries with a tolerance level of over 70% and the other parts of Europe, especially in southern Europe, to which Macedonia geographically belongs, as well as Turkey, Greece and Italy, where a higher percentage of people are affected.

P002
NIVO GALEKTINA-3 U SERUMU PACIJENATA SA DIJABETES MELITUSOM TIP 2

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Klinička primena galektina-3 u serumu pacijenata obolelih od dijabetes melitusa tip 2 (T2D) zahteva dodatna ispitivanja s obzirom na oprečne rezultate prethodnih studija. Stoga je cilj ovog istraživanja bio da ispita nivo galektina-3 u serumu pacijenata obolelih od T2D u Crnoj Gori. Ukupno 35 T2D pacijenata i 36 zdravih ispitanika (kontrolna grupa) je uključeno u istraživanje. Serumske vrednosti galektina-3 merene su ELISA metodom. Univarijantna i multivarijantna binarna logistička regresiona analiza su primenjene da bi ispitale potencijalnu povezanost između galektina-3 i T2D. Utvrđene su veće vrednosti galektina-3 u serumu pacijenata sa T2D u poređenju sa kontrolnom grupom (p=0,016). Galektin-3 je pokazao korelaciju sa sistolnim krvnim pritiskom u bivarijantnoj Spearman-ovoj korelacionoj analizi (ρ=0,293; p=0,013), ali ne i sa ostalim kardiometaboličkim parametrima. Univarijantna binarna logistička regresiona analiza je pokazala da je sa porastom vrednosti galektina-3 za 1 ng/mL verovatnoća pojave T2D porasla za 7,3% (OR=1.073; p=0,008). Slično, porast vrednosti galektina-3 za 1 ng/mL pokazao je 8,5% veću verovatnoću za pojavu T2D (OR=1.085; p=0,015) u multivarijantnoj binarnoj logističkoj regresionoj analizi. U zaključku, galektin-3 u serumu može biti koristan biomarker kod pacijenata obolelih od T2D.

P002
SERUM GALECTIN-3 LEVELS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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The clinical benefit of serum galectin-3 in patients with type 2 diabetes (T2D) needs to be furtherinvestigated in view of the consistent results of previous studies. Therefore, the aim of this study was to investigate serum galectin-3 levels in relation to T2D in adult Montenegrins. A total of 35 T2D patients and 36 control subjects were included. Serum galectin-3 levels were measured by ELISA. Univariate and multivariate binary logistic regression analyses were used to test possible associations between serum galectin-3 and T2D. Patients with T2D had higher serum galectin-3 levels compared to the control group (p=0.016). Serum galectin-3 correlated with systolic blood pressure in the bivariate Spearman correlation analysis (ρ=0.293, p=0.013), but not with other cardiometabolic parameters. Univariate binary logistic regression analysis showed that with a 1 ng/mL increase in galectin-3 concentration, the probability of T2D increased by 7.3% (OR=1.073, p=0.008). Similarly, the increase in galectin-3 concentration by 1 ng/ml showed an 8.5% higher probability of T2D occurrence (OR=1.085, p=0.015) in the multivariate binary logistic regression analysis. In conclusion, serum galectin-3 may be a useful biomarker in T2D patients.

P003
MALE GUSTE LDL ČESTICE KAO BIOMARKER KOMPLIKACIJA U TRUDNOĆI

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Trudnoća je metabolički izazov za organizam majke, te ukoliko ne uspe da isprati rastuće potrebe fetusa, može da dovede do razvoja komplikacija. Pojava dislipidemije je zajednička karakteristika komplikovanih trudnoća i razvoja kardiovaskularnih oboljenja. Cilj ovog istraživanja bio je ispitati da li se na osnovu parametara lipidnog statusa i profila LDL subfrakcija može predvideti rizik za razvoj komplikacija u trudnoći. U istraživanju je učestvovalo 107 trudnica, od kojih je 87 imalo normalnu trudnoću, a 20 trudnoću sa komplikacijama. Parametri lipidnog statusa su određeni rutinskim metodama, a LDL subfrakcije su razdvojene metodom elektroforeze na poliakrilamidnom gradijent (3–31%) gelu. Relativni udeo malih gustih LDL (sdLDL) čestica je izračunat kao zbir relativnih udela LDL III i LDL IV subfrakcija. Utvrđeni su značajno niži relativni udeli LDL II subfrakcija, a viši relativni udeli LDL IV subfrakcija kod trudnica sa komplikacijama u drugom trimestru. Učestalost trudnica sa povišenim sdLDL česticama (>50%) je bila značajno viša kod trudnica sa komplikacijama u drugom i trećem trimestru. Utvrdili smo da je verovatnoća za razvoj komplikacija u trudnoći 3,4 puta veća ukoliko je u drugom trimestru relativni udeo sdLDL veći od 50% (OR=3,40; 95% CI:1,25–9,25; P<0,05). Naši rezultati ukazuju da bi se udeo sdLDL čestica mogao koristiti kao prognostički biomarker za razvoj komplikacija u trudnoći.

P003
SMALL DENSE LDL PARTICLES AS A BIOMARKER OF PREGNANCY COMPLICATIONS

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Pregnancy represents a metabolic challenge for the mother's organism, and if she does not meet the growing needs of the fetus, this can lead to the development of complications. The occurrence of dyslipidemia is a common feature of complicated pregnancies and the development of cardiovascular disease. The aim of this study was to investigate whether the risk of developing complications during pregnancy can be predicted by parameters of lipid status and LDL subfraction profile. The study involved 107 pregnant women, 87 of whom had a normal pregnancy and 20 of whom had a pregnancy with complications. Lipid status parameters were determined by routine methods, while LDL subfractions were separated by polyacrylamide gradient gel electrophoresis (3–31%). The relative proportion of small dense LDL (sdLDL) particles was calculated as the sum of the relative proportions of LDL III and LDL IV subfractions. Pregnant women with second trimester complications were found to have significantly lower relative proportions of LDL II subfractions but higher relative proportions of LDL IV subfractions. The prevalence of pregnant women with elevated sdLDL particles (>50%) was significantly higher in pregnant women with second- and third-trimester complications. We found that the odds of developing complications in pregnancy were 3.4 times higher when the relative proportion of sdLDL in the second trimester was more than 50% (OR=3.40; 95% CI:1.25–9.25; P<0.05). Our results suggest that the proportion of sdLDL particles could be used as a prognostic biomarker for the development of complications in pregnancy.

P004
OKSIDATIVNI STRES U POPULACIJI PACIJENATA SA METABOLIČKIM SINDROMOM

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Smatra se da oksidativni stres igra ključnu ulogu u patogenezi metaboličkog sindroma (MetS). Međutim, prethodne studije pokazuju oprečne rezultate. Cilj ovog istraživanja je bio da se ispita nivo oksidativnog stresa meren produktima uznapredovale oksidacije proteina (AOPP), ksantin oksidazom (XO) malondialdehidom (MDA), produktima azot-monooksida (NOx) i katalazom (CAT)] i ispita potencijalna povezanost sa MetS. Ova studija slučajeva i kontrola je uključila 51 pacijenta sa MetS i 46 zdravih ispitanika uparenih po polu i starosnoj dobi. Određivani su biomarkeri inflamacije visokosenzitivni C-reaktivni protein (hsCRP)], metabolički i biomarkeri oksidativnog stresa. Vrednosti hsCRP, AOPP, XO i MDA ($p<0,01$, $p<0,001$, $p<0,05$, $p<0,05$) su veće kod pacijenata sa MetS u poređenju sa kontrolnom grupom. Nije utvrđeno postojanje statistički značajne razlike u nivou NOx i aktivnosti CAT među ispitivanim grupama ($p>0,05$). Prema ROC analizi, najbolji prediktor MetS-a je AOPP (sa dobrom diskriminatorskom sposobnošću, AUC=0.713), dok su drugi parametri pokazali AUC ispod 0.700, bez klinički značajne prediktivne sposobnosti (AUC za XO= 0.620, AUC za MDA=0.618, AUC za NOx=0.484, AUC za CAT=0.480). U zaključku, pacijenti sa MetS imaju veće vrednosti oksidativnog stresa i inflamacije, tj. veće vrednosti AOPP, XO, MDA i hsCRP. AOPP je pokazao dobru diskriminatornu sposobnost za pacijente sa MetS u odnosu na zdrave ispitanike.

P004
OXIDATIVE STRESS IN PATIENTS WITH METABOLIC SYNDROME

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It is assumed that oxidative stress plays a key role in the development of metabolic syndrome (MetS). Previous studies on this topic have shown contradictory results. The aim of this study was to investigate oxidative stress as measured by advanced oxidation protein products (AOPP), xanthine oxidase (XO), malondialdehyde (MDA), nitric oxide products (NOx) and catalase activity (CAT) and their possible association with MetS. This case-control study included 51 patients with MetS and 46 age- and sex-matched healthy controls. Inflammation levels i.e. high-sensitivity C-reactive protein (CRP)], metabolic levels and oxidative stress biomarkers were measured. The levels of hsCRP, AOPP, XO and MDA ($p<0.01$, $p<0.001$, $p<0.05$, $p<0.05$, respectively) were higher in MetS patients as compared with controls. There was no difference in NOx levels and CAT activity between examined groups ($p>0.05$ for both). After ROC analysis, the best predictor of MetS was AOPP with a good discriminatory power, AUC=0.713, while other parameters were with AUC below 0.700, i.e. without real predictive power (i.e. AUC for XO=0.620, AUC for MDA=0.618, AUC for NOx=0.484, AUC for CAT=0.480). In summary, patients with MetS have higher levels of oxidative stress and inflammation as reflected by higher AOPP, XO, MDA and hsCRP levels. AOPP has a good ability to differentiate patients with MetS from healthy individuals.

P005
LIPIDNI STATUS I PROFIL LDL SUBFRAKCIJA PACIJENATA SA LIMFOMOM

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Limfomi su heterogena grupa hematoloških malignoteta, a klasifikuju se kao Hočkinov limfom (HL) i ne-Hočkinov limfom (NHL). Moguća veza između hipoholesterolemije i limfoma kao uzroka ili posledice još uvek nije utvrđena. Cilj ovog rada je ispitivanje parametara lipidnog statusa i profila LDL subfrakcija kod pacijenata sa limfomom i njihove povezanosti sa stadijumom i ishodom bolesti. Studija je uključila 55 pacijenata, od čega 11 sa HL i 44 sa NHL, kao i 33 zdrave osobe, koje su činile kontrolnu grupu (KG). Koncentracije ukupnog holesterola, triglicerida i HDL-holesterola su određene standardnim enzimskim metodama, dok je koncentracija LDL-holesterola izračunata primenom Friedwald-ove formule. Razdvajanje LDL subfrakcija izvršeno metodom vertikalne elektroforeze na gradijent gelu poliakrilamida. Obe grupe pacijenata su imale značajno niže koncentracije ukupnog i HDL-holesterola u odnosu na KG. Pacijenati sa HL su imali značajno niže koncentracije LDL-holesterola, kao i niži udeo LDL II subfrakcija, a viši udeo LDL IV subfrakcija u poređenju sa druge dve grupe ispitanika. Preživeli pacijenti sa NHL imali su značajno više koncentracije ukupnog i LDL-holesterola, a manji udeo LDL I subfrakcija. Naši rezultati ukazuju na izmenjen metabolizam holesterola i potencijalnu ulogu malih LDL čestica u razvoju i progresiji limfoma, što može biti važna tema budućih istraživanja.

P005
LIPID STATUS AND PROFILE OF LDL SUBFRACTIONS IN PATIENTS WITH LYMPHOMA

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Lymphomas are a heterogeneous group of hematological malignancies classified as Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL). A possible association between hypocholesterolemia and lymphoma as a cause or consequence has not been established. The aim of this study is to evaluate the lipid status and LDL subfractions profile in patients with lymphoma and their association with disease stage and outcome. The study included 55 patients, 11 with HL and 44 with NHL, and 33 healthy individuals as a control group (CG). Total cholesterol, triglycerides and HDL cholesterol were determined using standard enzymatic methods, while the concentration of LDL-cholesterol was calculated using the Friedwald formula. LDL subfractions were separated by vertical polyacrylamide gradient gel electrophoresis. Both patient groups had significantly lower concentrations of total and HDL-cholesterol compared to CG. Patients with HL had significantly lower concentrations of LDL cholesterol and a lower proportion of LDL II subfractions, but a higher proportion of LDL IV subfractions compared to the other two groups. Surviving patients with NHL had significantly higher concentrations of total and LDL-cholesterol and a lower proportion of LDL I subfractions. Our results point to altered cholesterol metabolism and the potential role of small LDL particles in the development and progression of lymphoma, which may be an important topic for future research.

P006
OKSIDATIVNI STRES U POST-KOVID SINDROMU

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Post-Kovid sindrom javlja se kod približno 10% pacijenata koji preleže akutnu SARS-CoV-2 infekciju, a karakterišu ga simptomi slični mijalgičnom encefalomijelitisu/sindromu hroničnog umora. Dosadašnje studije predložile su mitohondrijalnu disfunkciju i posledično prekomerno formiranje reaktivnih kiseoničnih vrsta kao jednog od ključnih mehanizama patogeneze post-Kovida. Cilj ovog rada jeste određivanje oksidativno-stresnog statusa kod post-Kovid pacijenata i ispitivanje njegove moguće veze sa manifestacijom post-Kovid simptoma. U studiji su ispitani uzorci seruma 60 post-Kovid pacijenata. U svakom uzorku spektrofotometrijski su određeni sledeći parametri oksidativno-stresnog statusa: totalni oksidativni status (TOS), totalni antioksidativni status (TAS), aktivnost superoksid dismutaze (SOD), koncentracija uznapredovalih produkata oksidacije proteina (AOPP), sadržaj ukupnih sulfhidrilnih grupa (SHG), prooksidativno-antioksidativni balans (PAB), ishemijom modifikovani albumin (IMA), paraoksonazna aktivnost paraoksonaze 1 (PON1) i koncentracija malondialdehida (MDA). Statistička analiza podataka pokazala je da su PAB, TOS, MDA, IMA i AOPP značajno viši ($p<0,01$), odnosno da su SHG ($p<0,05$), TAS, SOD, PON1 ($p<0,01$) značajno niži kod post-Kovid pacijenata nego u kontrolnoj grupi. Naš rad je pokazao povećan oksidativni stres i smanjenu antioksidativnu zaštitu kod pacijenata sa post-Kovid sindromom.

P006
OXIDATIVE STRESS IN POST-COVID-19 SYNDROME

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Post-COVID-19 syndrome affects approximately 10% of patients who have recovered from acute SARS-CoV-2 infection and is characterised by symptoms similar to those of myalgic encephalomyelitis/chronic fatigue syndrome. Recent studies have suggested mitochondrial dysfunction and consequently excessive generation of reactive oxygen species as one of the key mechanisms in post-COVID-19 pathogenesis. The aim of this study was to measure the main parameters of oxidative stress in post-COVID-19 patients and to evaluate their possible association with post-COVID-19 symptoms. Serum samples were collected from a total of 60 patients with post-COVID-19 symptoms. Oxidative stress status was determined in each sample by spectrophotometric measurements: total oxidative status (TOS), total antioxidant status (TAS), superoxide dismutase activity (SOD), advanced oxidation protein product (AOPP) concentration, total sulfhydryl groups (SHG), prooxidant-antioxidant balance (PAB), ischemic modified albumin (IMA), paraoxonase activity of paraoxonase 1 (PON1), and malondialdehyde concentration (MDA). Statistical analysis showed that PAB, TOS, MDA, IMA and AOPP were significantly higher in post-COVID-19 patients ($p<0.01$), while SHG ($p<0.05$), TAS, SOD and PON1 ($p<0.01$) were significantly lower than in the control group. Our study shows that patients with post-COVID-19 syndrome have increased oxidative stress and decreased antioxidant defences.

P007
ISPITIVANJE UTICAJA GOJAZNOSTI NA EKSPRESIJU SCARB1, ABCA1 I ABCG1 GENA U TOKU TRUDNOĆE

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SCARB1, ABCA1 and ABCG1 transporteri su lokalizovani na placenti, gde posreduju u preuzimanju holesterola sa HDL čestica iz majčine cirkulacije i njegovom transferu ka fetusu. Ekspresije gena koji kodiraju njihovu sintezu su dobro ispitane u tkivu placente, ali manje se zna o nivoima ovih ekspresija u ćelijama krvi majke. U ovom istraživanju smo ispitivali uticaj gojaznosti pre trudnoće na ekspresiju SCARB1, ABCA1 i ABCG1 gena u mononuklearnim ćelijama periferne krvi (MĆPK) trudnica. Studija je obuhvatila longitudinalno praćenje 125 trudnica kroz trimester trudnoće. Kvantifikacija genske ekspresije SCARB1, ABCA1 i ABCG1 izvedena je primenom quantitative polymerase chain reaction (qPCR) metode u uzorcima RNK izolovane iz MĆPK. Ekspresija ABCA1 značajno je rasla kroz trimestre trudnoće ($P<0,05$). Naši rezultati pokazali su da su ekspresije ABCA1 i ABCG1 gena u drugom trimestru bile više kod žena koje su trudnoću započele sa preporučenom telesnom masom, u odnosu na trudnice koje su pre trudnoće imale prekomernu telesnu masu ili bile gojazne ($P<0,05$ u oba slučaja). Negativna korelacija uočena je između koncentracije triglicerida i genske ekspresije SCARB1 ($P<0,05$), ABCA1 ($P<0,01$) i ABCG1 gena ($P<0,01$) u prvom trimestru trudnoće. Rezultati ove studije pokazali su da su ekspresije ABCA1 i ABCG1 gena u MĆPK gojaznih trudnica smanjene u odnosu na trudnice koje su imale preporučenu telesnu masu pre trudnoće. Ove promene mogu imati uticaja i na ishode trudnoće.

P007
EXAMINING THE INFLUENCE OF OBESITY ON THE EXPRESSION OF SCARB1, ABCA1 AND ABCG1 GENES DURING PREGNANCY

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SCARB1, ABCA1 and ABCG1 transporters are localized on the placenta, where they mediate the uptake of maternal HDL-cholesterol and its transfer to the fetal circulation. Placental expressions of the corresponding genes have already been extensively explored, but less is known about changes of their expressions in maternal blood cells during pregnancy. In this study we explored the influence of pregestational obesity on the expression of SCARB1, ABCA1 and ABCG1 genes in peripheral blood mononuclear cells (PBMC) of pregnant women. The study comprised longitudinal monitoring of 125 pregnant women during the entire course of pregnancy. Quantification of SCARB1, ABCA1 and ABCG1 gene expression was performed using the quantitative polymerase chain reaction (qPCR) method in total RNA isolated from PBMC. ABCA1 gene expression significantly increased through trimesters of pregnancy ($P<0.05$). Our results have shown that gene expression of ABCA1 and ABCG1 in the second trimester were higher in pregnant women who started pregnancy with normal body weight, when compared to pre-pregnancy overweight and obese pregnant women ($P<0.05$, respectively). A negative correlation was obtained between the concentration of triglycerides and the expression of SCARB1 ($P<0.05$), ABCA1 ($P<0.01$) and ABCG1 ($P<0.01$) genes in the first trimester. In this study, we demonstrated that the expression of ABCA1 and ABCG1 genes in PBMC of obese pregnant women is reduced when compared to pregnant women with normal body weight before pregnancy. These changes could be associated with pregnancy outcomes as well.

P008
UPRAVLJANJE RIZIKOM UKUPNOG PROCESA TESTIRANJA I PREANALITIČKE GREŠKE U DVE LABORATORIJE: NOVA OPCIJA ZA BUDUĆNOST 2024?

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Neadekvatna priprema pacijenata i nedovoljne veštine medicinskih flebotomista su izvori grešaka u preanalitičkoj fazi. Netačni rezultati laboratorijskih ispitivanja su rezultat uglavnom posledica upravo preanalitičke faze. Standardizacija procesa po principima dobre kliničke prakse (GCP), praćenje savremene medicine prema medicini zasnovanoj na dokazima (EBM) i dobra laboratorijska praksa (GLP) smanjuje mogućnost uticaja različitih faktora u upravljanju rizikom. Cilj ove retrospektivne studije je praćenje, dokumentovanje i prevencija grešaka u preanalitičkoj fazi u vezi flebotomije u dve laboratorije: kliničko-biohemijskoj laboratoriji primarne zdravstvene zaštite Zavoda za zdravstvenu zaštitu studenata Novi Sad i Specijalnoj neuropsihijatrijskoj bolnici Kovin na sekundarnom nivou radi bolje zdravstvene zaštite pacijenata. Studija je urađena od 2019. do 2023. godine tokom pandemije korone i obuhvata praćenje, dokumentovanje i prevenciju grešaka sa aspekta flebotomije u dve laboratorije. Greške su klasifikovane kao indikatori kvaliteta prema preporuci IFCC-a: nedovoljna zapremina uzorka, neodgovarajuće obeležen uzorak i neadekvatan uzorak. Studija je pokazala da su najčešće greške nedovoljna količina i neadekvatan uzorak u primarnoj zdravstvenoj zaštiti studenata (0,97% prema 0,95% u specijalnoj psihijatrijskoj klinici). Neodgovarajuće obeleženih uzoraka je bilo značajno manje i potpuno su eliminisani tokom perioda istraživanja (2019: 0,24%, 2023: 0%; p<0, 01) u obe laboratorije. Nije uočeno značajno smanjenje broja neadekvatnih uzoraka na nivou primarne zdravstvene zaštite (2019: 0,40%, 2023: 0,30%) kao ni na nivou sekundarne zdravstvene zaštite (2019: 0,45%, 2023: 0,30%) i nedovoljna količina uzorka na nivou primarne zdravstvene zaštite (2019: 0,33%, 2023: 0,22%) i u specijalnoj psihijatrijskoj bolnici (2019: 0,43%, 2023: 0,32%) je ostala konstantna tokom perioda ispitivanja. Kroz kontinuirano unapređenje sistema upravljanja kvalitetom – QMS, sprovođenje sertifikacije i akreditacije laboratorija po standardu ISO15189:2022 (QM/QA) za medicinske laboratorije sa posebnim zahtevom na celokupan proces laboratorijskog ispitivanja i implementaciju LIS-a (Laboratorijski informacioni sistem), standarda za POCT-ISO22870:2006 POCT testiranje, jasne,

P008
RISK MANAGEMENT OF TOTAL TESTING PROCESS AND PREANALYTICAL ERRORS IN A TWO LABS: A NOVEL FUTURE OPTION IN 2024?

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Inadequate preparation of patients and insufficient skills of medical phlebotomists are sources of error in the pre-analytical phases. Inaccurate results of laboratory tests are mostly due to errors in the pre-analytical phase. The standardization of conditions according to the principles of good clinical practice (GCP), the monitoring of modern medicine according to evidence-based medicine (EBM) and good laboratory practice (GLP) reduces the possibility of the influence of various factors in risk management The aim of this retrospective study is to monitor, document and prevent errors in the pre-analytical phase with regard to phlebotomy in two laboratories: the primary health care clinical biochemistry laboratory, the student health protection institute and the secondary level neuropsychiatric specialty hospital for better patient health care. The study was conducted from 2019 to 2023 during the Corona pandemic and includes the monitoring, documentation and prevention of errors related to phlebotomy in two laboratories at different levels of patient health care. The errors are classified as quality indicators according to the IFCC recommendation: insufficient sample volume, improperly labeled sample and sample damage. The study showed that the most common errors were insufficient specimen volume and specimen damage in the primary health care of students (0.97% compared to 0.95% in the psychiatric specialty clinic). Improperly labeled specimens were significantly less frequent in both laboratories and were completely eliminated during the study period (2019: 0.24%, 2023: 0%; p<0.01). The number of damaged samples in the student laboratory (2019: 0.40%, 2023: 0.30%) could not be significantly reduced in the secondary laboratory either (2019: 0.45%, 2023: 0.30%) and insufficient sample quantities in the student laboratory (2019: 0.33%, 2023: 0.22%) and in the psychiatric specialist hospital (2019: 0.43%, 2023: 0.32%) remained constant during the study period. Through the continuous improvement of the quality management system – QMS, the introduction of certification and accreditation of laboratories according to the ISO15189:2022 (QM/QA) standard for medical laboratories with a special requirement for the entire laboratory examination and the introduction of LIS

transparentne i dostupne procedure, greške iz preanalitičke faze u primarnoj i sekundarnoj laboratoriji mogu biti svedene na minimum. Posebnu pažnju treba obratiti na greške koje se i dalje javljaju tokom studije. Upravljanje rizikom za manji broj grešaka u preanalitičkoj fazi podrazumeva tačnije, preciznije i validnije rezultate, tačnu i brzu dijagnozu, zadovoljne pacijente i princip isplativosti uz smernice: »bolje nijedan uzorak krvi nego loš uzorak krvi« i »više je bolje«.

P009
STAROSNO ZAVISNA VARIJABILNOST NIVOA INR KOD STARIJIH PACIJENATA SA ATRIJALNOM FIBRILACIJOM NA ACENOKUMAROLU

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Atrijalna fibrilacija je uobičajena srčana aritmija koja povećava rizik moždanog udara, posebno kod starijih osoba, što zahteva efikasan tretman antikoagulansa. Međunarodni normalizovani odnos (INR) je ključan za praćenje efikasnosti i bezbednosti antikoagulantne terapije, ali na njegovu varijabilnost može uticati starost, što potencijalno komplikuje lečenje kod starijih odraslih osoba. Studija ima za cilj da kvantifikuje uticaj starosti na varijabilnost INR-a i povezane potrebe za prilagođavanjem doze kod starijih pacijenata sa atrijalnom fibrilacijom lečenih acetokumarolom. Ova retrospektivna kohortna studija, sprovedena u Opštoj bolnici Strumica, obuhvatila je 60 starijih pacijenata (30 muškaraca, 30 žena) starosti 60 i više godina, sa dijagnozom atrijalne fibrilacije, od januara do marta 2024. Praćeni su nivoi INR, a podaci su analizirani linearnom i nelinearnom regresionom analizom za procenu korelacije između starosti i varijabilnosti INR-a. Model kvadratne regresije je posebno korišćen za upravljanje potencijalnim nelinearnim vezama. Analize su otkrile značajan uticaj starosti na varijabilnost INR ($p<0,01$), pri čemu kvadratni odnos ukazuje na nesrazmerno veću varijabilnost kod pacijenata starijih od 75 godina ($F(2,57)=5,36$, $p<0,05$). Prosečan INR je bio $2,5\pm0,4$ u ovoj starijoj podgrupi naspram $2,2\pm0,3$ kod mlađih pacijenata. Hi-kvadrat test je potvrdio da

(Laboratory Information System), standard for POCT-ISO22870:2006 Point of care testing, clear, transparent and available procedures, errors from the pre-analytical phase in a primary and secondary laboratory can be minimized. Particular attention should be paid to errors that still occur during the study. Risk management for a lower number of errors in the pre-analytical phase means more accurate, precise and valid results, a correct and fast diagnosis, satisfied patients and the principle of the cost-benefit ratio with guidelines: »no blood sample is better than a bad blood sample« and »more is better«.

P009
AGE-RELATED VARIABILITY IN INR LEVELS AMONG ELDERLY PATIENTS WITH ATRIAL FIBRILATION ON ACENOCUMAROL

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Atrial fibrillation is a common cardiac arrhythmia that increases the risk of stroke, especially in elderly, and requires effective anticoagulation. The International Normalized Ratio (INR) is crucial for monitoring the efficacy and safety of anticoagulation therapy, but its variability may be influenced by age, potentially complicating management in older adults. The aim of the study is to quantify the impact of age on INR variability and the associated need for dose adjustments in older patients with atrial fibrillation treated with acenocoumarol. This retrospective cohort study was conducted at Strumica General Hospital and included 60 elderly patients (30 men, 30 women) aged 60 years and older who were diagnosed with atrial fibrillation between January and March 2024. INR values were monitored and data were analyzed using linear and non-linear regression analyses to assess the correlation between age and INR variability. A quadratic regression model was specifically used to account for possible non-linear relationships. The analyses revealed a significant effect of age on INR variability ($p<0.01$), with a quadratic relationship indicating a disproportionately higher variability in patients over 75 years of age ($F(2,57)=5.36$, $p<0.05$). The mean INR value was 2.5 ± 0.4 in this older subgroup compared to 2.2 ± 0.3 in younger patients. The chi-square test confirmed that older patients required

su starijim pacijentima potrebna češća prilagođavanja doze zbog nestabilnosti INR-a (χ^2=12,3, p <0,001). Ovi nalazi naglašavaju neophodnost praćenja specifičnu za uzrast i prilagođavanje doziranja kako bi se efikasno upravljalo nivoima INR-a kod starijih pacijenata sa atrijalnom fibrilacijom. Intenzivnije i prilagođene strategije antikoagulacije preporučuju se osobama starijim od 75 godina kako bi se održali optimalni terapijski rasponi i smanjio rizik od neželjenih ishoda.

P010
POREĐENJE ANALIZATORA ADVIA 2120 I MEK-1305 NA OSNOVU REZULTATA HEMATOLOŠKIH PARAMETARA

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Svako uvođenje nove metode ili novog analizatora u rutinski rad laboratorije zahteva prethodnu verifikaciju koja se često sprovodi poređenjem njihovih rezultata sa rezultatima dobijenim prethodno korišćenom metodom ili analizatorom. U ovu svrhu koristi se provera odstupanja od ukupne dozvoljene greške (TEa) ili različiti statistički testovi poput t-testa parova, linearne regresione analize, Passing and Bablok regresije ili Blad-Altman grafika. Svaki od ovih testova ima prednosti i nedostatke, a ispravan izbor testa u velikoj meri presuđuje rezultate verifikacije. TEa je procenat dozvoljenog odstupanja koji se izvodi iz biološke varijacije i za određeni parametar i koji uzima u obzir bias i nepreciznost i osetljiv je na sistematsku i slučajnu grešku. Passing and Bablok regresija omogućava proveru slaganja između dve grupe podataka koji nisu normalno distribuirani i njeni rezultati ukazuju na prisustvo sistematske greške dajući uvid u to da li je ona konstantna ili procentualna. U ovom radu smo ispitivali slaganje rezultata dobijenih na dva hematološka analizatora računajući odstupanje od TEa i koristeći Passing-Bablok analizu. Hematološki parametri, broj leukocita (WBC), broj eritrocita (RBC), koncentracija hemoglobina (HGB), vrednost hematokrita (HCT) i broj trombocita (PLT) određivani su upotrebom dva hematološka analizatora, Advia 2120 (Siemens, Minhen, Nemačka) i MEK-1305 (Nihon Kohden, Tokio, Japan). Za svaki parametar je računato odstupanje od TEa vrednosti koje su preuzete iz Westgardove baze Desirable Biological Variation Database i more frequent dose adjustments due to INR instability (χ^2=12.3, p<0.001). These results emphasize the need for age-specific monitoring and dosage adjustment to effectively control INR values in elderly patients with atrial fibrillation. More intensive and tailored anticoagulation strategies are recommended in over 75-year-olds to maintain optimal therapeutic ranges and minimize the risk of adverse outcomes.

P010
COMPARISON OF THE ADVIA 2120 AND MEK-1305 ANALYZERS BASED ON THE RESULTS OF HAEMATOLOGY PARAMETERS

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Every introduction of a new method or a new analyzer to the routine work of a laboratory requires a previous verification that is often carried out by comparing their results with the results obtained by the previously used method or analyzer. For this purpose, examining the deviation of the allowable total error (TEa), or the different statistic tests such as paired t-test, linear regression analysis, Passing and Bablok regression or Blad-Altman chart can be used. Each of these tests has its advantages and setbacks, and the right choice of a test decides the results of the verification to a significant degree. TEa is the percent of allowed deviation derived from the biological variance for a specific parameter and it takes into account both bias and unprecision, while it is sensitive to both systematic and random error. Passing and Bablok regression allows testing the agreement between the two groups of data with non-normal distribution and its results point to the presence of a systematic error, giving insight to wheter it is constatnt or proportional. In this study we examined the agreement between the results obtained by two different haematology analyzers calculating the deviation from TEa and using the Passing and Bablok analysis. Haematology parameters, white blood cell count (WBC), red blood cell count (RBC), haemoglobin concentration (HGB), haematocrit value (HCT), and platelet count (PLT) were assessed using two haematology analyzers, Advia 2120 (Siemens, Munich, Germany) i MEK-1305 (Nihon Kohden, Tokyo, Japan). The calculation of the deviation from TEa value taken from the Westgard Desirable

primenjena je Passing and Bablok regresiona analiza upotrebom softvera MedCalc (Medcalc Software Ltd, Osten, Belgija). Passing-Bablok analiza je pokazala zadovoljavajuću linearnost definisanu kao p>0,05 za CUSUM test, i odsustvo konstantne greške za sve parametre. Proporcionalna greška je bila prisutna za WBC i HCT pri čemu rezultati WBC između dve metode nisu pokazali značajno odstupanje od TEa (TEa<14,6%), dok rezultati HCT jesu (TEa>4,1%). Sa druge strane, velika odstupanja od TEa su bila prisutna i za RBC (TEa>4,4%) i PLT (TEa>13,4%), dok Passing and Bablok analiza nije pokazala da postoji značajna razlika u rezultatima ovih parametara između dve metode. Primenom Passing-Bablok analize pokazano je dobro slaganje u vrednostima parametara određivanih na dva različita hematološka analizatora. Uzimajući u obzir da su za neke od parametara uočena veća odstupanja od TEa, preporučuje se korišćenje oba načina provere prilikom verifikacije novog analizatora.

Biological Variation Database and Passing and Bablok regression analysis using MedCalc (Medcalc Software Ltd, Osten, Belgium) were performed for each parameter. Passing and Bablok analysis showed satisfying linearity defined as p>0.05 for CUSUM test, and the absence of the constant error for all parameters. The proportional error was present for WBC and HCT, whereas the WBC results didn't show significant deviation from TEa (TEa<14.6%), while the HCT results did (TEa>4.1%). On the other hand, great deviations from TEa were present for both RBC (TEa>4.4%) and PLT (TEa>13.4%), while Passing and Bablok analysis showed no significant difference in the results between the methods for these parameters. Using the Passing and Bablok analysis showed a good agreement in the values of the parameters measured on the two different haematology analysers. Considering the fact that the greater deviations from TEa were noticed for a few parameters, the use of both these methods for the verification of a new analyser is recommended.

P011
VREDNOSTI TUMORSKIH MARKERA AFP I CA19-9 U PRIMARNOM HEPATOCELULARNOM KARCINOMU I METASTATSKOJ BOLESTI JETRE

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Hepatocelularni karcinom (HCC) predstavlja jedan od najučestalijih karcinoma na svetu i među vodećim je uzrocima smrti. Povišene koncentracije AFP i CA19-9 povezane su sa visokim rizikom za nastanak HCC i ukazuju na lošiju stopu preživljavanja. Cilj ovog rada bio je ispitivanje koncentracija tumorskih markera AFP i CA19-9 i biohemijskih parametara kod pacijenata sa HCC i metastazama u jetri i njihovo poređenje sa kontrolnom grupom. Studija je uključila 20 pacijenata obolelih od HCC, 20 pacijenata sa dijagnozom prisustva sekundarnih metastaza u jetri i 20 ispitanika u kontrolnoj grupi. U serumu su spektrofotometrijski određivani biohemijski parametri: direktni i ukupni bilirubin, ukupan holesterol, albumin, AST, ALT, ALP, GGT, LDH, i CRP. Za određivanje ovih parametara korišćen je OLYMPUS AU400 (Beckman Coulter, USA). Imuno-hemiluminiscentnim »sendvič« testom određivani su tumorski markeri AFP i CA19-9 na automatskom analizatoru Access 2

P011
VALUES OF TUMOR MARKERS AFP AND CA19-9 IN PRIMARY HEPATOCELLULAR CARCINOMA AND METASTATIC LIVER DISEASE

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Hepatocellular carcinoma (HCC) is one of the most common types of cancer in the world and is one of the most frequent causes of death. Elevated levels of AFP and CA19-9 are associated with a high risk of developing HCC and indicate a poorer survival rate. The aim of this study was to investigate the concentrations of the tumor markers AFP and CA19-9 as well as biochemical parameters in patients with HCC and liver metastases and to compare them with the control group. The study included 20 patients with HCC, 20 patients with secondary liver metastases and 20 subjects in the control group. The biochemical parameters were determined spectrophotometrically in the serum: direct and total bilirubin, total cholesterol, albumin, AST, ALT, ALP, GGT, LDH and CRP. The OLYMPUS AU400 (Beckman Coulter, USA) was used to determine these parameters. The immunochemiluminescent »sandwich« test was used to determine the tumor markers AFP and CA19-9 on

Immunoassay Systems (Beckman Coulter, USA). Primenom statističkih testova utvrđeno je da postoji statistički značajna razlika između grupe sa HCC i kontrolne grupe za AFP i CA19-9 (p=0,001 i p=0,013) dok je razlika između kontrolne grupe i grupe sa sekundarnim karcinomom evidentna samo za parametar CA19-9 (p=0,035). AFP je jedini parametar koji je pokazao značajnu razliku između grupe sa HCC i grupe sa sekundarnim karcinomom (p=0,001). Rezultati našeg istraživanja pokazali su povišene vrednosti tumorskih markera CA19-9 i AFP kod pacijenata obolelih od karcinoma jetre, a AFP je pokazao i značajnu razliku između hepatocelularnog karcinoma i sekundarnih metastaza. Iako ovi markeri pokazuju suboptimalnu specifičnost i osetljivost, i dalje predstavljaju korisne biomarkere koji mogu pomoći kliničarima u postavljanju dijagnoze i prognoziranju bolesti.

the automatic analyzer Access 2 Immunoassay Systems (Beckman Coulter, USA). Using statistical tests, it was found that there was a statistically significant difference between the HCC group and the control group for AFP and CA19-9 (p=0.001 and p=0.013) while the difference between the control group and the secondary cancer group was only significant for the CA19-9 (p=0.035). AFP was the only parameter that showed a significant difference between the HCC group and the secondary cancer group (p=0.001). Results of our study showed elevated levels of the tumor markers CA19-9 and AFP in patients with liver cancer, and AFP showed a significant difference between hepatocellular carcinoma and secondary metastases. Although these markers have suboptimal specificity and sensitivity, they are still useful biomarkers that can help clinicians in the diagnosis and prognosis of the disease.

P012
KORELACIJA PENTRAKSINA-3 I LAKTATA SA LIPIDNIM STATUSOM KOD PACIJENATA SA ZNAČAJNOM STENOZOM KAROTIDNE ARTERIJE

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Stenoza karotidne arterije je jedan od glavnih uzroka akutnog ishemijskog moždanog udara i povezana je sa razvojem aterosklerotskog plaka u karotidnoj arteriji. Za procenu stepena stenoze od najvećeg značaja su »imaging« testovi. Međutim, nedavna istraživanja ukazuju na potencijalni značaj biohemijskih parametara u dijagnostici stenoze. Cilj ovog rada bio je da se ispita korelacija pentraksina-3 (PTX-3) kao specifičnog markera zapaljenja i aterosklerotskih promena, i laktata koji nastaju usled razvoja mitohondrijalne disfunkcije, koja je u osnovi ateroskleroze, sa parametrima lipidnog statusa. U ovoj studiji učestvovalo je 40 pacijenata sa stenozom karotidne arterije i 20 ispitanika koji su činili kontrolnu grupu. Određivani su parametri lipidnog statusa i laktati, spektrofotometrijskom metodom, korišćenjem komercijalnih reagenasa na biohemijskom analizatoru Olympus AU 400 i i PTX-3 koji je određen komercijalnim ELISA testom (FineTest, Wuhan, Kina). Rezultati ukazuju da su kod pušača značajno niže vrednosti laktata (P=0,016) i više vrednosti PTX-3 (P=0,001). U grupi pacijenata sa preporučenim vrednostima ukupnog i LDL-holesterola dobijena je značajna negativna korelacija laktata sa ukupnim i LDL-holesterolom (P<0,05), i pozitivna korelacija PTX-3 sa

P012
CORRELATION OF PENTRAXIN-3 AND LACTATE WITH LIPID STATUS IN PATIENTS WITH SIGNIFICANT STENOSIS OF THE CAROTID ARTERY

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Carotid artery stenosis is one of the main causes of acute ischemic stroke and is associated with the development of atherosclerotic plaques in the carotid artery. »Imaging« studies are of paramount importance in assessing the extent of stenosis. However, recent research points to the potential importance of biochemical parameters in the diagnosis of stenosis. The aim of this work was to investigate the correlation of pentraxin-3 (PTX-3), as a specific marker of inflammation and atherosclerotic changes, and lactate, resulting from the development of mitochondrial dysfunction, which is the basis of atherosclerosis, with lipid status parameters. 40 patients with carotid stenosis and 20 control subjects participated in this study. The parameters of lipid status and lactate were determined by the spectrophotometric method using commercially available reagents on the Olympus AU 400 and PTX-3 biochemical analyzer, which was determined by a commercially available ELISA test (FineTest, Wuhan, China). The results show that smokers have significantly lower lactate levels (P=0.016) and higher PTX-3 levels (P=0.001). In the group of patients with recommended levels of total and LDL cholesterol, a significant negative correlation of lactate with total and LDL cholesterol

LDL-holesterolom (P<0,05). Kod pacijenata sa preporučenim LDL-holesterolom dobijena je značajna pozitivna korelacija PTX-3 sa ukupnim holesterolom (P<0,001). Koncentracije laktata su bile u značajno negativnoj korelaciji sa PTX-3 (P=0,045). Dodatno je važno napomenuti da u kontrolnoj grupi korelacije ispitivanih parametara nisu dobijene. Rezultati ove studije ukazuju na značajnu korelaciju laktata i PTX-3 sa parametrima lipidnog statusa kod pacijenata sa karotidnom stenozom, ali i njihovu potencijalnu primenu kao biomarkera kod ove bolesti.

P013
ISPITIVANJE BIOHEMIJSKIH MARKERA KARDIOMETABOLIČKOG RIZIKA KOD GOJAZNE DECE PRE I POSLE LEČENJA U CENTRU ZA PREVENCIJU I LEČENJE GOJAZNOSTI »ČIGOTA«

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Gojaznost, najčešće hronično oboljenje među decom i adolescentima, je stanje prouzrokovano prekomernom akumulacijom masnog tkiva. Imajući u vidu rapidno rastući broj gojazne dece, kako u svetu, tako i u Srbiji, veliki napori se ulažu u rad na prevenciji i lečenju gojaznosti i njenih komorbiditeta. Trajne promene životnog stila, koje podrazumevaju promenu navika u odabiru namirnica u ishrani i povećanje stepena fizičke aktivnosti su se pokazale najefikasnijima u smislu tretmana gojaznosti u dečijoj populaciji, kao i gojaznosti uopšte. Cilj ovog rada bio je analizirati biohemijske markere kardiometaboličkog rizika gojazne dece pre i posle dvadesetjednodnevnog ciklusa lečenja u Centru za dečiju gojaznost u okviru multidisciplinarnog programa »Čigotica«. Takođe, ispitivali smo status i uticaj vitamina D na kardiometaboličke faktore rizika, kao i parametre statusa štitaste žlezde kod svih polaznika programa. Ukupan broj ispitanika bio je 74, od čega su 29 bili dečaci, a 45 devojčice. Većina adolescenata imala je indeks telesne mase (ITM) ≥30 kg/m^2 (68,9%), a skoro svaki peti imao je metabolički sindrom. Na početku programa uočeno je da veliki procenat ispitanika ima vrednosti bihemijskih markera koje su više od optimalnih. Značajan broj njih imao je dislipidemiju, a najveća odstupanja od preporučenih vrednosti zabeležena su u koncentracijama LDL holesterola (62% ispitanika). Parametri lipidnog statusa se nisu značajno razlikovali po polu, sa izuzetkom koncentracije triglicerida, koja je bila značajno viša kod dečaka. Analiza statusa vitamina D je pokazala da svi

(P<0.05) and a positive correlation of PTX-3 with LDL cholesterol (P<0.05) was found. In patients with recommended LDL cholesterol, a significant positive correlation of PTX-3 with total cholesterol was found (P<0.001). Lactate concentrations were significantly negatively correlated with PTX-3 (P=0.045). It is also important to note that in the control group, no correlations were found between the parameters studied. The results of this study indicate a significant correlation of lactate and PTX-3 with parameters of lipid status in patients with carotid stenosis, but also their potential application as biomarkers in this disease.

P013
EXAMINATION OF BIOCHEMICAL MARKERS OF CARDIOMETABOLIC RISK IN OBESE CHILDREN BEFORE AND AFTER TREATMENT AT THE CENTER FOR PREVENTION AND TREATMENT OF OBESITY »ČIGOTA«

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Obesity, the most common chronic disease among children and adolescents, is a condition caused by excessive accumulation of fat tissue. Bearing in mind the rapidly growing number of obese children, both in the world and in Serbia, great efforts are being made in the prevention and treatment of obesity and its comorbidities. Permanent changes in lifestyle, which include changing habits in the food selection and increasing the level of physical activity, have proven to be the most effective in terms of treating obesity in pediatric population, as well as obesity in general. The aim of this work was to analyze biochemical markers of cardiometabolic risks in obese children before and after a twenty-one-day treatment cycle at the Center for Childhood Obesity within the multidisciplinary program »Čigotica«. We also investigated the status and influence of vitamin D on cardiometabolic risk factors and thyroid status parameters in all participants of the program. The study included 74 participants, 29 boys and 45 girls. The majority of adolescents had a BMI≥30 kg/m^2 (68.9%), and almost one in five had metabolic syndrome. At the beginning of the program, a large percentage of participants were found to have values of bi chemical markers that were above optimal. A considerable number of them had dyslipidemia, and the largest deviations from the recommended values were found in LDL cholesterol concentrations (62% of subjects). Lipid status parameters did not differ significantly by gender, with the exception of triglyceride concentration, which were significantly higher in

ispitanici imaju suboptimalne koncentracije, a 15% deficijenciju ovog vitamina. U grupi sa deficijencijom vitamina D uočen je trend ka višim koncentracijama ukupnog i LDL holesterola. Oko 5% ispitanika imalo je vrednosti TSH koje odgovaraju subkliničkom hipotireoidizmu. Na kraju programa utvrdili smo da je došlo do značajnog smanjenja vrednosti antropometrijskih parametara i biohemijskih markera kardiometaboličkog rizika. Broj ispitanika sa metaboličkim sindromom se takođe značajno smanjio (9,5%; P<0,05). Osim toga, značajno je smanjen broj ispitanika sa povišenim koncentracijama ukupnog holesterola i triglicerida (P<0,01), kao i LDL holesterola (P<0,001). Veći procenat redukcije kardiometaboličkih faktora rizika uočen je kod dečaka, a statistički značajna razlika u odnosu na devojčice utvrđena je za vrednost ITM (P<0,01) i koncentracije ukupnog holesterola (P<0,01) i triglicerida (P<0,05). Ispitanici sa deficijencijom vitamina D na početku programa imali su veći procenat smanjenja koncentracija glukoze i parametara lipidnog statusa. Ovo istraživanje povrđuje ulogu i značaj analiza koje se rade u biohemijskoj laboratoriji Specijalne bolnice za lečenje bolesti štitaste žlezde i metabolizma »Čigota« za procenu kardiometaboličkog rizika i praćenje postignutih rezultata programa.

boys. An analysis of vitamin D status revealed that all subjects had suboptimal concentrations, while 15% had vitamin D deficiency. A trend towards higher concentrations of total and LDL cholesterol was found in the vitamin D deficient group. Approximately 5% of participants had TSH levels consistent with subclinical hypothyroidism. At the end of the program, a significant decrease in the values of anthropometric parameters and biochemical markers of cardiometabolic risk was observed. The number of subjects with metabolic syndrome also decreased significantly (9.5%; P<0.05). In addition, the number of subjects with elevated concentrations of total cholesterol and triglycerides (P<0.01) and LDL cholesterol (P<0.001) was significantly reduced. A higher percentage of reduction in cardiometabolic risk factors was observed in boys, and a statistically significant difference compared to girls was found for BMI (P<0.01) and concentrations of total cholesterol (P<0.01) and triglycerides (P<0.05). In subjects who were vitamin D deficient at the beginning of the program, the percentage reduction in glucose concentration and lipid status parameters was higher. The results of this study confirm the importance of the analyzes performed in the biochemical laboratory of the Special Hospital for the Treatment of Thyroid and Metabolic Diseases »Čigota« for the assessment of cardiometabolic risk and the monitoring of the results achieved by the program.

P014
ZNAČAJ ODREĐIVANJA GENSKE EKSPRESIJE MIR-122 I CD36 RECEPTORA U METABOLIČKOM SINDROMU

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Metabolički sindrom (MetS) predstavlja skup simptoma i znakova usko povezanih sa povećanim rizikom od razvoja kardiovaskularnih bolesti. MetS objedinjuje brojne međusobno povezane kardiometaboličke faktore rizika, kao što su insulinska rezistencija, dislipidemija, hipertenzija i gojaznost. Cluster of Differentiation 36 (CD36) kao receptor hvatač vezuje oksidovane lipoproteinske čestice, ali omogućava i transport dugolančanih masnih kiselina u ćelije.

P014
THE SIGNIFICANCE OF MIR-122 AND CD36 RECEPTOR GENE EXPRESSION IN PATIENTS WITH METABOLIC SYNDROME

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Metabolic syndrome (MetS) represents a cluster of symptoms and signs closely linked to an increased risk for cardiovascular disease development. MetS encompasses numerous interconnected cardiometabolic risk factors such as insulin resistance, dyslipidemia, hypertension, and obesity. The Cluster of Differentiation 36 (CD36) as a scavenger receptor binds oxidized lipoprotein particles, but also facilitates the transport of long-chain fatty acids into cells.

Mikro ribonukleinska kiselina 122 (miR-122) utiče na ekspresiju gena uključenih u metabolizam masnih kiselina i holesterola. Cilj našeg istraživanja je bio da ispitamo povezanost genskih ekspresija CD36 i miR-122 sa MetS-om. U studiji je učestvovalo 108 ispitanika bez i 57 sa MetS-om. Nivoi miR-122 određivani su u plazmi osiromašenoj trombocitima, dok su nivoi informacione ribonukleinske kiseline (iRNK) CD36 određivani u limfocitima i monocitima kvantitativnom lančanom reakcijom polimerizacije. Određivani su i osnovni klinički i biohemijski markeri. Nivoi iRNK CD36 i miR-122 su značajno bili veći kod ispitanika sa u odnosu na ispitanike bez MetS-a (p=0,001 i p=0,008, respektivno). Statistički značajna pozitivna korelacija pokazana je između miR-122 i iRNK CD36. Indeks telesne mase (ITM) i trigliceridi su značajno pozitivno korelirali sa miR-122. Sa nivoima iRNK CD36 značajno pozitivno su korelirali ITM i obim struka. Binarnom regresionom logističkom analizom utvrđena je značajna pozitivna asocijacija iRNK CD36 i MetS-a OR: 4,382 (1,726–11,125; p=0,002], dok je kod miR-122 prisutna pozitivna asocijacija sa hipertrigliceridemijom kao jednom od komponenti MetS-a OR: 2,635 (1,420–4,890; p=0,002], ali ne i sa MetS-om.

Micro ribonucleic acid 122 (miR-122) influences the expression of genes involved in fatty acids and cholesterol metabolism. The aim of our research was to investigate the association of CD36 and miR-122 gene expressions with MetS. The study included 108 participants without and 57 with MetS. MiR-122 levels were determined in platelet-poor plasma, while CD36 messenger ribonucleic acid (mRNA) levels were determined in lymphocytes and monocytes using quantitative polymerase chain reaction. Basic clinical and biochemical markers were also determined. CD36 mRNA and miR-122 levels were significantly higher in participants with MetS compared to those without MetS (p=0.001 and p=0.008, respectively). A statistically significant positive correlation was shown between miR-122 and CD36 mRNA. Body mass index (BMI) and triglycerides were significantly positively correlated with miR-122. BMI and waist circumference significantly positively correlated with CD36 mRNA. Binary logistic regression analysis showed a significant positive association of CD36 mRNA with MetS OR: 4.382 (1.726–11.125); p=0.002], while miR-122 was positively associated with hypertriglyceridemia as one of the components of MetS OR: 2.635 (1.420–4.890); p=0.002], but not with MetS itself.

P015
POVEZANOST SOLUBILNOG RECEPTORA UROKINAZNOG AKTIVATORA PLAZMINOGENA SA KLINIČKIM KARAKTERISTIKAMA GREJVSOVE ORBITOPATIJE

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Solubilni receptor urokinaznog aktivatora plazminogena (engl. Soluble Urokinase Plasminogen Activator Receptor, suPAR) se dovodi u vezu sa mnogim autoimunskim poremećajima, između ostalog i Grejvsovom bolešću. Ovo istraživanje je imalo za cilj da utvrdi da li postoji povezanost između koncentracije suPAR-a i Grejvsove orbitopatije (GO). Ispitivanjem je obuhvaćeno 40 pacijenata, 10 muškaraca i 30 žena, prosečne starosti 54,23±12,34 godina sa potvrđenim prisustvom Grejvsove orbitopatije. Koncentracije suPAR-a su određene korišćenjem komercijalnog nekompetitivnog Enzyme-linked Immunosorbent Assay (ELISA) testa proizvođača Fine Test. Univarijantnom binarnom logističkom regresijom je dokazano da pacijenti sa teškim oblikom GO imaju 6,9 puta veću

P015
RELATIONSHIP OF SOLUBLE UROKINASE ACTIVATOR PLASMINOGEN RECEPTOR WITH CLINICAL CHARACTERISTICS OF GRAVES' ORBITOPATHY

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The soluble urokinase plasminogen activator receptor (suPAR) is associated with many autoimmune diseases, including Graves' disease. The purpose of this study was to determine whether there is a relationship between suPAR levels and Graves' orbitopathy (GO). The study included 40 patients, 10 men and 30 women, mean age 54.23±12.34 years, with confirmed Graves' orbitopathy. SuPAR levels were determined using a commercial non-competitive enzyme-linked immunosorbent assay (ELISA) from Fine Test. Univariate binary logistic regression showed that patients with a severe form of GO have a 6.9 times higher chance of having suPAR serum concentration above the median for the studied population (p=0.012). A correlation was also found between

šansu da će imati koncentraciju suPAR-a u serumu iznad medijane za ispitivanu populaciju (p=0,012). Takođe uočena je korelacija između porasta koncentracije suPAR-a i smanjenjem motiliteta oka. Univarijantnom regresionom analizom je utvrđena statistički granično značajna povezanost aktivnosti GO sa koncentracijom suPAR-a u serumu ispitanika. Dokazano da pacijenti sa aktivnim oblikom GO imaju 3,7 puta veću šansu da će imati koncentraciju suPAR-a u serumu iznad medijane za ispitivanu populaciju (p=0,058). Na osnovu rezultata dobijenih ovim istraživanjem pokazano je da postoji povezanost kliničkog oblika GO i serumskih koncentracija suPAR-a, što ukazuje potencijal suPAR-a kao biomarkera čija bi se koncentracija mogla u budućnosti koristiti u cilju praćenja težine Grejvsove orbitopatije.

P016
POVEZANOST TIROIDNOG I NUTRITIVNOG STATUSA U POPULACIJI STUDENATA

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Tiroidna žlezda zauzima centralnu ulogu u održavanju homeostaze organizma. Međutim, poremećaji funkcije tiroidne žlezde su sve učestaliji i predstavljaju problem za sve veći procenat populacije. Pored autoimunih poremećaja, način ishrane u velikoj meri utiče na rad žlezde. Optimalan unos hranljivih materija, vitamina i esencijalnih elemenata, znatno poboljšava funkciju tiroidne žlezde. Nedostatak tiroidnih hormona u detinjstvu može dovesti do značajnih smetnji u razvoju deteta, dok je kod odraslih poremećaj tiroidnog statusa povezan sa dislipidemijama i oboljenjima jetre i bubrega. Cilj ovog rada je bio da se proceni tiroidni i nutritivni status u populaciji od 20 zdravih dobrovoljaca (studentkinje, starosne dobi: 20–25 godina), Farmaceutskog fakulteta Univerziteta u Beogradu i u tom smislu ispitivana je raspodela podataka i korelacija biohemijskih parametara sa nutritivnim statusom. Informacije o načinu ishrane ispitanika dobijene su na osnovu dnevnika ishrane, koji je svaki ispitanik vodio za sebe sa detaljnim opisima vrste, količine i sastava namirnice (dva radna dana i jedan dan vikenda). Pored nutritivnog statusa i biohemijskih parametara, određivane su i antropo-

the increase in suPAR concentration and the decrease in ocular motility. A univariate regression analysis revealed a statistically borderline significant relationship between GO activity and the concentration of suPAR in the subjects' serum. It was demonstrated that patients with an active form of GO were 3.7 times more likely to have a serum suPAR concentration above the median of the population studied (p=0.058). Based on the results obtained in this study, it was shown that there is a correlation between the clinical form of GO and the serum concentration of suPAR, indicating the potential of suPAR as a biomarker whose concentration could be used in the future to monitor the severity of Graves' orbitopathy.

P016
ASSOCIATION BETWEEN THYROID GLAND AND NUTRITIONAL STATUS IN THE STUDENT POPULATION

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The thyroid gland plays a central role in maintaining the body's homeostasis. However, thyroid dysfunction is becoming increasingly common and is a problem for a growing percentage of the population. In addition to autoimmune disorders, the type of diet also has a major influence on the functioning of the gland. An optimal intake of nutrients, vitamins and essential elements significantly improves the function of the thyroid gland. A deficiency of thyroid hormones in childhood can lead to significant disorders in child development, while in adults the disruption of thyroid status is associated with dyslipidemia and liver and kidney disease. The aim of this work was to evaluate the thyroid and nutritional status in the population of 20 healthy students (female students, 20–25 years old) from the Faculty of Pharmacy at the University of Belgrade and, in this sense, to investigate the distribution of data and the correlation of biochemical parameters with nutritional status. The information about the subjects' diet was obtained from a food diary which each subject kept for herself and in which the type, quantity and composition of food were described in detail (two days of the week

metrijske karakteristike. Pokazana je statistički značajna korelacija između parametara tiroidnog statusa i parametara lipidnog statusa (FT4 i HDL: Rho= 0,459, p<0,05), kao i parametara tiroidnog statusa sa količinom unetih šećera, masti i masnih kiselina (TSH i šećeri: Rho=0,462, p<0,05; FT4 i masti Rho=0,552, p<0,05; FT4 i masne kiseline Rho=0,609; p<0,01). Rezultati ove studije mogu da ukažu da je za prevenciju tiroidnih poremećaja od značaja kontrola i optimizacija lipidnog statusa organizma, kao i unosa adekvatne količine šećera i masti u populaciji studentkinja.

P017
ČESTI UZROCI NEUSPEHA AUTOVALIDACIJE REZULTATA LABORATORIJSKIH TESTOVA U PERIODU OD ŠEST MESECI

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Autovalidacija (AV) rezultata je važan proces u laboratorijama koji smanjuje broj grešaka i povećava efikasnost izveštavanja o rezultatima. Kriterijumi za autovalidaciju treba da budu striktno definisani i lako se menjaju ako je potrebno. Cilj ove studije bio je da se uporede najčešći razlozi blokiranja autovalidacije u vreme implementacije i 6 meseci nakon. AV je implementiran u Laboratorijskom informacionom sistemu (LIS) Odeljenja za kliničku hemiju, Univerzitetski bolnički centar Sestre milosrdnice u septembru 2023. za biohemijske testove na Alinity ci (Abbott Laboratories, SAD), za testove gasova krvi na ABL90 Flex (Radiometer, Danska) i za analizu urina na Atellica UAS1500 (Siemens Healthineers, Nemačka). Delta provera, kritične vrednosti, vrednosti izvan referentnog i mernog opsega, uzorci nepotvrđene kontrole kvaliteta (KK), KK koji nije sproveden duže od 24 sata i različiti komentari su postavljeni kao kriterijumi za AV za svaki test. Posle septembra, neka pravila su izmenjena u narednih 6 meseci (kritične i vrednosti delta provere, komentari su standardizovani) da bi se bolje detektovali rezultati van kriterijuma i da bi se zaustavila AV ako je potrebno. Od oktobra 2023. do aprila 2024. zabeležen je broj

and one day at the weekend). In addition to the nutritional status and biochemical parameters, the anthropometric characteristics were also determined. A statistically significant correlation was found between parameters of thyroid status and lipid status (FT4 and HDL: Rho=0.459, p<0.05) and between parameters of thyroid status and the amount of sugars, fats and fatty acids ingested (TSH and sugars: Rho=0.462, p<0.05; FT4 and fats: Rho=0.552, p<0.05; FT4 and fatty acids: Rho=0.609; p<0.01). The results of this study may suggest that in order to prevent thyroid disease, it is important to control and optimise the body's lipid status, as well as the intake of adequate amounts of sugar and fat in the student population.

P017
COMMON CAUSES OF LABORATORY TEST RESULT AUTOVALIDATION FAILURE OVER A SIX-MONTH PERIOD

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Autovalidation (AV) of results is an important process in laboratories that reduces the number of errors and increases the efficiency of result reporting. The criteria for autovalidation should be strictly defined and easy to change if necessary. The aim of this study was to compare the most common reasons of blocking autovalidation at the time of implementation and 6 months after. AV was implemented in the Laboratory Information System (LIS) of the Department of Clinical Chemistry, Sestre milosrdnice University Hospital Center in September 2023 for biochemical tests on the Alinity ci (Abbott Laboratories, USA), for blood gas tests on the ABL90 Flex (Radiometer, Denmark) and for urinalysis on the Atellica UAS1500 (Siemens Healthineers, Germany). Delta check, critical values, values outside the reference and measurement ranges, unvalidated quality control (QC) samples, QC not performed for more than 24 hours and various comments were set as criteria for AV for each test. After September, some rules were modified in the next 6 months (critical and delta check values, comments were standardized) to better detect results beyond the criteria and to stop AV if necessary. From October 2023 to April 2024, number of autovalidat-

automatski potvrđenih rezultata i razloga za neuspešne AV rezultate. Rezultati su predstavljeni brojevima i procentima. Razlike u procentima nakon prvog (verifikacionog) meseca izračunate su u Medcalc statističkom programu. Sigma vrednosti su izračunate za sve ne-AV rezultate za svaki mesec i kriterijum. Tokom analiziranog perioda, procenat ne-AV rezultata značajno se smanjio sa 20% u septembru 2023. na 15,8% u aprilu 2024. (P<0,001). Od svih rezultata koji nisu uspeli u AV, provera delta iznad unapred definisanih kriterijuma, nepotvrđene vrednosti KK i kritične vrednosti bili su najčešći razlozi tokom svih analiziranih meseci (prosek 27,3%; 23,0% i 7,7%, redom) sa najnižim odgovarajućim sigma vrednostima (medijana 2,15, 2.3 i 2.9, redom). Neuspeh AV je bio manji za rezultate van mernog i referentnog opsega (4,64 i 3,60 sa 0,11% i 2,05%, redom). Procenat različitih komentara koji su prekinuli AV značajno se smanjio nakon implementacije AV (20,1% u 9/23 i 17,0% u proseku u ostalim mesecima, P<0,001). Kriterijumi za autovalidaciju koji su postavljeni prema ekspertima doveli su do autovalidacije u više od 80% rezultata. Vremenom nakon implementacije, kriterijumi su blago modifikovani, značajno smanjujući procenat neautovalidovanih rezultata iznad unapred definisanih kriterijuma. Proces autovalidacije bi trebalo da može da se modifikuje kako bi se obezbedilo više AV rezultata u velikim laboratorijama.

ed results and reasons for failed AV results were recorded. The results are presented as numbers and percentages. The differences in percentages after the first (verification) month were calculated in Medcalc. Sigma values were calculated for all non-AV results for each month and criterion. Over the period analyzed, the percentage of non-AV results decreased significantly from 20% in September 2023 to 15.8% in April 2024 (P<0.001). Of all results that failed AV, delta check above predefined criteria, unvalidated QC values and critical values were the most common reasons during all months analyzed (average 27.3%, 23.0% and 7.7%, respectively) with the lowest corresponding sigma values (median 2.15, 2.3 and 2.9, respectively). Failure of AV was less likely for results outside the measurement and reference ranges (4.64 and 3.60 with 0.11% and 2.05%, respectively). The percentage of various comments that discontinued AV decreased significantly after the implementation of AV (20.1% in 9/23 and 17.0% on average in other months, P<0.001). The criteria for autovalidation which were set according to the experts, led to autovalidation in more than 80% of results. Over time after implementation, the criteria were modified slightly, significantly decreasing the percentage of non-autovalidated results beyond the predefined criteria. The autovalidation process should be modifiable to provide more AV results in large laboratories.

P018
ISPITIVANJE RIZIKA ZA NASTANAK KARDIOVASKULARNIH BOLESTI I PRIDRUŽENIH MARKERA KOD PACIJENATA SA STEATOZOM

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Nealkoholna masna bolest jetre, koja obuhvata širok spektar histopatoloških promena u jetri, se smatra hepatičnom manifestacijom metaboličkog sindroma. Većinu pacijenata sa ovom bolešću prati gojaznost, dislipidemija, tip 2 dijabetesa, hipertenzija, kao i povećani rizik za nastanak kardiovaskularnih bolesti (KVB). Cilj ovog istraživanja je bio da se utvrdi da li postoji povećani rizik za nastanak KVB kod ispitanika sa stea-

P018
INVESTIGATION OF THE RISK FOR THE OCCURENCE OF CARDIOVASCULAR DISEASES AND MARKERS ASSOCIATED WITH IT IN PATIENTS WITH STEATOSIS

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Non-alcoholic fatty liver disease, which encompasses a broad spectrum of histopathological changes in the liver, is considered a hepatic manifestation of metabolic syndrome. Most patients with this disease are accompanied by obesity, dyslipidemia, type 2 diabetes, hypertension and an increased risk of cardiovascular disease (CVD). The aim of this study was to determine whether patients with steatosis are at

tozom, pri čemu se rizik ispitivao Reynolds risk skorom. U istraživanje je bilo uključeno 159 ispitanika (58 ispitanika kontrolne grupe i 101 pacijent sa steatozom). Ispitanici sa steatozom imali više vrednosti Reynolds risk skora u odnosu na kontrolnu grupu. Takođe, ispitanici sa steatozom su imali više koncentracije glukoze, C-reaktivnog proteina (CRP), triglicerida (TG), glikohemoglobina (HbA1c), a niže koncentracije holesterola u lipoproteinskim česticama visoke gustine (HDL-h). Prisustvo steatoze, koncentracije glukoze, CRP, TG, mokraćne kiseline, aktivnosti γ-glutamil transferaze, su bili u značajnoj pozitivnoj, dok je HDL-h bio u značajnoj negativnoj asocijaciji sa Reynolds risk skorom. Prisustvo steatoze 2,4 puta povećava rizik za nastanka KVB kada je taj rizik preračunat preko Reynolds risk skora (OR=2,422 [(1,021–5,741)]; p=0,045). Od svih ispitivanih markera kada su testirani u modelu binarnom logističkom regresijom kod ispitanika sa steatozom, HDL-h se pokazao kao nezavisan negativan prediktor za nastanak KVB (OR=0,088 (0,160–0,485)]; p=0,005). Prisustvo steatoze, kao i sniženje koncentracije HDL-h predstavljaju faktore rizika za nastanak KVB.

increased risk of CVD. The risk was assessed using the Reynolds risk score. 159 participants (58 participants in the control group and 101 patients with steatosis) were included in the study. Patients with steatosis had higher Reynolds risk score values compared to the control group. In addition, patients with steatosis had higher levels of glucose, C-reactive protein (CRP), triglycerides (TG) and glycohemoglobin (HbA1c) and lower levels of high-density lipoprotein cholesterol (HDL-c). The presence of steatosis, glucose, CRP, TG, uric acid and γ-glutamyltransferase activity were significantly positive, while HDL-c was significantly negatively associated with the Reynolds risk score. The presence of steatosis increased the risk of CVD by 2.4-fold when this risk was calculated using the Reynolds risk score (OR=2.422 [(1.021–5.741)]; p=0.045). Of all the markers examined, HDL-c was found to be an independent negative predictor of CVD in a binary logistic regression analysis in patients with steatosis (OR=0.088 0.160–0.485]; p=0.005). The presence of steatosis and a decrease in HDL-c levels are risk factors for the development of CVD.

P019
UTICAJ TERAPIJE KOJA MODIFIKUJE PRIRODNI TOK BOLESTI NA NIVO KINURENINSKE I HINOLINSKE KISELINE KOD PACIJENATA SA RELAPSNO-REMITENTNOM MULTIPLOM SKLEROZOM

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U patogenezi relapsno-remitentne multiple skleroze (RRMS) prisutni su poremećaji kinureninskog puta metabolizma triptofana. Neuroprotektivna uloga kinureninske kiseline (KA) se, između ostalog, odnosi na njene antioksidativne osobine, sprečavajući oštećenje tkiva izazvano prekomernim inflamatornim odgovorom. Neurotoksični potencijal hinolinske kiseline (QA) se može pripisati stvaranju reaktivnih vrsta kiseonika koji učestvuju u lipidnoj peroksidaciji i sniženju endogenih antioksidanasa. Cilj ovog istraživanja je bio da se ispitaju efekti terapije koja modifikuje prirodni tok bolesti (eng. disease modyfing

P019
THE INFLUENCE OF DISEASE MODYFING THERAPY ON THE LEVEL OF KYNURENIC AND QUINOLINIC ACID IN PATIENTS WITH RELAPSING-REMITTING MULTIPLE SCLEROSIS

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Disorders in the kynurenine pathway of tryptophan metabolism play a role in the development of relapsing-remitting multiple sclerosis (RRMS). The neuroprotective role of kynurenic acid (KA) is related, among other things, to its antioxidant properties, which prevent tissue damage caused by an excessive inflammatory reaction. The neurotoxic potential of quinolinic acid (QA) can be attributed to the generation of reactive oxygen species involved in lipid peroxidation and the reduction of endogenous antioxidants. The aim of this study was to investigate the effects of disease-modifying therapy (DMT): Betaferon®,

therapy-DMT): Betaferon®, Rebif®, Copaxone®, S1PR-modulatori, na nivoe KA, QA i indeksa QA/KA kod 111 pacijenata sa RRMS pre i posle šestomesečne terapije. KA i QA su određivani u serumu, komercijalnim kompetitivnim ELISA testom (Immusmol SAS, Bordeaux, France). Rezultati pokazuju sniženje indeksa QA/KA nakon šestomesečnog praćenja DMT (p= 0,032). Utvrđena je značajna razlika između četiri leka koji su ispoljili pad indeksa QA/KA (p=0,010). Pokazano je da šestomesečna terapija lekom Copaxone® dovodi do porasta KA (p=0,048), i pada indeksa QA/KA (p=0,006). Ukljanjanjem uticaja početnih vrednosti KA i QA, uočava se značajan pad indeksa QA/KA kod primene lekova Copaxone® i S1PR-modulatora nakon šestomesečne terapije u odnosu na početne vrednosti. Postojala je tendencija porasta indeksa QA/KA kod pacijenata na terapiji iz grupe Interferona- (Betaferon® i Rebif®). Dobijeni rezultati ukazuju da šestomesečna DMT, dovodi do značajnog pada indeksa QA/KA koji bi mogao biti koristan parametar za praćenje efikasnosti terapije lekovima Copaxone® i S1PR- modulatora kod RRMS pacijenata.

Rebif®, Copaxone®, S1PR modulators on KA, QA and the QA/KA index in 111 RRMS patients before and after six months of therapy. The concentrations of KA and QA in serum were measured using a commercial competitive ELISA (Immusmol SAS, Bordeaux, France). After six months of DMT treatment, the QA/KA index decreased (p= 0.032). A significant difference was found between the four drugs that showed a decrease in the QA/KA index (p=0.010). It was observed that six months of therapy with Copaxone® led to an increase in KA (p=0.048) and a decrease in the QA/KA index (p=0.006). When Copaxone® and S1PR modulators were used, a decrease in the QA/KA index was observed after six months of therapy compared to baseline values (when the influence of baseline values of KA and QA was eliminated). Patients who received therapy from the interferon-β group (Betaferon® and Rebif®) experienced an increase in QA/KA. The study shows that six months of DMT leads to a significant decrease in the QA/KA index, which could be a useful parameter for monitoring the efficacy of Copaxone® and S1PR modulator therapy in RRMS patients.

P020
MOGUĆNOSTI VEŠTAČKE INTELIGENCIJE U AUTOMATIZOVANOJ DIGITALNOJ MIKROSKOPIJI RAZMAZA PERIFERNE KRVI – PRIKAZ SLUČAJA

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Veštačka inteligencija u današnje vreme nalazi primenu u različitim granama medicine, pa i u području laboratorijske hematologije. Razlozi za to su brojni: ograničenja hematoloških analiazatora kada su u pitanju patološki uzorci, veliki broj uzoraka, nedostatak vremena, nedovoljan broj iskusnih hematoloških morfologa itd. Zbog toga u praksi, danas značajno mesto zauzimaju napredni hematološki sistemi, koji pored klasičnih analiza, imaju integrisane sisteme za automatsko pravljenje, bojenje i pregled razmaza periferne krvi i vrše klasifikaciju leukocita u 17 subpopu-

P020
POSSIBILITIES OF ARTIFICIAL INTELLIGENCE IN AUTOMATED DIGITAL MICROSCOPY OF PERIPHERAL BLOOD SMEAR – CASE REPORT

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Today, artificial intelligence is used in various areas of medicine, including laboratory hematology. The reasons for this are manifold: limitations of hematology analyzers for pathological samples, large number of samples, lack of time, insufficient number of experienced hematology morphologists, etc. Therefore, in practice, advanced hematology systems, which, in addition to classical analyzes, have integrated systems for the automatic preparation, staining and examination of peripheral blood smears and perform the classification of leukocytes into 17 subpopulations, the

lacija, karakterizaciju eritocita u 6 morfoloških oblika kao i pregled trombocita. Zato je cilj ovog rada prikazati mogućnosti automatskog hematološkog analizatora u klasifikaciji ćelija krvi kod stanja hronične mijeloične leukemije (CML), te dobijene rezultate uporediti sa rezultatima svetlosne mikroskopije. Analiziran je uzorak krvi pacijenta starosti 62 godine sa suspektnom dijagnozom CML. Uzorak je analiziran na automatskom hematološkom analizatoru Sysmex XN 3100 te su dobijeni rezultati upoređeni sa rezultatima manuelnog pregleda razmaza periferne krvi koji je obojen MGG (May Grunwald Giemsa) bojom, te analiziran svetlosnom mikroskopijom. Hematološki analizator je pokazao izrazitu leukocitozu, nepotpunu diferencijaciju leukocita uz niz upozorenja, zbog čega je uzorak analiziran u delu aparata koji ima digitalnu mikroskopiju, gde koristi CellaVision software u čijoj osnovi je veštačka inteligencija. Dobijeni su sledeći rezultati: segmentirani neutrofilni granulociti 35%, nesegmentirani neutrofili 16%, eozinofilni granulociti 2,5%, bazofilni granulociti 10%, limfociti 18%, monociti 5,5%, metamijelociti 4,5%, mijelociti 5,5%, blasti 1,0%, plazma ćelije 2,0%, 11,0% nedifinisanih ćelija, uz 19,5% eritroblasta. Manuelnom mikroskopijom su dobijeni sledeći rezultati: 20% segmentirani neutrofili, 16% nesegmentirani neutrofili, 4% eozinofili, 12% limfociti, 8% monociti, 13% bazofili, 9% metamijelociti, 10% mijelociti, 5% promijelociti, 3% blasti uz 18% acidofilnih, 12% polihromatofilnih i 1% bazofilnih eriroblasta. Dobijeni rezultati pokazuju zadovoljavajuće podudaranje analiziranih metoda, te daju prednost upotrebi veštačke inteligencije u hematološkim laboratorijama, ali svakako potpunu implementaciju automatskog analizatora u rutinskom radu moguće je izvršiti nakon detaljne evaluacije na velikom broju uzoraka.

characterization of erythrocytes into 6 morphological forms and the examination of thrombocytes, now occupy an important place. The aim of this work is therefore to demonstrate the capabilities of the automated hematology analyzer in the classification of blood cells in chronic myeloid leukemia (CML) and to compare the results obtained with the results of light microscopy. A blood sample from a 62-year-old patient with a suspected diagnosis of CML was analyzed. The sample was analyzed using the Sysmex XN 3100 automated hematology analyzer, and the results obtained were compared with the results of a manual examination of the peripheral blood smear stained with the dye MGG (May Grunwald Giemsa) and analyzed by light microscopy. The hematology analyzer showed marked leukocytosis, incomplete differentiation of leukocytes with a series of alerts, so the sample was analyzed in the part of the device that has digital microscopy, where the CellaVision artificial intelligence- based software is used. The following results were obtained: segmented neutrophil granulocytes 35%, band neutrophils 16%, eosinophil granulocytes 2.5%, basophil granulocytes 10%, lymphocytes 18%, monocytes 5.5%, metamyelocytes 4.5%, myelocytes 5.5%, blasts 1.0%, plasma cells 2.0%, 11.0% undefined cells, with 19.5% erythroblasts. Manual microscopy yielded the following results: 20% segmented neutrophils, 16% unsegmented neutrophils, 4% eosinophils, 12% lymphocytes, 8% monocytes, 13% basophils, 9% metamyelocytes, 10% myelocytes, 5% promyelocytes, 3% blasts with 18% acidophilic, 12% polychromatophilic and 1% basophilic erythroblasts. The results obtained show a satisfactory agreement of the analyzed methods and give preference to the use of artificial intelligence in hematology laboratories, but the full implementation of the automatic analyzer in routine work is certainly only possible after a detailed evaluation of a large number of samples.

P021
PROCENA KVALITETA ODREĐIVANJA BIOHEMIJSKIH, IMUNOHEMIJSKIH I HEMATOLOŠKIH PARAMETARA ZA DIJAGNOZU, PRAĆENJE I DIFERENCIJACIJU ANEMIJE PRIMENOM PRINCIPA »ŠEST SIGMA«

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Šest sigma princip predstavlja metodu evaluacije kvaliteta, kojom se identifikuju i kvantifikuju defekti procesa. Ukoliko je izračunata sigma vrednost ≥3,

P021
QUALITY ASSESSMENT OF BIOCHEMICAL, IMMUNOCHEMICAL AND HEMATOLOGICAL PARAMETERS DETERMINATION FOR DIAGNOSIS, MONITORING AND DIFFERENTIATION OF ANEMIA USING THE »SIX SIGMA« PRINCIPLE

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»Six sigma« principle is a method for quality evaluation, which can identify and quantify defects in the process. This method can lead the analyst into solv-

metoda može da se koristi u medicinsko-biohemijskoj laboratoriji, uz odgovarajući plan kontrole kvaliteta. Cilj ovog rada jeste evaluacija kvaliteta određivanja parametra koji se koriste u dijagnostici, praćenju i diferencijaciji anemije, korišćenjem metode šest sigma. U ovom radu, sprovedena je retrospektivna analiza unutrašnje i spoljašnje kontrole kvaliteta određivanja hematoloških (hemoglobin, hematokrit, MCV, MCH, MCHC i RDW-CV), biohemijskih (nezasićeni kapacitet vezivanja gvožđa (UIBC), gvožđe, laktat dehidrogenaza (LDH), ukupan i konjugovani bilirubin) i imunohemijskih parametara (haptoglobin, transferin, feritin i folna kiselina), u medicinsko-biohemijskoj laboratoriji. Izračunavanje sigma vrednosti, obavljeno je na osnovu koeficijenta varijacije, vrednosti bias-a i ukupne dozvoljene greške. Od ukupno 77 izračunatih sigma vrednosti za 16 odabranih parametara, 44 je imalo vrednost manju od tri. Ovakve vrednosti su indikator neprihvatljivih performansi. Pojedini kontrolisani parametri imaju prihvatljivu sigma vrednost, i pouzdano mogu da se koriste u laboratorijskoj obradi patološkog stanja anemije. Ipak, određivanje pojedinih parametara nema prihvatljive performanse. Sigma vrednosti se umnogome razlikuju u zavisnosti od baze podataka koja je korišćena za ukupnu dozvoljenu grešku.

ing the systemic problem, and it can give the insight into the importance of the problem. If sigma value of the method is ≥3, than the method can be used in clinical laboratory, along with suitable quality control program. The aim of this study is quality evaluation of the parameters that are used for diagnosis, monitoring and differentiation of anemia, using the »six sigma« method. In this study, a retrospective analysis of internal and external quality control of haematological (haemoglobin, haematocrit, MCV, MCH, MCHC and RDW-CV), biochemical (unsaturated iron binding capacity (UIBC), iron, lactate dehydrogenase (LDH), total and conjugated bilirubin) and immunochemical parameters (haptoglobin, transferrin, ferritin and folic acid) was conducted in a clinical laboratory. Sigma value is calculated based on coefficient of variation, value of bias and value of total allowable error. Out of 77 calculated sigma values for 16 chosen parameters, 44 sigmas have the value of less than three. These sigma values are indicators of unacceptable performance. Some of the controlled parameters have acceptable sigma value and are suitable for laboratory use for processing anemia. However, some of the parameters have unacceptable performance. Sigma values highly vary based on the data base which is used for total allowable error.

P022
POVEZANOST MIR-21-5P I GPX1 IRNK U NEALKOHOLNOJ MASNOJ BOLESTI JETRE (NAFLD)

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Nealkoholna masna bolest jetre (eng. nonalcoholic fatty liver disease, NAFLD) je najčešće hronično oboljenje jetre, u čijoj osnovi je akumulacija triglicerida u hepatocitima – steatoza. Procenjuje se da ovo stanje pogađa između 20% i 30% stanovništva zapadnih zemalja. NAFLD se dovodi u vezu sa izmenjenom ekspresijom mikro ribonukleinskih kiselina (mikroRNK, miRNA), ali i sa oksidativnim stresom. Cilj ovog istraživanja bio je da se utvrdi da li su ekspresija miR-21-5p, kao i ekspresija gena za glutation peroksidazu 1 (GPX1) odgovarajući markeri za procenu rizika za nastanak steatoze. Studija je obuhvatila 130 ispitanika, podeljenih u dve grupe: 84 pacijenata sa steatozom i 46 zdravih ispitanika (KG), na osnovu nalaza ultrazvuka abdomena dobijenih u Kliničko-bolničkim centrima »Zvezdara« i »Zemun«. U uzorcima plazme

P022
ASSOCIATION OF MIR-21-5P AND GPX1 MRNA IN NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

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Non-alcoholic fatty liver disease (NAFLD) is the predominant chronic liver disease characterised by the accumulation of triglycerides in the liver cells – steatosis. It is estimated that between 20 and 30% of the population in Western countries are affected by this disease today. NAFLD is associated with dysregulated expression of micro-ribonucleic acids (microRNAs/miRNAs) and oxidative stress. This study aimed to investigate whether the expression levels of miR-21-5p and the glutathione peroxidase 1 (GPX1) gene can serve as markers for steatosis risk assessment. The study included 130 participants divided into two cohorts: 84 NAFLD patients and 46 healthy controls (HC), who were examined by abdominal ultrasound at the University Medical Centres »Zvezdara« and »Zemun«. Expression of miR-21-5p

osiromašene trombocitima određena je ekspresija miR-21-5p, dok u mononuklearnim ćelijama periferne krvi ekspresija gena za GPX1 primenom reakcije lančane polimerizacije u realnom vremenu (real-time PCR). Ekspresija miR-21-5p je bila značajno viša, dok GPX1 gena značajno niža u grupi pacijenata u poređenju sa KG. Spearman-ova korelaciona analiza pokazala je da je miR-21-5p bila u značajnoj negativnoj korelaciji sa informacionom RNK (iRNK) GPX1 kod svih ispitanika. Sa povećanjem ekspresije miR-21-5p za 1 ekspresionu jedinicu raste verovatnoća za pojavu steatoze 5,3 puta, dok sa smanjenjem nivoa GPX1 iRNK za 1 ekspresionu jedinicu raste verovatnoća za pojavu steatoze 58,2%. Ekspresija miR-21-5p, koja delovanjem na ciljne gene utiče na biosintezu holesterola i regulaciju metabolizma lipida u jetri, kao i ekspresija gena za GPX1, jednog od enzima antioksidativne zaštite, su značajni prediktori za razvoj steatoze.

P023
EVALUACIJA METILACIJE DNK KOD PACIJENATA SA NEADEKVATNO REGULISANIM DIJABETES MELITUSOM TIP 2

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U osnovi dijabetes melitusa tip 2 (DM2) su insulinska rezistencija i perzistentna hiperglikemija, koje doprinose razvoju hroničnih komplikacija, poput kardiovaskularnih bolesti i mikrovaskularnih komplikacija. Novije studije sugerišu da epigenetske modifikacije, a posebno metilacija DNK, igraju ključnu ulogu u patogenezi DM2 i pridruženih komplikacija. Cilj ove studije je bio ispitivanje veze između globalne metilacije DNK i kliničkih i biohemijskih parametara kod pacijenata sa neadekvatno regulisanim DM2. U studiji je učestvovalo 107 pacijenata sa DM2 i 56 zdravih ispitanika. Globalna DNK metilacija je određena HPLC-UV metodom i izražena kao procentualna zastupljenost 5-metilcitozina (5mC) u ukupnom sadržaju nukleozida u molekulu DNK. Biomarkeri oksidativ-

was measured in platelet-depleted plasma, while GPX1 gene expression was determined in peripheral blood mononuclear cells by real-time PCR. The miR-21-5p expression was significantly higher in the patient group, while GPX1 gene expression was significantly lower compared to HC. Spearman correlation analysis showed that miR-21-5p was significantly negatively correlated with GPX1 messenger RNA (mRNA) in all subjects. A one-unit increase in miR-21-5p expression corresponded to a 5.3-fold increase in the likelihood of developing steatosis, while a one-unit decrease in GPX1 mRNA levels resulted in a 58.2 percent increase in the likelihood of developing steatosis. The expression of miR-21-5p, which is known to influence cholesterol biosynthesis and regulate lipid metabolism in the liver, and the expression of the GPX1 gene, an enzyme of the antioxidant defence system, are significant predictors for the development of steatosis.

P023
EVALUATION OF DNA METHYLATION IN POORLY REGULATED TYPE 2 DIABETES MELLITUS

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Diabetes mellitus type 2 (T2DM) is characterized by insulin resistance and hyperglycemia, contributing to development of chronic complications such as cardiovascular disease and microvascular complications. Emerging evidence suggests that epigenetic modifications, particularly DNA methylation, play a pivotal role in the pathogenesis of T2DM and its associated complications. This study aimed to investigate global DNA methylation levels and their associations with clinical and biochemical parameters in patients with poorly regulated T2DM compared to healthy controls. The study included 107 patients with T2DM and 56 healthy controls. Global DNA methylation was quantified by the HPLC-UV method and expressed as the proportion of 5-methylcytosine

nog stresa su određeni spektrofotometrijskim metodama. Pacijenti sa DM2 su imali značajno niži nivo globalne DNK metilacije (3,56±0,31%) u poređenju sa zdravim kontrolama (4,00±0,68%, p<0,001). Globalna DNK metilacija je bila u negativnoj korelaciji sa godinama starosti i koncentracijom glukoze u serumu, a u pozitivnoj sa koncentracijom HDL- holesterola. Primenom multivarijantne analize potvrđena je nezavisna povezanost globalne DNK metilacije sa starošću, ukupnim antioksidativnim statusom i koncentracijom sulfhidrilnih grupa u serumu. Možemo zaključiti da pacijenti sa DM2 imaju globalnu hipometilaciju DNK, povezanu sa starošću, glikemijskom kontrolom i redoks statusom. Naši rezultati sugerišu da promene u metilaciji DNK mogu imati ulogu u patogenezi DM2 i razvoja komplikacija.

(5mC) within DNA nucleosides. Biomarkers of oxidative stress were evaluated by spectrophotometric methods. Patients with T2DM had significantly lower global DNA methylation levels (3.56±0.31%) compared to healthy controls (4.00±0.68%, p<0.001). Global DNA methylation was inversely associated with age and serum glucose concentration, and positively with HDL-cholesterol levels. Multivariate analysis revealed that global DNA methylation was independently associated with age, total antioxidant status, and levels of sulfhydryl groups. In conclusion, patients with T2DM exhibit global DNA hypomethylation, which is related to age, glycemic control, and antioxidant status. These findings suggest that altered DNA methylation may play a role in the pathogenesis of T2DM and its complications.

P024
ODREĐIVANJE AUTOANTITELA G KLASE NA OKSIDOVANI LDL KOD PACIJENATA SA AKUTNIM INFARKTOM MIOKARDA SA ELEVACIJOM ST SEGMENTA

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Studije novijeg datuma sugerišu da pored dislipidemije, inflamacije i oksidativnog stresa, u nastanku i razvoju ateroskleroze učestvuju i imunski procesi. Dobro je poznato da oksidovani lipoprotein niske gustine (oxLDL) podstiče proizvodnju autoantitela, što rezultira formiranjem imunskih kompleksa koji sadrže oxLDL. Izolovana autoantitela pretežno su G izotipa i usmerena su protiv različitih epitopa na oxLDL. U praksi se smatraju markerima prisustva oxLDL, ali njihova uloga u nastanku i progresiji ateroskleroze još uvek nije rasvetljena. Ova studija imala je za cilj da odredi koncentracije autoantitela G klase na oxLDL (IgG anti-oxLDL antitela) u serumu kod bolesnika sa akutnim infarktom miokarda sa elevacijom ST segmenta (STEMI) i da istraži njihovu povezanost sa rizičnim LDL i HDL fenotipom. Kod 69 STEMI bolesnika i 67 zdravih kontrolnih ispitanika, IgG anti-oxLDL antitela su određena korišćenjem komercijalno dostupnog ELISA kita (OLAB IgG Anti Oxidized Low Density Lipoprotein, Biomedica Medizinprodukte GmbH & Co KG, A-1210 Wien, Austria) prema preporukama proizvođača. Razdvajanje HDL i LDL subklasa izvršeno je poliakrilamid gradijent gel elektroforezom. Osim toga, određeni su i relativni

P024
DETERMINATION OF IGG ANTI-OXLDL ANTIBODY LEVELS IN PATIENTS WITH ST-SEGMENT ELEVATION ACUTE MYOCARDIAL INFARCTION

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Recent studies suggest that in addition to dyslipidemia, inflammation and oxidative stress, immune processes are also involved in the formation and development of atherosclerosis. It is known that circulating oxidized low-density lipoprotein (oxLDL) triggers the production of autoantibodies, leading to the formation of oxLDL-containing immune complexes (oxLDL-IC). Isolated human anti-oxLDL antibodies are predominantly of the IgG isotype and are directed against various epitopes in oxLDL. In practice, these antibodies are considered markers for oxLDL, but their role in the development and progression of atherosclerosis has remained controversial to date. The aim of this study was to determine serum levels of human class G antibodies against oxLDL (IgG anti- oxLDL antibodies) in patients with ST-segment elevation acute myocardial infarction (STEMI) and to investigate their association with low-density lipoprotein (LDL) and high-density lipoprotein (HDL) risk phenotypes. IgG anti-oxLDL antibodies were determined in 69 STEMI patients and 67 healthy volunteers using a commercially available ELISA kit (OLAB IgG Anti Oxidized Low Density Lipoprotein, Biomedica Medizinprodukte GmbH & Co KG, A-1210 Vienna, Austria). The separation of HDL

udeli četiri LDL subklase (LDL I, LDL II, LDL III, LDL IV) i dve glavne HDL subklase (HDL 2, HDL 3). Kriterijum za definisanje rizičnog LDL B fenotipa i fenotipa malih HDL čestica bio je dominantni prečnik LDL čestica ≤ 25,5 nm, odn. dominantni prečnik HDL čestica ≤ 8,8 nm. Istovremeno, primenom standardnih biohemijskih metoda određeni su i drugi biohemijski parametri (glukoza, trigliceridi, ukupni holesterol, HDL-holesterol i LDL-holesterol). Rezultati ove studije nisu pokazali postojanje statistički značajne razlike u koncentracijama IgG anti-oxLDL antitela između STEMI bolesnika i zdravih kontrolnih ispitanika ($p>0,05$). Međutim, nađeno je da su kontrolni ispitanici sa povećanim udelom malih HDL i LDL čestica imali više koncentracije IgG anti-oxLDL antitela ($p<0,05$), što sugeriše da se oksidativna modifikacija LDL čestica i posledično povećana produkcija IgG anti-oxLDL antitela lakše dešavaju kod zdravih osoba sa nepovoljnim lipidnim profilom. Istovetna analiza kod STEMI bolesnika nije otkrila razlike u koncentracijama IgG anti-oxLDL antitela ($p>0,05$), što bi se moglo objasniti naknadnim formiranjem nerastvorljivih imunskih kompleksa koji sadrže oxLDL i njihovim deponovanjem u tkivima. Dobijeni rezultati podržavaju hipotezu dualističke uloge IgG anti-oxLDL antitela, odnosno mogućnost da ova antitela imaju zaštitnu ulogu kod zdravih osoba i proaterogenu ulogu u uznapredovaloj aterosklerozi karakterističnoj za STEMI.

and LDL subclasses was performed by polyacrylamide gradient gel electrophoresis. In addition, the relative proportions of four LDL subclasses (LDL I, LDL II, LDL III and LDL IV) and two main HDL subclasses (HDL 2 and HDL 3) were determined. LDL phenotype B was defined when the dominant LDL particle diameter was ≤ 25.5 nm. Similarly, the predominant HDL particle diameter ≤ 8.8 nm was the criterion for defining a small-format HDL phenotype (HDL 3 phenotype). At the same time, other biochemical parameters (glucose, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low- density lipoprotein cholesterol (LDL-C)) were measured using standard biochemical methods. The results showed no significant difference in IgG anti-oxLDL antibody levels between patients and the control group ($p>0.05$). However, our study provided the interesting finding that control subjects with a higher proportion of small HDL and LDL particles had increased IgG anti-oxLDL antibody levels ($p<0.05$), suggesting that oxidative modifications of LDL and the consequent increased production of IgG anti-oxLDL antibodies occur more readily in healthy individuals with an unfavorable lipid profile. The same analysis in STEMI patients revealed no differences in IgG anti-oxLDL antibody levels ($p>0.05$), which could be explained by the subsequent formation of insoluble immune complexes, oxLDL-IC, which are deposited in the tissue. The results obtained support the concept of the dualistic role of IgG anti-oxLDL antibodies, i.e. the possibility that these antibodies play a protective role in healthy individuals and a proatherogenic role in advanced atherosclerosis, as is the case in our STEMI group.

P025
PREDNOSTI, NEDOSTACI I PRIMENE RAZLIČITIH TEHNIKA PCR TEHNOLOGIJE

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Osnovna analiza lančane reakcije polimeraze (PCR) pored konvencionalne PCR je reakcija plus/minus test, gde analize pokazuju prisustvo ili odsustvo PCR amplifikacije fragmenta molekula DNK. »Real time« PCR može pratiti amplifikaciju PCR proizvoda u »stvarnom vremenu« pomoću određenih fluorogenih signala. Dve najpoznatije metode su SYBR green, koja je zapravo fluorescentna boja koja se interkalarno veže na DNK, i TaqMan sistem sondi, koji se sastoji od oligonukleotidne sonde koja je komplementarna određenom delu DNK od interesa. Cilj ove studije je da se procene, uporede i definišu karakteristike konvencionalne PCR sa SYBR green

P025
ADVANTAGES, DISADVANTAGES AND APPLICATION OF DIFFERENT TECHNICS IN PCR TECHNOLOGY

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The basic analysis beside the conventional PCR reaction is a plus/minus assay, where the analyses indicates the presence or absence of PCR amplification of a fragment of a DNA molecule. Real-time PCR can monitor the amplification of the PCR product in »real time« with certain fluorogenic reporters. Two the most well-known reporters are SYBR green, which is actually a fluorescent dye that intercalarily binds to DNA, and the TaqMan probe system, which consists of an oligonucleotide probe that is complementary to a certain part of the DNA of interest. The aim of this study was to evaluate, compare and define the specifies of conventional PCR with SYBR green real-time PCR

PCR analizama u stvarnom vremenu i TaqMan sistemom sondi. SYBR green u stvarnom vremenu ima nekoliko nedostataka i prednosti u poređenju sa TaqMan sistemom sonde. Najveća prednost SYBR green metode je što se u njoj može koristiti bilo koji par prajmera, s time da se prajmeri mogu prilagoditi PCR tehnologiji u realnom vremenu. Takođe, metoda je mnogo robusnija i finansijski isplativija od TaqMan sistema sonda. Nedostatak ove metode povezuje se sa manjom specifičnošću od TaqMan sondi koje su usko vezane za određeni deo DNK. Zato je potrebno izvršiti analizu krive topljenja nakon PCR-a u stvarnom vremenu sa SYBR green metodom. SYBR green je relativno isplativ i jednostavan za korišćenje i zasniva se na vezivanju fluorescentne boje na dvolančanu DNK. TaqMan metoda je skuplja i zasniva se na dvostruko obeleženom oligonukleotidu i egzonukleaznoj aktivnosti enzima Taq polimeraze. Specifičnost je najvažnija kod upotrebe bilo koje nespecifične boje za vezanje DNA kao što je SYBR green, međutim više specifičnosti pokazuje metoda označenih oligonukleotida kao što je TaqMan. Rezultati ove studije ukazuju da su razvijeni konvencionalni PCR i SYBR green PCR testovi u stvarnom vremenu visoke osetljivosti, specifičnosti, tačnosti i da se mogu primeniti kao efikasan alat za skrining u različitim područjima laboratorijske dijagnostike.

P026
SKRINING ZA MIKROALBUMINURIJU

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Mikroalbuminurija predstavlja izlučivanje malih količina albumina u urin, i to između 30 i 300 mg/24h. Studija je sprovedena kao početna faza skrininga u kojoj su odabrani oni pacijenti koji su imali mikroalbuminuriju u jednom uzorku urina. Pacijenati su birani na osnovu radne dijagnoze na uputu koji su sa sobom poneli prilikom dolaska u Centar za laboratorijsku dijagnostiku JZU Doma zdravlja Herceg Novi, od oktobra 2023. godine do aprila 2024. godine, a birani su oni sa hipertenzijom, Diabetes Mellitus-om, kardiovaskularnim oboljenjma, ili samo na osnovu godišta (rođeni pre 1960. godine) onda kada radna dijagnoza nije upućivala ni ja jedan od navedenih poremećaja. Uzorak izbora je bio prvi jutarnji urin. Mirkoalbuminurija u uzorcima je određena korišćenjem test traka. Analizirano je 550 uzoraka od kojih je 118 (21,45%) imalo pozitivan rezultat na prisustvo albumina u urinu, što je novootrkivena disfunkcija bubrega. U 71 uzorku je vrednost mikroalbuminurije bila preko 20 mg/L, u 29 uzoraka od 50 do 100 mg/L, a u 18 uzoraka vrednost je bila preko 100

assays and TaqMan probe system. Real-time SYBR green has several disadvantages and advantages compared to the TaqMan probe system. The biggest advantage of the SYBR green is that any pair of primers can be used in this method, with the fact that the primers can be be adapted to real-time PCR technology. Also, the method is much more robust and financially profitable than the TaqMan assay system. The disadvantage of this method is linked to the advantage, because the method is less specific than TaqMan probes that are narrowly bound to a certain part of the DNA. Therefore, it is necessary to perform melting curve analysis after real-time PCR with SYBR green method. SYBR Green is relatively cost benefit and easy to use and technically based on binding the fluorescent dye to double stranded deoxyribonucleic acid (dsDNA) where TaqMan method has more expensive and based on dual labeled oligonucleotide and exonuclease activity of Taq polymerase enzyme. Specificity is the most important concern with the usage of any non- specific dsDNA-binding Dyes such as SYBR green whiles more specificity showed by labeled oligonucleotide method such as TaqMan. The results of this study indicated that the developed conventional PCR and SYBR green real-time PCR assays are high sensitivity, specificity, accuracy and could be applied as an effective screening tool in different field of laboratory diagnostics.

P026
MICROALBUMINURIA SCREENING

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Microalbuminuria is the excretion of small amounts of albumin in the urine, between 30 and 300 mg/24h. The study was conducted in an initial screening phase in which patients with microalbuminuria were selected in a single urine sample. Patients were selected on the basis of the working diagnosis they brought with them when they visited the Center for Laboratory Diagnostics of the JZU of the Herceg Novi Health Center from October 2023 to April 2024, and those with hypertension, diabetes mellitus, cardiovascular disease or only on the basis of age (born before 1960) were selected if the working diagnosis did not indicate one of the mentioned diseases. The sample of choice was the first morning urine. The microalbumin in the samples was determined using test strips. 550 samples were analyzed, of which 118 (21.45%) gave a positive result for the presence of albumin in the urine, being newly detected renal dysfunction. 71 samples had a microalbuminuria value of over 20 mg/L, 29 had a microalbuminuria value of 50 to 100 mg/L and 18 patients

mg/L. Svaka grupa je podeljena prema starosti (31 do 50, 51 do 65 i preko 65) i polu. Većina pacijenata je u ranoj fazi oštećenja bubrega kada bi trebalo da se preduzmu korektivne mere za očuvanje funkcije buubrega. Test je potrebno ponoviti za 3 do 6 meseci, a ukoliko se i tada nađe pozitivan nalaz potrebno je korigovati antihipertenzivnu i antidijabetičnu terapiju, redovno kontrolisati krvni pritisak, smanjiti telesnu masu i dnevni unos proteina.

P027
POVEZANOST EKSPRESIJA MIR-34A I TOLL-LIKE RECEPTORA 9 I 4 SA GOJAZNOŠĆU

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Mikro ribonukleinska kiselina 34a (miR-34a) i Toll-like receptori (TLR) 9 i 4 u dosadašnjim radovima bili su povezivani sa bolestima jetre, insulinskom rezistencijom i oksidativnim stresom. miR-34a je uključena u poremećaj metabolizma holesterola, pri čemu joj je ciljno mesto dejstva jetrena nikotinamid-adenin-dinukleotid – deacetilaza, Sirtuin 1, koja je uključena u modulaciju apoptoze ćelija jetre, metaboličke bolesti i nastanak karcinoma. Aktivacija TLR 9 i 4 urođenim imunološkim odgovorom promoviše inflamaciju u adipoznom tkivu kod gojaznih. Cilj našeg rada bio je da ispitamo povezanost ekspresija miR-34a i informacione ribonukleinske kiseline (iRNK) TLR 9 i 4 sa gojaznošću kao jednim od poremećaja koji je povezan sa svim gore navedenim komorbiditetima. Kontrolnu grupu (KG) činilo je 116 ispitanika, dok je gojaznih ispitanika bilo 46. Nivoi miR-34a određivani su u plazmi osiromašenoj trombocitima, a nivoi iRNK TLR9 i TLR4 u mononuklearnim ćelijama periferne krvi kvantitativnom lančanom reakcijom polimerizacije. Gojazni pacijenati su imali značajno viši nivo miR-34a (p=0,006) i značajno niže nivoe iRNK LR9 (p=0,003) i iRNK LR4 (p=0,022) nego ispitanici KG. Pokazana je statistički značajna negativna korelacija nivoa miR-34a i ITM-a, a pozitivna korelacija sa nivoima iRNK TLR 9 i 4. Takođe, utvrđena je i pozitivna korelacija između nivoa iRNK TLR 9 i 4. Binarnom logističkom regresionom analizom pokazana je pozitivna asocijacija nivoa miR-34a sa

had a value of over 100 mg/L. Each group was subdivided according to age (31 to 50, 51 to 65 and over 65) and gender. Most patients are in the early stages of kidney damage, when corrective measures should be taken to preserve kidney function. It is necessary to repeat the test in 3 to 6 months, and if a positive result is found even then, it is necessary to correct antihypertensive and antidiabetic therapy, control blood pressure regularly, reduce body weight and daily protein intake.

P027
ASSOCIATION OF MIR-34A AND TOLL-LIKE RECEPTORS 9 AND 4 WITH OBESITY

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Micro ribonucleic acid 34a (miR-34a) and Toll-like receptors (TLR) 9 and 4 have been associated with liver diseases, insulin resistance, and oxidative stress in previous studies. miR-34a is involved in cholesterol metabolism disorder, targeting hepatic nicotinamide adenine dinucleotide – deacetylase, Sirtuin 1, which is implicated in the modulation of liver cell apoptosis, metabolic diseases, and carcinogenesis. The activation TLR 9 and 4 through innate immune response promotes inflammation in adipose tissue in obese individuals. Our study aimed to investigate the association between the expression of miR-34a and TLR 9 and 4 messenger ribonucleic acid (mRNA) with obesity as a disorder associated with the above-mentioned comorbidities. The control group (CG) consisted of 116 participants, while there were 46 obese participants. MiR-34a levels were determined in platelet-poor plasma, and TLR9 and TLR4 mRNA levels in peripheral blood mononuclear cells by quantitative polymerase chain reaction. Obese patients had significantly higher miR-34a levels (p=0.006), significantly lower TLR9 mRNA (p=0.003) and TLR4 mRNA (p=0.022) than participants from CG. A significant negative correlation was found between miR-34a and BMI, and a positive correlation with TLR 9 and 4 mRNA levels. Additionally, a positive correlation was found between TLR 9 and 4 mRNA levels. Binary logistic regression analysis showed a positive association between miR- 34a levels and BMI

ITM-om (OR=1,153 (1,046–1,271); p=0,004) i negativna asocijacija nivoa iRNK LR9 sa ITM-om (OR=0,914 (0,844–0,989); p=0,026), što znači da se sa povećanjem ITM-a povećava i nivo miR-34a, a da se nivo iRNK LR9 povećava sa smanjenjem ITM-a. U našoj studiji nije dokazana značajna povezanost iRNK LR4 sa ITM-om.

P028
PREDIKTIVNE KARAKTERISTIKE SPECIFIČNIH CITOKINA KOD PACIJENATA NA HEMODIJALIZI

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IL1-Ra i TNF-α su proinflamatorni citokini koji pokazuju porast koncentracije kod pacijenata na hemodijalizi, u odgovoru na inflamaciju. Uprkos napretku tehnologije, pacijenti na hemodijalizi i dalje imaju značajan kratkoročni i dugoročni morbiditet povezan za dijalizom. Glavni cilj studije je da ispita da li IL1-Ra i TNF-α mogu biti prediktori mortaliteta kod pacijenata na hemodijalizi, ali i uticaj različitih faktora na stepen preživljavanja. U studiji je učestvovalo 45 pacijenta sa različitim uzrocima hronične bubrežne bolesti. Koncentracije IL1-Ra i TNF-α određene su ELISA ImmunoAssay metodom, a statistički podaci obrađeni u programu PASW Statistics 18. Koncentracije oba ispitivana parametra bile su značajno veće kod preminulih u odnosu na žive pacijente (P<0,05). Trajanje dijalize bilo je značajno duže kod preminulih pacijenata, dok su vrednosti BMI na granici statističke značajnosti. Određeni su i biohemijski parametri, od kojih je samo LDH pokazao statistički značajno povećanje koncentracije kod preminulih. Primenjena Kaplan-Majerova analiza preživljavanja je pokazala da su visoke koncentracije TNF-α (>5 pg/mL) statistički značajni prediktori kraćeg preživljavanja kod pacijenata na hemodijalizi (LogRank=4,21, P=0,040). Rezultati studije su pokazali da su proinflamatorni citokini TNF-α i antagoniste receptora za IL1 (IL1-Ra) značajni prediktori mortaliteta kod pacijenata na hemodijalizi.

(OR=1.153 (1.046–1.271); p=0.004) and a negative association between TLR9 mRNA levels and BMI (OR=0.914 (0.844–0.989); p=0.026), indicating that an increase in BMI is associated with an increase in miR-34a levels, while increase in BMI is associated with decrease in TLR9 mRNA levels. Our study did not demonstrate a significant association between TLR4 mRNA and BMI.

P028
PREDICTIVE CHARACTERISTICS OF SPECIFIC CYTOKINES IN HEMODIALYSIS PATIENTS

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IL1-Ra and TNF-α are proinflammatory cytokines that show increased concentrations in hemodialysis patients in response to inflammation. Despite technological advances, hemodialysis patients continue to suffer from significant short- and long-term dialysis-related morbidity. The main objective of the study is to investigate whether IL1-Ra and TNF-α can be predictors of mortality in hemodialysis patients, but also the influence of various factors on survival. The study involved 45 patients with various causes of chronic kidney disease. The IL1-Ra and TNF-α concentrations were determined using the ELISA-ImmunoAssay method, and the statistical data were processed using the PASW Statistics 18 program. The concentrations of the two parameters studied were significantly higher in deceased compared to living patients (P<0.05). The duration of dialysis was significantly longer in deceased patients, while BMI values are at the limit of statistical significance. Biochemical parameters were also determined, of which only LDH showed a statistically significant increase in concentration in the deceased. The applied Kaplan-Meier survival analysis showed that high concentrations of TNF-α (>5 pg/mL) and are statistically significant predictors of shorter survival in hemodialysis patients (Log Rank=4.21, P=0.040). The results of the study show that the proinflammatory cytokines TNF-α and IL1 receptor antagonists (IL1-Ra) are significant predictors of mortality in hemodialysis patients.

P029
POVEZANOST VITAMINA D I PARAMETARA OKSIDATIVNOG STRESA KOD OBOLELIH OD MULTIPLE SKLEROZE

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Niske koncentracije vitamina D su faktor rizika za nastanak multiple skleroze (MS). Oksidativni stres (OS) ima značajnu ulogu u patogenezi MS. Cilj studije je bio ispitivanje povezanosti vitamina D i parametara OS kod obolelih od multiple skleroze. U studiji je učestvovalo 178 pacijenata obolelih od relapsno-remitentnog oblika multiple skleroze, 117 u fazi remisije i 61 u relapsu bolesti. 112 pacijenata je primalo suplementaciju vitaminom D, dok je 66 bilo bez suplementacije. Od parametara OS statusa su analizirani prooksidansi (proteinski produkti uznapredovale oksidacije, prooksidativno-antioksidativni balans, malondialdehid i azot-monoksid) i antioksidansi (superoksid-dizmutaza, katalaza, totalni antioksidativni status, paraoksonaza 1, ukupne sulfhidrilne grupe, ukupni bilirubin, mokraćna kiselina, feritin i transferin), u serumu i u heparinizirаnoj plazmi, metodom spektrofotometrije. Vitamin D, u obliku 25(OH)D, je kvantifikovan hemiluminiscentnim imunoodređivanjem na mikročesticama (CMIA), u serumu. Utvrđena je pozitivna Pirsonova korelacija vitamina D sa azot-monoksidom ($r=0,289$, $p<0,05$) i negativna korelacija vitamina D i malondialdehida ($r=-0,282$, $p<0,05$) u MS grupi bez suplementacije vitaminom D. Spirmanovom korelacionom analizom vitamina D i parametara OS kod MS ispitanika na suplementaciji vitaminom D utvrđena je statistički značajna negativna korelacija logaritmovane vrednosti vitamina D i uznapredovalih proizvoda oksidacije proteina ($\rho=-0,243$, $p<0,01$). Vitamin D smanjuje lipidnu peroksidaciju i povećava azot monoksid pacijentima bez suplementacije, istovremeno suprimirajući oksidaciju proteina kod obolelih od MS koji su na suplementaciji vitaminom D. Protektivna uloga vitamina D kod obolelih od MS je posledica i antioksidativnog dejstva, koje vitamin D ostvaruje mehanizmom strukturne mimikrije sa holesterolom i inhibicijom ekspresije receptora za krajnji proizvod glikacije proteina.

P029
RELATIONSHIP BETWEEN VITAMIN D AND PARAMETERS OF OXIDATIVE STRESS IN PATIENTS WITH MULTIPLE SCLEROSIS

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Vitamin D deficiency is a known risk factor for multiple sclerosis (MS). Oxidative stress (OS) is associated with the pathophysiology of MS. The aim of the study was to investigate the relationship between vitamin D and oxidative stress parameters in MS patients. 178 patients with relapsing- remitting MS were included in the study, 117 in remission and 61 in the relapse phase of the disease. 112 patients received vitamin D supplementation, while 66 did not. We analyzed prooxidants (advanced oxidation protein products, prooxidant-antioxidant balance, malodialdehyde and nitric oxide) and antioxidants (superoxide dismutase, catalase, total antioxidant status, paraoxonase 1, sulfhydril groups, total bilirubin, uric acid, ferritin and transferrin) in serum and heparinized plasma by spectrophotometry. Vitamin D, as 25(OH)D, was analyzed in serum samples by chemiluminescence microparticle immunoassay (CMIA). After performing person-to-person correlation, we found that vitamin D correlated positively with nitric oxide concentration ($r=0.289$, $p<0.05$) and negatively with malondialdehyde ($r=-0.282$, $p<0.05$) in the MS group without vitamin D supplementation. On the other hand, Spearman correlation revealed a statistically significant negative correlation between vitamin D and the logarithmic value of advanced protein oxidation products ($\rho=-0.243$, $p<0.01$) in MS patients who received vitamin D supplementation. Vitamin D decreased lipid peroxidation and increased nitric oxide in patients without supplementation, while the highest protein oxidation occurred in MS patients with vitamin D supplementation. The protective role of vitamin D in MS may be due to its antioxidant effect as a result of structural mimicry with cholesterol and higher than average expression of Receptor for Advanced Glycation End Product.

P030
UTICAJ PREGESTACIONOG INDEKSA TELESNE MASE NA SFINGOLIPIDNI PROFIL

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Sfingolipidi su strukturne komponente bioloških membrana, ulaze u sastav lipoproteinskih čestica i učestvuju u međućelijskoj signalizaciji kao sekundarni glasnici. U trudnoći imaju fiziološku ulogu, ali mogu biti povezani i sa razvojem komplikacija, pre svega preeklampsijom. U ovom istraživanju ispitivali smo promene sfingolipidnih parametara kod trudnica sa prekomernom težinom i gojaznošću pre trudnoće. Studija je obuhvatila 131 trudnicu, 99 sa indeksom telesne mase pre trudnoće (ITM) <25 kg/m^2 i 32 sa ITM≥25 kg/m^2. Koncentracije sfingolipida (sfingomijelina, sfingozina, sfinganina, sfingozin-1-fosfata, sfinganin-1-fosfata, ceramida C16:0 i ceramida C24:0) određene su HPLC-MS/MS metodom, nakon pripremnog koraka ekstrakcije uzorka. Uočene su značajno niže koncentracije sfingomijelina tokom drugog i trećeg trimestra u grupi trudnica sa povećanim ITM pre trudnoće (P<0,05). Uočena je pozitivna korelacija između koncentracije ceramida i težine novorođenčadi majki koje su pre trudnoće imale prekomernu telesnu masu ili su bile gojazne (P<0,05). Sa druge strane, nivo sfingozin-1-fosfata negativno je korelirao sa dužinom novorođenčadi majki sa povećanim ITM (P<0,05), dok je u grupi žena koje su imale normalnu telesnu masu pre trudnoće ova korelacija bila pozitivna (P<0,05). Rezultati ove studije pokazali su da pregestaciona prekomerna težina i gojaznost dovode do promena u profilu sfingolipidnih parametara, što dalje može imati uticaj i na karakteristike novorođenčadi.

P030
IMPACT OF PREGESTATIONAL BODY MASS INDEX ON THE SPHINGOLIPID PROFILE

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Sphingolipids are structural components of biological membranes, they are components of lipoprotein particles and participate in intercellular signaling as secondary messengers. They have a physiological role in pregnancy, but can also be associated with the development of complications, primarily preeclampsia. In this research, we examined changes in sphingolipid parameters in pre-pregnancy obese and overweight women. The study included 131 pregnant women, 99 with pre-pregnancy body mass index (BMI) <25 kg/m^2 and 32 with BMI≥25 kg/m^2. Concentrations of sphingolipids (sphingomyelin, sphingosine, sphinganine, sphingosine-1-phosphate, sphinganine-1-phosphate, ceramide C16:0 and ceramide C24:0) were determined by the HPLC-MS/MS procedure, after a preparatory sample extraction step. Significantly lower levels of sphingomyelin were recorded during the second and third trimesters in the group of pregnant women with increased BMI before pregnancy (P<0.05). A positive correlation was observed between the concentration of ceramide and the weight of newborns of mothers who were overweight or obese before pregnancy (P<0.05). On the other hand, the level of sphingosine-1-phosphate negatively correlated with the length of newborns of mothers with increased BMI (P<0.05), while in the group of pregnant women who started their pregnancies with healthy weight this correlation was positive (P<0.05). The results of this study imply that pregestational overweight and obesity can lead to changes in gestational sphingolipid profile, which can further affect neonatal characteristics.

P031
ZNAČAJ ISPITIVANJA VELIČINE I PROTEOMA HDL ČESTICA TOKOM TRUDNOĆE

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Veličina i sastav HDL proteoma mogu biti značajno izmenjeni u stanjima praćenim inflamacijom, razvojem oksidativnog stresa i dislipidemije, pa i u trudnoći sa komplikacijama. Cilj rada je bio da se ispita povezanost veličine i sastava proteoma HDL čestica sa ishodom trudnoće. U istraživanju je učestvovalo 107 trudnica, pri čemu je 87 imalo normalnu trudnoću, a 20 trudnoću sa komplikacijama. Koncentracija HDL-holesterola je određena rutinskom enzimskom metodom, a apoA-I imunoturbidimetrijski na automatskom analizatoru. Veličina i raspodela HDL subfrakcija su određene metodom vertikalne elektroforeze na poliakrilamidnom gradijent gelu. Koncentracije PON-1, SAA i MCP-1 su određene ELISA metodom. Trudnice sa optimalnim koncentracijama HDL-holesterola u prvom trimestru imale su veći udeo HDL 2b subfrakcija ($P<0,05$), a manji udeo HDL 3b čestica ($P<0,05$). U ovoj grupi trudnica su takođe bile značajno više koncentracije apoA-I i PON-1 ($P<0,01$), a niža koncentracija MCP-1 ($P<0,01$). U drugom trimestru, kod trudnica bez komplikacija udeo HDL 2b subfrakcija je bio značajno viši ($P<0,05$), kao i koncentracija apoA-I, PON-1 i SAA ($P<0,01$), dok su udeo HDL 3a subfrakcija i koncentracija MCP-1 bili značajno niži ($P<0,01$). Ove promene nisu utvrđene u grupi trudnica koje su razvile komplikacije. Dobijeni rezultati ukazuju na značaj ispitivanja veličine i HDL proteoma za prognozu ishoda trudnoće, kao i rizika za razvoj kardiovaskularnih oboljenja kasnije tokom života.

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SIGNIFICANCE OF HDL PARTICLES SIZE AND PROTEOME EVALUATION IN PREGNANCY

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HDL size and proteome composition may be significantly altered under conditions associated with inflammation, oxidative stress and dyslipidemia, such as pregnancy with complications. Our aim was to investigate the association between HDL size and proteome composition and pregnancy outcome. 107 women were included in the study, 87 of whom had a normal pregnancy and 20 of whom had a pregnancy with complications. The concentration of HDL-cholesterol was determined by a routine enzymatic method and that of apoA-I by immunoturbidimetry on an automated analyzer. The size and distribution of HDL subfractions were determined by vertical polyacrylamide gradient gel electrophoresis. The concentrations of PON-1, SAA and MCP-1 were determined by ELISA. Pregnant women with optimal HDL cholesterol concentrations in the first trimester had a higher proportion of HDL 2b ($P<0.05$) and a lower proportion of HDL 3b ($P<0.05$) subfractions. This group was also characterised by significantly higher levels of apoA-I and PON-1 ($P<0.01$) and lower levels of MCP-1 ($P<0.01$). In the second trimester, the percentage of HDL-2b subfractions was significantly higher in the pregnant women without complications ($P<0.05$), as was the concentration of apoA-I, PON-1 and SAA ($P<0.01$), while the percentage of HDL-3a subfractions and the concentration of MCP-1 were significantly lower ($P<0.01$). These changes were not found in the group of pregnant women who developed complications. The results indicate the importance of HDL size and proteome assessment in predicting pregnancy outcome as well as the risk of developing cardiovascular disease later in life.